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Symposium 1: Organelle biogenesis

Immunolocalization and biosynthesis of the oligosaccharide processing enzyme glucosidase II in hepatocytes

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Glucosidase II cleaves the two inner α 1,3-linked glucose residues on the oligosaccharide precursor chains of N-glycosylated glycoproteins. By immunoelectron microscopy, the enzyme was found in the lumen of the rough and smooth endoplasmic reticulum. Golgi apparatus cisternae were not labeled but smooth-surfaced vesicles in the proximity of the cisternal stack contained labeling. These findings are in harmony with biochemical data on the biosynthesis of glucosidase II. It is synthesized as a larger precursor containing a cleavable signal sequence. Glucosidase II is a hydrophilic glycoprotein of high mannose – type loosely associated with the inner face of the membrane. Its half-life of 50 min is surprisingly short. The enzyme is most probably degraded in autophagosomes.

Energetics of mitochondrial protein import

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Translocation of proteins across both procaryotic and eucaryotic membranes requires energy. This energy can be supplied by a transmembrane potential, in form of ATP or by a combination of both. The import of proteins into mitochondria has been shown to depend on an energized inner membrane (Gasser et al., J. biol. Chem. *257* (1982) 13034; and Pfanner and Neubert, EMBO J. *4* (1985) 2819).

We now find that in addition to the membrane potential external ATP is required to import a purified precursor protein (Eilers and Schatz, Nature *322* (1986) 228) into isolated yeast mitochondria. Non-hydrolyzable analogues of ATP do not support import of this protein.

Experiments are under way to learn more about the role of ATP during this protein transport.

Degradation of the precursor of mitochondrial aspartate aminotransferase in chicken embryo fibroblasts

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The higher MW precursor of mitochondrial aspartate aminotransferase (pre-mAspAT) accumulates under conditions of import block in the cytosol of cultured chicken embryo fibroblasts (CEF). However, its accumulation is limited by rapid degradation ($t_{½} \sim 5$ min; see J. biol. Chem. *257* (1982) 13334). The degradation was almost completely prevented by thiolspecific reagents. Inhibitors of serine proteases had little effect whereas specific inhibitors of cysteine proteases or intralysosomal proteolysis decreased the rate of degradation by ~ 30%. On depleting CEF of ATP, degradation was lowered by ~ 70%. Metal-depleted cells showed a degradative activity of 10% of the initial value which was increased to 24 or 107% on reconstitution with Ca^{2+} or Mg^{2+}, respectively. Apparently, the bulk of accumulated pre-mAspAT in CEF is degraded by a presumably cytosolic protease dependent on Mg·ATP as well as nonactive site thiol groups; the rest of pre-mAspAT seems to be degraded by intralysosomal cysteine proteases.

Role of the Golgi apparatus in the transport of human intestinal microvillar hydrolases to the cell surface

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Newly synthesized microvillar peptidases (e.g. dipeptidylpeptidase IV) and disaccharidases (e.g. sucrase-isomaltase) are known to migrate to the cell surface at different rates in the intestinal human adenocarcinoma cell line Caco-2. The asynchronism was found to be due to the endoplasmic reticulum to trans-Golgi pathway (Hauri et al., J. Cell Biol. *101* (1985) 838). To further dissect this asynchronous transport we have now purified Golgi membranes from Caco-2 cells by a novel procedure. Pulse-chase experiments suggest (1) that the rate-limiting step for efficient vectorial hydrolase transport is the maturation in and the transit through the Golgi apparatus and that (2) the Golgi compartment constitutes a pool of newly synthesized hydrolases (disaccharidases and peptidases) that is not immediately expoerted to the cell surface. The Golgi apparatus may thus regulate the expression of brush border hydrolases at a posttranslational level.

The translocation of polyoma virus middle T antigen from the cytoplasm to cellular membranes

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Middle T antigen (mT) is one of the early gene products of Polyoma virus (Py) and capable of transforming established tissue culture cells. mT associates with the inner face of the plasma membrane through a stretch of 22 hydrophobic amino-acids located close to the carboxyterminus. Membrane insertion does not require an aminoterminal leader sequence and passage through the ER and Golgi. mT associates with $pp60^{c\text{-}src}$, a kinase specific for tyrosine residues, in the plasma membrane. Complex formation leads to activation of $pp60^{c\text{-}src}$ and is well correlated with cell transformation by Py. We studied the synthesis and membrane insertion of mT in vitro. mT was cloned into a SP6 expression vector and translated in a wheat germ lysate followed by insertion of the protein into ER or plasma membrane fractions. We found that wild type mT but not a mutant form lacking the membrane anchor associates with membranes. When the mT gene is supplemented with a leader peptide sequence derived from the hemagglutinin gene of influenza virus, a fraction of the protein product is routed through the ER and Golgi and is glycosylated. Most of the material still associates with $pp60^{c\text{-}src}$ in the plasma membrane and is not glycosylated.

Novel photoactivatable fluorescent dyes designed for studies of the intracellular membrane traffic

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Fluorescent dyes are widely used as fluid phase markers for endocytosis and related intracellular traffic. We now have developed two photoactivatable derivatives of the water soluble dye Lucifer Yellow CH which, upon illumination become covalently bound to biological targets. This opens new experimental possibilities. To demonstrate covalent binding of these derivatives to membranes, human erythrocyte ghosts were used as a model system. Following photoactivation of the reagents in the presence of these membranes, labeling of proteins and lipids could be easily detected upon SDS polyacrylamide gel electrophoresis. The membrane associated dye could also be clearly demonstrated by conventional fluorescence microscopy. In addition, we were able to trace the membrane bound Lucifer Yellow derivatives by electron microscopy using immuno-gold labeling.

Conformational states of a precursor fusion protein in translocation across mitochondrial membranes

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Recent evidence has suggested that tightly folded proteins cannot be translocated across biological membranes. An unfolded or loosely folded state may be necessary for this event to occur. In our laboratory, a fusion protein consisting of the presequence of subunit IV of cytochrome oxidase (a mitochondrial protein) and dihydrofolate reductase (a cytosolic protein) is available to study the conformation of proteins before and during the process of translocation into the mitochondrion. We are currently using protease digestion sensitivity as a marker for the foldedness of this fusion protein during translocation.

An in vitro modelsystem for exocytosis: II. Inhibition

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Stimulus evoked exocytosis of hormones and neurotransmitters is initiated by a receptor mediated calcium influx leading to the fusion of the storage vesicles with the plasma membrane (PM). In order to evaluate this process at the molecular level we established an in vitro release system using bovine adrenal chromaffin cell (CC) membranes. Chromaffin granules (CG) specifically interact with CC plasma membranes pretreated with phospholipase A_2 (PLA_2) to release their content upon elevating the free calcium concentration to the micromolar level, as demonstrated in a preceding poster.

Here we show that incubation of the PM with proteases or the akylating agent N-ethylmaleimide leads to an almost complete inhibition of hormone release. Two PLA_2 blockers, P-bromophenacylbromide and mepacrine, as well as Na_2CO_3 treatment, known to release peripheral membrane proteins, are all potent inhibitors, too. Our experiments indicate that the action of PLA_2 is necessary but not sufficient for hormone release; another membrane associated protein seems to be involved as well, either in the recognition process of the two membranes or in fusion itself.

Protein transport to the cell surface: effect of N-methyl-1-deoxynojirimycin (MDNM)

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Caco-2, a differentiated intestinal adenocarcinoma cell line, can serve as a model to study migration of newly synthesized hydrolases to the brush border membrane. A fast transport, represented by dipeptidylpeptidase IV, and a slow transport, represented by sucraseisomaltase, have been uncovered previously (Hauri et al., J. Cell Biol. *101* (1985) 838). In the present study we have investigated the role of carbohydrate trimming for these processes using the glucosidase I inhibitor MDNM in conjunction with subcellular fractionation and the pulse-chase technique. The results suggest that MDNM (1) does not inhibit transport to the Golgi apparatus, (2) has no effect on the asynchronous transport, but (3) reduces the efficiency of the Golgi-to-brush border pathway by 60–70%.

Transferrin receptor endocytosis requires an intact cytoplasmic domain but not phosphorylation sites

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Cellular iron uptake involves receptor-mediated endocytosis of transferrin. The structural features of the transferrin receptor (TR) that lead to its clustering within coated pits and endocytosis are not known. We have investigated whether the cytoplasmic domain of the receptor or serine phosphorylation sites within this domain are required for TR internalization. By site-directed mutagenesis we constructed human TR cDNAs having either a deletion of 36 of the 65 amino acids comprising the cytoplasmic domain or point mutations at five cytoplasmic serine residues. Wild type and mutant TR cDNAs were stably transfected into mouse L-cells. Cell lines with high numbers of human TR were isolated by cell-sorting and subcloning. Both the wild type human TR and serine TR mutants were as efficient as endogenous mouse TR in binding and internalizing transferrin. In contrast the deletion TR mutant bound transferrin normally but was not endocytosed, demonstrating that the cytoplasmic domain is required for receptor internalization.

Functional expression of the rabbit pIGR in a homologous epithelial cell line

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The polymeric immunoglobulin receptor transports IgA_2 across epithelial cells. We would like to identify the signals targetting the receptor correctly on its way through the polarized cells. The rabbit mammary gland cell line RMC-SV40 was transfected with the cDNA of the rabbit pIgR, inserted into the expression vector pSV2 and cotransfected with the hygromycin resistance gene. The stably transfected cells can be grown on Millipore filters and have a transepithelial resistance of 400–500 Ω/cm^2. The rbpIgR is expressed as a N-linked glycosylated and phosphorylated protein of 85 kD. It is cleaved and a 60 kD protein, the secretory component, is found in the apical medium. This system allows us to study the transcytosis of IgA_2. We analyzed the phosphorylated variants of the pIgR by 2-d gel electrophoresis and identified the phosphorylated amino acids. We are in the process of identifying the cellular compartments in which the receptor is phosphorylated during its routing in the polarized epithelial cell.

An in vitro modelsystem for exocytosis: I. Characterization and specificity

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Stimulation of bovine adrenal chromaffin cells leads to an increase in intracellular calcium triggering the fusion of hormone containing granules with the plasma membrane (PM). The elucidation of this exocytotic process is hampered by the inaccessability of the intracellular compartment in intact cells. An in vitro model system for the fusion of purified chromaffin granules with the plasma membrane has been briefly reported by Izumi et al. (FEBS Lett. *196* (1986) 349). Chromaffin granules release their content upon elevation of the calcium concentration to the micromolar level if incubated with chromaffin cell plasma membranes digested by phospholipase A_2. We could show that this release is time- and temperature-dependent and PM specific. Calcium induced leakage of hormones from the granules can be excluded bysed on the finding that liver cell membranes or a fraction of chromaffin cell membranes containing only a few plasma membrane impurities fail to induce any hormone release. This specificity might reflect the existence of receptors and/or fusion molecules on the chromaffin cell PM. The influence of various agents on this release system is discussed in an accompanying poster.

The *trans*-tubular network of the Golgi apparatus of hepatocytes and enterocytes

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We have investigated the *trans*-tubular network (TTN) of the Golgi apparatus in rat liver hepatocytes and human small intestinal enterocytes with respect to its structure and function. In both cell types, the TTN is continuous with two *trans* Golgi apparatus cisternae and forms a complex, extensive network. It can be seen to connect widely separated cisternal stacks, illustrating its extensive nature. Cytochemically, the TTN displays both acid phosphatase and thiamine pyrophosphatase activity. By immunocytochemistry, it was found to contain the terminal glycosyltransferases sialyl- and blood group A transferase, as well as their products, and secretory proteins. Collectively, these data demonstrate that the TTN is structurally and functionally an integral part of the Golgi apparatus and includes elements of the so-called GERL system.

Characterization of a mitochondrial assembly mutant

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Import of proteins into mitochondria is a keystep in the biogenesis of mitochondria. Most of the proteins are made as larger precursors in the cytoplasm and get cleaved upon import. To investigate the components of the cell necessary for this import temperature-sensitive mutants of *S. cerevisiae* were obtained that fail to import proteins into mitochondria. Isolated mitochondria from one of the mutants called 'masl' import in vitro synthesized precursors as efficiently as wild type mitochondria, yet fail to process them to their mature form. This suggests that the mutant is deficient either in the mitochondrial processing protease or in a component necessary for its activity. We now have cloned and sequenced a piece of DNA complementing the defect and raised antibodies against part of the protein encoded by that gene. With the help of these antibodies we hope to identify the role of this protein.

Allophenic chimeras of autoimmune MRL-*lpr/lpr* and normal mice

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Mice homozygous for the autosomal recessive mutation *lpr* develop massive lymphoproliferation, hypergammaglobulinemia, and autoimmunity. Outgrowth of cells of the T lymphocyte lineage with a surface phenotype unique to these mice accounts for most of the lymph node enlargement. To test whether genetically normal cells could 'cure' the disease or be recruited to participate in the lymphoproliferation when allowed to develop within the *lpr* environment, embryo aggregation chimeras were constructed between MRL/MpJ *lpr/lpr* and DBA/2 Ha (a 'wild type' strain with respect to lymphoproliferation). All 17 chimeras analyzed had massive lymphoproliferation. In all cases, including those in which > 90% of both the bone marrow cells and skin were of the DBA/2 Ha type, virtually all of the Thyl-bearing lymph node cells were of teh *lpr* type. These results suggest that the lpr gene directly controls the proliferating cells and does not function indirectly through a putative regulating cell.

Regulation of IL-2 receptor expression in an inducible rat x mouse T cell hybrid

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PC 60 is a hybrid between an IL-2 dependent mouse CTL and a rat thymoma. It proliferates independently of IL-2 and does not have detectable IL-2 receptors on its cell surface. When cultured in presence of IL-1 or IL-2, PC60 starts to express both rat and mouse IL-2 receptors as can be monitored with anti IL-2 Rec antibodies. A strong synergistic effect on IL-2 receptor expression can be observed when the cells are induced simultaneously with the IL-1 and IL-2.

The number of receptors at the surface correlates well with the level of IL-2 Rec mRNA, as detected by RNA blot analysis, which suggests a pretranslational control mechanism. The synergistic effect of IL-1 and IL-2 may be due to the fact that they influence the IL-2 Rec mRNA steady state level via different mechanisms. This is supported by the finding that the kinetics of appearance of mRNA induced by these two lymphokines is different.

Stimulation and detection of $\alpha_2$macroglobulin (α_2M) in the rat

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α_2M is an acute phase protein of the rat. It is produced as a consequence of monokine synthesis. We describe the isolation from rat serum after stimulation with LPS and the production of antibodies in the rabbit. In a longitudinal study we analysed, by means of immune-rocket-electrophoresis, the influence of different stimuli upon the synthesis of α_2M. In acute in in vivo tests (carrageenin paw edema, LPS fever) the protein is increased 5–10 times in 24–48 h resp. In adjuvant arthritis the level increases 40–60 times after 2 weeks and is maintained for at least 6 weeks. Rat strains which are refractory to adjuvant arthritis, do not produce significant amounts of α_2M. In arthritis produced by bovine collagen II the protein did not increase even when paw swelling had developed. IL-1 (recomb. human, Roche) showed a dose-dependent stimulation. Non-storoidal antiphlogistic drugs had no influence. Dexamethasone (a steroidal antiphlogistic) alone produced a signficant increase in 24 h.

Isolation and characterization of human platelet membrane CD9 antigen and glycoprotein 17

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CD9 and glycoprotein (GP) 17 are surface glycoproteins of human platelets with similar Mr and pI. CD9 is poorly glycosylated and is strongly labelled by iodination with ^{125}I whereas GP17 contains *N*-linked oligosaccharides and is poorly labelled by iodination. CD9 is found also in other cell-types particularly cALL cells whereas GP17 appears to be restricted to platelets. Platelets are activated by many antibodies to CD9 which appears to be involved in Ca^{2+} channels. CD9 was isolated from the hydrophobic phase of a Triton X-114 separation of platelets by affinity chromatography with a monoclonal antibody against CD9 and gel filtration. GP 17 forms part of the GPIb receptor complex and can be isolated from the aqueous phase of a Triton X-114 separation of platelets by wheat germ agglutinin affinity chromatography followed by gel filtration. Both glycoproteins have been investigated using proteolysis and labelling techniques to determine extracellular, membrane-bound and cytoplasmic domains.

Cloning of the gene coding for a human lymphocyte Fc_ε receptor

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The overproduction of immunoglobulins of the IgE isotype is a major cause of allergic disease. Lymphocytes bearing low-affinity IgE receptors ($Fc_\varepsilon R$) have been shown to secrete IgE-binding factors (IgE-BF) which either enhance or suppress the synthesis of IgE. We have cloned the gene coding for a human lymphocyte Fc_ε receptor from human genomic λ-libraries. A good correlation between the intron/exon arrangement and putative functional domains was seen. The 5'-end of the mRNA was mapped by primer extension and S_1-mapping. A promoter region was identified containing two closely spaced TATA boxes which correlate with two mRNA start sites. A 188 bp palindromic sequence was found 5' of the TATA boxes and in the first intron. Its role in gene regulation is under investigation.

Induction of the serine esterase specific for cytotoxic T-cells is coupled to cell proliferation in the T-cell line PC60

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One of the two serine esterases found in T-cells, the benzyllysylthioesterase (BLT esterase) has been shown to be induced concomitantly with cytotoxicity in PC60 cells by supernatant of spleen cells induced with Con A (Con A Sup) or by the combination of IL-1 (P388 Sup) and IL-2 (100 U/ml).

We show that this induction can be enhanced by derivatives of cAMP (8BrcAMP, Bt_2cAMP) or by stimulators of adenylate cyclase (forskolin, prostaglandin E_2). Together with this enhancement, cell growth is inhibted, On the other hand, when growth is inhibited (low serum content of culture media), the induction power of Con A Sup or IL-1 plus IL-2 is enhanced several folds. Low serum alone is a very weak inducer of BLT esterase activity. Furthermore, it has been observed, that IL-1 plus 0,2 mM 8BrcAMP can replace the combination of IL-1 plus IL-2, whereas IL-2 plus 8BrcAMP gives only a minor stimulation of BLT esterase activity.

Significance of pterin metabolism in the cellular immune response

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The cellular origin and the control of neopterin (N) release associated with immune stimulation, i.e., viral infections, cancer, and AIDS, was studied in vitro. The intracellular change in concentrations of pterins and of GTP, the starting substrate in tetrahydrobiopterin (BH_3) biosynthesis, was measured using cultures of highly purified human T cells and monocytes. Three enzymes involved in BH_4 biosynthesis, GTP cyclohydrolase I (GTPCH), 6-pyruvoyl tetrahydropterin synthase (PTS), and sepiapterin reductase (SR) were also determined. The main producers of N were macrophages (MØ). When stimulated by culture supernatants (SN) of activated T cells, GTPCH activity was elevated due to high GTP levels in these cells, but they lack PTS activity. In MØ, contrary to T cells, BH_4 biosynthesis is stopped at the first intermediate, dihydronepterin triphosphate, which accumulated and was degraded to N. Stimulation of MØ with recombinant interferon-gamma (IFN_γ) or addition of human IFN_γ-specific monoclonal antibody to SN of activated T cells, showed that IFN_γ induced the changes in GTPCH activity and N levels. These results suggest that N is an unspecific marker for the activation of the cellular immune system.

In vitro stimulation of human B cells and generation of human monoclonal antibodies to bee venom phospholipase A2

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Phospholipase A2 (PLA) is the major antigenic compound of bee venom. In general, individuals frequently stung by bees produce mainly anti-PLA IgG_4 antibodies, whereas most patients allergic to bee venom show increased serum levels of anti-PLA IgE. To analyze the mechanism of this shift, we established a culture system to induce anti-PLA antibodies. Peripheral blood mononuclear cells (PBMC) from bee keepers were stimulated in a 2-step procedure with PLA and/or pokeweed mitogen (PWM). Specific antibodies and total IgG were measured by solid phase ELISA. Highest antibody levels were obtained in culture after an incubation period of 10 days. For the establishment of monoclonal antibodies, PBMC were immortalized by transformation with Epstein-Barr virus (EBV) after 2 days of stimulation or after 6 days by fusion with a mous × human heteromyeloma. Furthermore, hybridomas were established by fusion of EBV transformed cells with a heteromyeloma.

Isolation and characterization of human platelet membrane glycoproteins Ia and IIa

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GPIa and IIa are relatively minor surface components of the platelet surface with similar molecular properties. Evidence exists suggesting that GPIa is a collagen receptor. The role of GPIIa is still unknown but a similar molecule has also been found on endothelial cells. They show only slight differences in molecular weight, pI and affinity for various lectins. They also show similar shift to higher Mr on reduction suggesting that both are rich in intramolecular disulphide bridges. It has been suggested that GPIa and IIa are complexed with a further membrane component GPIc. However, GPIa always associates with the cytoskeleton in resting platelets while GPIIa may but is often not. After solubilisation in Triton X-114 and centrifugation most of the GPIa was in the pellet whereas the GPIIa was mostly in the supernatant. These fractions were further purified by wheat germ agglutinin and lentil lectin affinity chromatography and by preparative SDS-PAGE using electroelution into a membrane trap.

Symposium 2: Lymphocyte differentiation

Analysis of LCMV-specific T cell clones

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Several cytotoxic T cell clones specific for lymphocytic choriomeningitis virus (LCMV) have been established. We analyzed their biological activities in vitro and in vivo. All the examined clones of C57BL/10 or C57BL/6 (H-2^b) origin have been found to be restricted to the H-$2D^b$ antigen coded by the major histocompatibility gene complex (MHC), whereas all clones of B10.BR (H-2^k) origin showed restriction to the H-$2K^k$ antigen. One clone (50.1; H-$2D^b$ restricted) was assessed for its activity in vivo. Upon intracerebral administration, this clone mediated immunopathological LCM-disease in infected, immunosuppressed recipients in a H-2 restricted manner.

For some of these clones the cDNAs encoding the α and β chain of the T cell receptor have been cloned and characterized by Southern-analysis.

Mammalian cell mutants deficient in the addition of glycolipid anchors to surface glycoproteins

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Recent evidence shows that mature Thy-1 glycoprotein lacks amino acid 113 to 143 predicted form the cDNA sequence and is anchored to the plasma membrane by a phosphatidylinositol-containing glycolipid attached to amino acid 112. We analysed previously characterized Thy-1-deficient mutant lymphoma lines of several complementation classes. They make detergent binding Thy-1 precursors but in contrast to wild type, the detergent binding moiety cannot be removed by phospholipase C. Moreover, tryptophan which only occurs at position 124 is incorporated into mutant but not parental Thy-1. This suggests that the mutants make a Thy-1 precursor of 143 amino acids but fail to replace its C-terminal end by a glycolipid anchor. We present evidence that these mutants are deficient in the enzymes required for the attachment of glycolipid anchors.

Biological characterization of tetanus toxin derived antigenic structures generated by tetanus toxin specific B cells

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The antigen specific stimulation of T helper lymphocytes (Th) is triggered by the cellular interaction between Th and antigen presenting cells (APC). Prior recognition by the antigen T cell receptor, antigens (Ag) have to be processed by APC. The biochemical events which take place during processing and the chemical nature of the antigenic structure seen by the T cells are unknown.

Tetanus toxin (TTI) specific Epstein-Barr virus transformed human B cell clones present very efficiently TT derived antigenic structure seen by the KT-4 T cell clone, two experimental procedures were followed:

1. Purification and sequencing of a TT chymotryptic peptide which could be presented to KT-4 cells by glutaraldehyde fixed B cells.
2. Production of monoclonal antibodies which are directed against TT derived antigenic structures which appear after uptake of TT by B cells.

Development regulated transcription termination and protein binding sites in the immunoglobulin membrane poly (A) addition region

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The expression of immunoglobulin μ and δ heavy chain mRNAs from the μ/δ transcription unit changes during B cell development. We have used transcription assays to examine the role of transcriptional controls in determining the levels of μ and δ gene expressionl. In IgM-secreting cells the δ gene is transcribed at a much lower level than in mature B cells. Transcription termination occurs within 500 nucleotides immediately following the μ membrane (μ m) pol (A) site and blocks δ gene expression. We defined nuclear protein factor binding sites within the region of active transcription termination which coincide with highly conserved sequences between murine and human μ genes. Experiments of DNA transfection into plasma cells confirmed that the region μm poly (A) addition is a major control site active in developmentally regulated transcription termination and in the processing of μ m mRNA.

In vitro biosynthesis of Thy-1: a membrane protein anchored via a glycolipid

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The Thy-1 glycoprotein is a major surface constituant of mouse thymocytes and neurons. During biosynthesis a hydrophobic C-terminal peptide is rapidly replaced on the precursor molecule by a glycolipid covalently attached to the protein and which anchors the mature molecule in the plasma membrane.

In order to further investigate the various steps of processing of this membrane protein, a full length precursor has synthesized using cell free transcription/translation systems. Moreover, parts of the Thy-1 cDNA have been joined with the cDNA coding for the rabbit polymeric Ig receptor in order to study the signal(s) required for this peculiar mode of processing of membrane proteins.

Hybrids between cytolytic T lymphocytes and IL2 receptor negative thymomas express the IL2 receptor genes of both parental cells

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We have analysed the species of the IL2 receptors (IL2 Rec) expressed by rat × mouse hybrids between an IL2 Rec negative, IL 2 independent mouse AKR thymoma, BW5147, and rat CTL. In this cross IL2 dependence is dominant and IL2 Rec are expressed constitutively. These two characteristics cosegregate with another marker of mature T cells, i.e. resistance to glucocorticoids in these hybrids.

The presence of IL2 receptors was determined with ^{125}I-IL2 binding assays. The species of the IL2 Rec was analysed with mouse anti-rat (OX39) and rat anti-mouse (PC61) monoclonal antibodies against the IL2 Rec, respectively. The results show that the IL2 dependent hybrids express not only the IL2 Rec gene inherited from the CTL parent but also the gene of the IL2 Rec negative thymoma parent. The receptors of both species form high as well as low affinity binding sites and mediate internalization of IL2. These results suggest that IL2 Rec expression in lymphoid cells is controlled by transacting regulatory elements.

The serine esterase granzyme A is present in both cytolitic and helper T cells

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Granzyme A is a protease contained in cytoplasmic granules of activated cytolytic T lymphocytes (CTL). It is secreted upon antigenic stimulation. The screening of established cell lines suggested that expression of the enzyme is restricted to CTL.

We have studied the expression of granzyme A in normal purified Lyt 2^+ (CTL) and L3T4$^+$ (T helper) cells activated with alloantigen. Both populations were > 95% pure. Granzyme A activity and its mRNA was present in equal amounts in both cell types, whereas only the cytolytically active, Lyt 2^+ cells contained the pore-forming protein perforin. In both populations granzyme A is contained in granules of the same density than the

granules containing perforin, and it is secreted as a result of antigenic stimulation.
These results suggest that granzyme A is not specifically involved in target cell killing but plays a role in functions shared by both CTL and T helper cells.

B-cell differentiation in *Xenopus laevis*

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Double immunofluorescence was used to study B-cell differentiation in metamorphic, postmetamorphic and adult *Xenopus*. Two successive waves of $slg^-c\mu^+$ pre-B cells were observed in the liver and spleen of developing *Xenopus*, while none were detected in the bone marrow at any age. Pre-B cells were first seen (0.25%) in the liver of small larvae (stage 46), increased to peak value (0.81%) at st.49, then disappeared at st.58, the climax of metamorphosis. Pre-B cells were again detected (0.02%) at st.60, increased to peak value (0.20%) at the end of metamorphosis, then dropped to 0.10% at which frequency they were maintained. Two comparable but slightly delayed waves of pre-B cell development were observed in the spleen. The formation of μ^+ pre-B cells persists at a low frequency in the spleen through postmetamorphic and adult life. Beside pre-B cells, *Xenopus* spleen contains 20–28% sIg^+ lymphocytes and 0.20–0.80% sIg^+cIg^+ plasma cells. The three successive stages of B-cell differentiation suggest that *Xenopus* spleen has the features of both primary and secondary lymphoid tissues.

cDNA cloning of complement C8β and its sequence homology to C9

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Efficient lysis of biological membranes by complement is due to the sequential assembly of the terminal complement components into the pore-forming complex, $C5b.C6.C7.C8.(C9)_n$. C5b-7 forms the first membrane inserting complex to which C8 must be added before polymerisation of C9 at the site of the C5b-8 complex causes major membrane damage and cell lysis. Human C8 is a glycoprotein comprising the disulfide linked α-γ and the β polypeptide chains.
The complete amino acid sequence of mature C8β has been derived from the DNA sequence. A clone with a 1.7 kb cDNA insert was identified by expression screening of a human liver cDNA library and found to contain the entire coding region for the mature subunit (1614 bp). The 5'sequence preceding the coding region contains no clear evidence of a leader peptide. Comparison with the amino acid sequence of C9 shows an overall homology with few deletions and insertions. In particular the cysteine-rich domains and membrane inserting regions of C9 are well conserved. These findings are discussed in relation to a possible mechanism of membrane attack complex (MAC) formation.

Structural characterization of the rabbit κ locus controlling the expression of immunoglobulin λ-light chains

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Seven cDNA clones encoding complete or partial variable and constant regions have been characterized. A comparative analysis of this data indicates: 1. The length of the complementarity-determining region 3 appears to be heterogeneous, as with rabbit κ chains and unlike light chains of other species. 2. Two distinct λ-chain constant domains are represented and the germ-line genes encoding these sequences have been identified. The cDNA encoding one of these C-regions, when inserted in an expression vector, could be immunoprecipitated with an anti-C21 alloantiserum. This suggests that at least some of the C21 allotopes are located within the C-region. Southern blot analysis of genomic DNA from rabbits typed for C7 and C21 allotypes indicates a correlation between specific Vλ genes and the two allotypes. Genetic implications will be discussed.

Structural analysis of molecules involved in antigen recognition and cytolysis by cytotoxic T lymphocytes

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To evaluate the possible structural contributions of the variable domains of the α-, β- and γ-chains to antigen X and H-2 antigen recognition, we have sequenced T-cell receptor cDNA-clones of alloreactive, hapten- and LCMV-specific cytotoxic T-cell clones. Up to now we could not find any obvious correlations between the utilization of V, D and J gene segments and antigen or restriction specificities.
By using a rabbit-anti-mouse perforin 1 (P-1)-antiserum we localized P-1 by EM. We immunoscreened a λgtll expression library from an alloreactive cytotoxic T-cell line and selected a cDNA clone with sequence homologies to complement C9, suggesting structural similarities between molecules involved in different cytolytic mechanisms.

A factor that binds specifically to the recognition sequence flanking the immunoglobulin J gene segment of the mouse

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Immunoglobulin gene segments are flanked by conserved sequences thought to be involved in DNA rearrangements. It is postulated that a hypothetical recombinase binds to these recognition sequences thereby mediating DNA rearrangements. To detect such binding activity in nuclear extracts of mouse pre B cells (38B9) we applied an electrophoretic mobility shift assay using a synthetic oligonucleotide probe. The probe consisted of the nonamer/23 bp spacer/heptamer recognition motif flanking the J segment on the 5' side. With this assay we detected and partially purified a factor which bound this recognition sequence specifically.

PMA induces surface expression of T3 on human immature T cell lines with and without concomitant expression of the T cell antigen receptor complex

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Five immature, surface T3 negative, T cell leukemia lines (Molt-4, HSB-2, 8402, CEM, Ichikawa) were shown by immunoperoxidase staining and S-35 biosynthetic labeling to contain high levels of T3 in their cytoplasm. β-chain transcripts of the T cell receptor molecule (TCR) could be demonstrated in all cell lines,

whereas the α-chain transcripts were only found in Ichikawa and CEM.
Immunoprecipitation of S-35 biosynthetically labeled Ichikawa and CEM cells by a monoclonal antibody (mAb) directed against a common determinant of the TCR confirmed at the protein level, the synthesis of fully mature α and β-chains. Treatment of these two cell lines with phorbol 12-myristate 13-acetate (PMA) induced the surface expression of a complete T3/TCR complex on Ichikawa and of exclusively T3 on CEM cells. These results were confirmed by using a digitonin extraction procedure of surface labeled PMA treated cells: the anti-T3 mAb, Leu4, precipitated the whole T3/TCR complex from Ichikawa cells and T3 only from CEM cells.

Expression of the lymphotoxin gene is neither necessary nor sufficient for T-cell cytotoxicity

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Lymphotoxin (LT) and tumor necrosis factor (TNF) are two related cystokines that are toxic to a number of tumor cell lines. LT is secreted by activated T-lymphocytes as well as by some B-cell populations. Because it seems to induce DNA degradation in its target cells, similarly to cytotoxic T-lymphocytes (CTL), LT has been tentatively implicated in CTL-mediated target cell killing. We show here that there are highly active CTL clones that do not express detectable mRNA for LT. Furthermore, stimuli that mimic antigen presentation and induce blast transformation do not necessarily increase LT mRNA levels. A cell line (PC60) that can be induced to become cytotoxic and express many CTL markers by a combination of IL-1 and IL-2 fails to accumulate LT mRNA after stimulation. In contrast, a non-cytotoxic T-cell clone (CTLL) contains large amounts of LT mRNA. We conclude that expression of LT is neither necessary nor sufficient for the CTL phenotype.

Identification of the C-terminal α-chain remnant of human platelet membrane glycoprotein Ib

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Glycoprotein Ib (GPIb) is a major receptor on the human blood platelet membrane consisting of two subunits, GPIbα and GPIbβ, linked via a disulfide bridge and involved in platelet activation. The N-terminal extracellular part of GPIb is easily split off by proteases. The C-terminal part was previously unidentified. SDS-PAGE of chymotrypsin-treated solubilized platelets under reducing and nonreducing conditions followed by blotting with rabbit GPIb antibodies demonstrated the existence of a 20 kDa fragment of GPIbα, linked to GPIbβ. The C-terminal remnant of GPIb was identified by chromatography of chymotrypsin-treated, solubilized platelets on an anti GPIbβ-Sepharose 4B column and analysis of the eluate by 2-D nonreduced/reduced SDS-PAGE, followed by silver-staining. After reduction it was purified to homogeneity by gel filtration on an AcA34 column. Its size and the fact that it is protected from further cleavage on the intact platelet suggest that it spans the plasma membrane.

T cell growth regulation by T90/44

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The T90/44 glycoprotein functions as a membrane signal transducing unit in human T cell growth control, since the anti-T90/44 monoclonal antibody 9.3 mAb in various culture systems is found to regulate cell growth. Resting T cells are directly activated by 5–10 μg/ml 9.3 mAb in the presence of monocytes whose F_c receptors play a role. 9.3 mAb at low concentration (> 1–10 ng/ml) enhance mitogenic T cell responses to pha, PPD, TT, etc. up to 30-fold. In contrast to these activations, 9.3 mAb inhibits the activation of class II-restricted cloned T_H cells derived from leprosy patients and reactive with M. leprae antigen. Both the inhibitory and activating effects of 9.3 mAb are regularly dose-dependent. The activation of T cells is paralleled by a strong induction of T90/44 expression. Effector functions of class I-restricted CTL were not modulated by 9.3 mAb. It is proposed that T90/44 functions as receptor for a factor or in intercellular contact in antigen-independent T cell growth control.

Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor

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Low-affinity receptors (FcεR) and secreted factors (IgE-BF) which bind to immunoglobulins of the IgE isotype play a key role in the regulation of human IgE synthesis. We have cloned a cDNA coding for the FcεR of the human B-lymphoblast cell line RPMI 8866. The nucleotide sequence of this cDNA predicts a polypeptide with 321 amino acids and a molecular weight of 36281 Daltons. Expression of the cDNA in Chinese hamster ovary cells produces a membrane protein which binds IgE as shown by the IgE-rosette formation. The N-terminal amino acid sequence of the IgE-BF produced by the RPMI 8866 cells corresponds to a portion of the receptor. We conclude that IgE-BF released by RPMI 8866 cells is encoded by the same gene as the low-affinity membrane FCε receptor. A computer search with the translated amino acid sequence of the FcεR revealed a domain of 120 amino acids having striking homology to the human asialoglycoprotein receptors.

Early events in intrathymic T cell differentiation

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The development of functionally mature T lymphocytes from immature precursor cells involves the coordinate expression of structures involved in antigen recognition such as the T cell receptor complex (TCR) and the CD4 and CD8 molecules, as well as the presumptive elimination of self-reactive clones. In mammals, these differentiation events are believed to occur predominantly (if not exclusively) within the thymus. Attempts to understand early events in T cell development thus depend upon the identification and characterization of immature thymocyte subpopulations. We have used multiparameter flow microfluorometry to identify at least 4 discrete subpopulations of immature (ie $CD4^-$ $CD8^-$) mouse thymocytes as dfined by surface phenotype, size and cell cycle status. Furthermore, we have correlated these phenotypes with an analysis of in vitro growth requirements as well as the degree of re-arrangement and expression of TCR alpha, beta and gamma genes. Collectively, these data demonstrate the existence of a previously undetected subset of immature thymocytes that express TCR proteins and respond to a variety of stimuli capable of activating mature T cells. The developmental relationship of these cells to other known thymocyte subpopulations, including the mature $CD4^+$ and $CD8^+$ subsets, is currently under investigation.

A family of serine esterases in lytic granules of cytolytic T-lymphocytes

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Cytoplasmic granules of cytolytic T-lymphocytes (CTL) contain, besides the poreforming lytic protein perforin, a family of highly homologous serine esterases, designated granzymes A to H. The serine esterase affinity label ^{3}H-DFP reacts strongly with granzymes A and D and to a lesser extent with granzymes B, E, F, G and H. Granzymes A and D cleave trypsin-like substrates. Antibodies raised against purified proteins show strong cross-reactions between different granzymes. Homology is supported by N-terminal sequence analysis, which also revealed the identity of granzyme A with H-factor, a protease predicted from a CTL-specific cDNA clone (Gershenfeld et al., Science *232* (1986) 854) and of granzymes B, G or H with a protein encoded by the CTL-specific cDNA clones CTLA 1/CCPI (Brunet et al., Nature *322* (1986) 268; Lobe et al., PNAS *83* (1986) 1448).

Cell type-specificity of immunoglobulin enhancers and promoters

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Immunoglobulin genes are expressed in B lymphocytes only. This expression is regulated transcriptionally (by the enhancer and the promoter) and posttranscriptionally (by intragenic sequences). To characterize the elements (DNA modules/proteins) which confer cell-type specificity we are dissecting the heavy chain (IgH) enhancer and the kappa light chain promoter. In vitro binding studies with nuclear cell extracts showed that a conserved decanucleotide (TAATTTGCAT) found in the IgH enhancer and also in the Ig gene promoters binds to both ubiquitous and lymphoid cell-specific factors. In vivo transfection studies demonstrated that this decanucleotide is an essential element for B cell-specific activity of the IgH enhancer. This activity is presumably mediated by the lymphoid-specific forms of the interacting factor, whose isolation is currently underway. A transcriptional preference for B cells was also found in vitro: The kappa promoter linked to a test gene is better transcribed in lymphoid extracts than in a HeLa extract.

Abnormal expression of T cell receptor (TCR) genes in *lpr* mice

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The thymus dependent lymphoproliferation of *lpr* mice is characterised by the accumulation of Lyt-2$^-$ L3T4$^-$ T cells resembling most closely a minor subpopulation of normal thymocytes. We have compared the expression of TCR genes in these two populations as well as in normal lymph node T cells. The unique features of the *lpr* population were 1) normal mRNA expression for the TCR-α and -β genes, 2) extensive rearrangement of 2 of the three Vβ8 genes, 3) expression of low density surface antigen receptor and 4) low levels of TCR-γ message. Cultivation with PMA and IL2 did not induce drastic changes in TCR expression in the immature thymocyte and T cell populations. The cultivated and fresh *lpr* cells showed similar patterns of Vβ8 and TCR-γ rearrangements, however, striking changes in cultured *lpr* cells were 1) increased density of surface antigen receptor to normal levels and 2) over 200-fold induction of TCR-γ mRNA. We are now examining whether the induced TCR-γ mRNA is derived from loci that are generally not transcribed or from loci that are known to be expressed at the protein level in immature thymocytes.

Experimental models for the regulation of gene expression during T lymphocyte activation

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T lymphocyte activation and proliferation is a complex process controlled by stimulation with antigen and hormon-like factors such as interleukin (IL) 1 and 2. Only a few aspects of the molecular mechanisms involved are understood. We have been analyzing the regulation by IL1 and IL2 of the expression of the IL2 receptor (IL2Rec) and of a serine esterase which is a specific marker of activated T cells. For this purpose we have used cell lines in which the appearance of both proteins can be induced with these lymphokines. The evidence obtained so far suggests that this regulation depends on trans-acting factors which control the level of IL2Rec and esterase mRNA.

Symposium 3: DNA transposition

Mutational studies on the recombinational enhancer in the site-specific DNA inversion of phage P1

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The site-specific C-inversion system of phage P1 comprises the Cin recombinase, the two inversely repeated crossover sites (*cix*L and *cix*R) and the recombinational enhancer *sis*. The *sis* sequence, a 70-bp segment forming a part of the 5' terminus of the *cin* gene, stimulates inversion 100-fold. We mutagenized the *sis* region and assayed both *sis* and Cin functions in vivo. One mutant at position 9 in the Cin protein alters the Ser codon TCA to the amber codon TAG. This mutation abolished the *sis* activity. Using hosts carrying amber suppressors, the Ser residue in Cin was substituted by Gln, Leu or Tyr. The resulting Cin enzymes were all inactive. The results show that this Ser residue in Cin is important for the enzymatic activity and that the sequence TCA is also essential for the *sis* function.

Insertion elements which can generate target duplications of variable length

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One of the characteristics of prokaryotic transposable elements is the generation of a target duplication upon their integration. The insertion element IS*1* residing in the *E. coli* chromosome is known to integrate into AT-rich regions and to generate 9-bp target duplications. We have shown that IS*1* can intrinsically generate not only 9-bp but also 8-bp or 10-bp target duplications. IS*186*, another IS element on the *E. coli* chromosome, transposes preferentially into GC-rich sequences and generates duplications varying in length between 8 and 12 bp, even at the same target site. Comparisons with other IS elements known to generate duplications of variable length and implications to transposition mechanisms are also presented.

Characterization of a multiple invertible segment of the P1 prophage-related plasmid P15B

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Phage P1 encodes the site-specific C-inversion system which controls its host range by rearranging structural genes for the tail fibers. C-inversion belongs to a family of closely related DNA inversion systems found also in phage Mu and in bacterial chromosomes. While DNA inversion in these systems is brought about by site-specific recombination at two specific crossover sites, a newly investigated, functionally related inversion system carried on the plasmid P15B is more complex. This inversion system comprises within its 4.7-kb sequence an active recombinase gene, six crossover sites and a pseudogene which is an inactive 5' half part of the recombinase gene. Due to inversions at any pair of these six crossover sites, a plasmid carrying this inversion system has 240 different isomeric configurations.

The target specificity of the procaryotic mobile genetic element IS*30*

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The mobile genetic element IS*30* of *E. coli* was originally isolated from 3 independent defective P1 phages. In all three derivatives the insertion was at the same nucleotide, suggesting that the insertion site serves as a hot target for IS*30*. In order to investigate the target specificity of the element, an artificial transposon (Tn*2700*) was made with two copies of IS*30* flanking a chloramphenicol resistance gene. It was shown that the transposon Tn2700 has the same specificity as the element alone. The P1 fragment in which the element was originally found was then cloned in phage λ. In this derivative the P1 site still served as a hot target for IS30 insertion, and another hot target ws found on phage λ DNA. Both hot targets were used with high frequency, and sequence analysis showed that independent insertions were always at the same nucleotide. The two target sequences have partial homology. The hot λ sequence shows quite strong homology to the termini of IS*30*, whereas for the P1 hot target this homology is weak.

Symposium 4: Growth factors and signal transduction

Activation of phosphoinositide metabolism in rat mesangial cell membranes: different effects of phorbol ester on angiotensin II and GTP-analogue induced activation

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Angiotensin II stimulates a rapid and concentration-dependent rise in inositol phosphate formation in rat mesangial cells. The half-maximal potency of angiotensin II in stimulating inositol-trisphosphate (IP_3) production is 3.5 nM. Angiotensin II is also able to stimulate IP_3 formation from membrane preparations of [^{3}H]inositol labeled mesangial cells with a half-maximal potency of 1.1 nM. The angiotensin II-induced formation of IP_3 is enhanced by GTP. Guanosine 5'-[γthio triphosphate ($GTP_\gamma S$) and guanosine 5'[βγ-imido triphosphate (Gpp[NH]p), nonhydrolyzable analogs of GTP, stimulated IP_3 production in the absence of angiotensin II with K_d values of 0.19 μM and 2.4 μM, respectively. Angiotensin II augmented the increase in IP_3 formation induced by $GTP_\gamma S$. However, when mesangial cells were pretreated with 12-O-tetradecanoylphorbol 13-acetate, there was a dose-dependent inhibition of the synergistic action of angiotensin II on $GTP_\gamma S$-induced IP_3 production, while the inactive phorbol ester 4α-phorbos 12, 13-didecanoate was without effect. These results suggest that activaiton of protein kinase C in mesangial cells does not impair phosphoinositide hydrolysis by stable GTP-analogs but strongly inhibits the synergistic action of angiotensin II on inositol phosphate formation.

Growth factor like domains in laminin

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Laminin a multifunctional glycoprotein present in all basement membranes mediates between cells and other components of the extracellular matrix and plays an important role in cellular organisation and morphogenesis. The recent detection of cystein rich motifs in the sequence in one (B_1) of the three chains of laminin (Sasaki et al., 1986) which are in part related to epidermal growth factor (EGF) prompted us to search for possible functional and structural relations with EGF. It was found that laminin as well as fragments derived from it containing the EGF-like domains show antigenetic crossreactivity with EGF, exhibit mitogenic effects on Swiss 3T3 cells, stimulate a transient rise in cytoplasmatic free Ca^{2+} in A431 cells, and produce other growth factor dependent effects in a similar dose response as EGF. A peptide comprising the part of the cystein rich motif which exhibit the highest degree of homology with EGF was synthesized and tested for EGF-activity.

Interferon increases the cholinergic properties of cultured human spinal cord neurons

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In human embryonic spinal cord cultures, we found that gamma-IFN can increase the choline acetyltransferase (CAT) activity without altering the level of glutamic acid decarboxylase (GAD) or the neuronal survival; an antibody to IFN can prevent these effects. Gamma-IFN appears to mediate these effects via the nonneuronal cells since in the absence of non-neuronal cells, gamma-IFN has no effect on the cholinergic properties; the non-neuronal cells alone have no CAT or GAD activity. Astrocytes may be responsible for these changes since gamma-IFN increases the development of GFAP immunoreactivity in cultures of 6-7 week old spinal cord cells and it causes no visible change in the Thyl immunoreactivity of the fibroblasts. Thus we propose that IFN acts on non-neuronal cells, possibly the astrocytes, which in turn stimulate neuronal cholinergic traits either by means of a diffusible factor or via cell-cell contact. These studies could be relevant in understanding the effect of the immune system on the nervous system and also in the search for new drugs which act specifically on cholinergic neurons.

Growth hormone (GH) dependent stimulation of bone cells in culture is mediated partially by local synthesis of insulin-like growth factor (IGF) I

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According to the IGF-hypothesis, GH is thought to act indirectly on bone in vivo via the stimulation of hepatic IGF I synthesis.

Osteoblastic cells (OB) prepared from new born rat calvaria were cultured serum-free in viscous medium for 21 days. Single OB selectively divide into clonally derived cell clusters of spherical morphology. Cell growth was monitored on line by counting the number of cells per cluster.
Number and size of clusters increased with IGF I (physiological range) up to 140% above basal growth of control cultures. GH stimulated in physiological concentrations up to 145% of control with a maximum at 15 ng/ml. This effect is in part due to local production of IGF I-like material in the medium, since: 1) GH-stimulation is partially inhibited by the addition of IGF I-specific antibody. 2) Radioimmunoassays indicate GH-dependent IGF I accumulation. In contrast, the basal growth of control cultures was not caused by local IGF I production. Since the relative amount of collagen synthesis is only stimulated by IGF I and both hormones combined stimulated OB proliferation at least additively, it might well be that GH and IGF I act at different stages of OB differentiation.

Tumor necrosis factor-alpha inhibits endothelial cell proliferation in vitro, but is angiogenic in vivo

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Tumor necrosis factor (TNF) causes hemorrhagic necrosis and regression of tumors and modulates some endothelial cell functions. The possibility that TNF might influence endothelial cell proliferation and neovascularization (of which endothelial cell proliferation is an element) was therefore investigated. Human TNF potently inhibits endothelial cell proliferation in vitro of bovine aorta and capillary endothelial cells in a dose-dependent manner from 0.14-14 ng/ml (half-maximal inhibition at 0.5–1.0 ng/ml). TNF also inhibits endothelial cell growth stimulated by exogenously added fibroblast growth factor (FGF). TNF is a noncompetitive antagonist of FGF-stimulated growth. Its action on endothelial cells is reversible and non-cytotoxic. Surprisingly, TNF does not appear to inhibit endothelial cell proliferation in vivo. On the contrary, in the rabbit cornea-angiogenesis assay, TNF induce formation of new blood vessels, but it does not affect the angiogenic activity of FGF, a known angiogenic factor. As TNF appears to cause an inflammatory response in this assay, it is possible that the angiogenic activity of TNF is a consequence of white cell infiltration.

Heterogeneous distribution of phospholamban phosphorylation in cardiac SR

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Cardiac phospholamban (PLB) is composed of 5 identical monomers. Each monomer contains a phosphorylation site for the cAMP-dependent (cAMP-PK) and one for the Ca^{2+}-calmodulin-dependent (calm-PK) protein kinase. Phosphorylation of PLB by either kinase correlates with the stimulation of the Ca^{2+}-pump. SR membranes from dog hearts were subfractioned on a density gradient to separate vesicles originating from the longitudinal system (LSR) from those deriving from the cisternal compartments (CSR). The action of the cAMP-PK and the calm-PK on these two subfractions was investigated. Maximal cAMP-dependent P_i-incorporation into PLB was found in LSR fractions (2100 pmoles P_i/mg SR), whereas maximal calm-dependent phosphorylation was found in CSR fractions (1220 pmoles/mg). This agrees with the observation that Ca^{2+}-uptake activity was maximally stimulated by cAMP in the LSR fractions while calmodulin stimulated mostly the CSR fractions. The results indicate that the cAMP-PK and Ca^{2+}/calm-PK dependent regulation of the Ca^{2+}-pump through phosphorylation of PLB is distributed heterogeneously among the different regions of the SR network.

Comparing the effect of H- and N-*ras* on the transmission of the mitogenic signal of interleukin-3

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We are studying the effects of the various *ras* oncogenes on the interleukin-3 (IL-3) requirement of PB-3c, an IL-3-dependent, non-tumorigenic mastocyte cell line. The normal as well as the mutated (codon 12) human cellular H-*ras* and N-*ras* genes were introduced into the retroviral ZIP Neo SV(X) shuttle vector, carrying the selectable neo marker. Following transfection using the packaging 2 cell line virus titers from 5.10^2 to 5.10^4/ml were obtained. NIH 3T3 cells infected with both mutated H- or N-*ras* showed a transformed phenotype indicating that the *ras* p21 proteins are expressed. PB-3c was then infected with these viruses. Infectants were selected with G418 and tested for their IL-3 requirement. It was found that the mutated H-*ras* but not the mutated N-*ras* nor the normal genes caused a ten-fold reduction of the IL-3 requirement. This result suggests that H-*ras*, but not N-*ras*, is specifically involved in transmitting the growth response of IL-3. Experiments are in progress determining the metabolism of the inositol lipids in H-*ras* and N-*ras* infected PB-3c cell lines.

Tissue specific expression of IGF II

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IGF II cDNA has been cloned from human brain. The cDNA contains the complete coding sequence and a part of the 5' untranslated region. The coding sequence for prepro-IGF II is identical with the pulished cDNA sequence from human liver, but the 5' untranslated sequence is different.
Northern blot analysis shows a length heterogeneity of the transcripts for IGF II in different tissues. Most of the tissues express a 6 kb transcript. Liver expresses a 5.3 kb transcript and a pheochromocytoma expresses in addition to the 6 kb transcript a 5 kb transcript. The 6 kb transcript contains a 5' untranslated sequence encoded by exon 3 and the 5.3 kb transcript contains a 5' untranslated sequence encoded by exon 1+2. The 5' extension of the untranslated sequence of the mRNAs has been determined using RNAse H experiments.
The different transcripts could arise by at least two separate mechanisms: Two different promotors could be present, one upstream of exon 1 and one upstream of exon 3, or the IGF II gene has a single promotor upstream of exon 1 and the different transcripts are generated by differential splicing.

Characterization of the binding sites of insulin-like growth factor I (IGF I) to the type I IGF receptor

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The three-dimensional structure of IGF I can be predicted by modelbuilding operations. We used the method of differential surface labeling to determine protected amino acids on IGF I in the type I IGF receptor-IGF I complex. These data give us the first direct information about the three-dimensional organisation of the receptor binding site on the IGF I molecule.

All three tyrosine residues in the IGF I molecule can be labeled with ^{125}I iodine (chloramine T), analysed by Edman-degradation of the radioactive labeled peptides. In the type I IGF receptor-IGF I complex all tyrosine residues of IGF I are protected against modification, indicating that the receptor binding site is homologous to the predicted insulin receptor-insulin interface. As a control, the differential labeling of monoclonal antibody 43-IGF I complex modifies TYR B25 and C2 only, whereas Tyr A19 is protected. A model of IGF I with the receptor-binding site will be presented.

A phorbol ester inhibits EGTA-induced detachment of chicken embryo fibroblasts from the substrate

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The spreading and attachment of cells onto a substrate are known to depend on extracellular calcium. Removal of calcium induces detachment of cells, concomitant with a reorganization of the cytoskeleton. The nature of the signal transduction across the plasma membrane during these processes has not yet been clarified. Recently the second messenger diacylglycerol has been implied to be involved in the organization of the platelet actin-cytoskeleton (Burn et al., Nature *314* (1985) 469). Possibly, diacylglycerol also plays a role in cell attachment. To test this hypothesis, the effect of phorbol-12-myristate-13-acetate (PMA, which can substitute for diacylglycerol in protein kinase C activation) on the detachment of chicken embryo fibroblasts in EGTA-containing medium has been studied. PMA (80 nM) reduces the amount of rounded and detached cells after 10 min incubation in EGTA by 77 ± 7% (6 experiments). 5–10 nM PMA is the half-maximally effective dose. The inhibition is transient, as most of the PMA-treated cells detach after prolonged incubation in EGTA. Inactive phorbol derivatives have no effect. PMA therefore appears to prevent transiently cell detachment in EGTA, implying that diacylglycerol may play a role in cell attachment.

Effect of interleukin 3 (IL3) perfusion in genetically hematopoietic deficient W/W^v mice

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W/W^v mice have a genetic defect in the proliferative potential of their hematopoietic stem cells, expressed by a lack of CFU-s cells, a severe macrocytic anemia and a total absence of subcutaneous mast cells. Under murine recombinant IL3 perfusion, these mice show an appearance of CFU-s colonies, a vast increase in all hematopoietic progenitor cells, appearance of mast cells in the skin and various organs, but only a slight increase in red blood cell count and hematocrit values (1.6 and 1.3 times higher than control W/W^v mice resp.). These results show that IL3 in vivo partially corrects the defective proliferative potential of W/W^v stem cells, but without allowing a complete cure of the anemia. This might result from a defective response of their hematopoietic cells not only to a stimulating factor (which can be counteracted by excess IL3) but to erythropoietin as well.

Cloned granulocyte-macrophage progenitors (CFU-GM) in serum-free cultures show limited proliferation in the absence of colony stimulating factor

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The proliferation and differentiation of hemopoietic progenitors depend on specific growth factors called colony stimulating factors. Mouse bone marrow cells were cultured in serum-free, semi-solid medium containing recombinant granulocyte-macrophage colony stimulating factor (GM-CSF) for 1 or 2 days, when progenitors had divided 1–5 times. Cells from these clusters were transferred to wells containing fresh medium with or without factor. After a further 3–4 days, proliferation was seen in 18% of the wells containing GM-CSF (up to approximately 7 divisions), whereas 21% of the unstimulated wells showed 1–4 divisions. Under both conditions, mature granulocytes and macrophages were detected in wells to which single cells were transferred. This apparent GM-CSF independant growth and differentiation of CFU-GM may result from continued stimulation by a pool of tightly bound or internalized GM-CSF accumulated during preculture.

Diacylglycerol catabolism in plasmamembranes of chromaffin cells from bovine adrenal medulla

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Rapid turnover of phosphatidylinositol (PI) is one of the initial events in signal transmission. PI is degraded to 1,2-diacylglycerol (DG) by a PI-specific phospholipase C. DG can be further catabolyzed by the action of lipases. In plasmamembranes of chromaffin cells from bovine adrenal medulla we found two sequentially acting enzymes. DG was hydrolysed to a sn-2-monoacylglycerol (MG). By the subsequent release of the fatty acid the existence of a MG-lipase could be demonstrated. Both lipases are calcium-independent, but they differ in pH-dependence of their activity and in their extractability from the membranes by detergents. The MG-lipase has a preference for arachidonate.

An alternative Ca^{++}-sensitive metabolic pathway of phosphoinositols in vascular smooth muscle cells

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Angiotensin II and vasopressin are known stimulators of phosphoinositides turnover in vascular smooth muscle cells (VSMC). The metabolism of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] was studied in rat VSMC permeabilized by electric field. Addition of ^{3}H-labelled Ins(1,4,5)P_3 to the leaky cells led to the formation of several phosphoinositols with various kinetics. About 85% was degraded to inositol bis- and monophosphate. Inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] and inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] were also formed in a Ca^{++}-sensitive manner at physiological intracellular concentrations. The amounts of Ins(1,3,4,5)P_4 present at 2 minutes and those of Ins (1,3,4)P_3 at 10 minutes were correlated. The degradation of Ins (1,4,5)P_3 was partly (25%) due to external phosphatases. These results suggest that, in VSMC, Ins (1,4,5)P_3 is metabolized via: 1) a dephosphorylation pathway, 2) a Ca^{++}-sensitive phosphorylation/dephosphorylation pathway generating the 1,3,4-isomer.

A low molecular weight growth inhibitor secreted in cultures of chicken embryo fibroblasts

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A novel type of growth inhibitor is found in conditioned medium of chicken embryo fibroblasts which inhibits both DNA synthesis and cell proliferation. The inhibitor does not compete with the growth factors present in the serum and its effect on the target cells is non-cytotoxic and reversible. The kinetics of the inhibition of thymidine incorporation indicate that the inhibitor

acts in the S-phase of the cell cycle. Comparison of the chemical and physico-chemical properties of the inhibitor with other recently described growth inhibitors of fibroblasts has demonstrated that it is different in many respects. The inhibitor has a M_r of less than 2000, is protease resistent, and acid- and heat-stable.

Applications of a new HPLC method separating the phosphoinositides: study of the mammalian nerve fibers phospholipids labelled in vitro by myo-(2-^{3}H) inositol or ^{32}P

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We have developed a HPLC method that allows the separation of phosphatidic acid (PA), phosphatidylinositol 4,5-bisphosphate (PIP2), phosphatidylinositol 4-phosphate (PIP), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) by the use of the gradient of two solvents containing hexane, isopropanol, water and H_2SO_4 and a silica 5µ column. This technique is applied to the study of the phospholipids turnover in rabbit vagus nerve; the nerve is incubated in a physiological medium during various times in presence of myo-[2-^{3}H]inositol of [^{32}P]phosphate or both. The effect of LiCl, tetracaine, electrical stimulation on the incorporation of the label is analyzed after extraction of the phospholipids, separation by HPLC and counting of the collected fractions.

Trophic factors and attachment-independent growth of colorectal carcinoma cells

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Cells from several tumor types (ovary, breast, neuroblastoma) respond to trophic factors such as epidermal growth factor and hydrocortisone with increased attachment-independent growth. In contrast, colorectal tumor cells grown in semi-solid medium exhibit a markedly polymorphic type of response. Hydrocortisone (10^{-6} M) decreased growth in 2 out of 28 cases tested: (−) = 2/28, increased growth in 5 (+) = 5/28 and had no effect in 21 (○) = 21/28. Similar results are observed with other factors: gastrin (10^{-8} M): (−) = 5/24,(+) = 1/24, (○) = 18/24; bombes in (10^{-8} M): (−) = 2/15, (+) = 1/15, (○) = 12/15, cholera toxin (10 ng/ml): (−) = 5/26, (+) = 3/26, (○) = 18/26; 17β-estradiol (10^{-8} M): (−) = 5/37, (+) = 6/37, (○) = 27/37; EGF (1 ng/ml): (−) = 5/38, (+) = 3/38, (○) = 30/38. Colorectal carcinoma cell lines are also poor responders to these factors. The reasons for this polymorphism are being investigated. They appear to be inherent to the tumor type rather than related to the culture conditions as such.

Growth factor, tumor promoter, and glucocorticoid hormone mediated modulation of plasminogen activator and plasminogen activator inhibitor gene expression

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We have analyzed the regulation of biosynthesis of plasminogen activators (PAs: tissue-type PA (tPA) and urokinase type PA (uPA)) and plasminogen activator inhibitors 1 and 2 (PAI 1 and PAI 2) by hormones and other effectors in several cell lines, employing radioimmuno-, Northern blot hybridization-, and 'run on' transcription assays. The results demonstrate that 1) in HeLa cells the tumor promoter phorbol 12-myristate 13-acetate (PMA) induces tPA gene transcription. On the level of tPA-mRNA augmentation, epidermal growth factor (EGF) cooperates with PMA. The PMA effect cannot be mimicked by diacylglycerol. 2) Dexamethasone induces PAI 1 transcription but suppresses pro-uPA transcription in HT-1080 (human fibrosarcoma) cells. 3) PMA induces a strong transient increase of PAI 2 gene transcription in the human histiocytic lymphoma line U-937. Our results suggest that transcriptional regulation of the genes of the plasminogen activating system may serve as a model for the analysis of protein kinase C, tyrosine specific protein kinase and glucocorticoid hormone mediated reprogramming of gene expression.

Bone collagen mRNA in rats is regulated by insulin-like growth factor I (IGF I) in vivo

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RNA extracted from parietal bones of rats was dotted onto nitrocellulose and analyzed for type I procollagen mRNA by hybridization with chain-specific ^{32}P-cDNAs. In hypophysectomized (hypox) rats, deficient in IGF, α1(I)mRNA was 4 times less abundant than in weight-matched (120 g) normal rats, and so was α2(I)mRNA. Hypox rats were infused sc. for 6 days with hGH (28mU/d) or recombinant human IGF I (120 µg/d) to raise serum IGF; α1(I)mRNA levels in bone were 2.5- and 4.2-fold higher, respectively, than in saline-treated rats. Starting within a few hours, IGF I appears to enhance coordinately the expression of the genes encoding the α1 and the α2 chains of the type I collagen triple helix, the major structural protein of bone.

Symposium 5: Tumor promoters and cancer genes

Do mice with inherited susceptibility to chromosomal damage develop cancer more readly?

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The objectives of the present study were to investigate whether mutagen sensitive mice were also more susceptible to certain carcinogens. An inbred strain of mice MS/Ae, which was shown to be highly susceptible to the induction of chromosomal aberrations by various classes of mutagens (Mut. Res. *164* (1986) 109; Mut. Res. *105* (1982) 253), was compared to OF1 outbred mice and the induction of neoplasms was recorded. Accordingly, both the MS/Ae and OF1 mice were injected with a single dose of methylnitrosourea (MNU) or diethylnitrosamine (DEN). With DEN a significantly higher incidence of total neoplastic lesions were observed in the sensitive inbred MS/Ae strain. With the direct acting MNU a greater early mortality were observed with MS/Ae mice.

These preliminary results suggest a clear disposition to develop neoplasms (at least for DEN) in mutagen sensitive mice.

Specific DNA-protein interaction and regulation of plasminogen activator gene expression in pig kidney cells

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In the pig kidney cell line LLC-PK_1 urokinase-type plasminogen activator (uPA) is induced by a cAMP-elevating hormone calcitonin and by a tumour promoter, phorbol ester. This induction is regulated mainly, perhaps entirely, at the level of transcription

of the uPA gene. Transcription of genes can be controlled by regulatory proteins that bind to specific sites of DNA. To analyse the interaction between cis-acting elements and trans-acting factors we improved a new method, employing a protein gel blotting procedure to search for cytoplasmic or nuclear proteins from the LLC-PK_1 cell line, that bind preferentially to the 5'-flanking region of the uPA gene. By incubating gel blots of whole cell extract or nuclear extract proteins with a ^{32}P-labelled uPA genomic probe, we detected two major binding proteins with molecular weights of approximately 70 and 100 kd. In order to define more precisely the regulatory cis-acting element we used a gel shift and a DNase I footprinting assay and showed specific interactions between nuclear protein(s) from LLC-PK_1 cells and 5'-flanking sequences of the uPA gene. Within the first 300 base pairs upstream of the transcription start site, we detected four protein binding sites with different affinities. Each binding site contains the Spl core sequence 5'-GGGCGG-3'. In these specific interactions we are investigating the effect of cAMP and phorbol esters.

A two-step tumor model involving H-ras and progression to autokrine IL-3 production

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As mutations of ras-protooncogenes are frequently seen in human leukemias, we examined the effect of ras on the action of the hemopoietic growth factor interleukin-3 (IL-3). The v-H-ras gene was introduced into a IL-3-dependent, non-tumorigenic mouse mastocyte line (PB-3c) using a Zip-neo vector and cells selected for G418 resistance were examined for IL-3 dependence of tumorgenicity. v-H-ras reduced the IL-3 requirement of these cells about 20-fold, but the cells remained factor-dependent. Following inoculation into syngeneic mice, these cells produced tumors after long and variable latencies. Tumor explants grew in vitro without added IL-3, indicating that a progression step had taken place in vivo. These ex vivo lines expressed viral p21 protein and 25/27 lines produced a mitogenic activity which could be identified serologically and biologically as IL-3. Cloning experiments in the presence and absence of IL-3 suggest that an autokrine mechanism is involved.

Thus, we present a novel two-step tumor model: IL-3-dependent cells are first converted by v-H-ras to reduced dependency, which is followed in vivo by a second alteration leading to autokrine IL-3 production.

Stable expression of human tumor associated antigen after DNA transfection into mouse cells

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DNA mediated gene transfer approach was used to clone genes encoding two cell surface human tumor associated antigens defined by the two monoclonal antibodies (MAb). MAb Me14-D12 recognizes a γ-interferon inducible, melanoma associated antigen (Ag) resembling MHC class II molecules and MAb LoVo-D1, a human colon carcinoma specific Ag. Mouse Ltk^- cells were cotransfected with genomic DNA isolated from colon carcinoma cell line and the thymidine kinase gene from Herpes simplex virus. Transfectants expressing the human surface Ag were isolated by selection in HAT medium and fluorescence activated cell sorting. Presence of human DNA in their genome was verify by southern blot and hybridization with nick translated human DNA as probe. Human inserts were enriched for the sequence coding for the Me14-D12 or the LoVo-D1 Ag by a second gene transfer with primary transfectants DNA into new Ltk^- cells. Two stable independent populations expressing the Me14-D12 Ag were obtained. Analysis of their genome by hybridization with human DNA revealed a shared set of restriction fragments considered to be candidates to encode Me14-D12 Ag. The size of the human insert was evaluated at about 25 to 30 kb. Independent populations of secondary transfectants expressing to LoVo-D1 Ag have been obtained and their DNA will be further characterized.

A novel form of membrane-bound PKC in breast cancer cells: possible involvement in growth inhibition by TPA

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Established human breast cancer cells display different sensitivities toward growth inhibition by TPA. No correlation was found between the degree of TPA-dependent growth inhibition and actual levels of PKC activity or the amounts of enzyme activity translocated to membranes in the corresponding cell line. Since the different levels of PKC activity can be either due to variable amounts of enzyme protein or different activity states of PKC, the actual cellular levels of PKC and its TPA-dependent translocation were monitored by an anti-PKC antibody. The amounts of immunodetectable 80 kD PKC matched the levels of enzyme activity. Prolonged treatment of cells with TPA resulted in a constant membrane association of the 80 kD PKC and appearance of a novel 72 kD PKC in the membranes. As predicted from PKC activity measurements, no loss of both the 80 kD holoenzyme as well as the 72 kD form of membrane-bound PKC was observed in those cell lines displaying the highest degree of TPA-dependent growth inhibition.

Transgenic mice harboring a neuroendocrine gene promoter fused with an oncogene develop thymic hyperplasia

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The neuroendocrine growth hormone releasing factor (GRF) stimulates the synthesis and secretion of growth hormone by cells in the anterior pituitary. The few thousand GRF producing neurons are located in the hypothalamus. Studies on the regulation of the GRF gene have been severely hampered since the hypothalamic cells have not been established in culture. To facilitate this and to study GRF gene regulation in vivo transgenic mice were generated that carry hybrid genes composed of the human GRF promoter fused with the gene encoding SV40T antigen. Unexpectedly, these mice suffered from severe thymic hyperplasia whereas abberrations of the hypothalamus were never found. The hyperplasia si due to expression of the oncogene in epithelial cells in the thymus which produce T-cell growth factors. Their imbalance apparently causes increased self renewal of T stem cells since the intra-thymic differentiation of T cells appears normal.

Mechanism of action of oxidant tumor promoters

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Prooxidant states appear to exert promotional effects in initiated cells. They can result from the direct exposure to oxidants, from the meatbolism of xenobiotics or indirectly from inflammation.

In mouse skin inflammation appears to be necessary for promotion. Phorbol-myristate-acetate (PMA) causes the infiltration of monocytes/macrophages which release clastogenic factors (CF). The CF produced by PMA-stimulated human monocytes contain among other components HETES (derived from HPETES), H_2O_2 and probably Fe. Catalase (CAT) and superoxide dismutase (SOD) inhibited the formation of CF.
Promoters could modulate gene expression of initiated cells (see Science *227* (1985) 375). Indeed, PMA induced a temporary decrease in SOD mRNA levels in mouse epidermal cells JB6. The potential role of antioxidant enzymes in promotion is currently being evaluated by overproducing them in cells transfected by recombinant genes. Furthermore, we find striking differences between promotable and non-promotable JB6 cell derivatives in their responses to speroxide, at the level of signal transduction and poly ADP-ribosylation of chromosomal proteins.

Malignant transformation of immortalized human skin keratinocytes by ras-oncogene transfection

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Two cell lines from human skin keratinocytes established spontaneously (HaCaT) or immortalized with SV-40 DNA (HaSV) were investigated. Both lines exhibited a transformed phenotype in vitro with abnormal heteroploid karyotype but remained non-tumorigenic. Upon transfection with the human cellular Ha-ras oncogene tumorigenic clones were obtained. Southern and Northern blot analysis of the HaCaT-ras clones indicated a positive correlation between the level of integration and expression of the ras-oncogene and the abnormal growth behaviour in vivo. Moreover, the reproducibility of the experiments and relative high frequency of tumorigenic clones (5 out of 35) strongly argue for a causal role of the ras oncogene in malignant conversion. Besides the common marker chromosomes of the parental HaCaT line, all ras-containing clones exhibited individual alterations. Although malignant conversion was accompanied by a reduction in differentiation capacity in vitro, in nude mice highly differentiated squamous cell carcinomas developed, expressing the differentation specific keratins. So far, the failure to transform normal human cells by transfection with ras-oncogenes, indicates that immortalization may be a prerequisite to malignant conversion by ras oncogenes, and that this step in carcinogenesis is not associated with major changes in cell proliferation and differentiation.

Expression of two 'pan T' antigens on solid tumor cell lines

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Two T-cells markers, that belong to cluster of differentation CD5 and CD7 identified by our monoclonal antibodies Lau-2D3 (gp67) and Lau-A1 (gp40), were found to be expressed not only on normal and malignant T cells but also on a large number of non lymphoid solid tumor cell lines, including melanomas, gliomas, colon carcinomas and a cervical and an endometrial carcinoma cell line. The expression of these two antigens was studied by antibody-binding radioimmunoassay and cell sorter analysis and confirmed at the biochemical level. Immunoprecipitation experiments showed a large variability in the molecular weight of these antigens in the different non lymphoid cell lines, probably due to different degrees of glycosylation. The expression of CD7 antigen was significantly enhanced on same melanoma cell lines or even de novo induced in a clone of the colon carcinoma line Lovo after recombinant interferon-gamma treatment. In order to further understand the function of this antigen and its regulation by gamma-interferon, mouse L-cells were transfected with DNA of human melanoma cells. Stable primary transformants expressing CD7 antigen were isolated.

Cellular responses to the activation of oncogenes

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The mechanism of action of oncogenes can be separated into events directly related to the biochemical capabilities of the oncogene products and mediated events characterised by changes in cellular gene expression, the state of cellular differentiation and the cellular growth potential. We have identified several levels of *ras* oncogene action in fibroblasts and epithelial cells. Enhancement of ornithine decarboxylase and c-*myc* gene expression was observed in NIH 3T3 fibroblasts which express the p21 *ras* oncogene product. The hormonally induced transcription of the MMTV LTR by glucocorticoid hormones is repressed. Interference with hormone action was also observed in mammary epithelial cells. Cells which respond to lactogenic hormones with the synthesis of the milk casein proteins are unable to do so when the ras oncogene is expressed. The *ras* oncogene also changes the growth parameters of fibroblasts and epithelial cells. Lactogenic hormones usually causing terminal differentiation and cessation of DNA synthesis exert a mitotic stimulus when the mammary epithelial cells express p21 *ras*. Fibroblasts respond with a constitutive increase in the internal pH and mitosis. Transgenic mice carrying an activated *ras* transoncogene develop tumors in organs where *ras* expression is observed and confirm its role in in vivo tumorigenesis.

Detection of dominant transforming genes by gene transfer into immortalized rat embryo cell lines

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The procedure of gene transfer into NIH3T3 cells has detected active transforming genes from approximately 20% of tumor DNA tested. The lack of transforming activity in the majority of specimens may be due to differentiation stage or tissue specific genetic restriction on the type of oncogenes which can be detected in the 3T3 assay. In an attempt to identify transforming genes from DNA which fail to score in the NIH3T3 assay, we have established a panel of immortalized rat embryo cells (REC) by transfection of early passage cells with immortalizing genes (i.e. constructs including c-myc, polyoma mT, Adenovirus Ela). Some of these lines have been characterized for integrated plasmid DNA and phenotype (morphology, anchorage independent growth and tumorigenicity). Preliminary testing of this test system using DNA from T24 bladder carcinoma and MCF-7 breast carcinoma transfected into a c-myc immortalized REC line has detected ras sequences in DNA isolated from primary foci.

Possible mechanism of myelopoiesis inhibition by acute leukemias

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The mechanisms responsible for neutropenia in patients with acute leukemia are poorly understood. We tried to determine if

the humoral regulation of the hemopoiesis is affected in this disease and if leukemic cells themselves produce an inhibitory factor of the normal hemopoetic stem cell proliferation. We investigated the in vitro influence of sera and fractions of sera (Sephadex G150) from normal donors or leukemia patients on the proliferation of the normal CFU-c (colony forming unit granulocyte/macrophage) in soft agar and on the proliferation of the normal CFU-c precursors in long term bone marrow cultures after 5 weeks. The influence of media conditioned by normal or leukemic mononuclear cells was also examined. We could demonstrate a disturbance in the balance between the action of normal inhibitory and stimulatory factors (CSFs) in patients' sera due to a lower concentration level (50%) of some of the CSFs. One of the missing CSFs is a serum protein with MW = 36000 and pluripoietin features. No inhibitory factor produced by leukemia cells could be identified. We explain the low CSF levels in leukaemic sera by consumption of these factors by the predominant leukemia cells.

DNA mismatch repair in *E. coli:* correlation between the binding of the *mutS* protein to the mismatched duplex in vitro and the efficiency of correction in vivo

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Purine 2'-deoxynucleoside analogues inosine (I), 7-deazainosine (DI), nebularine (N), 7-deazanebularine (DN) and tubercidin (Tu) were incorporated into synthetic oligonucleotides, which were complementary to the *Bam*HI/*Pst*I fragment of the polylinker of the filamentous bacteriophage M13mp9 and its opal mutant M13mp9/op7. Using standard methodology of oligonucleotide- directed mutagenesis, RF molecules were synthesized, which contained a single purine/pyrimidine mismatch in the polylinker region. Following transfection of these DNAs into mismatch repair-proficient (JM101) and -deficient (BMH71/18 *mutS*) strains, efficiency and direction of repair was monitored by the change in β-galactosidase phenotype of progeny phage. This repair efficiency could in general be correlated with the in vitro binding of the *E. coli* mismatch binding protein *mutS* to synthetic 16-mer duplexes carrying these mismatch analogues. The in vitro binding studies revealed the importance of flanking sequence effects and mismatch orientation on the efficiency of the protein/DNA interactions.

Transient changes in the protein pattern caused by the activated *ras* gene product

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Expression of the activated human H-*ras* gene leads to cell transformation. Some biochemical changes, provoked by the activated *ras* gene product (p21), are of a transient nature and no longer detectable in fully transformed cells. To study such effects we employed cell lines containing the activated and normal *ras* genes fused to the inducible promotors derived from MMTV or the hsp 70 gene. Following the induction of *ras* gene transcription we investigated the alteration in the patterns of newly synthesized proteins using 2D gel analysis. Expression of both, the normal and the mutated p21, results in numerous changes. In many cases the mutated p21 has a considerably stronger effect despite its much lower accumulation. Some changes are soley attributable to the mutated p21. Most changes are of a transient nature. Enhanced synthesis predominates while suppressed expression is rare.

The impairment of membrane fluidity, enzyme activities and ultrastructural properties of the liver plasma membrane during chemical carcinogenesis

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The effects of the chemical carcinogenesis on the membrane fluidity, the intramembrane glycoproteins frequency and on marker enzyme activities were evaluated in plasma membrane fractions isolated from normal liver and from neoplastic nodules induced by a combined regimen of diethylnitrosamine, 2-acetylaminofluorene and carbon tetrachloride. Plasma membranes of hyperplastic nodules were less fluid, as indicated by the increased order parameter S measured by means of spin labeled fatty acids and also had an altered distribution and density of intramembrane particles, as evaluated stereologically on freeze-fracture preparations. In these membranes, the 5'-nucleotidase and the basal adenylate cyclase activity were diminished. The latter, however, responded better to NaF than did that on normal plasma membranes, while the response to glucagon was lacking. Na-K-Mg Adenosine triphosphatase was also inhibited in neoplastic membranes while the ouabain – sensitive Na-K-Adenosine triphosphatase had a similar activity in both normal and carcinogen-treated rats.

Altered PKC in a malignant mast cell line

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PB-3c is a bone marrow-derived mast cell line which requires interleukin 3 (IL-3) for proliferation and antigen-mediated serotonin release. This antigen-mediated exocytosis is inhibited by TPA, a protein kinase C (PKC) activator. In contrast, the malignant cell variant PB-1 grows in the absence of IL-3 and is unable to undergo antigen-mediated exocytosis resembling TPA-treated PB-3c cells. The PB-1 cells showed a significantly higher membrane-bound specific PKC activity, displayed an unique class of phorbol ester receptors and exhibited altered IL-3 binding as compared with PB-3c. In addition to the normal 77 kD PKC holoenzyme, an immunologically related 72 kD PKC was predominantly found in the membrane fraction of PB-1. It is suggested that this altered and membrane-bound form of PKC may be involved in the blockage of the antigen-mediated exocytosis of PB-1 cells.

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Tumor cell deformability and metastasis in B16 melanoma cell variants

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To determine whether a correlation exists between tumor cell deformability and metastatic potential, 4 B16 melanoma variants of identical cell size, Fla, Nr. 1, Nr. 2 and Nr. 4 (in order of increasing metastatic capacity. Invasion Metastasis 5: 1–15 (1985)) were filtered through 10 µm Nuclepore filters. The filtration procedure was specifically designed to avoid cell aggregation or adhesion to the filters, and to ensure over 90% recovery

of cell input. Cell Fla with the lowest metastatic rate needed 44 s for 50% of the input to pass the filter; No. 1 cells with intermediate metastasis passed in 17s, whereas highly metastatic variants Nr. 2 and 4 required 12s. Pretreatment with cytochalasin B reduced these times to 11, 9 and 7s for Fla, Nos 1, 2 and 4 respectively. Colchicine led to a somewhat smaller reduction (19, 14 and 8 s). These findings imply a major involvement of the cytoskeleton in cell deformability as measured by filtration. The correlation of filtration rates to metastasis thus suggest that cytoskeleton and deformability play an important role in the metastatic process of the B16 melanoma.

Phosphorylation of two 27,000-dalton proteins in response to PKC activators in human mammary carcinoma cells

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Exposure of human mammary carcinoma cell lines to phorbol-12-myristate-13-acetate (PMA) leads to growth inhibition, which involves a rapid phosphorylation of two 27,000-dalton cytosolic proteins (pp27). The phosphor content of the pp27 increased about 3–4 times within 15 min and returned to the control level after 24 h PMA treatment. The phosphorylation level of the 27 kDa proteins was found to match the amount of protein kinase C (PKC) associated to the membranes. Other PKC activators like phospholipase C, the calcium ionophore ionomycin and fetal calf serum were also found to induce, in vivo, the phosphorylation of the pp27. In contrast, insulin, epidermal growth factor and dibutyryl-cAMP showed no effect. These results suggest that the enhanced pp27 phosphorylation mediated by PKC may be one of the early biochemical events leading to growth inhibition in human mammary carcinoma cells.

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Function of the *ras* gene in *Dictyostelium* and its introduction in mammalian cells

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Ras genes containing a missense mutation at codon 12 alter *Dictyostelium* development. In aggregation competent cells, mutated *ras* influences the cGMP response after cAMP stimulation and seems involved in adaptation. Binding of cAMP to cell extracts show that ras affects receptor number in the presence of Ca^{2+} and ATP. Furthermore, addition of a phorbolesther (PMA) parallels the effect of the mutation at amino acid 12, thus suggesting the involvement of ras in a protein kinase C pathway.

In another set of experiments, we detect differences in cellular localization and partitioning in Triton X-114 detergent of α-actinin in mutated *ras* containing cells using a monoclonal antibody.

Dictyostelium ras genes were also transfected into NIH 3T3 cells. Even though foci number was not above controls, we obtained tumors in nude mice after injection of agar selected cells transfected with mutated *ras* genes. The presence and expression of *Dictyostelium ras* in these tumors is currently investigated. We obtained hygromycin resistant cells containing *ras* genes after co-transfection with pY3 vectors. Injection of such cells in nude mice has not resulted in the formation of tumors up to now.

Activation of N-*ras* oncogene in human leukemia

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Interested in the clinical relevance of activated *ras* oncogenes we have recently established an assay system, using synthetic oligonucleotides, which allows detection of activating point mutations of the N-*ras* gene directly in the DNA of cancer patients. A study of 18 patients with acute nonlymphocytic leukemia resulted in the detection of 5 N-*ras* point mutations. Four mutations uniformly involved codon 12 (GGT→GAT), 1 mutation was found a codon 61 (CAA→AAA). By monitoring the follow-up of some of those patients, it became obvious that during chemotherapeutically induced remission the mutation was not longer detectable by our method. A variant case which does not seem to follow this 'rule' will be discussed.

A novel point mutation at codon 13 (GGT-TGT) has been detected in the N-*ras* gene in T-cell lymphoma malignancy, using combination of the NIH/3T3-transfection technique with the oligonucleotide hybridization method.

The calcium-binding protein oncomodulin as marker in chemically transformed and in growth stimulated rat fibroblasts

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Oncomodulin (Mr 12 kD, pI 3.9), expressed in tumor cells but not in normal tissue (Oncodev. Biol. Med. *3* (1982) 79, was isolated from rat Morris hepatoma. The purified protein was used as a specific probe to study its expression in chemically transformed and in growth stimulated rat fibroblasts. A rapidly proliferating granulation tissue was exposed in vivo to N-methyl-N'nitro-N-nitroso-guanidine (MNNG, 0.3–3.6 mg/kg) or in vitro to aristolochic acid (10–20 μg/ml for 2 h). The mutagen-exposed cells were screened for colony growth in soft agar. Clones were picked up individually, expanded in culture, and 6×10^6 cells per animal injected s.c. into nude mice to check for tumor growth.

2-D-PAGE of chemically transformed fibroblasts showed an oncomodulin spot (as confirmed by co-migration experiments), whereas oncomodulin was absent in untreated fibroblasts. Solid tumors derived from treated cells, injected into nude mice, showed a prominent oncomodulin spot. An early expression of oncomodulin was found in chemically transformed and also in growth stimulated fibroblasts. This protein seems to be a useful tool in studying the early appearance of this marker during transformation.

An in vivo/in vitro assay for the chemical transformation of rat fibroblasts

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Fibroblasts from a rapidly proliferating granulation tissue of 30-day-old, male S/D rats were treated in vivo with MNNG (0.3–3.6 mg/kg; injected s.c. into an air pouch) or in vitro with aristolochic acid (10–20 μg/ml for 2 h). The mutagen-exposed cells were screened for colony growth in soft agar. Clones were picked up, expanded in cell culture, and 6×10^6 cells per animal injected s.c. into nude mice to check for tumor growth. The spontaneous transformation frequency of freshly isolated, untreated fibroblasts (no pouch cells) was found to be $4.0 \pm 0.57/10^6$ cells ($n = 40 \times 10^5$), and dropped from $3.0 \pm 0.64/10^6$ to $0/10^6$ cells ($n = 40 \times 10^5$) when these cells were cultured for 1 to 3

passages. Untreated, cultured pouch cells revealed an increased colony formation up to 7 clones per 10^6 cells when the cells were isolated 1 to 3 days after formation of the air pouch. Treated pouch fibroblasts showed colony formation in soft agar in a dose-dependent manner, but significantly higher than in untreated pouch cells. Solid tumors were obtained from treated cells after injection into nude mice. No tumor growth was observed from rapidly proliferating, untreated pouch cells and from normal, untreated fibroblasts.

A milk protein gene promoter directs c-myc oncogene expression to mammary glands in transgenic mice

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We have established transgenic mice expressing the human Ha-*ras* gene under the control of the whey acidic protein (WAP) promoter region. Due to the mammary-specific, hormone-regulated promoter, the *ras* gene is expressed in the lactating mammary glands. However, the expression of one oncogene was not sufficient to cause transformation of mammary epithelial cells. Tumor formation very likely requires additional genetic events, such as the expression of another oncogene. In order to provide a second event we are establishing transgenic mice expressing the mouse c-*myc* oncogene subjected to the WAP promoter. The transmission and expression of the WAP-*myc* hybrid gene in five lines is under investigation. As of now, *myc* transgene expression occurs in one line in mammary epithelial cells and is regulated by lactogenic hormones. Myc expression during several lactation periods did not cause tumors in this line. Mice expressing both oncogenes will enable us to study the cooperation of *ras* and *myc* in tumor formation in an in vivo model.

Symposium 6: Control of gene expression during early development and cell differentiation

The evolution of octamer and CAAT binding factors, but not Sp1 proteins predates the divergence of sea urchin and man

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The octamer ATTTGCAT is not only an important regulatory element of several vertebrate genes, but also of the sea urchin early, late and sperm histone H2B genes. We have identified an octamer binding activity which is present in nuclear extracts prepared from sea urchin testis and from blastula and gastrula embryos and which interacts with the octamer sequence of all sea urchin H2B genes analysed. This ubiquitous octamer binding factor of the sea urchin generates the same DNase I and methylation protection patterns as the respective HeLa cell factor and, in addition, recognizes the octamer sequence in the enhancer of the murine immunoglobulin heavy chain gene. These data therefore suggest that octamer binding factors have been highly conserved in their DNA binding domain over a large evolutionary distance. The CAAT binding activities present in the three sea urchin extracts and in the HeLa cell extract all differ however in the DNase I footprint pattern they generate on the CAAT sequence of the sperm H2B promoter, whereas the ubiquitous vertebrate transcription factor Sp1 could not be detected in sea urchin extracts. These results indicate that octamer and CAAT binding factors have evolved before the divergence of echinoderms and vertebrates in contrast to the Sp1 proteins.

Cis- and trans-acting elements of developmentally regulated sea urchin histone genes

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Nuclear extracts prepared from sea urchin testis and from blastula and gastrula embryos were analyzed for factors interacting with promoter elements of early, late and sperm histone genes. All three extracts contain an ubiquitous factor recognizing the octamer sequence ATTTGCAT of all H2B promoters analyzed. Different CAAT binding activities were detected in the three extracts and hence constitute a family of related, stage-specific proteins. The CAAT and octamer binding factors of the testis extract were shown to associate with each other through protein-protein interaction, suggesting that these two proteins and the particular topology of their recognition sites within the sperm H2B promoter might be responsible for the tissue-specific expression of the sperm H2B gene. A nuclear factor present in blastula and gastrula embryos binds with equal affinity to one upstream element in each promoter of two pairs of late H2A and H2B genes. This nuclear factor does not recognize early and sperm histone genes and is absent from testis indicating a possible role for it in the developmental regulation of late histone genes. The recognition sequence for this novel factor was shown to be required in addition to the octamer element for efficient in vitro transcription of a late H2B gene in the gastrula nuclear extract.

Modulation of *paired* gene expression patterns in segmentation mutants of *Drosophila*

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The transcription pattern of *paired (prd)*, a segmentation gene belonging to the pair rule class of *Drosophila*, exhibits initially a double-segment periodicity and switches to a single-segment repeat during syncitial blastoderm. We have examined the effects of mutations in genes of all segmentation gene classes on the spatial expression of the *paired* gene. The results are consistent with the idea of a hierarchy among segmentation genes and reveal that the establishment of position specific expression of *prd* involves combination of segmentation genes that act in distinct partially overlapping domains along the anterior-posterior axis of the embryo.

Combination of three different domains in the *Drosophila paired* gene in support of a gene network concept

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On the basis of evolutionary considerations, we have formulated the following gene network concept. Genes of an integrated system, such as those regulating early development, form a functional network. We assume that genes of an integrated system are composed primarily of a relatively small number of different domains and their structural homologues, each gene representing a specific combination of a few of these domains. In a first test of our concept, we found that an arbitrarily chosen developmental gene, the segmentation gene *paired* of *Drosophila*, consists of at least three different domains each of which appears to occur preferentially (or exclusively) in a modified version in a number of other genes also involved in developmental processes. In this test two new domains, the PRD-repeat and the paired box, as well as two new types of homeo boxes were discovered. The paired box, a highly conserved protein domain of about 130

amino acids, has been found to be present in at least three segmentation genes and may be linked specifically with genes required for proper segmentation of the embryo.

Transcriptional activity of the transposon-like element Tas in the chromatin eliminating nematode *Ascaris lumbricoides*

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Several copies of the retrovirus-like element Tas, approx. 7 kb in length and containing 256 bp inverted repeats, have been isolated from a genomic library of *Ascaris lumbricoides var. suum*. In order to analyze the transcriptional activity and biological significance of these Tas elements, we constructed two poly A cDNA libraries, namely from oocyte and 1–64 cell stage RNAs. Two further cDNA libraries were established by hybrid selecting total spermatid and oocyte RNAs with immobilized LTR and subsequently priming the eluted RNA with an internal LTR restriction fragment for cDNA synthesis. Intensive screening of the different gene libraries revealed the presence of 2.1 kb and 2.5 kb cDNAs homologous to a 5' Tas probe and that of 1.5 kb inserts hybridizing with a 3' Tas subfragment. At present we are mapping and testing the stage specificity of Tas transcription products.

Pattern formation in *Drosophila*. The *snake* gene appears to encode a serine protease

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Snake, a maternal effect gene, is required for the establishment of the dorsal-ventral axis during the embryonic development of *Drosophila*. We have cloned the *snake* locus and rescued the genetic defect of *snake* mutants by genetic transformation using the wild type gene. The deduced amino acid sequence of *snake* reveals that it encodes a protein which is structurally similar to proteases involved in blood clotting, peptide processing, and complement fixation pathways.

Structure of a component of a putative membrane protein complex mediating cell-cell adhesion in *Drosophila*

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In analogy to a recently characterized membrane protein complex mediating cell adhesion in sea urchin embryos, a glycoprotein complex was purified from third instar larvae of *Drosophila*. An antiserum raised against it detected three independent λgtll-recombinant cDNA clones containing sequences from a single coding region located in chromosomal section 100B. Its 3.5 kb transcript codes for a glycoprotein of about 100 amino acids consisting of nine homologous domains with cysteine residues, potential phosphorylation sites and N-linked glycosylation sites at characteristic positions. This feature of repeated cysteines with characteristic spacings is found in a number of recently described membrane receptors. Northern analysis reveals continuous transcription with higher levels during the first half of embryogenesis and in third instar larvae.

Expression of α-smooth muscle actin (SMA) mRNA in rat aortic smooth muscle cells (SMC)

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Northern hybridization using a general actin cRNA probe and a cRNA probe specific for α-SMA mRNA shows that in normal rat aortic media the relative expression of α-SMA mRNA increases from 0.47 in 5-day-old, 0.73 in 14-day-old to 1.0 in adult rats. α-SMA mRNA decreases to 0.32 in aortic intimal thickening 15 days after endothelial injury, when SMC replicate; 60 days after injury, SMC stopped to divide and have a content of α-SMA mRNA comparable to that of normal aortic media. In primary culture of aortic SMC, αSMA mRNA represents between 83 and 91% of total actin mRNA; however only low amounts of α-actin are synthesized and translated in these cells. α-SMA mRNA decreases to 21% in subconfluent SMC at the fifth passage, this percentage being comparable to that of synthesized proteins. Thus, actin mRNAs are modulated in vivo during development and pathological conditions and during culture. In SMC primary culture, the initial decrease in α-actin expression depends on changes of mRNA translation rather than transcription.

Multiple elements involved in the tissue-specific expression and developmental regulation of AFP genes in transgenic mice

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Alpha-fetoprotein (AFP) is the major serum protein in the developing mammalian fetus. It is produced at high levels in the visceral endoderm of the yolk sac and in the fetal liver, and to a lesser extent in the gastro-intestinal tract. Its levels are reduced several orders of magnitude in the neonatal liver. The AFP gene in the mouse is under the control of at least two unlinked, trans-acting loci, termed *raf* which acts in liver to regulate the adult basal level of AFP mRNA, and *rif* which determines the degree of inducibility of AFP mRNA during liver regeneration in response to injury.

To map the regulatory domains in the AFP gene which are responsible for its tissue-sepcific pattern of expression, its post-natal repression and its reinduction during liver regeneration, we have generated a series of transgenic mice carrying the AFP gene and varying amounts of the 5' flanking region. The transgenic mice display high levels of expression in all three cell lineages which express the gene. Three enhancer elements were identified which are capable of directing the post-natal repression of the AFP gene in liver, as well as the induction of expression during liver regeneration.

Differential expression of the *Drosophila caudal* gene

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The *caudal (cad)* gene of *Drosophila* encodes two mRNAs: a maternal RNA of 2.4 kb and a zygotic RNA of 2.6 kb. The two transcripts have different promoters and differ at their 3' ends as well. *Cad* consists of two exons separated by a large intron. Both mRNAs contain the same open reading frame of 472 amino acids including a homeo box. The maternal transcript is synthesized in the nurse cells and is deposited evenly into the oocyte. In situ hybridization analysis and antibody staining show that the RNA and the protein are localized in an antero-posterior gradient during syncitial blastoderm. The protein is found mainly in the nuclei and is also present in the nuclei of the pole cells. From the cellular blastoderm on, zygotic RNA and protein are local-

ized in the primordia of the terminal abdominal segment and in the hindgut and later during germband extension also in the posterior midgut rudiment. In 3rd instar larvae *cad* is expressed in the gut, defined regions of the genital discs, and in the gonads of both sexes. It is the first homeo box-containing gene found to the expressed in the germline of *Drosophila*. The protein expression in maternal and zygotic mutants will be discussed.

Comparison of protein synthesis between different tissues of early postimplantation mouse embryos

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Mouse embryos of the NMRI strain between the 7th and 9th day of gestation were isolated from the uterus and dissected into the various tissue derivatives. The day-7 embryo was fragmented into trophoblast and ectoplacental cone, extraembryonic and embryonic ectoderm, distal and proximal endoderm. The day-8 embryo was divided into trophoblast and placental anlage, yolk sac, amnion, allantois, as well as cranial, central and caudal embryonic tissue. The isolated fragments were incubated in DMEM in the presence of ^{35}S-methionine for 4 h and then further processed for 2-D gel electrophoresis. Protein synthesis of the isolated tissue derivatives was analysed and investigated for cell lineage specific patterns. Concerning the proteins with M_r-values in the range of 20000 to 90000, we have found several significant quantitative and qualitative differences between the various tissue fragments. In addition, we have observed further quantitative and qualitative differences in protein synthesis between the developmental stages analysed. We propose that these differences reflect some of the cell lineage and developmental stage specific changes in protein synthesis during early mammalian postimplantation.

Towards an anti-sense assay of gene activity in embryogenesis

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Probing gene function by the anti-sense RNA approach during embryogenesis has encountered difficulties due to poor stability of the RNA injected into zygotes, and to its uneven distribution among blastomeres. We have set up conditions to produce RNA in *Xenopus* embryos from heat-inducible expression vectors under the control of a human heat shock promoter. Circular or linear DNA injected into zygotes is replicated during cleavage and supports appreciable synthesis of RNA upon heat treatment of host embryos up to stage 20. Polyadenylated RNA has a halflife of over 6 h.

Heat-inducible vectors thus provide a means to produce meaningful amounts of anti-sense RNA in *Xenopus* embryos between midblastula (stage 10) and early tailbud (stage 24).

An in situ enhancer trap: the detection of cell-type-specific elements in *Drosophila melanogaster*

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A translational fusion of the *Escherichia coli lacZ* gene to the P-element transposase, under the control of the P-element promoter, has been used to detect elements in the *Drosophila* genome which can regulate expression of the P-*lacZ* fusion at a distance. Some 60 different transformant lines of flies carrying the construct have been isolated, and 60% of these show some type of tissue- or cell-type-specific expression of *lacZ* in embryos. The expression patterns recovered include two with a two-segment periodicity, and fourteen with various patterns of nervous system expression. The results indicate that the P-*lacZ* fusion should be a powerful tool for detecting and analysing at the genetic and molecular levels, regulated gene expression in *Drosophila*.

Structural characterization of two new heat inducible genes form the *Drosophila* locus 67B

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We have characterized two developmentally regulated and heat shock inducible genes from the *Drosophila melanogaster* locus 67B. They are called genes 2 and 3 since their products are so far not known. Gene 3 is expressed during the mid-embryo-genesis and the beginning of pupation. Its genomic sequence reveals an open reading frame of 170 amino-acids having a clear homology with the other small heat shock genes and the mammalian αcrystallin. Gene 2 produces three differents transcripts. The largest is heat inducible and not expressed during the development. The two small developmentally regulated mRNAs are not activated by a stress; one is expressed during the embryogenisis and the early pupation, the other during pupation and in the male testis. The comparison of the genomic sequence with those of several different cDNAs reveals the presence of several small introns. (Introns are in general absent from heat-shock genes). Furthermore, the putative protein of 102 amino-acids has no homology with the other small heat shock proteins.

Construction of a mosaic gene suited for assaying DNA sequence elements stimulating transcription in *X. laevis* embryos

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In order to be able to determine the influence of upstream sequences on gene expression during early development we have assembled, in the polylinker area of pUC19, a mosaic gene consisting of the promotor area of the xenopsin gene, the translation initiation area of the α-skeletal actin gene, and coding sequences of the SV40 T antigen gene. In front of the promotor a unique EcoRI site allows one to graft additional upstream sequences. This mosaic gene is slightly transcribed when injected into oocytes. We are presently studying the influence of the SV40 enhancer and putative stimulatory sequences on the transcription of this mosaic gene after microinjection into oocytes and fertilized eggs.

Monoclonal antibody against α-smooth muscle actin. A new probe for smooth muscle differentiation

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We selected and characterized a monoclonal antibody (anti-α-sm-1) recognizing exclusively α-smooth muscle (α-sm) actin. In the aortic media anti-α-sm-1 recognized a population of cells negative for α sm actin and for desmin. The number of these cells decreases during differentiation. In cultures of rat aortic media SMC, there is a progressive increase of this cell population together with a progressive decrease in the number of α-sm actin

containing stress fibers per cell. Double immunofluorescent studies carried out with anti-α-sm-1 and antidesmin antibodies in several organs revealed an heterogeneity of stromal cells: some of them being desmin negative and α-sm actin positive and the others desmin positive, α-sm actin negative. α-sm actin was also found in myoepithelial cells and in stromal cells of mammary carcinomas, previously considered fibroblastic in nature.

Differentiation specific expression of poly-N-acetyllactosamine containing molecules in mouse teratocarcinoma cells

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Poly-N-acetyllactosamines are developmentally regulated in several systems and may be involved in cellular interactions. In the F9 teratocarcinoma model system these high-molecular-weight glycans show differentiation induced structural changes. In order to study the nature of the molecules carrying these carbohydrates a cell surface labeling specific for poly-N-acetyllactosamines was performed. Three major glycoproteins showing a differentiation dependent expression were labeled. Two of them were identified as laminin subunit A and B on the basis of the molecular weight and immunoreactivity with anti-laminin antibodies. The nature of the third high-molecular-weight species is currently under investigation. Developmentally regulated expression of poly-N-acetyllactosamines in molecules like laminin may play a role in modulation of cell-matrix interactions during embryogenesis.

Characterization of an intronless sarcomeric actin gene expressed in *X. laevis* and *X. tropicalis*

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A sarcomeric actin gene devoid of introns has been isolated from a *X. laevis* genomic library. The corresponding gene from the more primitive species *X. tropicalis* has also been isolated. Both genes consist of a sequence of 1131 bp able to code for a functional sarcomeric actin. The nucleotide sequences diverge by 4% of their bases; 2 base substitutions generate amino acid exchanges. The amino acids encoded by the *X. laevis* gene are characteristic of an α skeletal actin, whereas in *X. tropicalis* they are typical of an α cardiac actin. Northern blot analysis and S1 mapping experiments show that both genes are expressed in embryos. The transcription profile however is different in adults. In *X. laevis*, the gene without introns is strongly expressed in adult skeletal muscle, and only slightly in adult heart; in *X. tropicalis*, on the contrary, it is transcribed in adult heart, but inactive in adult skeletal muscle.

Isolation of homeo box sequences from honeybees

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To study genes that control early development of the *Honeybee*, we have isolated seven homeo box-containing genes using homeo box sequences of *Drosophila melanogaster*. A *Honeybee* library in the EMBL4 vector was constructed and screened with *Antennapedia, engrailed,* and *msh* homeo box sequences of *Drosophila melanogaster*. Sequence analysis of all homeo boxes revealed that all seven *Honeybee* genes isolated have strong homology to their corresponding *Drosophila* genes, i.e. *Deformed, Sex combs reduced, Antennapedia, infra-abdominal 2, engrailed* and *msh*. The region of homology includes the homeobox itself and sequences 5' and 3' of the homeo box. Homologies outside the homeo box are 4–29 amino acids long depending on the gene. The degree of amino acid homology varies between 90% and 100%. To test if the isolated *honeybee* genes have similar functions as the corresponding *Drosophila* genes, in situ hybridization to sections of *Honeybee* embryos was performed. First results with the clone H42, the *Deformed* homologue, revealed a spatial and temporal equivalent hybridization in the *Honeybee* compared to hybridization of *Deformed* in *Drosophila*. This fact is a strong indication for a similar function of both genes.

Regulation of *Antp* gene expression and function of its gene product

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Segments in *Drosophila* are specified mainly by genes of the ANT-C and BX-C. We have shown that *Antp* protein distribution in the CNS of wildtype embryos was strongest in T1p, T2a, T3a and extends weakly posteriorly to A7a. Here, we present the effect of mutations in the three complementation groups of the BX-C on *Antp* expression: *Ubx, abdA, AbdB* – alone or in double and triple mutant combinations. The data suggest a non additive regulation of *Antp* gene expression by those genes. In nervous systems of embryos lacking both the genes of BX-C as well as *esc* the pattern of *Antp* protein distribution extends from the brain to the very posterior end of the CNS. Comparison to CNS of embryos lacking *esc* only suggests that repression of *Antp* in the very end of the CNS requires the concerted action of the gene products of both loci. The homeodomain, present in proteins encoded by many genes involved in segmentation and segment identification is thought to bind to DNA. We present evidence that the protein of the *Antp* gene binds specifically to DNA sequences in its own promoter region, as well as to sequences in its non translated leader.

Symposium 7: Control of gene expression by steroid hormones

Regulation of transcription in mouse mammary tumor virus (MMTV) mutants

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Using linker scanning mutagenesis we have identified four elements within the glucocorticoid regulatory region of MMTV DNA which are necessary for optimal hormone response (J. molec. Biol. *190* (1986) 367 and 379). Two elements overlap with regions to which glucocorticoid receptors bind in vitro. The third element contains a binding site for nuclear factor 1, and the fourth the TATA box. Insertion of a 123-bp DNA spacer between any two of them led to a 5- to 10-fold reduction of the hormone response in transfected cells, indicating that the relative distance between elements is important. Analysis of deletion- and double mutants showed that the nuclear factor 1 binding site is also involved in the basal level of transcription, and that it is functionally associated with the proximal receptor binding site for the hormone response.

The molecular basis for pancreas-specific gene expression in mouse

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The transcription of the α-amylase gene *Amy*-2^a, but not that of elastase II and trypsin genes, is modulated by glucocorticoid hormones. This gene-specific hormone effect is pancreas-specific and indirect. It is mediated by an enhancer-like upstream element of *Amy*-2^a. Closely related sequence motifs occur in front of many other pancreas-specific genes. Footprint analysis under conditions which allow accurate transcription of *Amy*-2^a in vitro reveals a pancreas-specific protein factor bound to the enhancer. Identical footprints are observed using nuclear proteins from pancreatic cells grown in the presence or absence of the hormone. The role of this protein for modulating transcription of *Amy*-2^a and other pancreas-specific genes is being discussed.

The early ecdysterone-controlled gene I-18C of *Chironomus tentans* gives rise to multiple transcripts

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The cloned ecdysterone-controlled gene I-18C of *C. tentans* produces at least three different polyA RNAs: two of them approx. 4.6 kb and one 1.8 kb. They have a common transcription initiation site and arise by differential splicing. The RNAs differ in their metabolism and in their tissue distribution: the 4.6 kb type was detected in embryos, larvae, and pupae, but it was not found in adults. Adults, however, express the 1.8 kb RNA which is absent in in the other stages. All three RNAs are made constitutively in an epithelial cell line of *C. tentans*. While the abundance of all 3 RNAs is increased by ecdysterone, only the 1.8 kb RNA is also induced by heat shock. It codes for a 16 kD heat shock protein (see abstract by B. Lutz and M. Lezzi). In line with these findings is the discovery of heat shock consensus sequences in the promotor region of the I-18C gene.

Rapid cellular responses induced by estradiol and EGF in human mammary tumor cells: S6 phosphorylation, protein synthesis and c-myc expression

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Growth of the human mammary tumor cell line ZR-75-1 is stimulated by estradiol (E_2), epidermal growth factor (EGF) or α-transforming growth factor (α-TGF) to a similar extent and in an additive manner. To define if E_2 and EGF (α-TGF) use converging pathways, some early cellular responses were studied in serum-deprived ZR-75 cells. EGF and α-TGF led to rapid activation of 40S ribosomal protein S6 kinase and S6 phosphorylation but E_2 did not induce these responses. The rates of onset of overall protein synthesis during the first 4 h of stimulation were distinctly slower with E_2 than with EGF.

The induction of the protooncogene c-myc being a response to growth factors, it was investigated whether E_2 also leads to its enhanced expression. Northern analysis of RNA from EGF (α-TGF) and E_2-treated ZR-75 cells revealed distinct kinetic differences in the stimulation of c-myc expression by either E_2 or EGF (αTGF). Induction by EGF was visible after 1 h of stimulation, whereas induction by E_2 was detected after 4 h.

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Interference of oncogene products with the hormone dependent MMTV-LTR transcription

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The H-*ras* and the v-*mos* oncogene proteins repress the glucocorticoid hormone-dependent transcription of the MMTV-LTR (EMBO J. *5* (1986) 2609). To probe the mechanism of this effect NIH cells transfected with a MMTV LTR gene construct or a chimeric gene construct consisting of only the hormone-responsive element of the MMTV-LTR (HRE) and the α-globin gene were infected with *ras*-, *mos*-, *scr*- or *myc*-expressing retroviruses. The transcriptional activity of the hormone-dependent MMTV-LTR was stimulated by dexamethasone in all cases. The transcription of the gene constructs was subsequently repressed in *ras*- and *mos*-infected cells, but not in cells infected with *src* and *myc* viral vectors. Stimulation with dexamethasone and treatment iwth cycloheximide did not change the pattern of repression of the HRE-α-globin gene transcription. We conclude that the hormone/receptor binding domain is instrumental in the repression of the MMTV-LTR transcription, and suggest that the inhibtion of the transcription is mediated by a modification of the activated glucocorticoid receptor complex.

The estrogen responsive DNA element; sequence requirements and interaction with trans-acting factors

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By transfection of vitellogenin A2-CAT gene constructs into an estrogen responsive human cell line we have localised an estrogen responsive DNA element (ERE) in the 5'flanking region of the frog gene. A 13 bp palindromic sequence (position –331/–319) is sufficient to confer estrogen inducibility to a heterologous promoter (HSV thymidine kinase, tk). A single point mutation within the 13 bp element abolishes estrogen inducibility. This correlates with a strongly reduced affinity of the regulatory element for the estrogen receptor as tested by an in vitro receptor binding assay. The ERE regulates transcription initiation at the tk promoter in a relatively position and orientation independent manner and thus behaves like an estrogen inducible enhancer. An estrogen independent regulatory element has been identified downstream (–134/–87) of the ERE which in contrast to the ERE acts only in its normal orientation.

The early ecdysterone-controlled gene I-18C of *Chironomus tentans* codes for a heat shock protein

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In larval salivary glands of *Chironomus tentans* the molting hormone ecdysterone induces a prominent puff at locus I-18C. A gene isolated and cloned from this region codes for several transcripts (see abstract K. Dorsch-Häsler et al.). Sequence data indicate that one of them, a 1.8 kb poly(A^+)-RNA, contains a 471 b open reading frame which could code for a 16.6 kD protein. A 300 bp segment of this has been cloned into the expression vector pEX (K. Stanley et al., EMBO J. (1984) 1429). By immunisation of rabbits with the fusion protein made thereof in *E. coli*, an antiserum was obtained recognizing the Chironomus portion of the protein. This serum precipitated a 16 kD protein from Chironomus tissue culture cells which exhibited a V8 protease peptide pattern identical to that of immunoprecipitated peptides made in vitro either of SP6-RNA, containing the whole open reading frame, or of Chironomus mRNA. Remarkably, this 16 kD protein is heat-inducible in tissue culture cells as well as in salivary glands.

Identification of regulatory DNA sequences flanking the transcription initiation site of the *Xenopus* vitellogenin gene B1

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The 5'-flanking region of the *Xenopus laevis* vitellogenin gene B1 can confer estrogen inducibility to the HSV thymidine kinase promoter (Tk) in a transient expression assay using chimeric CAT constructs transfected into the human estrogen-responsive MCF-7 cell line. The induction is observed both at the level of CAT activity and correctly initiated tk-CAT mRNAs. Using 5' and 3' deletion mutants, we have localized an estrogen regulatory element (ERE) that contains an essential 13 bp palindromic sequence normally found between positions −334 and −322 bp upstream of the gene B1 promoter. The ERE behaves like an estrogen-inducible enhancer since it functions in both orientations and also when placed far away, i.e. ~ 1.7 kb 3' to the Tk promoter. In the latter case, duplication of the ERE leads to an increase in the stimulation by estrogen. We are currently testing the role of other vitellogenin upstream sequences using a similar transient expression assay.

Minimal nucleotide sequence requirement for steroid hormone induction of MMTV transcription

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Using transfections of LTK$^-$ mouse cells with chimeric molecules containing a mutated MMTV LTR joined to the coding region of the HSV-TK gene (Buetti, Kühnel and Diggelmann, 1986, J. Mol. Biol. *190*, 367–378 and 379–389) we have shown that two elements upstream of the TATA-box are necessary for the hormone dependent enhancement of MMTV transcription: the distal element is delimited by mutations at positions −181/−172 and contains the sequence CAAACTGTTC with a 3 bp dyad symmetry. The proximal element, located at −120 bp is an incomplete version of the distal element (CAAATGTTC). We are examining the contribution of these sequences in the steroid regulated transcription by substituting the complete proximal and/or the distal regulatory region with an oligonucleotide containing the minimal element or multimers of it and quantifying the transcription in presence or absence of hormone by S1 nuclease protection analysis.

Functional analysis of the rat glucocorticoid receptor

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The glucocorticoid receptor activates transcription by sequence-specific binding to a class of hormone-dependent enhancers[1]. We have obtained cDNA clones encoding 6.4 kb corresponding to the rat glucocorticoid receptor mRNA. Complete DNA sequence analysis revealed the putative initiator codon followed by an open reading frame of 795 aa and an alternatively polyadenylated trailer[2]. In vitro expression of fusion proteins containing discrete regions of the receptor allowed localization of epitopes recognized by two distinct monoclonal antibodies, and of functional domains for DNA and hormone binding (residues 440–546 and 550–793, respectively)[3]. Further properties of the binding domains will be discussed in the light of the most recent results obtained from experiments in which the trans-activation potential of various receptor segments is determined in vivo in a transient cotransfection assay[4].

[1] Chandler, Maler and Yamamoto, Cell *33* (1983) 489.
[2] Miesfeld et al., Cell *46* (1986) 389.
[3] Rusconi and Yamamoto, EMBO J. (1987) in press.
[4] Godowski, Rusconi, Miesfeld and Yamamoto, Nature *325* (1987) 365.

Glucocorticoid regulation of expression of the tyrosine-aminotransferase gene

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In gene transfer experiments two glucocorticoid response elements (GRE) located 2500 pb upstream of the transcription initiation site were identified. These elements are binding sites of the glucocorticoid receptor as evidenced from in vitro and in vivo footprinting experiments. The two GREs induce expression of the TAT gene in a cooperative manner, the proximal element which in itself has no inducing capacity will strongly enhance glucocorticoid induction in the presence of the other. Cooperativity of these two elements is maintained when located upstream of a heterologous promoter which is not regulated by glucocorticoids. An oligonucleotide of 15 bp representing one of the footprint regions is sufficient to confer glucocorticoid inducibility to the thymidine kinase promoter. Interestingly the same oligonucleotide mediates induction by progesterone in progesterone receptor containing cells. Using the genomic footprinting technique it is shown that changes in vitro protein/DNA interactions at the GREs can only be detected after hormone treatment in hepatoma cells, but not in fibroblast cells, in which the TAT gene is not expressed. These data support the concept that glucocorticoids increase the affinity of the receptor to its target sequences.

Visualization and mapping of protein/DNA complexes in the promoter region of a *Xenopus* vitellogenin gene

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Using electron microscopy, we have visualized interactions between the 5' region of *Xenopus* vitellogenin gene and factors present in a soluble protein extract prepared from estrogen-stimulated frog liver nuclei. Protein-DNA complexes are found at 3 positions on a 2.7 kbp DNA fragment containing the promoter of the gene B2. A hormone-independent site corresponds to the transcription initiation site used in vivo. A second complex which is estrogen-dependent occurs ~ 400 bp downstream in intron 1. The third binding complex maps 300–350 bp upstream of the RNA start site and is also strongly dependent upon estrogen. A DNA fragment spanning this site contains the conserved sequence shown to be necessary for estrogen induction in MCF-7 cells, and correspondingly it can also confer hormone-responsiveness on the HSV TK promoter in this cell line, whereas the intron binding site cannot. We discuss the implications of these observations in particular in reference to the homologous and heterologous systems utilized.

The human estrogen receptor can confer hormone responsiveness to *Xenopus* tissue culture cells or oocytes

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In *Xenopus laevis*, vitellogenin gene expression in the liver is strictly controlled by estrogen. Therefore, these genes are useful

to study the mechanisms involved in the estrogen response. We have tested the expression of various chimeric genes that contain vitellogenin gene B1 upstream regions linked to the CAT gene, by coinjection into oocytes and cotransfection into a *Xenopus* kidney cell line with a human estrogen receptor cDNA.
In the absence of the receptor cDNA and in the absence of hormone, we can detect only a low level of expression, which can be increased about 10-fold in the presence of both the receptor cDNA and estrogen. In contrast, the genomic vitellogenin genes seem to remain silent. This implies that the human receptor alone is insufficient to activate the normally silent endogenous genes, while it can specifically stimulate the expression of the introduced genes in these non-mammalian cells. Transfection of deletion clones confirms that the same *Xenopus* 5' flanking sequences that are required for induction in transfected human MCF-7 cells are necessary in *Xenopus* cultured cells, along with the human estrogen receptor.

Symposium 8: Excitatory amino acids, a rapid growth area in neurosciences

Characterization of the glutamate-induced stimulation of phosphoinositide metabolism in hippocampal slices of the adult rat

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In hippocampal slices of the adult male rat glutamate (glu) dosedependently increased the formation of ^{3}H-inositol-1-phosphate (3–5-fold at 5 mM). This effect appeared to be specific, since it was still present when α_1-adrenergic, cholinergic, serotoninergic and histaminergic receptors were blocked. Aspartate also increased PI-turnover to a comparable extent. An increase in PI-turnover was also seen with homocysteic acid, but the effect was about ten times less than that of glu. It seems that the receptor involved in the glu-induced PI-turnover is of the quisqualate type. Of the selective exitatory amino acid agonists quisqualate and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) were most effective (quisqualate being approx. 100 times more potent than glu). Kainate only marginaly increased PI-turnover, whereas NMDA had no effect. Ibotenate also proved to very potently induce PI-turnover (7-fold at 1 mM). The effect of carbachol was additive to those of noradrenaline and histamine suggesting that the involved receptors do not belong to the some PI-metabolic unit. On the other hand, glu-induced PI-turnover was not additive to that induced by carbachol, noradrenaline and histamine. Therefore, the glu receptors may occur linked to common PI-metabolic units in combination with other receptor types.

Effect of GABA-T inhibitors on brain levels of the excitatory amino acid L-α-aminoadipic acid

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The lysine metabolite L-α-aminoadipic acid (LαAA) shows excitotoxic properties which are probably mediated through excitatory amino acid receptors. It therefore seemed important to determine whether LαAA was present in the mammalian CNS. We report here the occurrence of a compound eluting with the same retention time as αAA from an HPLC cation-exchange column and it is assumed that the L-isomer is the endogenously occurring isomer. We found a large rise (from 30–40 to 200 nmol/g) in brain levels of this αAA-like compound 20 h after injection of γvinyl GABA (1500 mg/kg i.p.) a potent inhibitor of GABA-T. On the other hand, gabaculine and valproate were without action on LαAA brain levels. These results suggest that γvinyl GABA may inhibit the 2-amino-adipate aminotransferase (EC 2.6.1.39).

Excitatory amino acid release and pathways

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Evidence indicating that the acidic amino acids glutamate and aspartate act as excitatory neurotransmitters in many corticofugal projections and in some other systems will be reviewed and discussed. However, the existence of at least three excitatory receptors as well as the fact that no known transmitters could be attributed to some major CNS pathways incited us to screen for endogenous neuroactive substances. Sulfur containing amino acids, in particular homocysteic acid (HCA), have been found to be released upon K^+ depolarisation in a Ca^{++}-dependent manner from rat brain slices. This release is highest in cortex and hippocampus. The released HCA can be labeled after incubation of the slices with ^{35}S-methionine. Electrophysiological observations suggest that L-HCA acts preferentially on the NMDA-receptor. Other endogenous candidates will be discussed.

What has the radioligand binding approach told us about excitatory amino acid receptors in the brain?

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Work from several groups indicates that the three major excitatory amino acid receptors observed in physiological experiments (NMDA, quisqualate, kainate sub-types) can be labeled and studied using membrane binding and autoradiographic approaches. Each is enriched in synaptic membranes and differentially distributed in the brain. The NMDA receptor, of high interest due to its involvement in learning mechanisms and in several neurological diseases, is located primarily in cortical and limbic areas. Current research is aimed at defining the structural selectivity of the NMDA receptor recognition site, its interaction with other membrane components, and its regulation following lesions or in disease. Of interest are recent results indicating that TCP (an analogue of the psychomimetic drug, phencyclidine) binds to the 'open' configuration of the NMDA receptor-channel complex (and blocks NMDA receptor function). Through such studies, a model of the NMDA receptor complex relevant to the pathophysiology of brain function is beginning to emerge.

Release of vasoactive intestinal peptide (VIP) by nicotine in the taenia of the guinea pig caecum

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The relaxation of the taenia in response to nicotine (Nic) is due to the stimulation of intrinsic nerves in which the inhibitory transmitter may be ATP or VIP. Since Nic has been shown to release ATP, it was of interest to study VIP release. Incubation of strips of the taenia in Krebs buffer at 36 °C resulted in a basal release of VIP-like immunoreactivity of 26.9 ± 2.4 pg/g/min (mean ± SEM, n = 14). Nicotine application for 1.5 min at 8, 16 and 32 μm dose-dependently increased VIP release 11 ± 10%, 52 ± 12% and 84 ± 8%, respectively, above control levels (n = 8–14). Contraction of the taenia with histamine (2 μM) had no effect on VIP release. VIP release evoked by Nic was suppressed in the absence of Ca^{2+} or in the presence of tetrodotoxin (1 μM), indicating a neuronal origin of the released VIP. In view

of the known stimulation by Nic of ATP release, we conclude that Nic is unable to discriminate between the two most likely candidates for the inhibitory transmitter in the taenia.

Investigation of muscarinic and nicotinic discriminative cues produced by physostigmine, RS86 and other cholinomimetics

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The cholinesterase inhibitor physostigmine (PHYSO) and muscarinic agonist RS86 have been used in clinical trials in Alzheimer's disease. Here their central cholinergic effects are assessed. In a two-lever drug-discrimination paradigm separate groups of rats were trained to discriminate either arecoline (AREC), pilocarpine (PILO), oxotremorine (OXO) or nicotine (NIC) from saline. The AREC, PILO and OXO cues were antagonized by low doses of scopolamine but only by high doses of methylscopolamine, and were not mimicked by NIC, pentobarbital, chlordiazepoxide, haloperidol or morphine. PHYSO and RS86 were discriminated as drug by the PILO and OXO trained groups. The NIC cue was antagonized by mecamylamine but not hexamethonium, and was not elicited by amphetamine, scopolamine, AREC, PILO, OXO, PHYSO or RS86. Thus AREC, PILO, OCO, PHYSO and RS86 produce centrally-mediated muscarinic but not nicotinic discriminative cues.

Phencyclidine/sigma receptor agonists modulate synaptic transmission mediated by excitatory amino acids

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On the basis of agonist and antagonist activity, it has been possible to subdivide glutamate receptors into those preferentially activated by N-methylaspartate (NMA), by quisqualate and by kainate. We have shown that dissociative anaesthetics (e.g. phencyclidine or PCP, ketamine and dexoxadrol) and sigma opiates (e.g. cyclazocine, N-allylnormetazocine and dextrorphan) selectively block excitation by NMA. This antagonism is not competitive probably occurring at the channel activated by the NMA receptor.

These drugs reduce polysynaptic reflexes in spinal cord, as well as synaptic excitation and epileptiform potentials in the cerebral cortex and other parts of the mammalian CNS. These synaptic effects are likely to be mediated via the NMA receptor-channel complex and could underlie some of the behavioural effects common to PCP and sigma opiates.

The good correlation, between the potency of these and other drugs as NMA antagonists with their potencies a) as inhibitors of PCP binding, b) as anticonvulsants and c) as substitutes for PCP in drug discrimination studies, suggests that block of synaptic excitations mediated by NMA receptors contributes to the behavioural properties of PCP/sigma receptor agonists.

Release of excitatory amino acids from pathways of the rat CNS, in vivo

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Important pathways of the mammalian CNS are thought to use excitatory amino acids (eAA) such as glutamate (Glu) and aspartate (Asp) as transmitters. Evidence concerning the attribution of eAA to certain pathways is indirect, involving biochemical, pharmacological and retrograde tracing studies. We have attempted to demonstrate a stimulation dependent release of eAA in areas of termination of putative eAA-ergic pathways.

During electrical stimulation of frontal cortex, a rise in extracellular concentration of Glu and Asp has been observed in the striatum perfused by means of a push-pull cannula. This is in agreement with many lines of evidence indicating that corticostriate neurons use eAA as transmitters.

In preliminary experiments, thalamic nuclei were electrically stimulated. Changes in concentrations of eAA in a solution, bath applied to the fronot-parietal cortex, suggest that in this system also neurons releasing eAA are involved.

Hypothermic effects of neurotensine (NT) and analogs. Comparison with NT receptor interaction

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NT, i.c.v. or locally applied, produces hypothermia in rodents. The aim of the present study was to see whether or not, this effect was NT specific. i.c.v. injection of NT to rats induced a decrease of the body temperature. The minimal effective dose was 0.3 µg/rat, the ED_{50} around 10–30 µg. Fragments or analogs (NT 8–13, L-Phe^{11}-NT, LANT 6 NT, D-Trp^{11}-NT), interacting at (^{3}H)NT binding sites, also produced hypothermia, whereas those inactive on binding (NT 1–8, NT 1–11) did not affect the body temperature. D-Trp^{11}-NT produced pronounced hypothermia (−4 °C) at very low dose (maximal effect at 1 µg) which lasted for more than 7 h. Finally, two natural analogs of NT, neuromedin N and xenopsin, were found active in (^{3}H)NT binding as well as in body temperature. These data indicate that this hypothermic effect is specific for NT and can be used to assess the in vivo activity of NT agonists or antagonists.

Symposium 9: Developmental neurobiology: integrated systems

Proprioretinal connections in birds

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Although long-range physiological interactions are known to occur in the retinas of birds and mammals, the anatomical basis of this is largely unknown. We here report long projections within the retina, shown by retrograde transport of an intensely fluorescent carbocyanine dye, small flecks of which were inserted in different parts of the retina in chicks and chick embryos. Long intraretinal connections originate from cell bodies in the inner half of the inner nuclear layer. These 'proprioretinal cells' are of two types, resembling Cajal's 'stratified amacrine cells of the first level', and his 'association amacrine cells'. Both types occur throughout the ventral half of the retina but are absent from the dorsal half. However, their main projection, which is topographically organized, is onto the dorsal half. Additional connections remain within the ventral half. The proprioretinal cells do not project to the brain. We speculate that they may be involved in switching visual attention between the upper and lower parts of the visual field.

Cortico-cortical neurons with long transitory axons form short permanent connections

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The kitten's auditory cortex (including AI, AII and post. ectosylvian) contains neurons which send a transitory axon either to

ipsi- or to contralateral visual areas 17 and 18 (GMI+SC'84). By pd 20 the contralateral and by pd 36 the ipsi-lateral transitory axons, but not the neurons of origin, are eliminated. To study where these neurons establish permanent connections, 21 kittens received early (pd 1–4) injections of long lasting retrograde fluorescent tracers in 17–18 and late (pd 31–67) injections of other fluorescent tracers elsewhere. No neurons in AI/AII were relabelled after late injections into the contra auditory areas, or area 7 on either side or the ipsi auditory areas on the post. ectosylvian gyrus. Injections in the ipsi ant. ectosylvian sulcus and gyrus relabelled few neurons. Highest rate (5–15%) of relabelling was obtained with (small) injections in ipsi AI/II.
Thus AI/II neurons which send a long transitory axon to ipsi or contra 17–18 form short permanent connections in their own area or in neighboring auditory areas.

Spatial orientation in young rats

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In experiments in which rats have to find a hole at a fixed spatial position relative to distant room cues, under 30 days of age rats do not reach the hole by a straight path, yet they recognize its position. Is the impairment relative to a deficit of perceptual or cognitive processes, or is it linked to juvenile locomotor hyperactivity?
In a second experiment an object is added inside the enclosure as a beacon signalling the position of the hole. When this mark is just beside the hole, all age groups go straight to it. When the same mark is shifted relative to the hole position, young rats show improved performance, but remain dependent on this cue all over the test, neglecting distant room cues. These results suggest a peculiar deficit for the 18–21-day-old rats in learning connections between two relative positions. In a third experiment, rats have to find a hole in a configuration of objects within the enclosure. The results indicate that immature rats do not show guidance on objects proximal to the hole but learn to reach the hole using routes.
In conclusion young rats still show spatial orientation impairment in condition in which they have no obvious perceptual deficit.

Dendritic development in the lateral geniculate nucleus of the marmoset monkey

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We studied the postnatal development of dendrites of multipolar neurons in the lateral geniculate nucleus of the marmoset (*Callithrix jacchus)* by quantitative three-dimensional analysis of Golgi sections. In adult marmosets, there are 4 stem dendrites on average per neuron and each dendrite divides into a mean of 14 segments. Between birth and 6 weeks the mean dendritic length doubles, mainly due to changes in terminal dendritic branches. There is then a significant decrease in dendritic length into adulthood. The total number of stem dendrites does not change after birth but during the first postnatal week dendrites loose segments, after which there is a significant increase in the number of segments. Thus during the period of most rapid visual development important morphological changes occur in neurons, involving essentially an overshoot in the mean length of dendrites.

Postnatal development of area 17 callosal connections in *Tupaia belangeri*

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The neurons of origin projecting via the corpus callosum between the two primary visual cortices were studied by horseradish peroxidase histochemistry (2% WGA-HRP). Multiple injections (4–9 injection sites per experiment) were made with micropipettes parallel to the longitudinal fissure. Tree shrews were sacrificed at postnatal days 13, 15, 17, 19 and 26.
At day 13, the neurons are found to be uniformly scattered almost over the entire dorsal part of area 17, extending the area of injection sites in any direction. Therefore, homo- and heterotopic callosal projections do exist in immature *Tupaia*. The majority of neurons are located in supragranular layers II and III, and their shape resembles typical pyramidal cells. Over the next 6 days the adult distribution pattern seems to be established. Day 19 animals – eye opening occurs at about day 18 – show a much more restricted distribution of homo- and heterotopic connections with a higher density along the area 17–18 border, undistinguishable from the pattern found in mature tree shrews.

Ornithine decarboxylase kinetics in the developing rat cerebellum

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Ornithine decarboxylase (ODC), the key enzyme in polyamine biosynthesis, plays an important role during brain development when its activity has been shown to increase very markedly.
The kinetics of ODC were studied in the cerebellum of 10-day-old rat pups, at the time of fastest growth. The following parameters were examined: substrate and coenzyme (pyridoxal-5'-phosphate) concentrations and effects of inhibitors. Non-linear regression were used to fit Michaelis-Menten (MM) plots and to estimate the kinetic parameters. However, our experimental data did not fit a simple MM model: the reaction was not saturated in presence of a high substrate concentration (up to 50 mM ornithine).
Our results show that the use of ODC activity measurements in brain development studies should include the estimation of all the kinetic parameters for a valid interpretation.

A critical age in the ontogeny of locomotion and ultrasonic vocalizations in the woodmouse, *Apodemus sylvaticus*

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We have shown that the young woodmouse is able to walk and to perform optimal responses in sensorimotor tests from PND 11 onwards. At this critical age, we have observed that ultrasonic production in response to a light cold, manipulation and isolation from other siblings becomes strongly related with locomotion, and disappears progressively in inactivity periods. In consequence, the differences in the total number of bouts of calls recorded from PND 11 onwards in two very different conditions (a plus-maze and a small cup) is due to different levels of activity. Because of the pattern of calling, the structure of ultrasounds and their relation to locomotion, we can wonder whether ultrasonic vocalizations are still distress calls at the end of the second postnatal week. They could serve a reciprocal communicatory function between the young themselves and we suggest that they could also be the precursors of adult calls.

Topographic projections in the nervous system: developmental mechanisms

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The nervous system is characterized by the presence of a great number of topographically organized connections. In order to detect the developmental mechanisms responsible for the formation of these projections, the retino-tectal system has been used as a model since several decades. Until recently it was assumed that the interaction between growing fibres and their target neurons was the main or the only important mechanism which was able to produce topographic maps. We were, however, able to show that in the chick retino-tectal system a number of ordering mechanisms can be detected organizing the projecting fibres long before these fibres arrive at their tectal target. After several transformations retinal fibres represent the retinal image in the optic tract in such a way that they can sequentially be projected onto the tectal surface and do not require major sorting processes. Based on these findings a 'hypothesis of multifactorial determination' is proposed to explain the formation of topographic maps.

Electrophysiological studies of cholinergic receptors in explant cultures of rat brain

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The hippocampus receives a strong cholinergic innervation from the medial septum; the presence of muscarinic cholinergic receptors (mAChR) on hippocampal neurons is reported by many investigators. Electrophysiological studies show that acetylcholine (ACh) acts on hippocampal pyramidal cells by increasing their excitability. Cultured explants of hippocampus and septum from young rats have been shown to be a useful tool in the study of cholinergic pathways (Gähwiler and Brown, Nature *313* (1985) 557). Our preliminary experiments with hippocampal cultures confirmed that ACh and its agonist carbachol lead to a depolarization of pyramidal cells associated with an increase of the firing rate. Further studies on this preparation will be performed using pirenzepine, an antagonist of ACh. On the basis of different binding affinities of this drug, subtypes of mAChR have been postulated.

Behavioral studies with rats pre- and postnatally exposed to low levels of lead carbonate

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Four groups of rats, each consisting of four dams, were fed with three different doses of lead carbonate eight weeks before pairing, until end of lactation. Two male and two female offspring per litter were fed with the same diet during the rest of the experiment. A 1 mg/kg amphetamine challenge in the wheel-shaped activity monitor showed a slightly smaller activity increase of the high dose females. An amphetamine discrimination experiment on the female rats did, however, not show any effect of lead on the discrimination threshold dose which ranged for all groups between 0.3 and 0.4 mg/kg. In a differential reinforcement of low rates schedule (DRL20) the males of the two higher dose groups showed a shorter interresponse time correlated with fewer reinforced responses. The responses per opportunity distribution showed an effect similar to the one observed in hippocampal lesioned animals (higher peak of the response-probability/interresponse-time curves).

Effects of selective fornix-fimbria (FF) lesions on substance P (SP) and cholecystokinin (CCK) levels in the rat hippocampus

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The neurotransmitters/modulators SP and CCK are present in the mammalian hippocampus. The SP projection to the hippocampus likely arises in the medial septum-diagnoal band (MS), whereas CCK appears to be intrinsic to the hippocampus. We determined the activity of choline acetyltransferase (ChAT), as well as SP and CCK levels in 3 hippocampal regions, in intact rats and in rats with selective FF lesions. SP and CCK, like ChAt, exhibited a marked ascending concentration gradient along the septotemporal axis of the hippocampus. One week after complete unilateral lesions, there was a pronounced decline in ChAT and SP in all hippocampal regions. This decrease was hippocampal region-specific after partial (medial/lateral) lesions. Thus the mediolateral organization of cholinergic and SP efferents from the MS appears similar. CCK levels in the hippocampus were not influenced by any of the lesions, supporting the view that neurons containing this peptide are intrinsic to the hippocampus.

Cerebellar grafting: migration and differentiation of embryonic Purkinje cells (PCs) transplanted to adult deficient cerebellum

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Neuronal replacement of missing neurons in adult CNS, by embryonic neural grafting, implies that interactions between embryonic and adult neurons can duplicate the ontogenic sequential stages. This hypothesis was tested as follows: pieces of cerebellar primordia were dissected from 12-day-old C57BL embryos and transplanted to the cerebellum of 3- to 4-month-old pcd mutant mice. The host cerebella were examined 4 to 30 days after. By 5 days, PCs leave the graft, and move horizontally along the cerebellar surface, between the pial basal membrane and the glial limiting membrane. By 6-7 days, the tangentially elongated PCs change polarity and penetrate the host molecular layer along the Bergmann fibers. During inward migration, PCs retract their vertical processes and start to build dendritic trees. By 12–15 days, PC perikarya are studded with filopodia, synaptically contacted by climbing fibers (phase of pericellular nests). Their growing dendrites give rise to long, spinous appendages, postsynaptic to parallel fibers. By 30 days, PC dendrites, have acquired their characteristic monoplanar disposition and a qualitative normal synaptic investment. Thus, embryonic PCs are selectively attracted by the deficient adult molecular layer. Despite this environment, they migrate, differentiate dendrites, and form synapses in the same time span as during cerebellar ontogeny, suggesting that embryonic PCs impose their own developmental timing. Moreover, adult cerebellar interneurons, confronted to embryonic PCs, seem to interact with the latter by mechanisms closely related to those operating during ontogeny.

Studies on central cholinergic and peptidergic neurons and behavior of the rat after prenatal lead exposure

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Epidemiologic studies in man and animal experiments indicate, that pre- and postnatal lead exposure can result in behavioral disturbances. The neurochemical basis for these developmental effects remains uncertain, however. We are investigating the effect of prenatal lead exposure on the development of central neurotransmitter systems in Long Evans rats. On the first day of

pregnancy, the dams received drinking water containing 0.25% Pb acetate or 0.125% Na acetate. Levels of lead in blood of treated dams were significantly increased (52 µg/100 ml as compared to 8 µg/100 ml in controls). Offspring showed a decreased body and brain weight and a slightly decreased body-brain ratio. Activity was reduced on postnatal day 3 (PN3) and increased on PN6. Neurochemical effects are under investigation.

Symposium 10: Developmental neurobiology: cellular aspects

Differentiation of postmitotic neuroblasts into substance P-immunoreactive sensory neurons in dissociated cultures of chick dorsal root ganglion

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In mixed dorsal root ganglion cell cultures (neuronal+non neuronal cells), the neuronal population increases between 2 and 6 days in culture without incorporation of 3_H-thymidine. The increase in the neuron number results from a delayed differentiation of postmitotic neuroblasts, which are intermingled with non-neuronal cells. The newly-differentiated neurons give rise exclusively to small cell bodies which exhibit an immunocytochemical reactivity to myelin-associated glycoprotein (MAG) and substance P; in contrast these cells are devoid of immunostaining for parvalbumin, somatostatin, calbindin, tyrosine-hydroxylase and of carbonic anhydrase activity. Thus post mitotic neuroblasts differentiating into neurons express restricted phenotypic characters of a defined subpopulation of class B sensory neurons.

Effects of directly vs nerve-evoked muscle activity on denervated endplate membrane

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In mouse soleus muscle, denervation or botulinus poisoning cause a loss in endplate membrane structure (Brown et al., 1983), suggesting that, unlike in rat, the maintenance of the endplate membrane depends on muscle activity. Examining this possibility electrophysiologically, we found that 1) in denervated immature ectopic endplates muscle stimulation causes an increase of junctional acetylcholine (ACh) sensitivity and development of adult type endplate channels, 2) at denervated original endplates in stimulated muscle ACh sensitivity and the adult endplate channel form are maintained and 3) at denervated original endplates of fibres kept active by distant (> 3 mm) ectopic reinnervation ACh sensitivity is reduced, with the endplate channels remaining of the adult type. Thus, directly evoked activity promotes the functional differentiation of nerve-free immature endplate membrane and maintains it. In contrast, activity evoked by distant innervation is unable to do so, suggesting that ectopic innervation eliminates distant nerve-free synaptic sites.

Serum-free primary cultures of fetal chick neuronal retina and brain cells

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In order to develop a short term assay for in vitro screening of behavioral and other teratogens, we started to cultivate fetal chick (stage 28) neuronal retina and brain cells under total serum-free conditions (MCDB 201+MEM+supplements) and without growth factors such as NGF. Cell isolation was optimized by gentle mechanical dissociation. Initial viability as determined by dye exclusion was around 85%. The survival in monolayer culture under serum-free conditions could be increased over that reported by others from 6 days to 10 days (brain cells) and from a few days to at least 3 weeks (retina). Brain cell cultures were found to be almost devoid of glia cells, whereas retina cultures consisted of flat (Muller) cells with neuronal cells on top of them.

Preliminary experiments with ethanol and diphenylhydantoin showed a dose-dependent effect on attachment and lateron on cell and cell-clump morphology. In order to investigate the chronic application of drugs we cultured aggregates of brain and retina cells in suspension under constant gyratory shaker conditions. Preliminary morphological criteria suggest that in these cultures glia and nerve proliferate and differentiate, resp., during the first six weeks of culture. Nerve outgrowth, synapse formation and cell migration to specific regions of the aggregate are the most evident signs of developing brain cells.

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Two membrane proteins of myelin and oligodendrocytes inhibit neurite outgrowth and fibroblast migration

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In cultures of dissociated rat optic nerves, glial cells with the characteristics of myelin-forming oligodendrocytes are strongly non-permissive for nerve fiber growth. This contact-mediated effect was seen with CNS and PNS neurons, with neuroblastoma cells, and also with migrating 3T3 fibroblasts. CNS myelin of rat spinal cord (but not of sciatic nerve) was equally effective, and the inhibition could not be overcome by trophic factors or cAMP. Proteins extracted from CNS myelin and reconstituted in liposomes retained the inhibitory activity. Trypsin or elastase converted these liposomes into a very favorable substrate for neurite growth and fibroblast spreading. Separation of myelin proteins by SDS-PAGE followed by liposome reconstitution showed, that two components with approximative molecular weights 30 kd and 250 kd, and differing from the main myelin proteins, were responsible for the inhibitory effects. These components may play a crucial role for the absence of regenerative fiber growth in the lesioned mammalian CNS.

Influence of central and peripheral connections on the expression of somatostatine (SS) in developing chick dorsal root ganglion (DRG)

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The role exerted by peripheral target tissues or spinal cord on SP-immunoreactive ganglion cells was tested in lumbosacral DRG: 1. by resection of one hindlimb at the embryonic day E6 prior to formation of specialized peripheral connections; 2. by cauterization of the caudal part of the neural tube at E6. The DRG were examined at E12. After resection of one hindlimb, the size of the homolateral DRG and the number of SS-immunoreactive ganglion cells decreased preferentially when compared with control DRG. After cauterization of the neural tube, the DRG, which were also reduced in size, contained numerous and intensely SS-immunostained ganglion cells.

These results provide evidence that central and peripheral connection cooperate and modulate the phenotypes of SS-neuroblasts in the developing DRG.

A monoclonal antibody against the plasma membrane of cholinergic nerve endings inhibits choline acetyltransferase

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Choline acetyltransferase (ChAT) catalyzes the biosynthesis of acetylcholine and is a marker enzyme for cholinergic neurons. In cholinergic nerve endings a soluble and a membrane-bound form of ChAT exist. Interaction of ChAT with Triton X-114 shwos that soluble and membrane-bound ChAT are hydrophilic and amphiphilic, respectively. A monoclonal antibody (McAB) raised against the plasma membrane of the cholinergic nerve endings isolated from the *Torpedo* electric organ, inhibits both forms of ChAT of *Torpedo*, rat and human. For both forms of the *Torpedo* enzyme the inhibition is progressively, but partially, reversed by increasing concentrations (0.1–3.3 mM) of the substrate AcCoA (K_m 20 uM) added during the incubation of ChAT with the McAB. Immunoblots of membrane-bound or of soluble ChAT of Torpedo show two labelled bands at 66 and 135 kd. Although membrane-bound ChAT has a lower specific activity (125 nmol/h/mg prot.) than does soluble ChAT (1000), it is more heavily labelled by the McAB.

In vitro antigenic modulation of human neuroblastoma cells induced by IFN-γ, retinoic acid and dibutyryl cyclic AMP

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Analyzed with a panel of 26 monoclonal antibodies, the antigenic profile of human neuroblastoma cell lines was found to be characterized by a general poor antigenic expression. Interferon-gamma, dibutyryl cyclic-AMP and retinoic acid were used to analyse the modulation of surface antigenic expression upon differentiation. Treatment of neuroblastoma cell lines with interferon-γ resulted mainly in an induction or increase of class I MHC antigenic expression. Induction of class II MHC antigens was obtained on only one out of 13 neuroblastoma cell line. An increase of some other antigens expressed by neuroblastoma cell lines was also observed. In contrast, in addition to a morphological maturation, treatment of the same cell lines with the differentiation inducer dibutyryl-cyclic-AMP, resulted in a general downmodulation of antigenic expression, specially pronounced for the neuroblastoma-associated antigen 5A7 or the Leu7 antigen. Retinoic acid had no significant effect on MHC antigens but decreased the expression of 5A7 and Leu7 antigens, and highly increased the expression of the melanoma associated antigen Me14-D12. The similarity between the antigenic profile of in vitro differentiated neuroblastoma cells and that of mature ganglioneuroma cells suggests that compounds like cyclic-AMP or Retinoic acid represent excellent tools for further investigations on the mecanisms of neuroblastoma differentiation and might have important clinical applications.

A monoclonal antibody against the cell surface of a subpopulation of astrocytes

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Monoclonal antibodies were raised against cultured cells of embryonic chicken spinal cord. One antibody, termed G2B3, was found to recognize a cell surface epitope of a subpopulation of GFAP-positive astrocytes. The G2B3-epitope is present only of GFAP-positive cells and is absent on Schwann cells, oligodendrocytes, fibroblasts and meningeal cells. In younger cultures, the G2B3-epitope is present on both, A2B5-positive and A2B5-negative cells, whereas in older cultures G2B3-positive cells are A2B5-negative. Hence, in mature, process-bearing, astrocytes the G2B3-epitope may be unique to the cell surface of the type-1 subpopulation and may be a useful marker in studies of astrocyte function during developmental and regenerative processes in the CNS.

Hypoxia in brain cell cultures

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Dissociated brain cell cultures were kept for 14 days in vitro (DIV) and then exposed to hypoxia. The effect of hypoxia on protein, DNA, the oligodendroglia enriched enzymes, cerebroside sulfotransferase (CST) and cyclic nucleotide phosphohydrolase were investigated. In addition the direct cellular effects of oligodendrocytes, characterized by specific markers, were studied. After hypoxia CST was 22% of that of controls and the number of oligodendrocytes reduced to 50%. Astrocytes characterised with the specific marker glia fibrillary acidic protein (GFAP) showed no effect.

In order to investigate possible regeneration, cells were kept after hypoxia at 14 DIV for an additional 7 DIV under normoxic conditions. This resulted in a clear recovery of the CST activity but not of the number of oligodendrocytes. The results indicate that under the experimental conditions the oligodendrocytes are more vulnerable to hypoxia than astrocytes and that there is a recovery of the enzyme activity but not of the number of cells.

cDNA cloning of microtubule-associated protein 3

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Microtubules are cytoskeletal elements which are involved in the establishment of neuronal morphology. They consist of tubulin and a variety of associated proteins, the MAPs. One of these MAPs, a heat-stable protein doublet of 180 kD is called MAP3a and MAP3b. In the adult rat brain. MAP3 is present in neurofilament-rich axons and glial processes. During neuronal differentiation, it is associated with all nascent axons, until, at postnatal day 13, it decreases about 10-fold and disappears from all axons but the neurofilament-rich ones. In order to study the relationship of MAP3a and b, their structure and function during brain development, a cDNA-expression library in λgtll was made and screened with a mixture of monoclonal and polyclonal antibodies against MAP3. Out of 150000 pfu, one clone was isolated which reacted with a monoclonal α-MAP3. IPTG-induced expression of the βgalactosidase-fusionprotein showed on SDS-PAGE an apparent increase in MW of 10 kD, indicating, that the cDNA-insert represents only a small fraction of the whole gene.

Developmental changes of neural cell adhesion molecule (N-CAM) in retinal afferents to optic tectum

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At which stage of ontogeny does the change from the so-called 'embryonic' (E) to the 'adult' (A) form of N-CAM occur in a well-defined 'in vivo' system, which allows analysis at the cellular level? Is there a correlation between the timing of this 'E to A conversion' and certain events in the development of the system investigated? – 35S-labeled methionine (35S-Met) was injected into one eye of chicken embryos at different stages of development. N-CAM was biosynthetically labeled with 35S-Met in the retinal ganglion cells and axonally transported in the retino-

tectal pathway. One day after injection, N-CAM was immunoprecipitated from detergent extracts of tectal tissue by means of a monoclonal antibody developed against both forms of the molecule. Labeled N-CAM was then autoradiographically analyzed following SDS-polyacrylamide gel-electrophoresis. – Labeling was found almost exclusively in the tectum contralateral to the injected eye in this mostly crossed projection. N-CAM's E to A change in retinal afferents seemed to take place after the time of synapse formation, namely around the sixteenth incubation day. Expression of the 'stickier' A form at this stage may help to stabilize newly formed connections.

Nerve growth factor (NGF) levels in rat neocortex and nucleus basalis of meynert (NBM) after neuronal degeneration

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We have investigated in adult rats whether degeneration of neurons modifies NGF-levels in neocortex and NBM. After an electrolytic lesion of the NBM, NGF-levels were significantly increased in the ipsilateral neocortex from day 7 to day 14. This lesion induced a very marked degeneration not only of ipsilateral cholinergic but also of monoaminergic cortical afferents. Electrolytic lesion of the medial forebrain bundle, which destroys selectively monoaminergic input to the ipsilateral cortex, failed to modify the cortical NGF content up to day 15. Furthermore, a bilateral suction lesion of the neocortex markedly increased NGF-levels in NBM (250% at day 14).
Thus, a cholinergic mechanism appears to regulate NGF production in adult rat basal forebrain.

Distribution of fibronectin (FN) and laminin (LN) in mouse embryos: preliminary results

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We studied the distribution and localization of FN and LN in the ECM of 8- to 10.5-day mouse embryos by immunohistochemical techniques with monospecific antibodies. Both FN and LN are mainly distributed along the basement membranes (BM). In the cephalic region, LN was also found throughout the mesenchyme at day 8.5. Later, it was restricted to the BM alone. FN was also detected in both BM and mesenchyme. However, in this latter tissue, the distribution was neither found constant nor homogeneous. In early stages or in more posterior regions, the FN tends to spread throughout the mesenchyme, whereas in older embryos or in more anterior regions, the FN showed a distribution restricted to more specific areas. In particular, from the 9th day of development, we found FN around and in the center of the somitic cell mass as well as in the BM and mesenchyme of the visceral arches. These observations are discussed in relation to the neural crest cell migratory processes in mouse and chicken.

Properties of an elastase-clipped glia derived neurite promoting factor (GdNPF)

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Glial cells release a serin protease inhibitor which promotes neurite extension in neuroblastoma cells. This protein has a heparin binding site.

Elastase cleaves GdNPF predominantly at one specific site leading to a two chain form of the protein, the chains remaining noncovalently attached to each other. This clipped form of GdNPF no longer promotes neurite extension but still retains its ability to bind to heparin-Sepharose. A concommitant loss of thrombin inhibitory activity is observed.
The peptide with the newly created N-terminus was partly sequenced. The cleavage point was determined to be on the COOH-terminal side of the P2 position of the inhibitor. This suggests that for the inhibition of thrombin additional amino-acid residues are required.
These results indicate: 1) Cleavage with elastate affects the inhibitory site of GdNPF. 2) An intact site of inhibition is important for neurite promotion. 3) The heparin binding site cannot by itself promote neurite outgrowth.

Regional pre- and postnatal ontogeny of central ^{3}H-nicotine binding sites in the rat

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The ontogeny of nicotinic cholinergic binding sites was studied in the brain of Long Evans rats at different developmental stages, by in vitro autoradiography using ^{3}H-nicotine.
Binding sites appeared first in spinal cord at gestational day 16 (GD 16). At GD 18 extensive labeling by ^{3}H-nicotine was oberved in spinal cord, lower brainstem, hypothalamus, thalamus and in the quadrigeminal plate. Through GD 20 binding sites became detectable in neocortex and cerebellum and increase in density in the remaining brain regions. During postnatal development, labeling in brainstem and cerebellum decreased progressively until adulthood. Highest densities are then seen in forebrain areas.

Regulatory mechanisms involved in beta-nerve growth factor (NGF) biosynthesis in brain and peripheral tissues

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NGF is essential for the development and maintenance of function of sensory and sympathetic neurons in the peripheral nervous system and for cholinergic neurons in the basal forebrain. However, the mechanisms involved in NGF-biosynthesis are still unknown.
We have used a sensitive enzyme-linked immunosorbent assay for determination of NGF-protein and an optimized Northern blot technique for quantification of $mRNA^{NGF}$ to characterize the regulatory mechanisms involved in NGF synthesis both in peripheral and central nervous system of adult rats. Our results indicate that, in addition to neurally-mediated mechanisms, other stimuli, e.g. inflammation, significantly contribute to NGF production.

C_6 rat glioblastoma cells use a specific mechanism to overcome the non-permissive substrate effect of mammalian CNS white matter

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Cultured oligodendrocytes with myelin-forming characteristics from rat optic nerves and CNS myelin of rat spinal cord represent a strongly non-permissive substrate for nerve fiber outgrowth and fibroblast migration. In contrast, C_6 cells rapidly

spread on CNS myelin and on living oligodendrocxytes, equally well as on e.g. poly-lysine (PLYS) coated culture dishes. The inhibitory effect of myelin towards fibroblasts is strongly diminished by pretreatment of the myelin with C_6 cells or C_6 plasma membranes. The metalloprotease inhibitor 1,10-phenanthroline reverses this effect and leads to inhibition of C_6 spreading on CNS myelin and oligodendrocytes but not on PLYS. These results suggest, that C_6 cells contain a specific, membrane-associated metalloprotease activity, which may play a crucial role for C_6-invasiveness in the mammalian CNS white matter.

Myelin basic protein gene dosage effect in mld heterozygotes does not alter myelination

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We have shown recently that the primary lesion in myelin-deficient (mld) mutant mice is a reduction of myelin basic protein (MBP) synthesis and is not located in the coding region of the MBP gene*. We also found that young mld heterozygotes have MBP and MBP mRNA levels in the brain about half of those found in the controls. We addressed here the question whether or not myelination itself is impaired in mld heterozygotes. Our results show that, inspite of reduced levels of MBP and MBP mRNA in the brain, mld heterozygotes had normal amounts of myelin and a normal concentration of MBP in myelin at different ages during development. These results indicate that the concentration of total MBP present in control brains represents a safety factor since a 30–50% reduction of this concentration alters neither the amount nor the composition of myelin.

* Roch, J.-M., et al., Molec. Brain Res. *1* (1986) 137.

Axonally secreted proteins: II. Purification of ASP-140 and determination of neurons as target cells

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A protein (ASP-140) previously identified to be secreted from axons of chick embryonic dorsal root ganglia and possibly from all neuronal subpopulations (see E.T. Stöckli et al.) has been purified from chick embryonic vitreous fluid by a four step chromatography procedure. Antibodies have been raised. The identity of the purified protein and the in vitro identified axonally secreted protein has been determined by immunoprecipitation. Exclusive presence of ASP-140 in embryonic, but not in adolescent or adult, vitreous fluid suggests a developmental function. Circumscribed, extracellular depositions of ASP-140 on neuronal somas and processes, but not on nonneuronal cells have been revealed by indirect immunfluorescence. Immunoelectron microscopy for an exact localization of these extracellular depositions is under way.

Chick brain GABA gated Cl^- channels expressed in *Xenopus* oocytes: effects of benzodiazepine agonists and inverse agonists

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mRNA was isolated from chick forebrain and injected into *Xenopus laevis* oocytes. Several types of ion channels typical for vertebrate brain were functionally expressed in the oocyte plasma membrane. The response to γ-aminobuyrate (GABA) was further investigated using the two electrode voltage clamp technique. At a holding potential of −100 mV perfusion with GABA resulted in a dose dependent inward current. Half maximal response was observed with 20 μM GABA. The response was sensitive to picrotoxin and had a reversal potential of −30 mV. The stimulatory effect of the benzodiazepine agonist clorazepate and the inhibitory effects of the inverse agonist compounds methylcarboline-3-carboxylate and methyl-6,7-dimethoxy-4-ethylcarboline-3-carboxylate on the GABA response were characterized quantitatively. Artificially induced ion channels may be a useful alternative to cell culture for the study of ion channels not easily accessible to experimentation in situ.

Sequence and homologies of a protease inhibitor with neurite-promoting activity released by rat glioma cells

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Rat glioma cells release a 43kD neurite-promoting factor which is a strong serine protease inhibitor (Guenther et al., EMBO J. *4*, (1985) 1963–1966). A cDNA library has been established from these C6 rat glioma cells. A first 800 bp cDNA probe identified by hybridization selected translation has been used to isolate a 2100 bp clone which was further characterized by sequencing. This cDNA clone contains the entire coding region, 135 bp of 5′ nontranslated sequence and about 750 bp of the 3′ region. The open reading frame coded for 397 amino acids including a signal peptide of 19 amino acid residues. The sequence, deduced from the cDNA sequence, was compared to the corresponding sequence obtained for the corresponding human protein (Gloor et al., Cell, in press), to human endothelial cell plasminogen activator inhibitor, to antithrombin III and to alpha l-antitrypsin. The data indicates that the rat protein belongs to the serpins and that it can also be classified as a protease nexin.

Development of calcium currents and of calcium-activated chloride current in cultured mesencephalic neural crest neurons

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Mesencephalic neural crest explants from quail embryos at the 8 to 12-somite stage were cultured for 1–5 days. The electrical properties of the neurons were studied in voltage clamp. The presence of calcium and calcium-activated chloride currents was investigated under conditions where the sodium and potassium currents were suppressed. All neurons cultured for two days or more had an inward current carried by calcium ions that was activated by a depolarization and which was made up of three components similar to those described as I_{CaT}, I_{CaS} and I_{CaN} by Nowycky et al. (Nature *316* (1985) 440). The calcium-activated chloride current ($I_{Cl(Ca)}$) was seen in ca 20% of the neurons cultured for less than 5 days and in 80% of the neurons cultured for 5 days. We conclude that the appearance of $I_{CL(Ca)}$is delayed with respect to the appearance of the calcium current. The three types of calcium currents seem to appear simultaneously.

Regional development of drug binding sites in human fetal brain

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In rat brain, beta-adrenergic (3H-dihydroalprenolol), muscarinic cholinergic (3H-(N)-methylscopolamin), nicotinic cholinergic (3H-nicotine) and benzodiazepine binding sites develop from gestational day 14 and 15 in a highly differentiated pattern. Using in vitro receptor autoradiography, these binding sites

were also found to be present in human fetal brain towards the end of the first trimester of pregnancy. At this early fetal stage, the benzodiazepine 3H-flunitrazepam and 3H-(N)-methylscopolamine are labeling regions in the lower and upper medulla oblongata. Eventually, these binding sites are also detected in forebrain areas, i.e. in the basal ganglia and in the neocortex. At the 25th week of gestation, the caudate-putamen area reveals a dotted immature pattern of binding sites for these drugs, while labeling with the dopamine D2 antagonist 3H-sulpiride is more evenly distributed. With regard to the developmental pattern of drug binding sites, there appear to exist some interesting similarities between rat and human brain.

Axonally secreted proteins: I. Identification

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Dorsal root ganglia neurons from chicken embryos were cultured in a compartmented cell culture system that allows separate access to neuronal cell somas and axons. The proteins secreted from the axons were metabolically labeled by addition of [^{35}S]methionine to the compartment containing the cell somas and harvested from the culture medium of the axonal compartment. Two-dimensional SDS-PAGE/fluorography, followed by computerized gel-image analysis revealed two axonally secreted proteins (ASPs). Both ASPs were subsequently found also to be secreted from a variety of neuronal cell cultures, but not from any of the non-neuronal cultures investigated, and hence, might be neuron-specific. Virtual absence of these proteins from the axonal protein pattern indicates constitutive secretion. ASPs may have a role as developmental signals, e.g. in glia differentiation and synaptogenesis.

Distribution of cytoskeletal and matrix components in the area opaca of the chick embryo

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The area opaca (AO), extraembryonal membrane of chick embryos, is a sheet of polarized ectodermal cells forming a finely woven extracellular matrix on which migrate the cells of mesodermal line. Localization and distribution of fibronectin and laminin in the matrix and of myosin and tubulin in the cells were studied in the AO using immunofluorescence techniques. During the period of gastrulation and neurulation, fibronectin staining appeared early, was intense and revealed a radially organized fibrillar network. Laminin staining was rather weak in the matrix but very intense in some cells. In early embryos, individual cells stained for myosin were dispersed through the AO, in later stages, they formed a compact ring-shaped zone. Tubulin immunoreactivity in cells was observed only during the initial phase of metabolic activation due to the increasing temperature of incubation. These results will be discussed in respect to changes in cell contraction state observed in this period of development.

Developmental change of prostaglandins (PG) biosynthesis in chick dorsal root ganglia (DRG)

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Formation of PG was studied in chick DRG during development. After homogenization, 2 main PGs (PGD_2, PGE_2) were synthetized from [1-^{14}C] arachidonic acid. The specific activity (SA) of PGD_2-isomerase was practically constant from E10 to 15 days after hatching. In contrast, the SA of PGE_2 isomerase, which was low at E_{10}–E_{14}, reached a high level at E_{18}, and after hatching. These results are consistent with the hypothesis that PGD_2 would be generated mainly by ganglion cells and to a lesser extent by Schwann cells while PGE_2 would be produced later by fibroblasts.

In vitro formation of myelin in minisegments of newborn rat optic nerve

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In order to study the in vitro development of CNS glial cells in the complete absence of neurons, we cultivated small explants, called minisegments, of newborn rat optic nerves during 2–21 days in medium with or without serum. With both media loose and compact myelin formed by processes of differentiated oligodendrocytes was observed after only 3 d in culture and persisted until 21 d. These myelin sheaths, which were immunocytochemically labeled for both MBP and MAG, surrounded empty spaces or even glial cell bodies. With serum we observed a particular cellular organization, which was absent in minisegments cultured without serum. The former consisted of a belt like peripheral part and a compact center. These results show that oligodendrocytes can differentiate structurally and immunocytochemically and faithfully form myelin in the total absence of neuronal elements.

Differences in surface and released proteins of sympathetic and parasympathetic neurons in culture

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Neuronal cell surface glycoproteins, as well as proteins released by those cells, could play an important role in cellular recognition and in the establishment of highly specific connections during development of the nervous system.

To study this hypothesis, we have decided to look for components specific either for sympathetic or parasympathetic neurons. Dissociated cell cultures of chick superior cervical and ciliary ganglion neurons (SCG and CG) were metabolically labelled with ^{3}H-Fucose or ^{35}S-Methionine, or surface labelled with the galactose oxidase-NaB^3H_4 method. The labelled proteins were analysed by two dimensional polyacrylamide gel electrophoresis.

Comparing SCG and CG neurons, some characteristic and reproducible differences were found. On the cell surface for instance, two fucosylated glycoproteins appear exclusively in CG cultures whereas a third one is present only on SCG neurons. Surface labeling after neuraminidase treatment reveals two different glycoproteins present on SCG neurons only. Differences were also detected in the proteins released into the medium and attached to the substrate.

A glia-derived neurite-promoting factor with protease inhibitory activity promotes neurite outgrowth in cultured chick sympathetic neurons

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A 43kD glia-derived neurite-promoting factor (GdNPF) has been purified from medium conditioned by C6 glioma cells. GdNPF is a potent serine protease inhibitor. It induces neurite outgrowth in cultured mouse neuroblastoma cells and inhibits granule cell migration in explants of mouse cerebellum. We demonstrate here that the 43kD protease inhibitor released by rat C6 glioma cells also promotes neurite outgrowth in primary

chick sympathetic neurons grown in serum-free medium. The percentage of neurons bearing neurites as well as the mean neurite length are increased several-fold after one or two days in culture in the presence of 1–3 µg/ml GdNPF. The presence of nerve growth factor, a molecule essential for the long-term survival of chick sympathetic neurons, is not required for the neurite-promoting action of GdNPF.

Symposium 11: Molecular approaches to plant development

Properties of a plant gene with oncogenic functions: complementation studies with a cloned hormone gene

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Wild type Havana 425 tobacco cells transformed with Ti plasmids containing T-DNA with functional cytokinin *(cyt)* and auxin *(aux)* loci proliferate in culture without added cytokinin and auxin, do not regenerate plants, and form tumors when grafted onto a host plant. Earlier we showed that a host cell gene, called *Hl-1*, could replace a defective *cyt* locus in expression of the tumor phenotype. Here we have compared wild type (*wt*) and *Hl-1/Hl-1* cells transformed with a *cyt* defective *(cyt^-)* plasma and with a plasmid in which the T-DNA region is replaced with the auxin locus and a selectable marker, kanamycin resistance *(aux Km^r)*. The transformants fell into 3 classes: 1) *wt cyt+* and *wt aux Km^r* cells were cytokinin auxotrophic, capable of plant regeneration, and weakly tumorigenic; 2) *Hl-1/Hl-1 cyt^-* cells were cytokinin autotrophic, incapable of regeneration, and tumorigenic; and 3) *Hl-1/Hl-1 aux Km^r* cells were cytokinin autotrophic, capable of regeneration, and moderately tumorigenic. These results indicate that loci on the T-DNA in addition to *aux* interact with Hl-1 to restore the tumor phenotype.

Effect of TIBA on maize root auxin-oxidases

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Root growth including cell division (meresis) and cell elongation (auxesis) is localised in the root tip and controlled by several growth regulators including auxin (indol-3yl-acetic acid, IAA). Endogenous IAA content of maize roots is supraoptimal and an inverse linear correlation had been found between the root growth and the IAA content in the tissues of the elongating zone. The content of IAA in these tissues is dependent of its transport, which is polar (from the base to the tip of the root) and its metabolism including its destruction by enzymatic oxidation catalysed by the IAA-oxidases. When IAA transport is inhibited by 2,3,5-triiodobenzoic acid (TIBA) a decrease of root growth is observed. Data presented here show that TIBA inhibits also IAA-oxidases. From these results, possible modes of regulation of the IAA content in the elongating zone of the root will be discussed in relation with the control of root growth by auxin.

ATPase activity and proton pumping of purified vacuoles obtained from *Rubus hispidus* cells cultured in vitro

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Widely used methods for vacuole isolation allowed the obtention of tonoplastic fractions showing very low ATPase and proton pumping activities. Comparisons of these activities and those present in other membrane fractions were then very difficult to perform. Highly purified vacuoles were isolated from in vitro cultures of *Rubus hispidus* using two methods. In a first, common, step of purification, all the cells released protoplasts within 30 min when incubated in presence of enzymes. Purified protoplasts were then bursted with no concomitant osmotic shock. Vacuoles were purified by using discontinuous Ficoll gradients and differential centrifugation. Continuous Dextran T-70 gradient was then performed and ATPase activity and H^+ pumping measured. Only the vacuoles obtained by differential centrifugation still showed these two activities and used for their characterization (specific inhibitors and activators).

Properties of aminopeptidases from bean seeds

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Aminopeptidase forms, extracted from ungerminated bean seeds (*Phaseolus vulgaris* L., var. 'Saxa'), were separated by ion exchange and gel chromatography. The activities were detected with the following L-amino acid-p-nitroanilides: gly, ala, lys, arg, leu, met and phe as substrates. Some properties of four forms differing in their substrate specificities (1: ala/lys/arg; 2: leu/met/phe; 3: gly; 4: phe) were further investigated. The pH-optima were broad and similar for forms 1 and 2 (around pH 7.25), while gly was most rapidly liberated about pH 8.0 and form 4 was most active around pH 7.0. The form liberating ala, lys and arg was highly sensitive to 1,10-phenanthroline, but the same concentration of this chelator had no major effect on the other forms. The activities of forms 2,3 and 4 decreased after addition of $MgCl_2$, while form 1 was more tolerant in this respect. The inactivation of forms 1,2 and 3 was accelerated by the addition of endopeptidases. The form acting on phe was less sensitive to these treatments.

Autolysis in vitro of cotton (*Gossypium hirsutum* L.) fibre cell walls

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Cotton fibre cell walls possess β-glucanase activities which have been characterized as endo-(1→3)-β-D-glucanase (EC 3.2.1.39), exo-(1→3)-β-D-glucanase (EC 3.1.1.58) and β-glucosidase (EC 3.2.1.21). The endo-activity remains more or less constant throughout the fibre cell development, whilst the latter two activities vary in parallel and show a maximum late on in the development of the fibres. The fibre cells also contain callose, a (1→3)-β-D-glucan localized in the cell wall, which is probably the endogenous substrate for the above enzymes. Labelling experiments have shown that the callose undergoes turnover during development of the cell. It is now shown that isolated cell wall fragments undergo autolysis in vitro upon incubation, releasing glucose, and that the rate of autolysis reaches a maximum with walls harvested at the onset of secondary wall formation. The absolute amount of callose in the cell wall in vivo also decreases in the later stages of fibre development. The β-glucanases may thus be involved in vivo in the observed turnover of callose.

Characterization of a phyrophosphate-dependent proton transport by microsomal membranes of maize roots

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Maize (*Zea mays* L. cv. LG 11) root membranes were fractionated in sucrose density gradients. Marker enzymes were used to study their distributions; NADH-Cyt c reductase for the ER, latent UDPase for the Golgi, UDPG-sterolglucosyltransferase

for the plasmalemma and Cyt c oxidase for the mitochondria. Two proton transports were localized, using the technique of quinacrine fluorescence quenching. The first activity was ATP-dependent and presented the characteristics of the tonoplast-type pump (Sze 1985, A. Rev. Plant Physiol. 175–208). The second activity was pyrophosphate-dependent. The two pumps were present on the tonoplast of isolated vacuoles. However, it was not possible to exclude their partial association with the ER or the Golgi membranes. The pyrophosphate-dependent proton pump was characterized using sucrose step gradients to prepare microsomal membranes.

Which polypeptides of inner and outer chloroplast envelope membranes are coded by chloroplast DNA?

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It is well established that chloroplast proteins are coded either by the chloroplast and/or the nuclear genome. Here, we investigate optimal conditions ($MgCl_2$, KCl, pH, temperature, time, etc.) under which protein synthesis takes place in intact spinach chloroplast in the presence of ^{35}S-methionine as protein precursor. After isolation of pure inner and outer envelope membranes, polypeptides of each fraction are separated by SDS-gel electrophoresis. Using fluorography and sectioning techniques, labeled polypeptides are tentatively identified in both membrane fractions arising from control and chloramphenicol-treated intact chloroplasts. The degree of radioactivity incorporation and the pattern of labeled polypeptides are presented for both inner and outer membrane fractions. Results will be discussed in terms of the chloroplast DNA contribution in building up envelope proteins compared to those of thylakoid membranes.

Influence of solutes on the inactivation of glutamine synthetase by endopeptidases

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Glutamine synthetase (GS) extracted from young wheat leaves (low endopeptidase activity) is relatively stable at pH 7.5 and 30 °C, but is rapidly inactivated when extract from senescing wheat leaves (high endopeptidase activity), purified papain or trypsin is added. The inactivation of GS in the presence of endopeptidases can be affected by low molecular weight compounds and pH (e.g. 1 mM ATP stabilized the enzyme). In our work several tested solutes had different influences on GS stability. KCl, sucrose, glycine and alanine had no major effect, whereas GS could be protected from inactivation with $MgSO_4$, $MgCl_2$ and lysine. The protective solutes acted in the same manner in the presence of extract from senescing wheat leaves as well as after adding purified papain or trypsin. We conclude that the results mentioned based on interactions with GS and not with endopeptidases. It must be considered that the stability of various enzymes may be affected in a different manner by the same solutes.

Isolation and culture of betaxanthins and betacyanins producing cells of red beet (*Beta vulgaris* L.)

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Our topic concerns the regulation of biosynthesis of secondary metabolites in tissue culture of red beet.

Several strains of red beet cells were selected for their ability to produce betaxanthins and/or betacyanins. They were set up by classical methods. Primary calli were obtained from cotyledon and leaf explants on solid media and selected upon color criteria during more than 1.5 years.

Five basic variant phenotypes have now been established, expressing green (non-pigmented), yellow, orange, red (unstable) and violet pigmentations; this pattern beeing similar to the one observed in the flowers of *Portulaca grandiflora*. The stability of those colored strains demonstrates that the regulation of betaxanthins and betacyanins biosynthesis in *Beta vulgaris* cells occurs through discrete stable states. We failed in fact to isolate a continuous spectrum of pigmentation as well as strains accumulating betalamic acid only.

HPLC analysis of pigment composition revealed a limited number of major betaxanthins in the yellow and orange phenotypes, one of them beeing common to these two strains.

Lipid biosynthesis in *Chlamydomonas reinhardtii*

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The lipid and fatty acid pattern of plant membranes is the result of different biosynthetic pathways: a) The plastidic pathway and b) the cytoplasmic pathway. The lipid pattern of *Chlamydomonas reinhardtii* is unique by the absence of PC and by the presence of the betaine lipid diacylglyceryltrimethylhomoserine (DGTS). This raises the quesiton as to what extent the two known pathways contribute to the formation of the major lipids of this green alga. Partial hydrolysis and analysis by GLC of the fatty acids in 2-position revealed that both MGDG and DGDG are exclusively of plastidic, DGTS instead, of cytoplasmic origin. Correspondingly, the separation by HPLC of the molecular species showed mainly 18:3/16:4, 18:3/16:3 and 18:2/16:4 combinations for MGDG; 18:2/16:0, 18:3/16.0 and 18:1/16.0 species for DGDG and 16:0/γ18:3, 16:0/18:4, 18:2/γ18:3 and 16:0/18:2 combinations for DGTS. The intracellular organization of lipid biosynthesis and the interconnection of the acylation and desaturation steps will be discussed.

Transport of polypeptides into isolated *Chlamydomonas reinhardtii* chloroplasts

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We have compared different methods for the preparation of intact chloroplasts from the cell wall deficient cw 15 mutant of *C. reinhardtii*. Transport into the chloroplasts was assayed with in vitro synthetized precursor to the small subunit of Ribulose – bisphosphate Carboxylase – Oxygenase according to the following criteria: 1) light dependence, 2) processing to mature size 3) protection against externally added protease and 4) reversal of this protection by detergent. Competent chloroplasts for in vitro transport were obtained with a modified procedure based on silica sol gradient centrifugation (C. A. Price & E. M. Reardon (1982) in: Methods in Chloroplast Biology, M. Edelman et al, eds, Elsevier, pp 189–209; L. Mendolia – Morgenthaler, S. Leu & A. Boschetti (1985) Plant Science *38*, 33–39). We are using this preparation to analyze the requirements and specificity of polypeptide import into *C. reinhardtii* chloroplasts.

Vacuoles biogenesis: specific incorporation of [^{14}C]-choline into the tonoplast of *Rubus hispidus*

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[^{14}C]-choline, added into in vitro cultures of the highly dividing cells of *Rubus hispidus*, was rapidly incorporated (10 min) in the

cells. In the early phases of the incorporation (3 h), cinetic experiments on the distribution of the radioactivity over the constituants of the cells demonstrated that [^{14}C]-choline first bound to the cell walls and was progressively released into the cytoplasm. In the same time, choline was incorporated in a single membrane system as assayed on continuous sucrose gradient. After 18 h of incubation with [^{14}C]-choline, highly purified vacuoles were prepared from protoplasts. A same order amount of incorporated radioactivity was shown in the phospholipids of the vacuoles and whole protoplasts. When purified vacuoles or the entire membrane fractions of the protoplasts were separated onto continuous sucrose gradients, a single peak of radioactivity jumping with the vacuolar ATPase was observed in the two cases.

Protein synthesis and mRNA content during the cell cycle in chloroplast of *Chlamydomonas reinhardtii*

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To study the regulation of protein synthesis in chloroplasts of *Chlamydomonas reinhardtii* we measured changes in the rate of protein synthesis and in the mRNA content of the 32 kD herbicide-binding-membrane protein and the large subunit of the soluble ribulose-1, 5-bisphosphat carboxylase (LS), respectively. Intact chloroplasts were isolated during the cell cycle. They were able to perform light-dependent incorporation of [^{35}S]-methionine into proteins and the ratio of the synthesis of the 32 kD-protein and the LS was calculated from fluorograms of SDS-PAGE. The relative mRNA contents were measured by hybridization with radioactively labelled DNA-fragments from cloned genes of the 32 kD-protein and of the LS.

Furthermore, membrane-bound and free polysomes of chloroplasts were isolated. The relative content of mRNA for the 32 kD protein and the LS was measured in these chloroplast fractions during the cell cycle.

Nuclear photosystem II mutants of *Chlamydomonas reinhardtii*

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To study the interaction of the nuclear and chloroplast genomes in the biogenesis of the photosynthetic apparatus, nuclear mutants of *Chlamydomonas reinhardtii* deficient in photosystem II (PSII) activity were analyzed. Two independently-isolated, allelic nuclear mutants show a pleiotrophic reduction in a set of functionally related PSII polypeptides. Immunoblot analysis reveals that the two mutants, uv-KI 1–8 and XR-KI 1—1, accumulate reduced amounts of certain chloroplast-encoded PSII core components (P5 and P6) and are completely deficient in others (D1 and D2). Polypeptides of the oxygen evolving complex are present, but at reduced levels. PSII polypeptide-encoding mRNAS were measured by Northern analysis. These results show that in the two nuclear mutants, only the level of D2 message is effected, increasing 2–3-fold over WT levels. However, when mutant cells are pulse-labeled for 10 minutes with ^{14}C-acetate, no synthesis of D2 protein was detected, while all other PSII polypeptides are synthesized at normal rates. Therefore, it appears that the mutations present in uv-KI 1–8 and XR-KI 1–1 affect a nuclear gene whose product specifically controls the translation of the chloroplast-encoded D2 polypeptide.

Effects of ozone on photosynthesis and yield of wheat

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Wheat was grown in open-top chambers ventilated with charcoal-filtered ambient air, unfiltered air and air enriched with ozone to study the effect of ozone on grain yield and different photosynthetic parameters of flag leaves. Both, grain yield and $^{14}CO_2$-fixation were highest in filtered air with low ozone levels. In unfiltered air and in air enriched with ozone (+50 µl l^{-1}), grain yield was reduced by 12% and 57%, respectively, and $^{14}CO_2$-fixation by 10% and 52%, respectively. With increasing O_3 concentration, net CO_2 uptake was decreased proportionally at limiting and saturating CO_2 partial pressures. Ozone-induced reductions in net CO_2 uptake were associated with lower contents of activatable RuBPCO and increased CO_2 compensation concentrations. While the degree of activation of RuBPCO *in vivo* increased with increasing O_3 concentration, contents of soluble protein and of chlorophyll, and stomatal conductance decreased. The results suggest: (1) that reduced net photosynthesis of flag leaves is linked to reductions in grain yield, (2) that the response of photosynthesis to ozone is due to limitations in the amount of RuBPCO present, but not due to RuBP synthesis, and (3) that leaves exposed to ozone stress age prematurely.

Synthesis and accumulation of photosystem II polypeptides and their mRNAs during light induced greening in the y-1 mutant of *Chlamydomonas reinhardtii*

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Higher plants grown in the dark are deficient in chlorophyll and photosystem I and II polypeptides and their corresponding mRNAs. Unlike higher plants the unicellular green alga *C. reinhardtii* is capable of chlorophyll synthesis in the dark, and dark grown cells accumulate both PS I and PS II polypeptides and their corresponding mRNAs. We have used a mutant of *C. reinhardtii* which is incapable of chlorophyll synthesis in the dark (y-1) to study the influence of light induced greening on the synthesis and accumulation of photosystems II polypeptides and their mRNAs. We find that the chlorophyll deficient y-1 contains the three proteins of the oxygen evolving complex but lacks all of the core PSII polypeptides as well as the polypeptides of the light haversting complex LHCII. The m-RNAs encoding the missing polypeptides are present in dark grown y-1 cells. The rate of synthesis of these polypeptides was measured during light induced greening by pulse labelling the cells with ^{14}C acetate. The synthesis and the accumulation of LHCII and PSII polypeptides depend on both chlorophyll accumulation and light.

Carriers for IAA transport in maize roots

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Indol-3yl-acetic acid (IAA) is one of a number of substances (plant growth regulators) involved in the control of plant growth and development. The mechanisms by which IAA moves from cell to cell have been studied using maize root tissue. The processes involved are diffusion and carrier-mediated transport across the plasmalemma. Two carriers are described. The first is involved in IAA uptake across the plasmalemma. Studies of ^{3}H(IAA) uptake have permitted the investigation of its properties and localisation. The second carrier mediates IAA efflux across the plasmalemma. Its action is inhibited by N-1-naphthylphthalamic acid (NPA). Studies of NPA-sensitive ^{3}H(IAA) efflux and of ^{3}H(NPA) binding have given information concern-

ing the properties and distribution of this carrier. The evidence presented suggests that the preferential localisation of both carriers in the growing region of the root may play an important part in the regulation of growth.

Herbicide effects on lipid states studied by chlorophyll A fluorescence in liposomes

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To get new insights in a possible interactions occuring between herbicides inhibiting photosystem II (PSII) and acyl lipids of the thylakoid membrane we have studied the fluorescence (F_{680}) variations of chlorophyll *a* (Chl *a*) incoroprated in phosphatidylcholine (PC) liposomes as a function of temperature. In a preliminary approach, two PC species were used: DPPC (di-$C_{16:0}$ PC) and DSPC (di-$C_{18:0}$ PC). The typical phase pretransition (T'c) and transition temperatures (Tc) of DPPC appeared at 31–32 and 41–42 °C respectively, whilst for DSPC only Tc was observed at 54–55 °C. In both liposomes DCMU cuased: (a) a broadening and a shift of Tc to lower temperature; (b) a F_{680} increase below 20 °C and a decrease above Tc. However, DCMU suppressed T'c in CPPC but induced it in DSPC liposomes. Modulation of F_{680} by cholesterol was also studied. Interestingly, atrazine had different effects than DCMU on the above phenomena. These results will be discussed in terms of membrane fluidity.

Effects of soilborne cadmium on glutamate dehydrogenase and proteolytic activity in wheat leaves

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Wheat was grown in the greenhouse in pots with a sandy loam soil to which increasing amounts of cadmium as $CdSO_4$, (0, 1, 4, 16 and 64 ppm of total cadmium) were added. The aim was to study changes in the activity of glutamate dehydrogenase (GlDH), nitrate reductase (NRa) and in proteolytic activity in different leaves in response to Cd pollution. Seven and ten weeks after sowing, plants were harvested and flag leaves, 2nd and 3rd leaves were excised and extracted. Enzyme activities were determined on the basis of leaf area and fresh weight. The activity of GlDH increased significantly at levels above 4 ppm Cd for the second at 16 ppm for the first sampling date. The activity of NRa decreased slowly with increasing amounts of Cd. Proteolytic activity in all leaves studied at the first sampling date was 5–8 times higher than at control levels of Cd. By ten weeks of exposure, a significant increase in this activity was also observed at 4 ppm. These results suggest that soilborne Cd induced premature ageing of wheat leaves at levels much lower than the 64 ppm Cd which decreased yield.

Induction of adventitious roots on aspen cuttings grown in vitro

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The adventitious rooting of herbaceous aspen cuttings (*Populus tremula* cv Astria), grown in vitro, is a spontaneous process. However, to accomplish the rooting of the basal stem part, the presence of the rest of the stem is needed during 3–6 days. Isolated internode segments do not root without an external influence. Treatment with IBA induces rooting, either by using a 24-h pretreatment or including the IBA in the culture medium. In the former case the minimum concentration is 10 µM and in the latter 0.5 µM in order to obtain rooting in a 7-day period.

Characterization of tobacco chitinase genes

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The pathogenesis-related enzyme, chitinase, can be regulated by the hormones auxin and cytokinin. When tobacco pith tissue is subcultured hormone-free medium, its chitinase content increases by ca. 5-fold to upto 8% of the soluble protein over a 7-day period. This induction is inhibited 90% by mistures of auxin and cytokinin added to the culture medium. Several cDNA clones of tobacco chitinase were isolated. Sequence analysis shows that there are at least 2 expressed genes. Southern blot analysis of genomic DNA provides evidence for 3 related chitinase genes. Two different genomic clones were isolated. At least one of these genes is expressed. Genomic Southern and Northern blot analysis of the ancestral species of *Nicotiana tabacum, N. sylvestris* and *N. tomentosiformis*, provided evidence for the origin of the chitinase genes in tobacco.

Transport of γ-butyrobetaine in an *Agrobacterium sp.* isolated from soil

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An *Agrobacterium sp.* isolated from soil by selective growth on γ-butyrobetaine (γ-trimethylaminobutyrate) as the sole carbon and nitrogen source, has been shown to possess an inducible transport system for γ-butyrobetaine. The transport is energy dependent and occurs against a concentration gradient. The uptake of γ-butyrobetaine is optimal at pH 8.5 and has Michaelis-Menten kinetics with K_t of 0.5 µM and V_{max} of 3.8 nmol/min/mg dry weight.

Specificity of the transport system was investigated using analogues of γ-butyrobetaine and it was found that the transport system is very specific for trimethylamino carboxylic acids of defined chain length. γ-Butyrobetaine uptake was significantly reduced in osmotically shocked cells and a γ-butyrobetaine binding activity was detected in the crude shock fluid. This suggests a transport mechanism involving a periplasmic γ-butyrobetaine binding protein.

Cadmium stimulates sulfate assimilation in roots of *Zea mays*

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Plants treated with cadmium produce large amounts of heavy metal binding peptides, called phytochelatins. These compounds have a high cysteine content. This prompted us to think that Cd could stimulate assimilatory sulfate reduction, the pathway of cysteine synthesis.

Using Sephadex G-50 gel filtration we demonstrated that roots of corn seedlings cultivated with 50 µM Cd formed Cd-binding compounds. Consistent with our idea we detected increased extractable activities of the first two enzymes of assimilatory sulfate reduction in these roots. Both ATP-sulfurylase- (EC 2.7.7.4) and adenosine-5'-phosphosulfate-sulfotransferase activity were at 250% of the control after one day and at 500% after 9 days.

These results can be best explained by assuming that the increased need for cysteine to form phytochelatins is covered by stimulating the pathway of assimilatory sulfate reduction.

Hypovirulent strains of *Endothia parasitica* isolated in Switzerland contain double-stranded RNA

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Two different types of the chestnut blight fungus *Endothia parasitica* were isolated in the southern part of Switzerland: An orange pigmented virulent type and a white type with reduced virulence (hypovirulence). Double-stranded RNA (dsRNA) was detected in hypovirulent (white) but not in virulent (orange) strains. The nature of the dsRNA was confirmed by selective resistance to nuclease digestions. Double-stranded RNA from 10 white strains was electrophoresed on agarose gels. 9 strains showed a single band of dsRNA and one strain showed 2 bands. The strain with the two bands is strongly hypovirulent whereas the other strains range from hypovirulent to weakly virulent. Doublestranded RNA from hypovirulent strains was transmitted by hyphal anastomosis to dsRNA free virulent strains which became hypovirulent as tested in the field.

Conditional auxin-auxotrophic *Nicotiana plumbaginifolia:* in vitro selection and characterization

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Using BUdR and FUdR a negative selection system for recessive plant cell mutants was developed. Starting with haploid *Nicotiana plumbaginifolia* mesophyll protoplasts the parameters of mutagenesis, protoplast culture and selection conditions are being modified to arrive at a procedure suitable for routine processing of large numbers: the frequency of ts auxin-auxotrophic clones can be estimated to be 1 in 10^{-7} regenerants surviving protoplasting and mutagenesis. Although it is still unclear why no straight ts lines have been selected, 6 auxin auxotrophs and 1 ts auxin-auxotroph have been selected so far. At the callus level reversion of the auxin-auxotrophic phenotype remains a problem. Whole plants regenerated from the ts auxin-auxotrophic line are consistently smaller then the wt; they flower but show no pollen production and set no seed after pollination with wt pollen. In vitro shootlets of the ts auxin-auxotrophic line remain temperature-sensitive.

The isolation of temperature-sensitive auxin auxothrophs of *Nicotiana plumbaginifolia*

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Following UV mutagenesis and BUdR enrichment, temperature-sensitive auxin auxotrophs have been isolated from populations of cell clones derived from haploid protoplasts. Experiments are underway to characterise the variants biochemically and genetically after plant regeneration.

Characterization of a temperature-sensitive auxin auxotroph of *Hyoscyamus muticus*

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A culture line of *H. muticus* has been isolated which fails to grow at temperatures above 29 °C unless supplied with an auxin. Shoots regenerated from this line bleach and die after transfer to high temperature. This trait is recessive in protoplast fusion. No revertants have been detected. IAA-ethyl ester at 0,25 µM gives 50% of normal growth at restrictive temperature. This line is not rescued by cytokinins, amino acids, purines, pyrimidines or vitamins.

Free IAA in the mutant at restrictive temperature was determined by RIA after HPLC purification. The IAA content (5–60 pmoles/g fresh weight) did not vary significantly from the wild type.

Tracer experiments feeding (2-^{14}C)-IAA to the mutant and analysis of metabolites by HPLC revealed differences between the wild type and the mutant that are being investigated further.

The ts phenotype can be cured by transformation of mutant cells with genes 1 and 2 of the T-DNA of *Agrobacterium tumefaciens.*

Growth kinetics of auxin auxotrophic and temperature-sensitive mutants of *Nicotiana plumbaginifolia* in restrictive conditions

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Mutants with a temperature-sensitive, auxin-auxotrophic phenotype could be valuable for improving our knowledge of the biosynthesis of plant hormones and their modes of action (see the Abstracts of other Posters in this section). As little is known about the site of involvement of auxin in the control of cell growth, examination of the effects of auxin starvation on macromolecular synthesis in the mutants so far isolated is one of the first steps in the characterisation of the mutants, and could provide useful information for improving the isolation procedure.

Auxin resistant mutants in *Nicotiana plumbaginifolia*

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Selection schemes are described which have been developed to screen haploid and diploid *Nicotiana plumbaginifolia* at the protoplast and whole plant level for resistance to a range of several auxins and auxin analogues.

Positive selection for auxin mutants

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Auxins, auxin analogues and auxin transport inhibitors have been shown to be toxic in the range of 10–100 µM when applied on colonies derived from haploid or diploid protoplasts of *Nicotiana plumbaginifolia.* A suitable selection protocol has been designed and used in a number of selection experiments but no NAA resistant colony has been isolated thus far (selective NAA concentration: 60 µM). Following the same protocol but using NPA, a potent inhibitor of auxin transport, as selective agent, tolerant (and requiring) colonies appear very frequent but are mostly unstable. However, several plants have been regenerated and progeny, explants and protoplasts will be tested for their sensitivity to NPA. More recently dose response tests with the same compounds were performed using seeds of *N. plumbaginifolia.* Sensitivity profiles were very similar to those obtained by colonies. Thus selection on the germination level seems to be a reasonable alternative to the cell culture system. Currently M_2 seeds are being produced and selection experiments will be started soon.

Characterization of endosperm specific DNA-binding proteins from barley

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Promoter specific transcription factors seem to play an important role in the regulation of eukaryotic gene expression. Sequ-

ence comparisons of the 5'-flanking region of seed storage protein genes from barley, wheat and maize showed highly conserved regions at about −300, which probably could act as protein binding sites (Forde et al., Nucl. Acids Res., 13, 1985). We are looking for nuclear proteins, which bind to the promoter region of a seed storage protein gene from barley. SDS-PAGE separated proteins were blotted to nitrocellulose filters and incubated with a labelled gene fragment, which contains the conserved region. We found two endosperm specific proteins with similar molecular weights of about 50'000 D which bind to the DNA-fragment. Both factors are soluble in 2% TCA. This behaviour, combined with SDS-PAGE electrophoresis, led to a first crude purification of these proteins. Their function will be tested using in vitro transcription techniques.

Modification of ferredoxin-thioredoxin reductase with thiol reagents

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The ferredoxin-thioredoxin reductase (FTR) from spinach chloroplasts contains a Fe-S center and several thiols. Thiol modifying reagents have been used to determine the number of such groups per molecule of protein and to test whether they are important for catalytic activity. FTR activity is progressively inhibited when accessible thiols on the native oxidized protein are titrated with DTNB or PCMB. A decrease in absorbance at 408 nm indicating a destruction of the Fe-S center paralleles the inhibition with PCMB. NEM blocks rapidly and completely FTR that has been reduced with chloroplasts and ferredoxin in the light. Oxidized FTR (kept in the dark) is not at all inhibited by NEM. This indicates that upon reduction probably vicinal dithiols, important for catalysis, appear on the protein molecule. The modified FTR has been separated by FPLC and its properties studied. Analogy with the NADP-thioredoxin reductase in E. coli lets assume that the active site, responsible for the reduction of thioredoxins by FTR, is a reducible disulfide bridge.

Hydrogen and methane production from effluents of agro- and food industries

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Whey rejected from cheese factories, like other effluents from agro- and food industries, should be eliminated and valorized by anaerobic digestion producing biogas. Acidogenic and methanogenic bacteria differ widely in their physicochemical requirements. Both will therefore operate under better conditions if separated in two different reactors. Mixed mesophilic bacterial cultures, started with filtered sewage sludge and cowpat, were grown continuously in a bench scale two-stages digestion plant loaded with synthetic wastewater containing glucose. The effect of different dilution rates on the production and yield of volatile fatty acids, H_2 and CH_4 was studied. The production of H_2 was maximal for a retention time of 18 h in the acidogenic reactor. A total retention time of 3–4 days was required for obtaining the highest yield of methane and an optimal removal of the organic carbon from the effluent. Since this two-stages system was efficient with glucose, the scale-up in a pilot plant is currently tested with industrial effluents.

Effects of soybean and bean phytoalexins and related isoflavonoids on the zoopathogen, *Candida albicans*

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The use of isoflavonoid phytoalexins in human and veterinary medicine has been suggested. Since the zoopathogenic yeast, *Candida albicans*, was among the fungi to be controlled by the phytoalexins, the effect of a series of structurally related isoflavonoids on this yeast was studied.

The isoflavonoids did not effectively control growth of *C. albicans*. Phaseollinisoflavan, 250 µM, inhibited cell proliferation for 3 days only. The corresponding pterocarpan as well as mono- and di-*O*-methylphaseollinisoflavans and the soybean pterocarpan, glyceollin, had little effect on yeast growth. The order of effectiveness of the compounds was identical with that found for a range of phytopathogenic fungi, indicating target site(s) of similar specificity in species of various taxonomic groups.

C. albicans adapted to high concentrations of phaseollinisoflavan. Tolerance was based on nondegradative mechanism(s), while tolerance to glyceollin appeared to be based on detoxification. Available data question the use of isoflavenoid phytoalexins to control *C. albicans*.

Genetic and biochemical analysis of betacyanine and betaxanthine pigments in *Portulaca grandiflora*

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Genetic variation in pigment pattern of *Portulaca grandiflora* has been investigated in a few genotypes. Our investigation has been mainly devoted to the identification of the yellow pigments (betaxanthines). Betalamic acid has been purified from red beet roots and used as a precursor for the chemical synthesis of yellow betaxanthines through conjugation with different aminoacids. Retention time on HPLC and spectra has been used for pigment identification. We will propose a preliminary scheme for the genetic of the pigments.

Localization of sulfate assimilating enzymes in the lichen *Parmelia sulcata*

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The high sensitivity of many lichen species to SO_2 is well established. We were therefore interested in the compartmentation and regulation of sulfur assimilation in the lichen *Parmelia sulcata* Tayl. A method was established for the separate extraction of the fungus and the algae. Measurements of several enzymes showed in the algal fraction an amount of 80 to 90% of the total activities. Adenosine 5'-phosphosulfate (APS) was mainly reduced by the algal fraction. APS is the substrate for adenosine 5'-phosphosulfate sulfotransferase (APSSTase) in the first reduction step in green algae and plants, while adenosine 3'-phosphate 5'-phosphosulfate (PAPS) serves as substrate in fungi. The PAPS reducing activity was 10 times lower than APSSTase activity and found to 90% in the algal fraction. It could be shown that the algae are capable to dephosphorylate PAPS to APS and thus use it as a substrate. Our results indicate that, corresponding to CO_2 assimilation, the phycobiont plays the major role in the production of reduced sulfur compounds.

Forest decline: endogenous monoterpenes as mediators and amplifyers of environmental stress

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Monoterpenes (e.g. pinene, camphene, borneole) are characteristic storage products in conifer needles normally kept well separated from the cytoplasm. If brought into contact with chloroplasts and mitochondria they can uncouple photosynthetic and

respiratory electron transport. It is hypothesized that atmospheric pollutants induce leakage of monoterpenes from the storage compartments in to the cytoplasm where they might influence cell organelles. Uncoupled electron transport leads to an accumulation of radicals and excited oxigen species and to oxidation of chloroplast pigments. The capacity of detoxifying systems is not sufficient to cope with this extra stress. Shortage of specific metal ions may reduce the activity of the detoxifying systems still further. In both *Picea abies* L. seedlings and in five-year-old plants, monoterpene mediated reduction of photosynthesis and oxidative destruction of chloroplast pigments could be shown. These effects of monoterpenes can be prevented by supplying the plants with antioxidants.

The chloroplast rps12 gene in soybean is split and divided: the mRNA is the result of trans- and cis-splicing events

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We showed (Experientia *41*, 814, 1985) that the split soybean chloroplast *rps*12 gene is clustered with *rps*7 and located in the inverted repeats IR_A and IR_B. However, the 5' *rps*12 region (start to codon 38) was not found within a 6 kbp DNA region upstream of 3' *rps*12. The 5' *rps*12 region could now be mapped and sequenced based on sequencing data from tobacco (Shinozaki et al., EMBO J. *5*, 2043, 1986) and Marchantia (Ohyama et al., Nature *322*, 572, 1986). The 5' *rps*12 maps about 30 kb downstream of 3' *rps*12 (IR_B) on the same strand or about 85 kbp downstream of 3' *rps*12 (IR_A) on the opposite strand, similar to tobacco. Using DNA probes from 5' *rps*12 and 3' *rps*12 we identify in Northern hybridisations transcripts hybridizing either with one or both probes, obviously representing intermediate pre-mRNA and mRNA (trans- and cis-splicing). Splice junctions are sequenced by primer extension. The 5' termini of intron 1 (transon) and intron 2 follow consensus sequences 5' GYGYG- as established for chloroplast protein coding genes.

Plant cell protoplasts: population pattern analysis using image processing: preliminary results

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Protoplasts have been isolated from red beet (*Beta vulgaris* sp.) cell suspensions and *Nicotiana sylvestris* mesophyll cells.

Image processing of a microscopic preparation of freshly isolated protoplasts was made using the following set up: one CCP Camera (512 × 384 pixels), a video frame store memory and a microcomputer.

This equipment and the related software is able to: a) count the number of isolated protoplast; b) count the viability of the preparation using FDA fluorescent staining; c) measure the concentration of betanine type pigments in red beet protoplasts. Preliminary results will be presented.

Symposium 12: Nutrition and metabolism during growth

Genetic dependent stress response in fast growing 'fatty' and 'lean' pigs

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A population of domestic pigs has been separated by breeding into 'fatty' and 'lean' lines. The pigs of the 'lean' line display an increased response to manifold stressors. Stimulation by small doses of CRF (per kg body weight: 0.2 µg and 1 µg) elicits in the 'lean' line a large increase of plasma ACTH. Dexamethasone suppresses this release in a dose dependent manner. In contrast to this, ACTH responses of 'fatty' animals to small doses of CRF is weak. Paradoxically, dexamethasone potentiates this response up to a dose of 0.01 mg dexamethasone/kg; larger doses cause then a complete suppression. Pituitary corticotropes are enlarged in the 'fatty' line. No differences between the lines are observed in ACTH response to a high CRF dose (5 µg). Cortisol levels are raised equally in both lines. Our data suggest a relation between genetic obesity and intensity of the stress response.

Effect of the yeast extract added in the growth medium on the continuous cultures of *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae was grown continuously in a medium containing glucose as a limiting factor and different concentrations of yeast extract under aerobic conditions. The metabolic changes were assessed by an analysis of overall culture parameters (biomass formation; glucose consumption; ethanol, pyruvate, glycerol production and gas exchange rates) and these were compared in the different experiences. The specific oxygen uptake rate reached a maximum value for each concentration of yeast extract and at high dilution rates there was reduction in the respiratory flux. Over a critical concentration of yeast extract the micro-organisms were unable to increase their respiratory rate. But in all the cases, we observed the catabolic repression and we could suggest that this phenomena was the consequence of the limitation of the respiratory capacity.

Symposium 13: Phagocytes

Impairment of macrophage oxidative metabolism by *Leishmania*

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Murine macrophages incubated with macrophage activating factor (MAF) and/or lipopolysaccharide (LPS) undergo a respiratory burst upon triggering by membrane-active agents. This burst was impaired in *Leishmania enriettii*-infected compared to -non infected macrophages, as measured by chemiluminescence, cytochrome c and nitroblue tetrazolium reduction, and hexose monophosphate shunt activity. The inhibition appears to be due to a unique parasite effect as inert particles, such as latex beads, did not impair but rather stimulated the macrophage oxidative metabolism. The metabolic inhibition was a function of the number of infecting parasites and was less pronounced in infected macrobphages activated with both MAF and LPS (i.e. conditions leading to parasite killing), or in cells infected with dead rather than live parasites. The mechanisms whereby *Leishmania* interfere with the macrophage oxidative metabolism are unknown but the inhibition observed may play an important role in the survival of the parasite within the host cell.

Formation of reactive oxygen intermediates in cultured murine glial cells: the role of brain macrophages

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Cultured murine glial ceells were found to generate luminol-dependent azide-resistant chemiluminescence (CL) in response to stimulation with antibody-coated red cells (Ec-IgG), zymosan, and phorbol myristate acetate but not to Sendai virus. Rosetting assays revealed that Ec-IgG bound to a population of cells positive for monoclonal antibodies F4/80 and Mac-1, indicating that the CL burst induced by Ec-IgG originated from macrophages. Macrophages isolated from the heterogeneous glial cell culture resembled microglial cells and generated CL signals similar to the primary culture. Our experiments indicate the presence in brain tissue of cells capable of ROI gneration. 'Toxic oxygen' produced by brain macrophages might contribute to defence against invading microorganisms and may also play a role in destruction of noninfected brain tissue.

Senescent human red blood cells (RBC) contain covalently linked IgG/C3b complexes

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Recognition of senescent as well as oxidatively damaged RBC by phagocytes is dependent on binding of naturally occurring anti-band 3 antibodies and C3b deposition. The amount of C3b deposited on oxidatively damaged RBC exceeds that of bound anti-band 3 by 2 orders of magnitude and the process is independent of Ca^{2+} ions. This suggest alternative complement pathway C3b deposition. The mode by which anti-band 3 stimulated C3b deposition was studied. Results imply that RBC-bound anti-band 3 acts as an acceptor for spontaneously generated C3b, since covalently linked C3b-IgG complexes were detected in SDS-solubilized membranes from senescent, but not young RBC. They were characterized by using ^{125}I-iodinated anti-human IgG and monoclonal anti-C3 antibodies on immunoblots. Since covalent complexes of antibody and C3b maintain convertase activity (Fries et al., J. exp. Med. *160* (1984) 1640), it is likely that these complexes are responsible for enhanced C3b deposition on RBC containing additional amounts of bound anti-band 3 antibodies.

Intracellular Ca^{2+}-handling by phagocytes: evidence for a new, not yet described subcellular structure

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Digitonin permeabilization of human phagocytes (neutrophiles, HL-60 cells) reveals the presence of a vesicular, non-mitochondrial, MgATP dependent Ca^{2+} pool, capable of lowering ambient $[Ca^{2+}]$ to values close to pysiological intracellular free $[Ca^{2+}]$. This pool release transiently Ca^{2+} in response to inositol 1,4,5-trisphosphate ($InsP_3$), the Ca^{2+} mobilizing second messenger.

The present studies were designed to identify and characterize this vesicular pool. Ca^{2+} pumping and response to $InsP_3$ was monitored with Ca^{2+} sensitive electrodes and by $^{45}Ca^{2+}$ uptake. Using density centrifugation techniques and density modification by low concentration of digitonin ('digitonin-shift'), the Ca^{2+} regulating organelle could be separated from nuclei, mitochondria granules, plasma membrane and endoplasmic reticulum, leaving a fraction, the contains the Ca^{2+} regulating organelle, golgi elements and endosomes. Free flow electrophoresis allowed to separate between these three structures.

We conclude, that there is a specialized intracellular Ca^{2+}-sequestring, $InsP_3$-responsive organelle, that can be resolved from all organelles described so far.

Role of prostaglandins in intracellular killing of *Leishmania* parasites by murine macrophages

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Mouse bone-marrow-derived macrophages, when activated with macrophage-activating factor (MAF) in the presence of ng amounts of lipopolysaccharide (LPS), acquire the capactiy to kill intracellular *Leishmania major* parasites in vitro. This microbicidal activity is accompanied by an increase in prostaglandin (PG) synthesis. Macrophages stimulated with LPS alone secrete lower although significant amounts of PGE_2 but are unable to destroy the parasites. The microbicidal activity of macrophages exposed to MAF+LPS is increased by addition of exogenous PGE_2 and decreased by inhibitors of PG synthesis. The intracellular parasites themselves appear to modulate PG synthesis as a larger amount of PGE_2 is always found in uninfected compared to infected cultures. These findings suggest that macrophage activation is at lest partly controlled by PG. Experiments are in progress to determine whether a correlation exists between PG synthesis and stimulation of the oxidative metabolic burst upon activation.

Isolation and expression of two human genes encoding myelocyte-monocyte specific proteins associated with the macrophage migration inhibition factor (MIF)

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MIF affects the immune regulatory functions of macrophages. To study the regulation of this mechanism, we have isolated two human genes encoding proteins of 93 and 114 amino acid (MRP8 and MRP14). They have a similar gene organisation and both proteins share homologies including two calcium ion binding domains or so called 'EF hand' structures. As revealed by primer extension experiments and Western blot analysis, the MRP8 and MRP14 genes were correctly expressed in human embryonic L-132 fibroblast cells under the control of their own promoter and a cytomegalovirus (CMV) IE1 gene enhancer. In contrast, no expression was observed in the same cells when the CMV enhancer was omitted. This result could be explained if these two genes are selectively expressed in the myelo-monocytic cell lineage, due to a tissue specific promoter.

Activation of the human neurophil NADPH oxidase: role of degranulation

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The NADPH oxidase has been postulated to be a multicomponent system consisting of at least a flavoprotein and a cytochrome b. Since cytochrome b has been mainly found in the specific granules, translocation of this redox component to the plasma membrane has been proposed as a necessary step for oxidase activation. 4,4'-diisothiocyano-2,2'stilbene disulfonate (DIDS), and anion channel blocker in red cells and an inhibitor of degranulation in neutrophils was found to substantially increase the ability of the chemotactic peptide FMLP to produce superoxide (O_2^-), but had only slight effect on opsonized zymosan (OZ) or phorbol myristate acetate (PMA)-stimulated cells. Kinetic studies on 48000 g particulate fraction oxidase activity show that k_m of DIDS-treated cells stimulated with OZ or PMA

were not different from control cells, whereas V_{max} was slightly decreased in fractions from OZ-stimulated cells. In contrast to its effect on whole cells, DIDS treatment inhibited the oxidase activity from FMLP-stimulated cells. Washing the cells free of DIDS prior stimulation reversed degranulation, but did not restore the oxidase activity, while it preserved potentiation of O_2^- production of FMLP. These results indicate that degranulation is not a required step for NADPH oxidase activation, and that DIDS exerts a stimulus specific effect distal to oxidase stimulation.

Secondary lysosomes as an integral part of the cytoskeleton: A morphological study of rat Kupffer cells

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The structural integration of secondary lysosomes into the cytoskeleton has been studied in isolated rat Kupffer cells (kindly provided by M. deLeeuw, TNO Rijswijk, The Netherlands) in culture by light and electron microscopic immunocytochemistry. Actin, tubulin and vimentin were found to be the major cytoskeletal components. Vimentin was found to predominate in the perinuclear region from where it radiated to the periphery. As the most stable component it produced a dense meshwork around the lysosomes; it might play a role in positioning these organelles. The microtubules were also found in close association with the lysosomes. They formed radial tracks through the cells and may be involved in a translational transport mechanism. In the cell periphery the actin was found to predominate, forming a highly interconnected meshwork. Numerous T- and Y-shaped interconnection were found as well as bridges of finest 3 nm filaments between the microfilaments. This meshwork is so dense that a disintegration of the actin filament crosslinks has to be considered during intracellular transport of these organelles.

Studies on the lipoxygenases of granulocytes

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Lipoxygenase activity in intact, homogenized and detergent-permeabilized leukocytes (90% neutrophils) was measured using HPLC and TLC. Species studied were: pig, cow and horse. Intact horse cells did not produce detectable amounts of 15-lipoxygenase products, but when homogenized their main product was 15-hydroxy-eicosatetraenoic acid (15-HETE). Preliminary enzyme characterizations were carried out on cells from all three species by varying substrate concentration and enzyme concentration. To probe into the reaction mechanism, experiments using reducing and oxidizing substances as well as radical scavengers were done. The results show that the granulocytic enzymes of the 3 species are unstable, show a non-linear concentration vs. activity profile and no substrate saturation. It may furthermore be suspected that the ferric/ferrous redox couple may play a role in the reaction cycle. The observed irreversible inhibition of the enzyme seems to be due to organic hydroperoxides rather than to reactive oxygen intermediates.

Cloning and expression of two complementary DNAs encoding proteins that are associated with specific stages of myeloid cell differentiation

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In an attempt to identify proteins that are involved in the differentiation of macrophages we seek to identify the molecular basis of macrophage migration inhibition factor (MIF). Therefore, we cloned and expressed two cDNAs that code for two proteins, MRP-8 and MRP-14, that were isolated with a MIF-binding antibody. These proteins have predicted molecular sizes of 93 aminoacids and 114 aminoacids respectively, and contain no signal sequence. Monospecific antisera raised against the recombinant proteins detect MRP-8 and MRP-14 exclusively in cells of the myeloid lineage and in a differentiation specific manner. Both proteins contain stretches of aminoacids characteristic for Ca^{++}-binding domains, Ca^{++}-binding proteins have been implicated in the activation of macrophages. Therefore, MRP-8 and MRP-14 might play an important role in macrophage-activation.

Reduced skin lysyl oxidase activity in Menkes' disease

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Menkes' disease is a lethal X-linked recessively inherited disorder in which a defect in copper handling appears to render copper unavailable for copper-dependent enzymes. It is characterized by progressive cerebral degeneration and connective tissue abnormalities. We report a patient with unusually marked connective tissue involvement who died at 11 months. Prominent features were osteoporosis, scurvylike bone changes, pathological fractures, Wormian bones, cutis laxa, bladder diverticulae and toruosity of arteries, which were most likely to heve been due to a deficiency of the copper-dependent lysyl oxidase which initiates the crosslinking of collagen and elastin. We and others previously demonstrated considerably reduced lysyl oxidase activity in the medium of fibroblast cultures derived from patients with Menkes' disease. We have now examined directly lysyl oxidase activity in extracts of skin obtained at autopsy and found it to be $\sim 20\%$ of that in extracts of skin from two age-matched control subjects, validating for the first time the in vitro observations.

Quantitative assessment of the kinetics of bacteria phagocytosis, intracellular killing, and oxidative burst generation in bovine PMNLs

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We investigated quantitatively the kinetics of oxidative burst generation, phagocytosis and intracellular killing of bacteria in peripheral blood polymorphonuclear leukocytes (PMNLs) of adult and young (< 8 weeks) animals of different Swiss diary cow breeds. The obtained data of oxygen metabolite formation (chemiluminescence, hydrogen peroxide, superoxide anion), phagocytosis and intracellular killing (acridine orange/cristal violet method) using different stimuli (PMA, opsonized zymosan, opsonized *E. coli* and *Staph. aureus*) were analyzed by the IBM-SAS computer program. Significant differences were found between the activation patterns obtained with PMNLs from adult and young animals. Results obtained with PMNLs of infected animals will also be presented.

Apparent requirement for a protein kinase C- (PKC) and Ca^{2+}-independent process in human neutrophil NADPH oxidase activation

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There is multiple evidence that receptor agonists activate the NADPH-oxidase in neutrophils by a PKC-dependent signal transduction pathway involving a GTP-binding protein, phospholipase C activation, and rise of cytosolic free Ca^{2+}. Kinetic studies of the onset of H_2O_2 production and intracellular Ca^{2+} changes during neutrophil activation suggest that an additional receptor-dependent sequence takes part in NADPH-oxidase activation. Two main lines of evidence were obtained: 1) When neutrophils were stimulated with the receptor agonists fMLP, C5a, PAF or LTB4, identical onset times for H_2O_2 production were observed (2.4 sec). The onset times were much longer when ionomycin and PMA were used separately or in combination. Thus neither PKC activation (by PMA), immediate elevation of cytosolic free Ca^{2+} (by ionomycin), nor both together are sufficient to activate the NADPH oxidase as fast as any of the agonists. 2) In neutrophils pre-activated with PMA, a further rise of H_2O_2 production could be induced by addition of fMLP or ionomycin. The response to fMLP was immediate (onset time 0.2 sec) while the Ca^{2+} rise was delayed by 1–2 sec. Ionomycin induced an immediate Ca^{2+} rise, but the onset time of H_2O_2 production was 6 sec. These observations indicate that receptor activation initiates an apparently Ca^{2+}-insensitive transduction process which enhances NADPH oxidase activity, and is faster than the one involving PKC.

Symposium 14: Eucaryotic chromosome replication

Proteins involved in control of SV40 DNA replication

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Initiation of Simian virus 40 (SV40) DNA replication is controlled by three elements: a 59 bp sequence whichs acts as the origin of DNA replication, a multifunctional viral protein T antigen, and an unidentified cellular factor(s) provided by primate cells but not by rodent cells. We have identified a cellular protein that binds specifically to sequences in the core origin of replication. Mutant origins that do not replicate have lower affinity for this protein. Studies of this monkey cell factor and the biochemical properties of mutant and wildtype T antigens suggest a working model for the interaction of these elements in control of initiation.

Organization of the eukaryotic chromosome

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The DNA of the eukaryotic chromosome appears to be arranged in looped domains of 30–100 kb that are restrained by a central array of nonhistone proteins called the scaffold. The major protein of the metaphase scaffold is topoisomerase II, which has been localized by immunoelectron microscopy to the central axis of both histone-depleted and intact chromosomes.

Using Li-3, 5-diiodosalicylate to extract histones (Mirkovitch et al, 1984) we find specific sequences (SARs) bound to the nuclear scaffold of Drosophila cells. SAR sequences are located 5' and 3' of highly transcribed developmentally regulated genes, forming small loops of 5–14 kb. In regions transcribed at low levels, they are found less frequently. Three sequence motifs characterize these SAR fragments, among which is the cleavage consensus for topoisomerase II. Current research aims to test the structural role of topoisomerase II in loop organization and the significance of these SAR fragments for the higher-order folding of DNA in yeast.

Specific in vitro initiation of DNA replication by a mammalian DNA polymerase α holoenzyme complex

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Porcine circo-virus (PCV) is the only mammalian virus so far identified that contains a single-stranded (ss) closed circular DNA (1.76 kb) as its genome. In this respect it resembles the small *E. coli* bacteriophages ØX174, G4, fd and M13, which have been used so successfully to unravel the mechanisms of DNA replication in prokaryotes. The PCV genome is only one third the size of the bacteriophage DNAs. Therefore, DNA replication in PCV can be expected to depend even more on host cell function than it does in the prokaryotic systems. This promoted us to use the PCV genome as a naturally occurring ssDNA template to study the sequence requirements for initiation of DNA replication by mammalian DNA polymerase α holoenzyme. This major eukaryotic replicase contains a tightly associated primase. Here we show that purified DNA polymerase α holoenzmye from calf thymus containing at least ten polypeptides primes DNA replication in vitro preferentially at one specific site on the PCV genome, possibly unveiling a consensus sequence of mammalian origins of DNA replication.

DNA comprising the origin of replication of the extrachromosomal ribosomal DNA of *P. polycephalum* (per DNA) can replicate extrachromosomally

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We combined selective markers with sequences containing the origins of replicaiton of the Per DNA using the *E. coli* vector pHC8. In yeast such constructs replicate autonomously as seen by Southern analysis and by rescue of the plasmid in *E. coli*. Stability and rearrangements of such constructs in yeast and plants are discussed.

Replication of the *E. coli* plasmid PSC101

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We are studying the plasmid pSC101 in order to elucidate the molecular mechanisms involved in the regulation of its replication. By insertional mutagenesis we have already mapped the origin of replication, an essential gene repA and regions implicated in the regulation of replication. We are also analyzing three *cis*-dominant copy mutants of pSC101; preliminary results indicate that the mutations map in the plasmid encoded repA gene, the sequencing of the mutants is in progress. Genes fusion techniques have enabled us to identify transcriptional events within the regions, upstream of the gene repA. We are currently characterizing these transcripts by northern analysis and protection experiments.

RNA 3' processing: a mechanism coupling histone gene expression to cell proliferation and DNA replication

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Sequences causing a reduction of histone H4 mRNA levels in Gl-arrested cells of a ts mouse mastocytoma cell cycle mutant are located at the 3' end of the gene, within the same 55 bp as the RNA 3' processing signal. Two lines of evidence demonstrate that RNA processing is affected: 1) elevated levels of nuclear H4 mRNA precursors in Gl-arrested cells and 2) reduced efficiency of nuclear extracts from Gl-arrested cells for RNA processing in vitro. The defect seems to be due to the virtual absence of a heat-labile activity (first identified by O. Gick and M.L. Birnstiel, pers. comm.), whereas snRNPs also involved in histone mRNA 3' processing are present in similar amounts in extracts from either exponentially dividing or Gl-arrested cells. Regulation by 3'-terminal sequences is also observed in mouse fibroblasts arrested by serum starvation, and thus seems to be a general mechanism to couple histone gene expression to cell proliferation and DNA replication.

Symposium 15: Slow diseases of the nervous system

High variability of measles virus RNA genomes derived from diseased human brains

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Measles virus (MV), a negative strand RNA virus, normally causes acute infections but rarely its persistence in the central nervous system induces the fatal disease subacute sclerosing panencephalitis (SSPE). The diversity of defects restricting MV gene expression in SSPE brains suggests that an error prone viral replication machinery allowing particularly rapid evolutionary changes underly these defects. In fact partially overlapping cDNA clones from the matrix gene of a defective MV replicating in the brain of a SSPE patient showed sequence variations in 1% of the bases (Cattaneo et al., Virology 154, 97). Now several full length cDNA clones of five MV genes were obtained from cultured cells habouring a MV genome derived from a SSPE brain (Sheppard et al., PNAS 83, 7913). In sibling clones of all compared genes about 0.25% of the bases differed. Thus on average each MV genome varied from every other in about 40 of its 16.000 bases.

Measles virus transcripts in diseased human brains

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The MV specific transcripts expressed in brain autopsies of two patients with the slow disease of the central nervous system SSPE (see preceding abstract) and of one patient with inclusion body encephalitis were analysed. In the three brains 1000–4000 transcripts of the first MV gene per average cell were detected. The amount of transcripts diminished progressively to 10–25 (1%) for the fifth gene, this reflecting strong transcriptional attenuation at every gene junction. In a lytic and a persistent MV infection of cultured cells 27,000 and 1500 copies respectively of the first transcript were detected. A less pronounced transcription gradient reflecting weaker transcriptional attenuation was monitored, and the fifth MV transcript was expressed at levels corresponding to 15% and 25% respectively of the first transcript. The MV transcription machinery in diseased brains might generally be defective, leading to an altered balance of the MV proteins.

PrP-protein polymerizes in detergent extracts of microsomes derived from scrapie infected hamster brains

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PrP 33–35^{Sc} is a membrane protein, only found in scrapie infected hambster brains. It polymerizes into rods after detergent extraction of the lipids (Meyer et al., PNAS *83* (1986) 2310). A centrifugation assay was developed to study the kinetics of this polymerization reaction. Brain extracts were cleared by high-speed centrifugation before incubation at 20 °C for various time intervals. Proteins polymerized during incubation were collected by a second centrifugation step. PrP protein in the resulting pellet was quantitated by an ELISA. Within the first three hours of polymerization, increasing amounts of antigen precipitated but eventually leveled off. The polymerization reaction was not affected by the presence of salt of EDTA but could be inhibited by guanidine-HCl or KSCN. Examination of the extracts by electron microscopy revealed prion rods whose average size increased with polymerization time, eventually reaching an average length of about 180 nm.

Prion rods, not digested by proteinase K, as in standard procedures (e.g. Prusiner et al., Cell *38* (1984) 127), were partially purified by sucrose step gradients. The molecular structural analysis and protein composition of these rods are currently under investigation.

Anatomy, Histology, Embryology

The initial role of cellular autophagy during isoproterenol induced growth of the submandibular gland

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In a short-term series, the influence of isoproterenol (IPR) on autophagic breakdown in the submandibular gland (SMG) was studied in adult male rats. 5 animals were given a single dose of IPR (3 mg/kg b.wt), 5 controls received phys. saline. After 10 min retrograde perfusion fixation was performed via the abdominal aorta. Cubes of tissue taken from the SMG were processed for electron microscopy and were morphometrically evaluated for volume fraction of early stages of autophagic vacuoles (AVs). IPR significantly reduced the AV volume fraction by 69% ($p < 0.01$). The present data indicate that inhibition of cellular autophagy in the SMG can be regarded as an early subcellular response. This anticatabolic mechanism may contribute to protein accretion known to occur during growth of the SMG induced by long-term adrenergic stimulation. It is concluded that inhibition of cellular autophagy could play an important role in establishing a new balance of cellular protein turnover in order to adapt to an enlargement of the SMG.

A model for morphofunctional studies on rat medio-basal hypothalami (MBH)

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Our model is based on two prerequisites: the tissue must be 1) well preserved and functionally unimpaired throughout the incubation, and 2) processed for morphology in optimum conditions. To test this model we have studied some aspects of the LHRH system in adult male rats. The MBH was isolated, put in 0.5 ml HEPES buffered Locke's medium gassed by 5 ml/min of O_2/CO_2 (95%/5%), and shaken at 37 °C. After a 10-min washing, the medium was changed twice at 20-min intervals. At the end of the incubation the tissue was satisfactorily preserved as judged by light and electron microscopy. LHRH, somatostatin and TRH could be demonstrated by immunohistochemistry. The LHRH release was close to basal values after 30 min (22.1 ± 4.8 pg/MBH) and then remained constant for other 20 min (17.6 ± 2.6 pg/MBH). During the second 20 min, LHRH release increased in presence of 61.6 mM K^+ (110.4 ± 8.7 pg/MBH). Thus the MBH was excitable, until the end of the incubation. We conclude that this model can be successfully used for morpho-functional studies.

Polyethylenglycol impregnation as a simple technic to produce flexible dry specimens

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Gross anatomical specimens which were fixated by formalin can be transferred to dry specimens by impregnation with the non noxious polyethylenglycol 400 (PEG). These specimens are completely free of formalin, are resistant against mould, stay elastic and can be stored without any storage fluid. This saves space and brings more clearness into the storage room. On the other hand the specimens can be used at any time immediately for teaching or studying. When a strong air suction pump with membranes is used the formalin will be evaporated and the water of the tissue fluid will be substituted by PEG at room temperature and a pressure of 25 to 15 mbar within some hours. The expenses for running costs and materials are very low. The costs of a whole well working and safe system are reasonable when air suction pumps and vacuum tanks are used which are produced in larger series.

Morphological aspects of clonal growth of human tumor cells in a semisolid medium (methylcellulose)

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Tumor cells from different human carcinomas (we received the cells from Dr Aapro, Geneva) were grown 'in vitro' using a semisolid medium (methylcellulose) for clonal growth (J.E. Eliason et al., Rec. Results in Cancer Res. *94* (1984) 269). The morphology and cell organisation of the formed colonies were studied by electron microscopy. The cells forming the colonies showed different organisation patterns. At times and apart from the size, any organotypic structure was observed within the colonies which consisted in a packed cluser of cells. At other times tumor cells were situated at the periphery, while in the middle of the colony rare cells or cell remnants were found. For some tumors within the colonies, among non-organised tumor cells we could observe small organotypic structures (duct-like). In the case of other tumors all the cells of the more voluminous colonies formed organotypic vesicular structures. From the wall of these vesicles small cell clusters, then developing into vesicles were seen. This different behaviour in the organising capacity of the cells from different human tumors in a three-dimensional culture, may indicate an element of prognosis for the clinical evolution in the whole organism, being this cells 'a priori' in different differentiation stages.

Cellular localization of perforin 1 in murine cloned cytotoxic T-lymphocytes

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Recently it has been shown that the cell mediated cytolysis by cytotoxic T cells (CTL) is mediated by a pore forming protein called perforin 1 (P1). In our study we examined the localization of P1 in cytotoxic cells by immunoelectron microscopy using a monospecific rabbit antiserum against highly purified mouse P1. P1 was found in specific granules of cloned CTL; other organelles remained unlabelled. Within the granules P1 antigen was localized in the fine granular matrix whereas the vesicular compartment remained free of gold particles. The amount of P1 antigen detectable by immuno-electron microscopy varied between different CTL clones. CTL with NK like activity had the highest level of P1 antigen. A cytotoxicity loss mutant of CTL had no detectable P1 antigen suggesting an important role of perforin 1 during cell mediated cytolysis. P1 antigen was undetectable also in bone marrow macrophages indicating a different cytolytic mechanism of these cells.

Cell differentiation and organogenesis by 'formative cells'

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When a fibroblast, wandering in a heart cell monolayer culture, enters the sphere of influence of a myocyte, contact between the two cell types occurs. To this effect the fibroblast changes its direction. The area of the muscle cell contacted by the fibroblast tends to dart out. The resulting myocyte process advances along the fibroblast and, as a rule, is guided to neighboring myocytes. Thin threads produced in like manner and often repeatedly contacted by the handling lamella of the fibroblast subsequently broaden and develop into intercellular bridges. A conspicuous reaction of myocytes after contact with fibroblasts is the accelerated initiation of differentiation in which the muscle cell polarizes and starts to beat. After contact, a myocyte can be transported to a neighboring muscle cell by a fibroblast. These six interactions result in the development of synchronously pulsating muscle cell units. Those formative cells have also been found functioning in organogenesis of dissociated mesonephros and embryonic liver cells.

Secondary lysosomes as an integral part of the cytoskeleton: a morphological study of rat Kupffer cells

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The structural integration of secondary lysosomes into the cytoskeleton has been studied in isolated rat Kupffer cells (kindly provided by M. deLeeuw, TNO Rijswijk, The Netherlands) in culture by light and electron microscopic immunocytochemistry. Actin, tubulin and vimentin were found to be the major cytoskeletal components. Vimentin was found to predominate in the perinuclear region from where it radiated to the periphery. As the most stable component it produced a dense meshwork around the lysosomes; it might play a role in posi-

tioning these organelles. The microtubules were also found in close association with the lysosomes. They formed radial tracks through the cells and may be involved in a translational transport mechanism. In the cell periphery the actin was found to predominate, forming a highly interconnected meshwork. Numerous T- and Y-shaped interconnection were found as well as bridges of finest 3-nm filaments between the microfilaments. This meshwork is so dense that a disintegration of the actin filament crosslinks has to be considered during intracellular transport of these organelles.

Whisker stimulation increases ketone body (KB) metabolism in infant mouse trigeminal subnuclei caudalis (SC) and interpolaris (SI)

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Is β-hydroxybutyrate (HB) metabolism related to stimulus-evoked neuronal activity in infant mice? Metal pieces were glued on left whiskers Cl-3 & E1 of restrained mice at P4 (birth at PO), which had been removed from their mothers for 12 h; other whiskers were clipped. 8 mice were injected i.p. with [^{14}C]HB and exposed to magnetic field bursts for 2, 5, 10 or 45 min. In all brains autoradiography showed ^{14}C incorporation to be higher in gray than in white matter. Only at 2 and 5 min, HB metabolism in SC & SI was higher on the left than on the right side; it covered the representations of more whiskers than stimulated. Conclusions: a) HB is carried across the bloodbrain barrier fast enough to be used by neurons as an energy source under acutely increased demands, b) [^{14}C]HB can be used as marker for stimulus-evoked neuronal activity in infant mice. Does increased KB metabolism reflect an increase in activity of enzymes metabolizing KBs, or an increase in blood flow?

Minisegments of adult rat optic nerve in vitro: myelin maintenance or remyelination?

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Small explants, called minisegments, of adult rat optic nerve were cultured in vitro in order to study degeneration and the potential for regeneration of CNS glial cells in the absence of neuronal elements. The explants were investigated morphologically after 2, 6, 14, 20, 25, 50, 64 and 70 days. The most surprising finding was that after 70 days in vitro we observed the presence of morphologically perfect compact myelin with alternating major dense and intraperiod lines. A complete understanding of these preliminary morphologically findings will require further interdisciplinary investigation. Nonetheless, these results invite a number of important questions: Are these myelin sheaths newly formed or have they been maintained by surviving oligodendrocytes? Supposing that these sheaths are newly formed it is interesting question whether the oligodendrocytes that produced this myelin were already differentiated or whether they differentiated from progenitor cells during the time in culture?

Asymmetrical brain oedema due to experimental carotid microthrombi in guinea pigs

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Twenty-two male guinea pigs received an intra-carotid injection of about 140000 latex beads (mean diameter: 25.7 µm). 10 animals were sacrificed by Nembutal after 24 h and 12 after 48 h. Five of the former and seven of the latter were treated with Neurotropin (a kinin-release inhibitor) between injection and sacrifice. The brains of the perfusion-fixed guinea-pigs were serially frozen-sectioned at 30 µm in a coronal plane. Every 10th section was collected, stained and photographed at low magnification. In all sections rostral to Foramina interventricularia, the following parameters were measured for each hemisphere: a) typical necrotic areas due to thrombi, b) total hemisphere area and c) ventricular area within hemisphere. A significant positive correlation was found between the mean surface density of necrotic lesions and the rate of swelling of the latex bead-injected hemisphere (compared with the non-injected one). The slope of the correlation curve was steeper in the animals killed after 24 h and also steeper in the non-treated animals, than in those killed after 48 h or treated with Neurotropin.

Behavior, choline acetyltransferase activity and brain calcium binding proteins in chronically D-hypervitaminotic animals

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Female rats were fed daily 20000 IU vitamin D per kg, by intragastric application over 4 months. Controls received 40 IU/animal/day. The treated animals showed a moderate hypervitaminosis as seen in blood parameters and clinically. Behavioral tests of randomly selected animals included openfield activity, shuttle-box learning and radial-maze performance test, but a difference was found only in the 'Morris water navigation task'. The pooled swimming time to the hidden platform 1, 2 and 5 weeks after the learning session was significantly shorter in the treated animals (76.4 sec $\pm$ 17.7 (SE) versus 132.5 $\pm$ 16.7; $p = 0.028$; df 13). Notwithstanding this behavioral improvement, the choline acetyltransferase activity in whole cerebral cortex, hippocampus, and caudatoputamen did not reveal differences between treated and control animals. Also the concentration of the calcium binding proteins (CaBP's) calmodulin and parvalbumin, as determined by RIA, were unchanged. Other CaBP's are now under investigation.

Biochemistry

Interaction of Substance P with charged lipid bilayer membranes

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The interaction of Substance P with charged planar lipid bilayers (phosphatidyl choline/phosphatidyl serine mixtures having a Gouy-Chapman surface potential of -56 mV) was measured using the capacitance minimization technique for monitoring the surface potential of the membrane. Substance P binds to these charged bilayers with a maximum binding density of 3.6×10^{-4} molecules/A^2 and a K_D of 2.8×10^{-6} M. Correcting for the Boltzmann accumulation of the triply positively charged Substance P molecule on the negatively charged membrane yields an intrinsic dissociation constant of 2.5×10^{-5} M.

The surface potential measurements yield both the surface charge density and the dipole potential. For Substance P we measured an effective dipole moment normal to the bilayer surface of 12.5 D/molecule. This is consistent with the presence of a short alpha-helix in the membrane bound conformation of Substance P as suggested by other physical chemical and theoretical studies in our laboratory.

Three Z-DNA-binding proteins from bull testis

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Three Z-DNA-binding proteins of Mr's 31K, 33K and 58K were isolated from mature bull testis in apparently homogeneous form. Testis extracts were fractionated by cation exchange chromatography on CM-Trisacryl M, affinity chromatography on brominated poly(dG-dC)·poly(dG-dC) and anion exchange FPLC on Mono Q. The fractions were analysed for Z-DNA- and B-DNA-binding by filter binding tests and by protein blotting followed by challenging of the transferred proteins with [^{32}P] end-labeled B-DNA [poly(dG-dC)·poly(dG-dC)] and Z-DNA [brominated poly(dG-dC)·poly(dG-dC)]. All three proteins exhibited greatly preferred binding to Z-DNA.

Application of FPLC-reversed phase chromatography for the purification of small hydrophobic light-harvesting polypeptides

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A method is described using reversed phase (RP) chromatography (FPLC, Pharmacia) for isolating very pure antenna apoproteins from several photosynthetic bacteria (e.g. *Rc. gelatinosus, Rp. acidophila*). The individual detergent-solubilised antenna complexes were purified on DE-52. Subsequent extraction with $CHCl_3$ (CH_2Cl_2)/CH_3OH/NH_4OAC (1:1, 0.1 M) and chromatography on LH-60 and DE-32 in the same solvent yielded partially pure depigmented and delipidated antenna polypeptides (α,β-heterodimer, for a review see H. Zuber, TIBS *11* (1986) 414). These column fractions were concentrated (5-10 times) in a rotary evaporator and loaded on the RP -support (ProRPC 5/10, PepRPC 5/5). Most of the antenna apoproteins elute between ca. 50–80% CH_3CN with a recovery yield of ca. 60–80%. Thus, a powerful technique is offered for accurately monitoring antenna polypeptides and their products derived from labelling, crosslinking and proteolytic digestion studies.

Influence of protease inhibitors on red cell vesiculation

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Release of vesicles from human red cell membranes was induced either by ATP-depletion or by incubation of the cells in presence of sonicated dimyristoylphosphatidylcholine (DMPC) vesicles. Both vesiculation processes were inhibited in a concentration dependent way by the protease inhibitor N-α-tosyl-L-lysine chloromethylketone (TLCK).

In ATP-depleted erythrocytes proteolytic breakdown products could be demonstrated which were not detected in cells incubated with DMPC. Proteolysis was not significantly affected by TLCK. These results suggest that the influence of TLCK on membrane vesiculation is not primarily due to inhibition of proteolysis but to a direct interaction of the inhibitor with the erythrocyte membrane.

TLCK has been described to affect the shape of virus transformed cells as well as protein secretion from liver cells, however, the mechanism of action is largely unexplained. In light of the above results it is well possible that TLCK – besides being a protease inhibitor – acts via a direct interaction with the membrane structures involved.

Characterization of calcitonin receptor metabolism

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In the porcine kidney cell line, LLC-PK_1, the hormones calcitonin and vasopressin activate the cAMP-dependent protein kinase (cAMP-PK) which in turn leads to a 200-fold increase in production of urokinase-type plasminogen activator (uPA). Recently, a number of mutant cell-lines derived from LLC-PK_1 cell-line have been isolated and found to be defective in biosynthesis of the receptors for calcitonin and vasopressin. These fall into two categories: Mutants such as FIB5N4 affected in the function of the calitonin receptor; and a second group represented by the M18 cell-line, which is affected in both calcitonin and vasopressin receptor function. Using gene rescue techniques we hope to isolate genes either for the receptor itself or for a processing enzyme necessary for correct expression of both calcitonin and vasopressin receptors. Genomic DNA from T47D cells (human epithelial breast cancer cell-line) which possess calcitonin receptors will be transfected into M18 and FIB5N4 to complement the mutant phenotype. The resultant hormone-responsive clones will be examined for functional calcitonin receptor expression and used for subsequent genomic cloning.

Purification and fragmentation of creatine kinase from rat heart mitochondria

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Recent work has established that cardiolipin is the receptor for mitochondrial creatine kinase at the outer side of the inner membrane. The enzyme has now been purified from rat heart mitochondria according to a modified version of a procedure designed by T. Wallimann (personal communication). Mitochondria are first swollen in distilled water. After centrifugation the pellet is resuspendet and incubated overnight in 100 mM NaP_i, pH 7.4. The suspension is centrifuged an brought to 25% ammonium sulfate. After centrifugation the ammonium sulfate concentration is raised to 50%. The precipitate is dissolved in 10 mM NaP_i, pH 6.8. The solution is applied to CM-Sephadex column, from which the creatine kinase is eluted with a salt gradient. The active fractions are precipitated in 50% ammonium sulfate and dissolved in the buffer described above. The solution is then applied to a DEAE-Sephacel column equilibrated in the same buffer at pH 6.8, and the creatine kinase was collected in the first fractions. The purified protein was submitted to chemical and enzymatical cleavage to identify the cardiolipin interactin domain. Trypsin, chymotrypsin and thrombin had only small effects on the protein even after prolonged incubation. By contrast, endoproteinase Lys c fragmented the enzyme in a large number of peptides. Four to five fragments were produced by CNBr cleavage.

Further characterization of the cleavage sites of insulin by insulin protease

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We have previously characterized a major product of the degradation of insulin by insulin protease. We now report further characterization of other cleavage sites produced by this enzyme on insulin. We have used ($Gly^{A1-3}H$) insulin and ($Phe^{B1-3}H$) insulin as tracers. The analysis consisted of paper electrophoresis and reversed-phase h.p.l.c. with and without prior oxidation in performic acid. The cleavage sites observed are between A13 and A14, A14 and A15, B9 and B10, B10 and B11, B13 and B14. The physiological significance of these results is diminued.

Myo-inositol containing lipids in *Trypanosoma brucei*

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Procyclic and blood stream forms of the parasite were grown in the presence of (2-^{3}H)*myo*-inositol in semi-synthetic media. Extracted lipids were separated by thin layer chromatography on silicagel impregnated with K-oxalate. In both forms, two main radioactive peaks were found. The slowest has the ame migration (Rf 0.4) as compared with that of phosphatidyl inositol from bovine brain. The fastet (Rf 0.64) does not correspond to known inositol-containing lipid. Deacylation products from both peaks showed similar migration upon paper electrophoresis corresponding to myo-inositol monophosphate. From these results, the latter lipid could be phosphatidyl inositol in which the cyclitol ring is further acylated. A small fraction of the first peak cannot be deacylated and the possible presence of sphingolipid or polyprenoid containing inositol is suspected. Minor low migrating peaks, different according to the parasitic form, are under investigation.

Refolding of porin, a membrane protein consisting of β-pleated sheets, from a random coil configuration

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In which conditions and by which mechanism can a charged and polar polypeptide be properly folded in presence of lipids, yielding a funcitonal membrane protein with anti-parallel β-pleated sheets? We report here the in vitro refolding of *E. coli* B^E outer membrane porin. The native protein (a trimer forming voltage-dependant channels in planar bilayers, resistant to SDS and proteases, 65% β-structure), was denatured in 6M Guanidinium-Cl. After removal of the chaotropic agent by dialysis, the precipitate was resuspended in 1% SDS. The denatured protein (monomer, α-helical structure, protease-sensitive), was added to a lipid solution in 2% cholate. After dialysis, most of the protein (up to 80%) was refolded as judged by structural and functional characteristics of native porin. If instead of SDS, neutral or zwitterionic detergents were used, refolding was $< 20\%$. With cationic detergents, none could be detected at all. This in vitro system should provide further insight into the mechanism of membrane protein insertion.

BH_4 biosynthesis: ^{1}H-NMR evidence for the pyruvoyl tetrahydropterin synthase catalyzed formation of 6-pyruvoyl tetrahydropterin from dihydroneopterin triphosphate

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The conversion of dihydroneopterin triphosphate (NH_2TP) was studied in the presence of Mg^{++}, DTE and purified pyruvoyl tetrahydropterin synthase by follwing the reaction with ^{1}H-NMR.

In this way we obtained direct experimental evidence for the chemical structure of the product which has been deduced to be that of 6-pyruvoyl tetrahydropterin. During the catalysis a new signal in the NMR spectra appeared at ~ 2.4 ppm, which is compatible with the presence of a carbonyl group at the α-position of the biopterin side chain. Similarly, the disappearance of the signals corresponding to the protons of NH_2TP (4.2–4.4 ppm), i.e., their shift to ~ 3.2–3.5 ppm, is compatible with the reduction of the imine, that is, with the conversion of a dihydro- to a tetrahydropterin species. Our results confirm the proposed structure of the first intermediate in BH_4 biosynthesis.

Relationship study of reversed micelles, normal micelles, vesicles and a hydrocarbon gel with the help of electron spin resonance

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The well-known reversed micellar system formed by AOT (bis(2-ethyl-hexyl) sulfosuccinate sodium salt), isooctane and water was investigated with electron spin resonance (ESR or EPR) using different spin-probes. The choice of the probe was such that the spin resides in different regions of the reversed micelles: 1) tempocholin in water; 2) -doxyl-stearic acid ($5 \leqslant X \leqslant 9$) in the surfactant layer; 3) 10- and 16-doxyl-stearic acid in the organic solvent. We investigated the microenvironment of the probes as a function of the water content of the reversed micelles and of the temperature. The obtained data (hyperfine splitting constant, order parameter, correlation times) were compared with other well-known lipid dispersions such as egg phosphatidyl choline multilayer liposomes and lysophosphatidyl choline micelles in water. Finally we investigated a hydrocarbon gel that was obtained by solubilizing gelatine in the water-pools of the AOT reversed micelles.

[^{3}H]PTPC, a photoactivatible carbene-generating phospholipid, labels influenza virus hemagglutinin in a pH-dependent manner

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Fusion of influenza virus with cells or with model membranes (liposomes) is mediated by the viral spike protein hemagglutinin (HA). Upon acidificaiton, this protein undergoes a conformational transition, exposing (a) fusion-active peptide-segment(s) which then insert(s) into the target bilayer.

When the water-soluble ectodomain (BHA) of HA was incubated with liposomes which had been prepared from a mixture of egg-lecithin and [^{3}H]1-palmitoyl-2-[11-[4-[(trifluoromethyl)-diazirinyl]phenyl]-undecanoyl]-sn-glycero-3-phosphocholine ([^{3}H]PTPC), photoactivation of the reagent then resulted in covalent binding of radioactivity to the protein. This labeling was found to be strongly pH dependent. At pH 5 labeling was more than 10 times stronger than at pH 7. Essentially all of the label was incorporated in one of the BHA subunits (BHA2) demonstrating that this subunit unteracts with the bilayer.

Tetrahydrobiopterin-producing enzyme activities in liver of animals and man

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In order to find animal species with high activities of the enzymes involved in the biosynthesis of tetrahydrobiopterin (BH_4), GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR) were measured in liver of man, monkey, pig, dog, rat, rabbit, sheep, cattle, horse, chicken, and trout. GTPCH activities were highest in trout, high in rat, chicken and man, and lowest in sheep. Trout also exhibited the highest activity of PTPS, whereas the lowest was found in man. SR activity was highest in monkey, man and rat, and lowest in trout. The corresponding enzyme activities varied up to 250 times (PTPS) between the different species. It is also noteworthy that the activity of the subsequent enzyme in the biochemical pathway was always higher than the preceeding one. The ratio of PTPS/GTPCH activities varied between 11 and 150, except for monkey and man, where it was lower. This observation explains that neopterin occurs in man and some monkeys, but practically not in other animals.

Multiple mitochondrial creatine kinases (Mi-CK) arise by a genetic mechanism

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Creatine kinase plays a key role in the energy metabolism of vertebrate tissues with high energy requirement. In these tissues a mitochondrial form (Mi-CK) is coexpressed with one of the cytosolic forms (M- or B-CK) and the different isoenzymes are involved in a phosphocreatine-shuttle. Mitochondrial preparations from heart, skeletal muscle and brain differ in their specific Mi-CK activity. Mi-CK from brain and muscle were different in electrophoretic mobility on SDS-PAGE and 2-D immunoblot analysis showed the presence of a distinct Mi-CK species in brain. While polyclonal antibody against heart Mi-CK reacted with Mi-CK of heart, muscle and brain, only 2 out of 22 mAb reacted with the brain isoprotein. Northern blots and S1-protection experiments provided evidence for the existence of multiple Mi-CK mRNAs. These results suggest that Mi-CK isoproteins arise by differential splicing events or multiple Mi-CK genes subject to tissue specific control.

An interlocking, single-strand model of fibrin polymerization based upon an electron microscopic investigation

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Electron microscopic examination of the fibrin polymerization process revealed that during the early stages oligomers are present as loose structures. Within these, fibrin monomer units are probably present in the des A-form i.e. with only one fibrinopeptide A cleaved off by thrombin. During completion of activation (to the des AA-form), molecular alignment into single-stranded chains was observed to occur. During this process, the fibrin monomer units were interpreted to interact by an interlocking mechanism, whereby α, γ-linked trinodular units with a periodicity of 230 Å were formed. This new model for fibrin polymer formation is characterized by a linear mass density identical to that for the old, double-stranded fibrin model. Moreover, it provides a new basis for explaining the early branch formation (according to the Flory-Stockmayer-theory of polymerization) and lateral association phenomena, occurring independently of fibrinopeptide B removal.

The effect of phosphate on the kinetics of the nonenzymatic glycosylation (glycation) reaction

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Nonenzymatic glycosylation seems to play a role in the development of the late complications of diabetes mellitus. It involves the reaction between an unprotonated amino group with an open-ringed glucose to form a labile aldimine or Schiff's base which slowly rearranges to a stable ketoamine by way of the Amadori rearrangement. We incubated albumin, RNase A and model peptides (10 mg/ml) with glucose (1 M,pH 7.4, 37 C) in the presence and absence of phosphate (0.1 M). The rates of glycation were increased about twofold in phosphate as compared to Tris or ethylmorpholine. Browning in albumin, and browning and crosslinking in RNase A were also enhanced by phosphate. The magnitude of this enhancement was similar to the rate increase in phosphate, suggesting that this was due to increased glycation rather than a specific effect on these reactions. Phosphate therefore seems to enhance glycation by catalyzing the Amadori rearrangement, similar to the acid-base catalysis of positively charged amino acid side chains, which cause 'site-specificity' of glycation in hemoglobin, albumin and RNase A.

X-ray crystallographic studies of phosphoserine aminotransferase from *E. coli*

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The enzyme is an α_2 dimer with 362 A.A. residues of known sequence per chain. It catalyses the reaction L-glutamate +3-phosphohydroxypyruvate $\rightleftharpoons$ 2-oxoglutarate+phophoserine. Comparison of the amino acid sequence with that of mitochondrial aspartate amino-transferase (AAT), taking into account the known spatial structure of the latter, suggests a distant evolutionary relationship, although the sequence identity is only $\sim 17\%$.

PSAT has been crystallized in space group $P2_12_12_1$, with a = 68.1 Å, b = 94.7 Å and c = 133.6Å. A crystallographic structure determination is underway. Reflections have been measured to 2.8 Å from native crystals and to low resolution from four heavy-atom derivatives. Attempts to find the heavy-atom positions are underway. A low resolution electron density map should reveal the relationship with AAT.

Purification and characterization of cytochrom P-450 from *Saccharomyces cerevisiae* (var. *uvarum*)

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Cytochrom P-450 is the terminal oxidase of a widely distributed monooxygenase. Due to its broad substrate specificity which is based on the occurrence of many isozymes, possibilities for practical applications have been discussed and evaluations have been initiated.

We investigated the system of *Saccharomyces cerevisiae* (var. *uvarum*). Purification to electrophoretic homogeneity was effected by a three-step liquid chromatography procedure. The properties of the protein were closely related to that of other yeast cytochromes P-450. Polyclonal antibodies raised against the protein exhibited the same immunological specificity as those from another *S. cerevisiae* strain.

The microsomal monooxygenase also catalyzed the 14 α-demethylation of lanosterol, the same reaction that was found characteristic for this system in other yeasts. Our results suggest that this cytochrome P-450 system is identical in different species of the genus *Saccharomyces*. The research performed forms the basis for the investigation of the regulatory mechanism governing the expression of the system in yeasts.

Enantiomeric error frequency of aspartate aminotransferase

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Enzyme-catalyzed transamination cycling of L-glutamate and 2-oxoglutarate in conjunction with derivatization of D- and L-glutamate to diastereoisomers and their separation by high performance liquid chromatography has been used to determine the enantiomeric specificity of aspartate aminotransferase (mitochondrial isoenzyme from chicken). The analytical protocol allows the detection of 0.5–1% D-glutamate in a mixture with L-glutamate. Prolonged transamination cycling in 50 mM HEPES, pH 7.5, at 25 °C resulted in a time-dependent conversion of L-glutamate to its D-isomer corresponding to an enantiomeric error frequency of 5×10^{-8}. If the enzyme was first converted to the pyridoxamine 5'-P form and 2-oxoglutarate

was omitted from the reaction mixture, the racemization did not occur. The racemizing activity of free pyridoxal 5'-P was 50 times lower. The enantiomeric error frequency provides a novel parameter characterizing the catalytic mechanism of aspartate aminotransferase.

The stereospecific labilization of the C-4' pro-S hydrogen of pyridoxamine 5'-phosphate is abolished in (Lys 258→Ala) aspartate aminotransferase

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Reconstitution of wild-type apoaspartate aminotransferase from *E. coli* with [C-4'-^{3}H] pyridoxamine 5'-P ($t_{½} < 1$ min) results in instantaneous stereospecific release of the pro-S C-4' ^{3}H into the solvent. The release follows first order kinetics ($t_{½} = 15$ min at pH 7.5 and 25 °C). Substituting alanine for the active site residue Lys 258 by site-directed mutagenesis yields a catalytically inactive enzyme (BBRC 132, 915, 1985). The mutant enzyme failed to release any measurable ^{3}H from bound [C-4'-^{3}H]pyridoxamine 5'-P. The data not only corroborate the conclusion that ^{3}H exchange from enzyme-bound pyridoxamine 5'-P in the absence of substrate mechanistically corresponds to the deprotonation at C-4' of the ketimine intermediate during the transamination reaction (JBC *261*, 7106, 1986), but also support earlier propositions that Lys 258 is indispensable for the ketimine/aldimine tautomerization.

The effect of free fatty acids on the proton pumping cytochrome c oxidase reconstituted into liposomes

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The effect of free fatty acids (FFA) has been studied in a model system consisting of cytochrome c oxidase reconstituted in phospholipid vesicles. Neither the proton pump of the redox complex nor the proton permeability of the vesicles resulted to be affected by concentrations of palmitic acid known to uncouple oxidative phosphorylation. Also the permeability for potassium ions was not affected by palmitic acid. A constant diminution of the respiratory control index (RCI) was however observed in the presence of the fatty acid due to an increased respiration rate in the absence of uncoupler and/or valinomycin. To investigate the binding site of FFA the photoactivable 12-[N-(4-azido-2-nitrophenyl)] aminododecanoic acid was synthesized. Its effect on the RCI was similar to the other FFA tested.

DDT degradation by the cytochrome P-450 model system hemin+cysteine and an artificial 24-residue DDT-binding peptide

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The highly stable insecticide DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichlorethane) was shown to be degraded rapidly by the cytochrome P-450 model system hemin+cysteine. Degradation at pH 7.7 was $\sim 7 \times 10^4$ times faster than in the absence of the model system. The major products could be separated by HPLC and their structures elucidated by GC/MS and NMR. They are listed in the order of increasing polarity: DDD (2,2-bis(p-chlorophenyl)-1,1-dichloroethane), DDA (2,2-bis(p-chlorophenyl) acetic acid), the 2-hydroxyl derivative of DDA, a DDE-cysteine conjugate (DDE, 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene) and a DDA-cysteine conjugate. At pH 5.2, DDT degradation was slower yielding mainly DDD and the DDE- and DDA-cysteine conjugates.

In the presence of a designed 24-residue DDT-binding peptide[1] the rate of DDT degradation by the hemin-cysteine cytochrome P-450 model system was increased indicating that the peptide-hemin-cysteine mixture might represent an early stage in the evolution of an enzyme.

[1] R. Moser, R. M. Thomas, B. Gutte, FEBS Lett. *157* (1983) 247.

Synthesis of peptide derivatives with penicillin acylase in reverse micelles and in aqueous media

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Penicillin Acylase (EC 3.5.1.11) has been successfully applied in a reverse micelle system as catalyst for the synthesis of the water-insoluble N-phenyl acetyl tyrosin ethyl ester from the water soluble educts tyrosin ethyl ester and phenyl acetic acid. Using cetyltrimethylammonium bromide (CTAB) as surfactant in Chloroform/Isooctane (1:1 v/v) at room temperature with $w = {}_o13$–15 and a pH of 5.5–6.5 a yield of 80 % was obtained. The product can be separated from the educts by using a flat membrane reactor.

In an aqueous medium (pH 6.0) the enzyme has also been used for the synthesis of N-phenyl acetyl derivatives of different di- and tripeptides of the type Gly-X (X = Gly, Leu, Tyr), Ala-Tyr or Ser-Tyr and Gly-Leu-Tyr or Gly-Tyr-Gly. The yields ranging from 10 to 30%. In the case of Gly-Leu the yield could be increased up to 70% using water – 1,4-butanediol (1:1 v/v) mixtures.

The synthesis of fluorescent dichlorotriazinylamino-fluorescein-concanavalin A and its use as a glycoprotein stain on sodium dodecylsulphate polyacrylamide gels

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Concanavalin A (con A), a specific glycoprotein probe, has been optimally labeled to a maximum stoichiometry of 0.4 mol DTAF/mol con A monomer under mild reaction conditions (pH 8.0, 6 h) and under these conditions the DTAF-con A preparation retains its carbohydrate binding ability and is able to penetrate 7.5–15% gradient SDS-polyacrylamide gels. DTAF-con A yields fluorescent bands for the glycoproteins transferrin, fetuin, and deoxyribonuclease and shows no fluorescent response for the non-glycoproteins BSA and soy bean trypsin inhibitor. The detection limit of sensitivity for DTAF-con A, which is similar to that of FITC-con A, is in the range of 5–25 µg of glycoprotein. DTAF-con A is a suitable probe for the detection of glycoproteins in higher percentage ($\geq 10\%$) SDS-polyacrylamide gels and will likely have other applications in, for example fluorescent energy transfer and other structure-function studies.

Interaction of δ-endotoxin of *Bacillus thuringiensis* with the midgut brush border membrane vesicles of *Pieris brassicae*

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The δ-endotoxin from *Bacillus thuringiensis* is a bacterial toxin specific for lepidopteran larvae. The protein is synthesized as a 130 kD protein and later processed in the larval gut to the 68 kD active toxin. We have investigated the action of the active toxin on lepidopteran midgut brush border membrane vesicles from *Pieris brassicae*. These vesicles exhibit properties similar to those

of brush border membrane vesicles from mammalian tissue. The addition of the toxin caused a 50% reduction of the ATPase activty. The inhibition of the ATPase by the toxin was diminished in the presence of NaCl. At a NaCl concentration of 50 mM the toxin action was completely abolished. The lectins concanavalin A and *Lens culinaris* lectin prevented the ATPase inhibition by the δ-endotoxin in a concentration dependent manner. The ultrastructure of the brush border membrane vesicles was also investigated. The intramembrane particles seen in freeze-fracture electron microscopy exhibited no significant changes in the presence or absence of the toxin, although severe damages are observed in lepidopteran midguts after ingestion of the toxin by living insects. We conclude that the postulated destruction of the potassium gradient in the gut system caused by the toxin is mediated by toxin-ATPase interaction, possibly by inhibition of a K^+-ATPase.

Uptake of macromolecules into electroporated cells under optimized conditions for protein synthesis

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The goal of this investigation is to study the presumed regulatory interaction of highly purified, microinjected Semliki Forest virus core(C)-protein with the protein synthesizing machinery of the intact host cell. To clarify optimal conditions for the uptake of macromolecules, the loading efficiency of FITC-dextrane (Mr 42 KD) and C-protein (Mr 33KD) into P3X63Ag8 suspension cells was determined. Both FITC-dextrane and C-protein molecules were efficiently incorporated into electroporated cells. Control cells, electroporated in the absence of added C-protein, were perfectly viable. However, after diffusion loading protein synthesis was shut off immediately. Overall protein synthesis recovered slowly to resume full activity only after about 4 h of incubation at 37 °C. We have determined optimal conditions for diffusion loading and resealing leading to a full recovery of protein synthesis within a few minutes after poreclosure.

Multiple low affinity carbohydrate-carbohydrate interactions as the basis for cell aggregation in marine sponges

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Reaggregation of dissociated cells from the marine sponge *Microciona prolifera* is mediated by a large proteoglycan-like aggregation factor (MAF) via two functional domains, a Ca^{2+} independent highly polyvalent cell binding site and a Ca^{2+} dependent self-interaction site. Isolation of monoclonal antibodies specifically blocking self-association of MAF enabled the localisation of the active site in a highly repetitive epitope of carbohydrate nature. The isolated glycans did not, however, display measurable self interaction activity in such a monomeric form. Cross-linking of these purified protein free glycans into the polymers of MAF size led to the reconstitution of the selfassociation activity. This indicated that the self-interaction of MAF molecules needed for cell aggregation is based on the multiple low affinity carbohydrate-carbohydrate interactions differing from high affinity associations of adhesion molecules characterized so far.

Press-stud protein conjugates

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Many problems in protein chemistry (e.g. the use of antibodies in cancer diagnosis and therapy) call for the covalent conjugation of a protein to another molecule. Such conjugations can be between one protein and another or between a protein and a non-protein molecule. In most cases there are likely to be advantages to limiting the number of attachment sites on the protein, and it is best to make sure that the linking groups on one of the partners in the coupling should be different to that on the other. Neither reactive group chosen should be able to react with itself, but only with its complementary partner. In this way, heterodimers can be formed without risk of contamination by homodimers. This is the protein-chemical equivalent of the mechanical principle of the press-stud.

Some examples of the above principle are already known in the literature. Also, some older protein-chemical techniques can now be combined with newer, enzymic ones and a number of novel examples will be discussed.

Clq and creatine phosphokinase share the same receptor on the inner mitochondrial membrane

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The inner mitochondrial (mt) membrane activates Cl (Peitsch et al., J. Immunol., in press) and contains the receptor for mt creatine phosphokinase (mtCPK) (Müller et al., J. biol. Chem. *260*, 3839). The latter also reported that mtCPK binds to cardiolipin (CL) and we showed that CL is an antibody-independent activator of Cl (Kovacsovics et al., J. Immunol., in press). To test if Clq and mtCPK bind to the same receptor, we performed competition experiments with rat heart MT deprived of their outer membrane (mitoplast; MP). 50% of the mtCPK is released with the addition of 120 µg Clq per mg MP. Furthermore, 50% of MP-bound Clq is displaced by 30 µg mtCPK or by 47 µg adriamycin per mg MP. Adriamycin has an effect similar to that described by Müller et al. in the mtCPK-MT interaction. These data strongly suggest that CL is a receptor for both Clq and mtCPK on inner mt membranes. CL of damaged MT from ischemic heart myocytes may therefore be involved in the complement consumption observed during myocardial infarction.

The semisynthesis of cytochrome c analogues with substitutions at residues 38 and 39

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Arginine-38 of horse cytochrome c is a residue that has remained invariant throughout the evolution of the eukaryotic protein, whilst lysine-39 is conserved throughout the Animal Kingdom. An important structural role is thus implied for both residues. In the case of Arg-38, specific chemical modification of the residue has confirmed this supposition.

We recently reported that a semisynthetic two-fragment complex having the native sequence, 1–37:38–104, has functional properties little different from the parent protein. This makes possible the preparation for structure-function studies of analogues modified in the region of the cleavage point.

The changes we observe in redox potential and biological activity between normal sequence and analogues support our proposals for the functional roles of these residues in cytochrome c.

Isolation of B-creatine kinase (B-CK) subspecies from chicken heart

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B-CK, known to migrate as two distinct spots on 2D-gels, was purified from adult chicken heart. The 50–65% ammonium sulfate cut of the initial heart extract was eluted from Blue Sepha-

rose by 5 mM ADP and applied to an FPLC Mono Q anion exchanger column. Native B-CK was eluted by a salt gradient plateau at 150 mM NaCl as two distinct protein peaks, designated type I (basic) and type II (acidic) B-CK. The molar ratio of the two protein subspecies, both shown to be pure by silver staining of SDS-gels, was 1:4. Type B-CK migrated as a single spot on 2D-gels, which corresponds to the more basic spot of the B-CK doublet, whereas type II B-CK still contained both spots. The acidic subspecies showed an elevated specific activity, indicating a possible regulatory mechanism by post-translational modification. Thus, it is possible by FPLC to isolate the basic subspecies of B-CK in pure form and to study post-translational modification of B-CK that may be relevant to physiological function.

Preparation of a nicotinic acetylcholine receptor lacking the 'ion channel amphipathic helices'

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The nicotinic acetylcholine receptor (nAChR) has a pentameric structure, consisting of two copies of the αchain and one copy of the β, γ, and δchains. The amino acid sequences of all chains from *Torpedo californica* have been determined and models for the insertion of the chains in the membrane have been proposed. In one of these, amphipathic helices from all chains constitute the ion channel.

By trypsinisation of membranes followed by affinity chromatography of solubilized nAChR on cobratoxin-Sepharose, we have produced preparations of nAChR lacking the 'amphipathic helices' of at least three of the four chains, as demonstrated by loss of reactivity with polyclonal and monoclonal antibodies to these and neighboring regions.

This material should be useful for studying the role of these regions by reconstitution and ion -flux experiments.

Properties of apical bile salt binding proteins in liver, intestine and kidney epithelial cells

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Previous studies have indicated that in rat liver a 100 kDa glycoprotein is involved in the canalicular excretion of bile salts. This protein has been isolated and monospecific antibodies raised against it (Hepatology *5* (1985) 958). In this study the antibodies were used to screen for the presence of similar proteins in other epithelial cells. Positive indirect immunofluorescence was found at the apical, but not basolateral domain of intestinal and kidney epithelial cells. The immunopositive proteins exhibited MWs of 100000 in ileal and renal membranes, and of 95000 in the duodenum and jejunum. These proteins selectively bound radioactively labeled bile salts, and their chemical deglycosylation resulted in a uniform decrease of the apparent MWs to 48000. These results indicate the occurrence of closely related bile salt transport systems in apical membranes of liver, intestine and kidney epithelial cells.

Crosslinking of C-phycocyanin with dimethyl-pimelimidate: localization of an intersubunit and an intrasubunit crosslink

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The light-harvesting pigment-protein C-phycocyanin (CPC) of the cyanobacterium *Mastigocladus laminosus* consists of an α- and a β-subunit with 162 and 172 residues, respectively. These two subunits form an α, β-monomer, three of which build up a disc-shaped trimer. This trimer was crosslinked with the homobifunktional reagent dimethyl-pimelimidate having a max. crosslinking distance of 10 Å. Two crosslinks could be identified: an intramonomer intersubunit crosslink with a yield of 48% and an intrasubunit crosslink within α-CPC (57%). These products were chemically and enzymatically fragmented and the small crosslinked peptides were isolated and identified by aminoacid-analysis. The following aminiacids were crosslinked: α-Val 1 with β-Ala 1 and α-Lys 62 with α-Lys 134. The position of the two crosslinks within the known three-dimensional structure of CPC will be shown.

Interaction of C1q and heparin

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Clq, a subcomponent of the pentamolecular complex of the first component of complement, expresses a natural binding activity towards various macromolecules. Addition of 400 µg/ml heparin to serum (devoid of Clq binding activity) and 80 ng of radiolabeled Clq causes the formation of complexes precipitable with 2.5% PEG. Heparin and the radiolabeled Clq alone do not form these complexes. We have characterized two serum components responsible for the formation of PEG-precipitable complexes: Clq itself and fibronectin (FN). Both macromolecules expressed similar dose-dependent preicipitating activities in their native and reduced/alkylated forms. Partially digested molecules lost their precipitating activity. Thus, for the formation of these complexes, intact secondary structures are not required; however the integrity of the individual polypeptide chains must be maintained. The collagen-like stalk of Clq by itself does not seem to be able to mediate complex formation.

Localization of type VI collagen and the basement membrane components laminin, nidogen and the low density proteoglycan in the mouse cornea

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Ultrathin cryosections of mouse cornea were labelled with affinity purified antibodies against type VI collagen (COL VI), laminin (LA), its P_1-fragment (P_1), nidogen (ND) and the low density proteoglcan (LDPG), and stained with protein-A gold. The P_1 and ND was identified in the lamina densa at the borderline to the lamina rara, where La and LDPG showed an even distribution. The codistribution of P_1 and ND supports the recent finding, that ND binds to the central part of LA (personal communication Mats Paulsson). A surprising result is the relatively large distance of the P_1-domain from the plasma membrane, because it was proposed to be involved in cell binding. According to the known dimensions of LA one position of its E_3-fragment should be the interface of the lamina densa and the corneal stroma. COL VI appears uniformly distributed in the corneal stroma. This correlates with the results, that COL VI is a major compo-

nent of the human cornea. The COL VI-fibrils (beaded filaments) are building an irregular network betwen the collagen-fibers.

Complete amino-acid sequence of the lactate dehydrogenase from the psychrophilic bacterium, *Bacillus psychrosaccharolyticus*

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Lactate dehydrogenase form the psychrophilic bacterium, *B. psychrosaccharolyticus* was isolated by a three-step purification procedure and its amino-acid sequence of 318 residues determined. Most of the primary structure was established by the analysis of peptides obtained upon chemical cleavages with BNPS-skatole and CNBr. Still unidentified parts within the sequence and at the C-terminal end of the protein were determined with shorter peptides generated by further chemical or enzymatical digestions.

The amino-acid sequence of the first psychrophilic lactate dehydrogenase should complement a couple of LDH sequences from mesophilic and thermophilic bacilli. Comparisons between these sequences demonstrated a high homology (more than 60%), particularly at the active site. In relation to the varying thermostability of the native enzymes, structural differences should indicate possible structural principles of psychrophilic and thermophilic LDH.

Evidences for multiple mitochondrial creatine kinase isoforms from different tissues of chicken

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In vertebrate tissues with high energy requirement mitochondrial creatine kinase (Mi-CK) is thought to play an important role as a partner of cytosolic CK isoenzymes in a phosphocreatine-shuttle. The fact that mitochondria from skeletal muscle, heart and brain display different specific Mi-CK activities and that Mi-CK from these tissues also differ slightly in their electrophoretic mobility on SDS gels as well as in their immunological reactivities with anti Mi-CK antibodies indicated the existence of tissue specific mitochondrial CK isoenzymes. Limited proteolysis by V-8 protease and trypsin of Mi-CK from heart and brain (purified by Blue-Sepharose affinity chromatography and FPLC on Mono S resin) resulted in different polypeptide patterns as judged by SDS-PAGE. These results confirm that not only cytosolic CK but also mitochondrial CK exists in different isoforms that are expressed in a tissue specific manner.

Probing of membrane protein surface domains by monoclonal antibodies

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Diazotized arsanilic acid has been chosen as surface label due to the availability of monoclonal anti-*azob*enzene-*ar*sonate antibodies. In conjunction with the surface modification, the hapten directed antibodies provide the specificity required for accurate probing of membrane protein folding. Several anti-azobenzene-resonate monoclonal antibodies have been tested and selected for their specific in interaction with surface-labeled human eryhtrocytes (solid phase-ELISA). Monoclonal antibody binding was inhibited by 10^{-6}–10^{-7} M (I_{50}) synthetic ABA-His (C-2), ABA-His (C-4), ABA-Tyr and the diazotized peptide Glu-Thr-Tyr-Ser-Lys.

Increased surface label accessibility has been found to occur in red cell membranes of HEMPAS patients (Congenital dysenteropoietic anemia type II). HEMPAS membrane glycoproteins have a reduced carbohydrate content. Based on the inherent specificity of the immunological interaction, the procedure provides a finely tuned diagnostic test for membrane structural anomalies.

Lateral diffusion of antibodies bound to supported phospholipid bilayers

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Planar phospholipid bilayers supported on silicon wafers have been used as a model membrane system to study the binding, lateral mobility and phase behavior of monoclonal antibodies directed against lipid haptens. The binding of fluoresceinated IgG to planar and vesicular bilayer membranes depends critically on the mole fraction of available lipid hapten. Lateral diffusion coefficients have been measured by pattern photobleaching as a function of surface concentration. At low lipid hapten and/or antibody concentrations antibody diffusion is fast (approx. 2.5×10^{-8} cm^2/s) and only 1.5–2 times slower than lipid diffusion. At higher concentrations patches of immobile antibodies coexist with mobile antibodies, and at still higher concentrations all bound antibodies are immobile, perhaps forming a two-dimensional membrane-bound protein crystal.

Lipid absorption in brush border membrane

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When brush border membrane vesicles prepared from rabbit small intestine are either incubated at 4 °C (pH 7.1) or digested with papain, proteins are released into the supernatant. These proteins turn out to be responsible for the incorporation of lipid (phosphatidylcholine, cholesterylester) into brush border membrane. The proteins catalyze the exchange of phosphatidylcholine between small unilamellar lipid vesicles as well as micelles and brush border vesicles and also between different populations of unilamellar lipid vesicles. Evidence is presented to show that the activity in the 100 000 × g supernatant is associated with proteins of M_r = 12 500 and 25 000: 1) Boiling the supernatant led to complete loss of the exchange activity. 2) Treatment of the supernatant with trypsin, proteinase K or well-known protein inhibitiors reduced the activity significantly. 3) The exchange activity (not dialyzable) followed Michaelis-Menten kinetics and exhibited a pH-optimum between 7 and 7.5. The proteins liberated from brush border membrane were partly purified by gel filtration on Sephadex G75.

Studies on proteolytic fragments of the thioredoxin from *E. coli*

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The thioredoxin (TRX) from *E. coli* can be cleaved at the single arginine residue (R73) by the action of clostripain in the presence of acetonitrile or by trypsin (following the chemical modification of lysine residues) to yield the two large fragments (TRX(1–73) and TRX(74–108). The protein can also be cleaved by carboxypeptidase Y in the presence of 0.5% SDS producing TRX(1–101) or by carboxypeptidase A in 1% SDS (at pH 8.5) giving TRX(1–106). We are currently characterizing TRX(1-73) and TRX(1–101) using physicochemical and biochemical methods. Neither fragment has enzymatic activity in a standard assay for

thioredoxin but while TRX(1-73) adopts a random structure at neutral pH (as judged by circular dichroism (CD)), TRX (1–101) has a CD spectrum identical to that of the intact protein. However, when side-chain carboxylate groups of TRX(1–73) are altered to the corresponding N-methyl amide by suitable chemical methods, the fragment appears to adopt a well defined and stable structure with a CD spectrum similar to that of the intact protein.

Cytochrome c catalyses its own resynthesis from trypsin-activated fragments

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Fragments of cytochrome c have a tendency to form complexes that mimic native structure and function. In the complex of CNBr fragments (1–65) and (66–104), the peptide bond between the two is spontaneously reformed. This reactivity is due to the weakly activated C-terminal (homoserine lactone), and conformational assistance from the structure, in which the reacting species are proximate. We have sought ways to induce this process elsewhere in the protein. By choosing the sites of endopeptidase cleavage, we can use the enzyme to activate the C-terminal of fragments. For the tryptic cleavage site, Arg-38, mild activation is not very efficient, but use of amino acid dichlorophenyl esters leads to rapid coupling. We have thus made structural analogues, and excised the bottom Ω-loop from cytochrome c. Despite the need for a moderately strong activating group in these cases, it is clear that the stucture of the complex does efficiently catalyse the reaction, since in non-complexing conditions reaction is much less efficient.

Bacteriorhodopsin, renatured from its V8 fragments, is active in proton translocation

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The staphylococcal protease V-8 cleaves the bacteriorhodopsin molecule between Glu 166 and Val 167, producing two fragments V-1 and V-2. The fragments contain the five N-terminal and the two C-terminal helices respectively. When separated by chromatography in either protic or non-protic organic solvents the two denatured fragments can be renatured and reassociated in lipid/detergent micelles in the presence of retinal. Non-protic solvents and the presence of ion-pairing counterions favor the regeneration of the native bacteriorhodopsin structure. The resulting ternary complex recovers the absorption spectrum and secondary structures of intact bacteriorhodopsin. Upon detergent removal, vesicles form and light-driven proton pumping can be demonstrated.

Glucose permease of *E. coli*: function of cysteines in catalysis, regulation, and structure stabilization

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The glucose permease of *Escherichia coli* mediates sugar transport concomitant with sugar phosphorylation. The purified protein exists as a dimer of two identical subuntis. Upon gentle oxidation the two subunits become crosslinked by one or several disulfide brudges. To investigate the function of the sulfhydryl residues in catalysis, regulation and structure stabilization of the permeasé, cysteines have been replaced, one at a time, by serine. The effects of these alterations on autocatalytic protein phosphorylation, sugar phosphorylation and transport, as well as the possible role of disulfides in regulation of enzyme activity will be discussed.

Cell and Molecular Biology

Binding of RNA to the La Crosse virus nucleocapsid protein

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La Crosse (LAC) virus contains as its genome three segments of negative-sence ss-RNA, each of which is tightly associated with the viral nucleocapsid (N) protein to form a helical nucleocapsid. Within an infected cell the complements of the genome segments, the anti-genomes, are also found in nucleocapsid structures. Several lines of evidence suggest that sequences at the 5' terminus of the RNA are required for its association with N protein. In order to examine the details of the association of RNA species with viral N protein in vitro, we are constructing a series of plasmids to be usd for the production of defined transcripts. The plasmid pPM1 is a cloning vector designed by P. Ahlquist (U. Wisconsin) such that sequences inserted into a unique Sma I site are transcribed under the control of the lambda Pr promoter using *E. coli* RNA polymerase. The vector is designed such that the first nucleotide of the transcript is the first base of the inserted sequence. A number of constructs are being produced which contain all or part of the La Crosse S virion RNA terminal sequence. We are examining the ability of these sequences to direct the formation of stable complexes between RNA transcripts and LAC N protein in an in vitro system.

Two mRNA species code for the catalytic subunit of cAMP-dependent protein kinase from LLC-PK_1 cells

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In order to gain an understanding of the role of the cAMP-dependent protein kinase in the regulation of cell differentiation and gene expression, we have undertaken the molecular cloning of the subunits of this enzyme from LLC-PK_1 cells. Aλ-gt-11 cDNA library was screened using a 1.5 kb EcoRl fragment from a bovine cDNA for the C subunit (Fed. Proc. *44* (3) 1704, 1985). Two independent cDNAs were identified on the basis of partial restriction map analysis and sequence data. These two cDNAs apparently represent two forms of C subunit proteins designated Cα and Cβ, respectively. The nucleotide sequence analysis of these cDNAs showed heterogeneity in the coding region and degeneracy in 3' untranslated region. However, the deducted amino acid sequence for both forms of the C subunit showed 93% homology. Using Cα and Cβ specific probes, RNA blot analysis showed a major mRNA species of 2.75 kb with the Cα probe, while the Cβ probe detected two mRNA species of 5.0 kb and 3.8 kb. This data was supported by genomic blot analysis which showed distinct hybridization patterns. These results suggest that two distinct genes code for the C subunits and are apparently expressed in LLC-PK_1 cells. The functional significance of Cα and Cβ is currently being investigated.

Identification of smooth muscle cells in cultured sheep myometrial cells

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Myometrial cells were prepared by a combined tryptic/collagenase treatment of myometrial layers. Uteri of young animals (slaughterhouse material) have been employed.

Fibroblasts were separated by 1 h preplating in Hams F 10-medium (10% calf serum). The non-attached cells were transferred

in a new culture dish and cultured for 3–14 days. The proteinic component of the intermediate filaments, the desmin (Palmberg and Thyberg, Cell Tissue Res. *246* (1986) 253), has been identified immunohistochemically using a monoclonal antibody to desmin (Boehringer) and a rhodamin-conjugated anti-mouse antibody (Cappel Labs, U.S.A.). The technique is suitable to detect smooth muscle cells in the monolayer cultures.

Exposure of cultured human monocytes to MTP-PE (muramyl-tripeptide-phosphatidylethanolamine) is required to maintain an optimal H_2O_2 response

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Human monocytes isolated by counterflow elutriation (> 90% pure), release large amounts of H_2O_2 upon stimulation by phrobol-myristate acetate (PMA) Monocytes cultured in RPMI 1640 20% human serum lose their ability to release H_2O_2 over the course of 3 days. When IFN_γ is present in the cultures from day 0 or is added at day 3, the H_2O_2 response to PMA is restored to maximal levels. Here we report a similar effect when a synthetic drug, MTP-PE, is used.

Drug added	H_2O_2 released nmoles/10^6 cells Test day 0	Test day 3	Test day 6
None	4.8	1	0
IFN γ added day 0	4.8	7.6	8.8
IFN γ added day 3		8	8.4
MTP-PE added day 0	4.8	5.2	8
MTP-PE added day 3		7.2	4.4

This result supports the fact that MTP-PE enhances the effector activity of monocyte-derived human macrophages, thus being important for clinical use.

Inhibition of protein synthesis in LLC-PK_1 cells increases calcitonin-induced plasminogen activator gene transcription and messenger RNA stability

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The peptide hormone calcitonin induces the accumulation of urokinasetype plasminogen activator (uPA) mRNA in pig kidney cells, LLC-PK1. Inhibition of protein synthesis led to two superinductive effects: an increase in calcitonin induced uPA mRNA accumulation over time, and a shift in the dose response curve so that lower calcitonin doses became more potent. To explain these effects, we demonstrated that inhibition of protein synthesis increased both calcitonin-induced uPA gene transcription and mRNA stability. The superinductive effects of protein synthesis inhibition could not be mimicked when a tumor promoter was used instead of calcitonin as an inducer. Calcitonin and the tumor promoter exert their effects through different pathways, suggesting a clue to the mechanism of superinduction. To understand why inhibition of protein synthesis extends uPA mRNA stability, we performed cell-free mRNA decay reactions. Preliminary results showed that calcitonin-induced uPA mRNA from cycloheximide-treated cells was more stable than that from cycloheximide-untreated cells. Further progress in understanding the mechanisms of superinduction and the factors involved in uPA mRNA stability is discussed.

Analysis of cDNA clones of cytochrome c in *Chlamydomonas reinhardtii*

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We have taken advantage of a highly conserved region of cytochrome c and of the biased codon usage in nuclear genes of *Chlamydomonas reinhardtii* to design a synthetic oligonucleotide. The latter has been used to screen a *C. reinhardtii* cDNA library constructed in the vector λgt10. A 662 bp fragment has been cloned, which contains the entire cytochrome c coding region. Preliminary southern blotting experiments suggest that the cytochrome c gene is unique. Northern blotting shows that the mRNA levels depend on the growth conditions. The amino acid sequence of *C. reinhardtii* cytochrome c displays peculiar features which could be of interest in studies on the structure-function relationship of the protein. This sequence was used for the construction of phylogenetic trees in which *C. reinhardtii* and *Enteromorpha intestinalis* are separated in two different phyla, in contrast with previous views.

Heterochromatin-like sequences in centromere-linked DNA of *S. pombe*

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We have isolated DNA segments around and including the tightly centromere-linked genes *lysl* and *cyhl* on chromosome I and *tps13* and *ranl* on chromosome II of *S. pombe*. By 'chromosome walking', a region of repeated DNA has been isolated. It occurs at centromere-linked locations on chromosome I, II and III. These repeats analysed in greatest detail on chromosome II, consist of several types of repeated sequences, the most striking of which is 6.4 kb in length (repeat K). Portions of repeat K occur at least twice on chromosome II, and some cosmid genomic clones from chromosome III contain up to three copies of this repeat in a tandem array. DNA sequence analysis within Repeat K has revealed groups of much smaller repeats, the most common of which is the sequence T/CACCAT/C (consensus) which is repeated 25 times in 215 bp. Furthermore, we have found that the ratio of physical to genetic distance in this region is substantially larger than the genomic average of 8 kb/cM.

Tissue specificity and transactivation of transcription of the minute virus of mice (MVM)

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Genomic mapping of the tissue specific determinant. Two strains of MVM have been isolated, one (MVMp) growing in fibroblasts and the other (MVMi) growing in lymphocytes; both strains are able to enter both types of cells. To map the region of the viral genome responsible for tissue specificity, we have constructed recombinant genomes by exchanging restriction fragments between infectious clones from the two viruses, and examined the phenotype of trhe viruses produced by transfection of the resulting clones. This allowed us to map the host range determinant on a 800 bp long fragment located in the capsid protein coding sequence.

Transactivation of a viral promoter by a viral gene product. MVM contains two transcription units, each with its own promoter. By assaying the activity of these promoters in transfected cells, we found that the first one of them is active independently while the other is activated in trans by a product of the first transcription unit. A corresponding result was found for parvovirus H1 (Rhode, J. Virol. *55* (1985) 886).

Direct observation of respiratory syncytial virus by video-microscopy

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Video-enhanced differential interference contrast microscopy was employed for the observation of living cells infected with respiratory syncytial (RS) virus. The morphogenesis of this paramyxoviurs takes place as a process of budding at the plasma membrane of the host cell resulting in the formation of predominantly filamentous forms of enveloped virus particles with a diameter of 10 nm and an average length of 2 µm. Digital processing of TV signals in real time allowed the visualization of the assembly of RS virus filaments, to determine the kinetics of growth (100 nm/sec) and of fusion. Furthermore, the distribution of viral surface antigens was studied by detection of anti-RS virus antibodies in conjunction with collodial gold and by comparison with immunofluorescence.

IS431, a new insertion element from staphylococcal penicillinase plasmid pI524

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We have characterized by DNA sequencing an insertion sequence-like element (IS431) located on the *Staphylococcus aureus* penicillinase plasmid pI524. Two IS431 copies, whose sequences are 99% homologous, flank the mercury resistance determinant. IS431 left is 800 bp long and has a perfect terminal inverted repeat (IR) of 22 bp; IS431 right is 786 bp long and has a terminal IR homologous to the IR of IS431 left except that the terminal 8 bp are absent. Both IR's share a 10 bp homology with the IR of IS26 from *Proteus vulgaris*. No flanking target sequence duplications were detected. An open reading frame (ORF) of 675 bp spans most of the IS431 sequence. Its deduced amino acid sequence shows 40% homology to the 234 aa putative transposase coded by ORFI of IS26. These results show that IS431 and IS26, although from distant organisms, must have a common origin.

An SV40-induced transcription factor activating the late promoter

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We devised a way to isolate transcriptional fctors from SV40 chromosomes. Using hydroxylapative we obtained a protein fraction which stimulated transcription from the SV40 late promoter in vitro. Proteins in the active fraction were shown earlier by a gel-retardation assay to bind to a DNA fragment encompassing the late promoter. The proteins eluted from hydroxylapatite were electrophoresed in SDS-urea polyacrylamide gels, transferred under renaturating conditions to nitrocellulose and probed with radioactive SV40 DNA or bacterial plasmid control DNA. Specific DNA-binding proteins were detected with estimated molecular weights of approximately 70 kD, 88 kD, 112 kD and 130 kD. The proteins are candidates for the late stimulatory factor. We are testing monoclonal antibodies against SV40 chromsomal proteins (with E. Harlow, Cold Spring Harbor) for their effects on in vitro transcription from te SV40 late promoter, as naked DNA and in chromatin.

Gravitational effects on Friend leukemia virus transformed cells (FLC)

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The behavior of FLC was investigated at 10 × g in a centrifuge and at simulated low-g in a clinostat during an incubation time of 6 days. Clone FBU-3b-B2 (derived from FBU-3b cells kindly provided by J. F. Conscience) was selected for this investigation based on its proliferation rate (16 h for one cell division) and its good inducibility by DMSO (1.1% v/v) to produce hemoglobin (HG). Cell number, HG-production, percent of HG-producing cells, and glucose consumption were determined in cultures with and without DMSO. In general, proliferation is enhanced by 30–40% at low-g and depressed by a similar extent at 10 × g. These results are in contrast with our observations made earlier on Con A activated lymphocytes. While glucose consumption correlates well with cell proliferation data, HG-biosynthesis induced by DMSO shows a different g-dependence. The opposite g-effects observed on lymphocytes (Science *225* (1984) 228; Naturwissenschaften *73* (1986) 400) are probably due to an altered binding of Con A to the cell membrane.

The human parvalbumin gene: chromosomal localization and isolation from a chromosome specific library

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The human parvalbumin gene was localized on chromosome 22 pter to q11.2 by the somatic cell hybrid technique combined with gene dosage analysis on two human cell lines containing haploid or triploid copy number of chromosome 22. This segment of chromosome 22 is deleted in patients with DiGeorge immunodeficiency syndrom. From a human chromosome 22 specific library an *Eco*RI DNA fragment was isolated which gave strong hybridization signals with different parts of rat parvalbumin cDNA and genomic sequences including putative promoter regions [Berchtold and Means, PNAS *82* (1985) 1414; Epstein et al., J. biol. Chem. *261* (1986) 5886]. This fragment is 7.5 kb in length which is identical to the size of the strongest signal obtained when total human genomic DNA was digested with *Eco*RI and probed with rat parvalbumin cDNA 9f on a Southern blot. These results prove that the human parvalbumin gene is located on chromosome 22. By comparing 5' nontranscribed sequences of the human and rat parvalbumin gene we hope to define conserved regions which might be important for the developmental and tissue specific regulation of the parvalbumin gene.

The structure of the basement membrane protein laminin is highly conserved in evolution

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Basement membranes (BM) are ubiquitous thin extracellular matrices separating different cell-layers from each other and from the connective tissue. In vertebrate BM a major noncollagenous glycoprotein is laminin which is well characterized for some mammilian tissues. Applying the extraction protocol developed for a mouse tumor laminin on embryonic sea urchin basal lamina preparations we isolated a structural and biochemically related molecule. In rotary shadowing electron micrographs it exhibits the shape of an asymmetric cross consisting of three short arms with 38 ± 3 nm [37 nm] in length and one 110 ± 5 nm [77 nm] long arm (numbers in [] refer to vertebrate laminin). This molecule and vertebrate laminin are both bearing terminal globes at each arm and inner globes within the short

arms. In SDS-PAGE the unreduced sea urchin molecule runs with an apparent $M_r = 10^6$; reduction results in bands of $M_r = 260\,000$ [220 000] and 480 000 [440 000], respectively. Thus the chain composition of both can be assumed as a 1:2 ratio for the heavy to light chains which are disulfid linked. In comparison to vertebrate laminin the increase in length of the long arm can be explained by assuming that the three chains are co-aligned in the long arm in coiled-coil conformation fitting with the ΔM_r of 40 000/chain.

Structure of active chromatin as studied in SV40 and recombinant minichromosomes

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By using psoralen crosslinking we were able to analyze the chromatin structure of individual in vivo replicating and transcribing SV40 minichromosomes at the level of single nucleosomes (Sogo et al. 1986, J.M.B. *189*, 189; De Bernardin et al. 1986, J.M.B. *191*, 469). Interestingly we observed nascent RNA strands on replicative intermediates suggesting that transcription and replication may proceed at the same time on the same SV40 minichromosome. Since under the appropriate ionic strength and pH-conditions the accessiblity of psoralen to the nucleosomal DNA depends on the presence or the absence of histone H1 (Conconi et al. 1984, J.M.B. *178*, 920), we are using this technique to investigate the distribution of histone H1 on the replicating SV40 minichromosomes. Moreover we are studying the chromatin structure of recombinant minichromosomes containing the heat-shock promoter hsp 70 after growth at normal temperature and after heat-shock.

Cloning and sequencing of chicken sulfite oxidase cDNA

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Sulfite oxidase is an enzyme of the mitochondrial intermembrane space. It catalyzes the oxidation of sulfite to sulfate with cytochrome *c* as electron acceptor. The enzyme contains a *b*-type heme and molybdenum as cofactors, located on separate polypeptide domains.

In a primer extension experiment a specifically primed cDNA bank of chicken liver polyA)-mRNA was produced. About 4×10^6 clones were screened with two different oligonucleotide probes (17mer's). Two clones hybridized with both oligonucleotides. They contained sequences that could be related to the already known N-terminal protein sequence (heme domain of the enzyme). These two clones were used to find further cDNA clones in a oligo(dT)-primed lambda phage cDNA library.

Search for precursors of the major membrane protein of *Tetrahymena thermophila*

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Lysates of pulse-labeled (^{35}S-methionine) *T. thermophila* cells were immunoprecipitated with monospecific antibodies directed against the major membrane protein (HAg) of the cells. The kinetics of labeling indicated that considerable amounts of a precursor were synthesyzed, whereas HAg only was detected on the fluorograms.

In parallel, translation of the cell poly(A)$^+$-RNAs was attempted in reticulocyte and in homologous *Tetrahymena* lysates. Although protein synthesis was efficient in both cases, no immunoprecipitable polypeptides were detected. Addition of homologous smooth and rough microsomes did not change the results. As the antibodies so far used were produced against native, mature HAg, we propose that pre-HAg acquires the corresponding epitopes in the course of a post-translational modification (probably not a glycosylation). Work in progress focuses in the use of antibodies with broader specificity.

Effect of monensin, ammoniumchloride and colchicine on the synthesis and processing of β-glucuronidase in hepatocytes

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Three β-glucoronidase forms of the same primary translation product are present in liver of C57Bl/6 mice: a precursor is either processed to an 'acidic' lysosomal (complex type oligosaccharide) or a microsomal form. The microsomal form, formed only when a second microsomal protein 'egasyn' is present, is processed to the 'basic' lysosomal enzyme (non-complex type). Monensin caused the excretion of both lysosomal and also of the microsomal enzyme suggesting that the impairment of modification of the microsomal enzyme resulted in its misdirection; synthesis was reduced by 70%. With ammoniumchloride, 70% of the lysosomal forms and some microsomal enzyme were lost within 24 h; synthesis was only slightly decreased. Colchicine induced a conversion of the microsomal into the 'acidic' lysosomal enzyme and an the excretion of 30% of both lysosomal forms; synthesis was reduced by 80%, due in part to decreased mRNA concentration.

Acetylcholinesterase and acetylcholine receptor genes of *Drosophila*

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We have isolated the genes encoding two components of the cholinergic synapse of *Drosophila melanogaster*.

The *Ace* (acetylcholinesterase) locus maps at 87E on the third chromosome. CDNA clones from the locus were isolated and sequenced. The deduced 649 amino acids sequence contains the active site sequence FGESAG and has extensive homology to the vertebrate sequence (*Torpedo*). The 5' leader is unusually long and contains 6 initiation sites. In yeast, this arrangement has been found to control translation.

A *Drosophila* gene homologous to the vertebrate nicotinic receptor gene has been isolated by cross-hybridization. Its amino acid sequence was deduced from cDNA clones. The gene encodes a protein structurally similar to the α-subunit of vertebrate acetylcholine receptors. It maps at 96A on the third chromosome.

A technique to measure beta-receptor internalization using displacement of CGP-12177 by ^{125}J-cyanopindolol

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We have investigated the effects of low agonist concentrations on the desensitization of beta-adrenergic receptors in S49 mouse lymphoma cells. Earlier studies indicated that the rate of receptor internalization is dependent on the concentration of agonists. For instance, after 10 min no receptor internalization was observed at 10^{-8} M isoproterenol, an agonist concentration that on the other hand sufficed to induce a 50% down-regulation overnight. In the present study we followed the time course of receptor internalization and down-regulation over several hours. To establish more accurately changes in receptor num-

bers we used a new approach to measure internalization by applying the ligands CGP-12177 and ^{125}J-labelled cyanopindolol in combination. This method requires relatively small numbers of cells to measure receptor internalization and is applicable to samples of human blood lymphocytes.

Mismatch correction in mammalian cells: maintenance of 5'-methylcytosine residues in DNA by preferential correction of GT mispairs in favor of G

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We introduced a specific GT mispair into the genome of SV40 and determined the fate of the mismatched bases in African Green Monkey (CV-1) cells. The mispair was incorporated as part of a 12 bp duplex formed by hybridizing two oligonucleotides synthesized in vitro. The duplex was ligated into SV40 between the TaqI and BstXI restriction sites. Sequences were chosen so that correction of the GT mispair to a GC or an AT pair would generate a BamHI or a BclI restriction site, respectively. Transfection of this modified viral DNA into host cells yielded plaques. Restriction analyses of DNA isolated from individual plaques revealed that mismatches were corrected in 133 of 134 cases, yielding DNA with a GC pair in 93% of the cases. Control experiments demonstrated that this GC pair confers no growth advantage to the modified SV40. Selective repair of GT mispairs in favor of G is observed when the mispair is in the potentially methylated configuration CG/GT. Thus, one role of mismatch repair in mammalian cells is to maintain 5'-methylcytosine residues that would otherwise be lost due to spontaneous deamination to form thymidine.

Transcriptional activity of the AAV genome in the absence of helper virus

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The human parvovirus AAV is a single-stranded DNA virus which requires helper adenovirus (Ad) for its propagation. In the absence of Ad no AAV-specific nucleic acid or protein synthesis can be detected, but the AAV genome can stably integrate into the host cell genome. However, experiments with AAV vectors have shown that foreign genes are expressed from AAV promoters even in the absence of Ad. To study the regulation of AAV gene expression we have analysed the transcripts obtained in human 293 or HeLa cells transfected with cloned wildtype or mutant AAV by northern blot analysis. Similarly, we analysed cell lines which had AAV vectors integrated in the cellular DNA. In 293 cells transcription from all three promoters was detected in the absence of Ad, but the relative amounts of the transcripts were different when compared to Ad-infected cells. Also, the fraction of spliced AAV mRNAs was strongly reduced in the absence of Ad. So far, the respective analysis in HeLa cells and in cells having the AAV DNA stably integrated in their genome failed wo show any transcriptional activity.

Tenascin: an extracellular matrix protein involved in skeletal development

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Tenascin is a novel disulfide-linked hexameric glycoprotein with a restricted distribution in selective fetal mesenchymal tissues, adult organs and tumors (Cell 47: 131; 1986). During embryogenesis it was detected in chondrogenic and osteogenic areas of the developing skeleton. It appeared at the time of cell condensation of prechondrocytes before overt chondrogenic or osteogenic differentiation. In vitro a tenascin substrate promoted cartilage differentiation of chick limb bud cultures. Tenascin in the medium induced cell-cell aggregation and inhibited cell-substrate attachment and spreading even on a fibronectin substrate. Since it is known that less adhesive substrates and the rounding up of embryonic limb bud cells induce chondrogenesis, we propose that tenascin allows the cells to detach from the fibronectin-containing extracellular matrix in vivo and promotes their aggregation thereby contributing to the chondrogenic differentiation.

Expression of intermediate filament proteins in human breast tissue derived cell cultures

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The expression of cytokeratins and vimentin was analyzed in primary cultures derived from breast carcinomas and non-malignant breast tissue by immunohistochemical methods. The epithelial nature of the outgrowing cells could be demonstrated by staining for several types of cytokeratins. Three days after plating all outgrowing cells in primary cultures expressed high amounts of vimentin. When paraffin sections of the original mammary tissues were analyzed, epithelial cells were deveoid of vimentin as expected for epithelial tissue in vivo. In contrast to primary cultures, cells of the established cell lines like MCF-7 and BT-20 expressed no or low amounts of vimentin and high amounts of low molecular weight cytokeratins.

Vimentin expression in epithelial cells might be controlled by factors such as culture conditions or destruction of the three-dimensional architecture.

Repetitive sequences at the 3' ends of cDNAS from *Chlamydomonas reinhardtii*

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Four cDNAs containing repetitive sequences were isolated from a λgt10 library (from M. Goldschmidt-Clermont, P. Malnoe and A. Shaw). Only 2 (Rpt 1 and 2) of these hybridize to multiple restriction fragments on Southern blots washed in 0.1 × SSC at 65 °C. These cDNAs correspond to two different repetitive DNA families. The repeats of both families have a similar copy number (1000 conserved copies/haploid genome, 0.1 X SSC at 63 °C) and size distribution (100–600 bp). The dispersed organization of some members of both families was suggested by analyses of genomic fragments cloned in λEMBL3 (from M. Goldschmidt-Clermont). Restriction fragment polymorphisms between *C. smithii* and *C. reinhardtii* that have been uncovered by the Rpt 1 and 2 probes are being used to assess genetic linkage between the repeat units themselves and between repeat units and other genetic loci. Discrete transcripts corresponding to the Rpt 1 and 2 cDNAs have been detected on Northern blots. The Rpt 1 and 2 cDNA sequences will be presented.

Establishment of isotype-switch variants of a hybridoma reacting with human neuroblastoma

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Isotype-switch variants of monoclonal antibodies occur spontaneously in low frequency in hybridoma cultures. We have established such variants expressing the γ2a or γ2b isotype from the

hybridoma CE7 which secretes $\gamma 1/\kappa$ antibodies. The CE7 antibody binds selectively to all human sympatho-adrenomedullary cells (Schönmann et al., Int. J. Cancer *37* (1986) 255). The switch variants were obtained by a 3-step cloning procedure followed by a limiting dilution. In this way, $\gamma 2b$ secreting cells were selected from the original CE7/$\gamma 1$ and $\gamma 2a$ from the $\gamma 2b$ variant, respectively. The selection was performed by an isotype-specific sandwich ELISA with a sensitivity of < 200 pg. The frequency of variants was $5.4 \times$ exp-4 for $\gamma 2b$ and $5.0 \times$ exp-4 for $\gamma 2a$, respectively. By using an ELISA technique with whole neuroblastoma cells and galactosidase-labeled anti-mouse isotype antibodies, it was demonstrated that the 3 different antibodies bind to the same epitope. Spontaneously arising isotype-switch variants of monoclonal antibodies possess distinct effector functions mediated by the Fc part, but display identical epitope-specificity.

Analysis of the myofibrillar structure of isolated adult rat heart cells

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Adult rat cardiac muscle was dissociated into single cells by retrograde coronary perfusion with collagenase. In culture, most of the originally rod shaped cardiocytes underwent extensive morphological alteration after attachment. This is first accompanied by an apparent loss of myofibrillar structure followed by a subsequent reformation of myofibrils. Using phalloidin as a stain for F-actin and antibodies against myofibrillar antigens (myomesin, cardiac C-protein and (a gift of Dr. Deschesne) ventricular MHC's), a change from a mainly perinuclear presence of short myofibrils in earlier stages to a distribution either throughout the whole cell or in a typical patchy localization of multiple sarcomeres along actin cables in the pseudopodia of the polymorphic cardiocyte could be demonstrated. Both ventricular myosin HC-isoproteins were present in newly dissociated cells (95% of cells: β-MHC, 100% of cells: α-MHC), while afterwards α-MHC presence in cultured cells decreased qualitatively and quantitatively as well.

Effects of N-acyl-dehydroalanine derivatives in protecting cell cultures from radiation damage

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N-acylated dehydroalanine derivatives (AD-compounds) have recently been shown to selectively inhibit the reactivity of oxygen species arising from radiation and to increase the survival of pretreated irradiated mice (Buc-Calderon et al., Archs int. Physiol. Biochim. *94*, B-114 (1986)). Our aim was to characterize and measure the effects on a molecular level and to provide alternative in vitro assays, which may partially replace animal testing. There is an inversely exponential relationship between the percentage of DNA remaining in the double stranded form after alkaline treatment and the radiation dose. Rapid and accurate estimation of DNA breaks in cell cultures has been derived from alkaline unwinding kinetics. We investigated the response of V 79-M3-1 Chinese Hamster Cells, as well as myoblasts and myotubes prepared from 12-day-old chick embryo breast muscle.

Broad spectrum insertion mutagenesis in gram-negative bacteria

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Our objective has been to develop techniques for the genetic analysis and manipulation of cloned genes in gram-negative soil and water bacteria. As part of this effort, we have constructed a set of derivatives of the Omega interposon (Prentki and Krisch, Gene *29* (1984): 303). Each of these DNA fragments carries a different antibiotic resistance gene (AP^r, Cm^r, Tc^r, Km^r or Hg^r) which is flanked, in inverted orientation, by transcription and translation termination signals and by synthetic polylinkers. The properties of these Omega derivatives were verified by in vitro mutagenesis of a broad host range plasmid which contains the entire meta-cleavage pathway of the toluene degradation plasmid pWWO. We have shown that the appropriate resistance genes of the Omega derivatives are usually expressed in gram-negatives bacterial species as diverse as *Agrobacterium, Rhizobium, Paracoccus* and *E. coli*, and that the interposon acts in these bacteria as a strong polar mutation.

Presence, type and physical state of human papillomavirus DNA in precancerous and cancerous genital lesions

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Certain types of human papillomavirus (HPV) have been suggested to play a role in the pathogensis of cancer of the lower female genital tract. Preliminary results indicate that HPV-DNA is harbored in a free and unintegrated state in benign and premalignant lesions, but is found integrated into the host cell chromosome in malignant lesions. Thus one key event in malignant conversion of these epithelial cells could be the integration of HPV-DNA into the host cell DNA. To elucidate this question premalignant and malignant patient tissue was analysed for the presence and type of papillomavirus DNA by slot blot hybridisation. The physical state of the HPV-DNA was then determined by restriction analysis followed by Southern blot hybridisation. The DNA analysed was either extracted from freshly taken cellular smears or from paraffin embedded tissue blocs of patients with a well documentated clincial history. First results are discussed.

Molecular characterization of the colicin D operon and identification of its products

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The colicin D operon of the plasmid ColD-CA23 encodes for colicin D (an antibiotic protein of 87 kD) *cda*, an autolysis function (identified as a 10 kD protein) *cdl* and an immunity function against colicin D *cdi*. These three genes are located on a 2.4 kb fragment of ColD. Insertion mutagenesis using the Ω interposon and the transposon Tn5 reveal that the colicin D and the lysis genes are co-transcribed on a SOS inducible operon, while the immunity gene, which is located between them, has its own transcriptional unit. Using RNA polymerase binding studies on ColD, we have mapped the sites of the two promoters. The direction of transcription of the colicin gene was established by the analysis of the truncated proteins in different Ω mutants. We have genetic evidence that a gene function, located on the msame operon modulates the expression of *cda* and *cdl* after SOS induction.

A homologous in vitro transcription system to analyze Igμ transcription

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In order to analyse tissue-specific factors involved in the regulation of Ig gene transcription, we have developed a homologous in vitro transcription system. Nuclear extracts from mouse B-cell hybridomas were found to transcribe a mouse Igμ HC gene more efficiently than fibroblast extracts. However, no HC enhancer dependent stimulation could be observed. THe nuclear extracts have been further fractionated and screened for the presence of specific DNA binding proteins, using a gel retardation assay. Addition of protein fractions containing the octamer-binding protein to a minimized transcription system did not result in any stimulation of Ig specific transcription, suggesting that this DNA binding factor is not limiting in our extracts. However, Ig specific transcription stimulation could be detected in other protein fractions, but again not dependent on the presence of the enhancer.

The polymorphism of HLA-DR: molecular basis, evolution and analysis by 'DNA-typing'

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HLA-class II genes of the MHC are remarkably polymorphic. We have sequenced a number of HLA-DR β chain genes and have compared different loci and different alleles. The polymorphic variations are clustered and short segments of DNA are shared by different genes ('patchwork'). From the characteristic pattern of sequence differences observed, we conclude that the HLA-DR polymorphism has been generated through a succession of gene duplication, gene conversion and genetic recombination. Groups of haplotypes can be recognized which result from distinct evolutionary lineages. These sequence studies have also identified new allelic series and 'splits' of known DR specificities. They have allowed the analysis of HLA polymorphism in population studies with the use of loci and allele-specific oligonucleotide probes (HLA 'DNA-typing'). We have thus determined the segretion of several new DR and DQ allelic series in the population. Such a large-scale analysis of HLA micropolymorphism becomes an essential tool for accurate matching in transplantation.

Genetic mapping in *Xenopus laevis*

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In order to get an insight into the genome organization of *Xenopus laevis*, linkage relationships between genetic markers (mostly enzymes and blood proteins) were determined by recombination analysis. Independent assortment was observed between the duplicate Alb (albumin) loci. This is true for the MPI (mannosephosphate isomerase) loci as well, suggesting that these genes have been duplicated as part of a genome duplication that presumably occurred during the evolutionary history of *X. laevis*.

Four linkage groups were established. The first contains Alb2, ADH1 (alcohol dehydrogenase), NP (nucleoside phosphorylase), and a^p (periodic albinism). The second contains MPI1, SDH (sorbitol dehydrogenase), and M-IDH1 (mitochondrial isocitrate dehydrogenase). The third consists of M-ME (mitochondrial malic enzyme) and sex. The fourth contains GPI2 (glucosephosphate isomerase) and PepD (peptidase D).

Mitosis in the cellular slime mold *Acytostelium leptosomum*

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Microtubules (MT) of *A. leptosomum* were visualized by indirect immunofluorescence with the antibody YOL 1/34 of Kilmartin et al. (J. Cell Biol. *93* (1982)); with the antibody YL 1/2 no immunoreaction was detectable. The metaphase spindle is barrel-shaped, with broad poles and the chromosomes (DAPI-stained) aligned at the spindle equator. In ana- and telophase, the chromosomes form two crescents facing the spindle poles. Interpolar MT appear as multiple strands during ana/telophase and later as a single shaft with an apparent zone of overlap. In live cells, chromosome condensation and congression can be clearly seen by phase contrast microscopy. Metaphase lasts > 6 min, but segregation during anaphase is completed within 2–3 min. It begins zipper-like from the edges of the metaphase plate, then proceeds at a constant velocity of 2.5–3.0 µm/min. The distance from chromosomes to pole remains $\sim$ equal, suggesting that poleward movement of chromosomes does not occur. These results await confirmation by detailed electron microscopy.

Transcription map of bovine herpesvirus 1

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Bovine herpesvirus 1 (BHV-1) isolates can be classified in 3 groups according to the DNA and polypeptide patterns, and antigenically by means of monoclonal antibodies. The third group, represented by neuropathogenic BHV-1 strains, differs markedly from the other 2 groups. As a first step to study the expression of viral genes we constructed a transcription map of a BHV-1 reference strain by hybridization of labeled DNA-fragments with membrane-bound RNA, which had been extracted from infected cells 2, 4 and 8 hours post infection. Thus we could show that BHV-1, like other herpesviruses, replicates in a temporal cascade. The 8 h-transcripts were found throughout the whole genome. In comparison, many of the 4 h-transcripts had different mobility, were less abundant, and were virtually absent from the middle third of the genome. The 2 h-transcripts were restricted to one particular region. In parallel, a partial transcription map of a neuropathogenic BHV-1 strain was established which revealed significant differences.

Molecular cloning of the cAMP-dependent protein kinase regulatory subunits

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As modulators of cAMP-dependent protein kinase (cAMP-PK) activity, the regulatory (R) subunits play an important role in the hormonal regulation of cellular metabolism. Until recently it was thought that two classes [type I (R_I) and type II (R_{II})] of R subunits existed, which are apparently expressed in a differentation specific manner; thus implying specific physiological roles for the two different R subunits. We have investigated the expression of the R subunits in the porcine epithelial cell line LLC-PK_1 using cDNAs for both R_I and R_II. Interestingly the R_I cDNA detected two major RNA species corresponding to 2.0 and 4.5 kb's while only a single transcript of 6.0 kb's was detected using the R_{II} cDNA. In order to understand the regulation of R subunit synthesis we have isolated the genes encoding R_I and R_{II}. Currently the structures of the promoters for both genes are being determined.

Lectin cytochemistry in scanning electron microscopy

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Lectin cytochemistry in SEM using the colloidal gold system enables to: investigate large tissue surface area; better visualize label localized in specific areas; study cells in different physiological staes. However, too dense a marking may obscure underlying structures and lead to artefacts. These statements will be illustrated with specific examples using rat ileum, human milk fat globules, yeast cells and crab chitin marked with soya bean, wheat germ or *Ricinus communis* lectins. Direct vs indirect marking procedures were compared using mouse mastocytes marked for concanavalin A and wheat germ lectin binding sites. It is concluded that: direct (one-step) procedures can be used when lectin binding sites extend far from the bilayer; indirect (two- or three-step) procedures increase marking density, especially when the interacting species have high affinities (however controls are less satisfactory); with some lectins, steric hindrance plays a major role.

Distribution of serotonergic axons forming pericellular arrays in the cerebral cortex of the marmoset monkey

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One class of serotonergic acon terminals with large varicosities has been shown to form pericellular arrays (baskets) surrounding non-pyramidal neurons mainly in layers 1, 2 and 3 of the cat cerebral cortex (Tork and Mulligan, 1984).

A search for similar baskets in the marmoset monkey using a monoclonal antibody raised against serotonin (Sera-Lab, England) revealed the existence of axons with large varicosities in all areas of neocortex. However, these axons were sparse in the occipital lobe and did not form baskets, whereas they were most numerous in the frontal lobe formin arrays around many layer 2/3 bipolar neurons. The pericellular arrays were also present, in reduced numbers, in the parietal and temporal lobes and in the hippocampus. These findings show that the serotoniergic basket fiber system is also present in primates, and its non uniform distribution may reflect its differential influence on specific cortical functions.

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First report of gene-size molecules in macronuclei of non-hypotrichous ciliated protozoa

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From several years we know that hypotrichous ciliates possess a germinal *Mic* ronucleus with chromosome-size DNA (high MW) and a vegetative *Mac* ronucleus with gene-size DNA(0.2–20 kb). After cell mating, the Mac is derived from a Mic, by means of drastic reorganization consisting of fragmentation-elimination-polyploïdization of DNA sequences. We show here electrophoretic analysis of native DNA from *Nassula, Furgasonia, Pseudomicrothorax* (2 species); *Trithigmostoma* (Hypostomatia) and *Halteria* (Spirotrichia). The electrophoretic pattern for *Furgasonia* DNA shows only molecules above the limit of resolution of gels and for the other species large- and gene-size molecules (discret bands) in a continuous spectrum ranging from 1 to 30 kb: We conclude from these results: 1) no fragmentation of DNA occurs for 1 species, 2) when major fragmentation occurs the molecules length range from 7–30 kb *(Nassula)* to 1–30 kb and the percentage of gene-size molecules from 10 *(Nassula)* to 90% *(Halteria)*, 3) only very small differences are found between 2 species of the same genus. These findings contribute to a better understanding of Mac genome organization.

Isolation and characterization of the murine MX-promoter

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Specific resistance of mice to influenza virus is mediated by interferon (IFN) α or β and is due to a single, IFN-induced protein designated Mx. The cDNA encoding Mx has been isolated (Staeheli et al., Cell *44* (1986) 147).

We isolated 2 non-overlapping genomic DNA fragments, one of which encodes the 27 nucleotide first exon only. The Mx-locus spans > 50 kb, and the first exon is separated from the second by an intron of at least 30 kb.

To locate the Mx-promoter, we fused sequences upstream of the first exon to the rabbit β-globin transcription unit. Murine L929 cells were transiently transformed with the hybrid genes and the transcripts were S1-mapped after induction with murine IFN-α1. 445 nucleotides preceding the cap-site were sufficient to elicit 40fold induction of the transcript level. A shorter, 175 nucleotide fragment still allowed full induction, however, the uninduced transcription level increased. A detailed analysis of the promoter sequences will be presented.

Tubulin gene transcription in *Trypanosoma brucei*

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The tubulin genes of the parasitic hemoflagellate *Trypanosoma brucei* are all clustered in a tightly packed array of alternating α- and β-genes (Seebeck et al., PNAS *80* (1983) 4634). Steady state mRNA contains one abundant mRNA each for α- and for β-tubulin, and each species contains an identical 35 b mini-exon sequence at its 5'-terminus (Imboden et al., J. molec. Biol. *188* (1986) 393). No potential regulatory sequences for initiation or transcription are found in the intergenic regions of the tubulin cluster, and no startpoint of transcription has yet been identified upstream of the first gene of the cluster. Different indirect observations suggest that the entire tubulin gene cluster, and presumably a considerable stretch of DNA upstream from its first gene, are transcribed into a continuous primary transcript, from which the individual mRNAs are rapidly excised and subsequently completed by addition of the mini-exon sequence via a trans-splicing reaction. We will present data which strongly support this model and which directly demonstrate the read-through transcription of the tubulin gene cluster.

Immunocytochemical staining of different cell types in rat brain for angiotensinogen

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By using affinity-purified anti-angiotensinogen antibodies (rabbit origin) it was possible to locate the renin substrate angiotensinogen in or at three different cell types in the rat brain.

These locations included 1) subpopulations of astrocytes, predominantly in hypothalamic area. Almost no staining for angiotensinogen was seen on the dorsal side of the corpus callosum. Of potential importance is the tendency of angiotensinogen-containing astrocytes to make contact with brain microvessels; 2) magnocellular neurones that costain for angiotensin II, e.g. with subpopulations of neurones in the paraventricular nucleus, the accessory magnocellulary nucleus and the supraoptic nucleus; 3) cells of the choroid plexus. The cells in 2) and 3) were characterized by punctate staining.

These findings are consistent with the hypothesis of multiple functions for brain angiotensinogen as a precursor for neuronal angiotensin II and as a potential source of angiotensin II locally produced in the brain.

Analysis of dicentric chromosome II in *S. cerevisiae*

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CEN6 wt DNA and the URA gene were inserted next to the LYS2 gene of chromosome II in a ura3 strain. Despite the presence of two functional centromeres in chromosome II, 88 Ura+ transformants were obtained. Two transformants were chosen for further analysis. Chromosomal DNA separation (Schwartz & Cantor 1984 Cell 37, 67–76; Carle & Olson PNAS USA 1985, 3756–3760) revealed the occurence of different fragments of chromsome II in these transformants. Hybridizations with different chromosome II geneprobes were done to locate the breakpoints on the chromosome and to group the corresponding fragments. Outgrowth for 50 generations in selective (SD) and fullmedium (YPD) revealed that in the latter case only one fragment CF850, which is only 40–50 kb shorter than the original chromosome II (approximately 900 kb) is transmitted stably during cell division. Data achieved so far suppose that CEN2 plus adjacent sequences were deleted. To investigate this further, hybridization experiments with a CEN2 probe and experimental analysis of the presumptive novel joint regions are in progress.

Codominant expression of mutations affecting the catalytic subunit of cAMP-dependent protein kinase in somatic cell hybrids

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The cAMP-resistant FIB4 and FIB6 mutants of the LLC-PK_1 porcine kidney epithelial cell line, possess altered cAMP-dependent protein kinase (cAMP-PK) resulting in a 90% reduction of activity compared to parental. The residual kinase activity from the mutants was found to be almost entirely associated with the Type II cAMP-PK isozyme, and to be similar to that of parental by several criteria: Ka for activation by cAMP and affinities for ATP and kemptide. However the levels of C subunit in FIB4 and FIB6 were similar to parental. Thus, the mutational lesion was concluded to most probably be a structural mutation in the cAMP-PK C-subunit, affecting V_{max} of the enzyme. Somatic cell hybrids were formed between mutant and LLC-PK_1 cells, to examine expression of the mutant phenotype. Hybrids possessed cAMP-PK activity intermediate between mutant and normal parents, associated with both type I and type II cAMP-PK. The loss of type I cAMP-PK in FIB4 and FIB6 was therefore concluded to be a direct consequence of the cAMP-PK C-subunit mutation. Since the mutation was expressed codominantly in the presence of the normal allele, the molecular basis of the mutations in FIB4 and FIB6 will be clearly of interest with respect to the cAMP-PK catalytic mechanism.

Expression of precursor and mature chicken mitochondrial aspartate aminotransferase in *E. coli*

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A cDNA encoding the precursor of mitochondrial aspartate aminotransferase (mAspAT) was cloned downstream of the lambda P_L promoter in the expression vector pOTS (provided by Dr M. Rosenberg). In a modification of this construct, the cDNA encoding the prepiece was deleted with the exception of the initiator codon. Only low level expression of the expected products was obtained with both constructs ($< 0.1\%$ of the total protein). Attempts to extract the precursor from the cells in native state failed so far. However, mature mAspAT could be readily extracted and showed the same specific activity as the enzyme isolated from chicken heart. Thus, mAspAT can assume the correct folding pattern independently of the prepiece or any of the maturation steps, e.g. membrane passage, or proteolytic processing.

Partial cloning and comparison of the genes of mitochondrial and cytosolic aspartate aminotransferase (AspAT)

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Chicken genomic DNA clones encoding mAspAT or cAspAT have been isolated. Two mAspAT clones were mapped and found to contain part of the gene including the 3' noncoding end with the poly(A) signal. The nucleotide sequence of this part of the gene has been determined. It shows 9 exons and 10 introns over 6 kb. The first exon starts with the codon of the amino-terminal serine of mature mAspAT. Thus, the amino-terminal part of the gene encoding the mature protein is separated from the exon(s) encoding the presequence suggesting that the prepeptide has been acquired during evolution as an independent functional unit. The length of the hybridizing fragments of the cAspAT clones add up to a total of 4 kb corresponding to the length of the respective segment of the mAspAT gene. Recent sequencing data point to a similar organization of exons and introns in the two genes.

Fluctuations of the Ca^{2+} binding proteins parvalbumin, calbindin D-28K, calmodulin and S-100 during rat testis development

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Hormone production and spermatogenesis are Ca^{2+} dependent processes in testis. Parvalbumin (PV), calbindin and S-100 proteins co-exist in Leydig cells, which elaborate testosterone.

The highest PV protein and mRNA levels occurred at two stages corresponding to periods of highest testosterone synthesis; prenatal day −3 to term and starting with puberty (postnatal day 21), reaching a maximum at day 50. This result suggested an involvement of PV in testosterone production. Different from PV, calbindin immunoreactivity persisted from term to puberty, reaching a maximum around postnatal day 30. Labeling of S-100 proteins was low during fetal stages, completely absent until puberty and increased thereafter. This different developmental expression of the latter proteins suggests different physiological roles.

Calmodulin-immunostaining, in contrast to that displayed by the above proteins, was most intense in the cytoplasm of maturing spermatids, indicating an important function in spermatogenesis.

Screening of potential neuroactive substances in brain: analysis of in vitro released material with FMOC-precolumn derivatization HPLC

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As no established neurotransmitters have been ascribed to some major CNS pathways, a screening of neuroactive substances has been performed by selecting compounds which are released from rat brain slices upon K^+ depolarization in a Ca^{++}-dependent manner. The perfusates have been analysed for amino acids and

peptides by reversed-phase HPLC following derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) (Einarsson et al. J. Chromatogr. 282 (1983) 609). By structural elucidation (amino acid analysis, fast atom bombardment mass spectrometry) one of these compounds was identified as a to date unknown side-product of FMOC-derivatization of glutamate. Furthermore, a substance termed 25C could be isolated in the presence of protease inhibitors. The substance contains at least Glx and a base labile group. As there is still uncertainty whether Asp and/or Glu are the only endogenous transmitters of all so called acidic amino acid pathways our results appear of interest.

Potassium cyanide inhibits Semliki forest virus induced cell-cell fusion

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Semliki Forest virus infected Aedes albopictus cells were used to investigate virus induced cell-cell fusion from within. In this system the fusion process can be triggered by mildly acidic extracellular pH. To monitor cell-cell fusion, infected cells were microinjected with the highly fluorescent, non-permeable dye Lucifer yellow after lowering the pH to 6. Then cell-cell fusion was detected as spreading of the dye from the injected cell into neighbouring cells. It was shown by this novel method that cell-cell fusion was completed within approximately 5 min after triggering the fusion event by low pH. In contrast, polykaryon fermation, the usually used criterion to measure cell-cell fusion, occurred only within 30 min. Furthermore, it was shown that potassium cyanide, a potent inhibitor of polykaryon formation in the described system, inhibits an early step of membrane-membrane fusion of neighbouring cells.

A novel approach for identification and purification of ion channels

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Vectorial transport of Na across tight epithelia is mediated by an apical amiloride-sensitive sodium channel and a basolateral Na,K-ATPase. We used a high affinity photoactive Na channel inhibitor (NMBA) and antibodies directed against the inhibitor to identify and purify the channel from bovine kidney and A6 cells. Immunoblots of A6 microsomes (derived from cells grown on collagen) and from bovine kidney cortical microsomes revealed a single 120 kD protein specifically labeled with NMBA. Since NMBA blocks sodium transport through the channel in the nanomolar range both in bovine kidney vesicles and A6 monolayers, and the protein is present at the luminal membrane, as shown by immunoprecipitation of radio-iodinated and photolabeled A6 apical membrane proteins, this protein is likely to be a component of the epithelial Na channel.

Multiple forms of nucleoside-phosphate kinases and potential markers for proliferation and differentation

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Nucleotidephosphate kinase activities from various human tissues and tumors, from leaf cells and from normal and transformed tissue cultures of *Nicotiana tabacum* were analysed for their triphosphate and monophosphate specificity.

The post-electrophoretic identification of the enzyme activities revealed a very similar pattern of adenylate kinase activities in cell tissue types. On the other hand tissue types showing cell proliferation had a very specific UMP-dependent banding pattern. The GMP-dependent isozyme pattern seemed to reflect the state of differentiation of a specific tissue. The results stress the importance of specific changes in energy metabolism during growth and differentiation and in transition to neoplastic development.

The effect of calcium on receptor-mediated endocytosis of vitellogenin in cockroach follicles in vitro

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Vitellogenins (vg) are the predominant yolk protein precursors in egg-laying animals. In insects they are synthesized in the fat body as multimeric phospholipoglycoproteins, secreted into the hemolymph and sequestered by the maturing oocytes. Scatchard analysis of specific vg binding to membrane preparations from whole follicles of the cockroach *Nauphoeta cinerea* indicated the presence of two classes of vg receptors with different affinities for the ligand. To analyze the distribution of the two receptor populations, follicle membranes were fractionated into follicle cells (fc) and an oocyte plasma membrane (opm) fraction. Scatchard analysis revealed that the high-affinity binding sites were located in the fc-fraction, whereas the opm-fraction contained the low-affinity binding sites. The concentration of Ca was critical for both binding and uptake of vg in vitro. Optimal binding to the opm-fraction and the fc-fraction was observed at 0.3 mM and 10 mM Ca respectively, whereas uptake was optimnal at 5 mM Ca. On the basis of these findings we propose a model for vg uptake.

A promoter segment from human $\alpha 1$ interferon gene confers virus-inducible transcription to the rabbit β-globin gene

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The minimal regulatory element (MRE-IFN) required for induction of the IFN-$\alpha 1$ gene is located between position -64 and -109 as defined by deletion analysis. It contains an internal repeat. The MRE-IFN was isolated, furnished with linkers and inserted in one or several copies into different positions relative to the β-globin promoter. In all cases virus-inducible transcription of the β-globin gene was observed independent of the orientation of the chip. The MRE-IFN was most active when placed 5' to the globin transcription unit. Insertion into the first intron or at the 3' end of the β-globin gene allowed induction albeit with very low efficiency. A synthetic oligonucleotide comprising the sequences from position -79 to -99, when used as a dimer showed inducibility comparable to that of the complete MRE-IFN. Similar results were obtained with both transient and stable transformation assays.

In cholesterol fed rabbits, the reduction by nifedipine of cholesterol and foam cell accumulation in the liver precedes reduction of aortic fatty streaks

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In rabbits a 2% cholesterol (CH), 6% coconut oil diet induces aortic macrophage foam cell (FC) lesions. Preceding these, FC are found in the liver sinusoids (Kuhn et al., Experientia *38* (1982) 743).

Nifedipine (Ni; 10 mg/kg b.i.d., p.o.) significantly inhibited CH and FC accumulation in both the liver and aorta. After 28 days on the atherogenic diet Ni reduced CH in the abdominal aorta from 18 ± 2 (n = 14) to 11 ± 1 μg/mg d.wt (n = 15; mean ± SE). Neointima in % of the media was reduced from 12 ± 1 to 5 ± 1 and the number of FC/cross section from 52 ± 12 to 2 ± 6.

After 7 days no aortic lesions were seen. However, liver CH increased to 11.9 ± 0.6 mg/g w.wt (n = 10) in controls, and was less, 8.5 ± 1.3 mg/g, in the Ni group (n = 10). The number of FC per mm^2 of sinusoids was reduced by Ni (686 ± 67 vs 474 ± 60). In conclusion, in CH fed rabbits Ni reduced CH and FC accumulation in the liver and later in the aorta. Whether the two events are related remains to be established.

Influence of sodium intake on the cardiac gene expression of artrial natriuretic peptide in normotensive rats

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The effect of short- and long-term Na load on the cardiac gene expression of atrial natriuretic factor (ANF) was evaluated in normotensive rats by determining ANF mRNA. Two groups of rats were kept on a high sodium intake for 1 and 3 weeks respectively. They received 1% saline as drinking water instead of tap water. They were compared to control rats kept on a regular sodium intake for 3 weeks. AFN mRNA was determined after pooling of atria and ventricles in the different groups of rats, by the Dot Blot technique. A synthetic oligonucleotide complementary to ANF mRNA was used as a probe. ANF mRNA levels (arbitrary units) are estimated for 1 μg of total RNA:

	Regular Na (n = 11)	High Na after 1 week (n = 12)	Regular Na (n = 11)	High Na after 3 weeks (n = 11)
Right atrium	591	650*	607	563
Left atrium	419	532*	558	486
Right ventricle	8.1	20.6*	12.7	21.0*
Left ventricle	5.2	6.4*	7.8	8.9

* $p < 0.05$ vs regular Na.

ANF mRNA levels were clearly increased by Na loading at the end of the 1st week in all cardiac cavities. After 3 weeks, the changes in ANF mRNA levels were not consistent. In conclusion, these data, obtained by RNA hybridization analysis, suggest that the transcription of ANF gene is influenced to a larger extent during the short- than the long-term adaptation to changes in sodium intake.

Cloning and sequencing of cDNA's coding for chicken nuclear lamins A and B_2

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The nuclear lamin proteins form a filamentous meshwork lining the inner nuclear membrane. They are believed to be important for nuclear envelope integrity and interphase chromatin organization. Among the three major mammalian lamin proteins, lamins A and C are thought to interact with chromatin, whereas lamin B is implicated in anchoring the lamina to the membrane. Based on cDNA sequence comparisons, human lamins A and C were recently shown to be related to intermediate filament proteins, but no sequence information by yet available for mammalian lamin B. From λgt11 libraries we have isolated partial cDNA clones encoding chicken lamins A and B_2. (Lamin B_2 is the major chicken lamin 'B' protein). Sequence data unequivocally show that not only chicken lamin A, but also lamin B_2, belong to the intermediate filament protein family. It remains to be examined, however, whether or not chicken lamin B_2 is functionally analogous to mammalian lamin B.

Cultivation of outer root sheath cells isolated from plucked human hair follicles

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The role which outer root sheath (ORS) cells play in the development and growth of hair is still elusive. To evaluate their differentiation potential and develop culture models for testing environmental modulation, we have isolated ORS cells from plucked human scalp hair follicles and optimized conditions for their growth in culture.

Trypsinization of one anagen hair follicle for 10 min at 37°C yielded approximately 1.5×10^4 ORS cells which were plated on a mitomycin C-arrested 3T3 feeder layer preformed in a 35-mm culture dish and grown in medium supplemented with growth factors.

Under these conditions, the ORS cells grew to confluence within 2–3 weeks, yielding approximately 10^6 cells. At this time, the ORS cells exhibited a stratified structure resembling much that formed by interfollicular keratinocytes grown under similar conditions. Further, the ORS cells could be serially propagated up to 4 passages expanding the population to approximately 10^9 cells, and cryopreserved.

Nucleo-mitochondrial interactions: characterization of mitochondrial *oxi2* mutations and cloning of a nuclear suppressor gene

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Most of the mitochondrial proteins are encoded by the nucleus. In the case of cytochrome c oxidase the three largest subunits are encoded in the mitochondria whereas the six remaining subunits are encoded in the nucleus. The complex of cytochrome c oxidase represents therefore a suitable system to study the interaction of nuclear and mitochondrial genes and the products thereof.

We have cloned a nuclear gene (see Yeast *2* (1986) S212) which is able to suppress two mutations in the mitochondrial gene *oxi2*. Suppression can only be observed if the gene is cloned on a multicopy plasmid stressing the importance of a gene dosage effect. Upon gene inactivation by disruption experiments no mutant phenotype can be observed. Moreover we have found by hybridization a second DNA sequence which is homologous to the isolated gene. These results indicate the presence of a second gene which overtakes the function of the first, inactivated, gene. At present we analyse the implication of the second gene in biogenesis of mitochondria.

Is the interaction between Con A and the lymphocyte membrane gravity-dependent?

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In several experiments performed under microgravity and hypergravity conditions we have demonstrated that activation of human lymphocytes by Con A is gravity dependent. In particular in whole-blood cultures (W-B), obtained by diluting fresh blood with medium 1:10, activation by Con A is enhanced by more than 400% at 10 × g. Conversely this effect is smaller in cultures of purified lymphocytes. The objective of this study was to explain the different G-effects between W-B and purified lymphocytes. The hyper-G effect on W-B could be quantitatively reproduced by incubating purified lymphocytes with erythrocytes coated with Con A, while no free Con A was present in the medium. The same effect was obtained with sepharose-coated Con A.

Our observations provide a strong indication that the difference in magnitude of the hyper-G effect between W-B and purified lymphocytes is probably due to an altered presentation of Con A to the lymphocyte membrane.

Cytosolic aspartate aminotransferase is encoded by two mRNAs with heterologous 3' noncoding regions

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Screening of a lambda gtll cDNA expression library from chicken embryonic muscle (supplied by Dr. U. Rosenberg) with a polyclonal antibody against cytosolic aspartate aminotransferase yielded a 450 bp long cDNA coding for amino acid residues 111 to 263. This cDNA was used as a probe to re-screen the library. We found 7 different clones spanning nearly the entire coding region of the protein. Sequencing showed that there were two different 3' noncoding regions probably due to differential splicing of the primary transcript. On Northern blots two mRNA species of 1.7 and 2.0 kb were detected. Currently, RNA from several embryonic and adult tissues is being inspected for differential occurrence of the two messengers.

Microinjection of an antibody to the interferon induced Mx protein inhibits the establishment of an antiviral state

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Interferon (IFN) alpha/beta induces in cells from influenza virus resistant mice a karyophilic protein, the Mx protein, whose presence is correlated with inhibition of influenza viral mRNA synthesis. In rat cells, rat IFN alpha/beta induces three proteins that crossreact with a monoclonal antibody, 2C12, specific for mouse Mx protein. These three rat proteins are encoded by mRNAs with homology to the mouse Mx cDNA clone. As in mouse cells, influenza virus replication is inhibited by IFN at the level of mRNA synthesis. When antibody 2C12 is microinjected into the cytoplasm of rat cells, IFN can non longer protect these cells against influenza virus, although it still induces the Mx homologous proteins. The antiviral state against other viruses such as VSV is not affected. We conclude that all or a subset of the Mx homologous proteins of rat cells are inhibitory to influenza virus and that their activity can be neutralized by specific antibodies in vivo.

Short introns with unconventional termini interrupt the leader, translated region and trailer of the chloroplast EFTu gene in *Euglena gracilis*

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The genes for ribosomal proteins S12, S7 and elongation Tu (EFTu) are clustered in the chloroplast genome of *Euglena*. *Rps*12 and *rps*7 are uninterrupted and co-transcribed. *Tuf*A is expressed as a 2kb mRNA which is the result of 4 cis-splicing events. Intron 1 interrupts a 5' untranslated leader, introns 2 and 3 interrupt the protein coding part and intron 4 interrupts a trailer segment. The 5' end of EFTu mRNA and the splice junctions of introns 1, 2 and 3 are established by primer extension sequencing. The 5' end of mRNA maps 19 b downstream of the last codon of the S7 gene and within the 173 bp spacer between the S7 termination and EFTu start codon. The intron termini do not follow chloroplast consensus sequences. Intron 2 cuts codon 141 of EFTu which borders the GTP-binding domain (Kohno et al., PNAS *83* (1986) 4978). Intron 3 of EFTu is located at about the same position as intron 4 in the homologous eukaryotic EF1α gene of Artemia (Lenstra et al., Eur. J. Biochem. *155* (1986) 475). This positional coincidence suggests a common ancestor gene.

On the mechanism of DNA-renaturation promoted by recA-protein

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RecA-protein catalyses the renaturation of homologous single-stranded DNA's. We analyzed the requirements for nucleotide cofactors and Mg^{++} for optimal reaction conditions. In presence and in absence of ATP, renaturation of DNA goes in parallel to aggregation of recA-protein/DNA complexes.

ATPγ-S, a non-hydrolyzable analogue of ATP abolishes renaturation in case of saturation of DNA with recA. However, it does not prevent aggregation. From this we conclude that renaturation occurs in 'dynamic' aggregates formed with or without ATP. The 'static' aggregates formed in presence of ATPγS do not promote renaturation.

Bacterial synthesis of specific antigens from *Echinococcus multilocularis*

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Alveolar echinococcosis is a zoonotic disease caused by the larval stage (metacestode) of *Echinococcus multilocularis*. The disease is prevalent in many areas of the Northern hemisphere. In Switzerland and neighbouring regions of France and Germany it is well known as a public health hazard of man. Immunodiagnosis and seroepidemiology were greatly complicated by the fact that for a long time no species-specific antigens were available. Recently such an antigen has been isolated from *Echinococcus* material by one of us (BG), and its diagnostic value has been proven in extensive field studies in endemic (Switzerland) and hyperendemic (Alaska) areas as well as in routine immunodiagnosis (lit. see Schantz & Gottstein, 1986). However, not enough antigen can be prepared biochemically to allow its general use in routine serology.

To overcome this problem, a project was initiated with the aim of producing the appropriate antigen(s) in bacteria. We have isolated a number of gene fragments form an *Echinococcus multilocularis* cDNA expression library, which code for proteins which are recognized by antibodies from sera of patients with

alveolar echinococcosis. The corresponding genes will be introduced into suitable expression vectors and the proteins synthesized in bacteria will then be tested for their suitability as immunodiagnostic reagents.

Diversity of glycosylation of the fusogenic E_1 molecule of Semliki Forest virus

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We have been studying the mechanism of fusion from within mediated by Semliki Forest virus in *Aedes albopictus* cells and found that the viral envelope protein E1 transmits the signal. In SDS-PAGE, the E1 displays several bands indicating that this protein undergoes diverse posttranslational modifications. We are interested to determine whether these structural differences play a role in the fusogenic activity of the protein. Thus, we have analysed the degree of N-glycosylation of this protein by applying several endo-glycosidases, mannose labelling and various lectins. Data will be presented showing heterogenious glycosylated populations or E1. Infact, low mannose as well as hybrid or complex type of carbohydrate chains were detected. This contradicts in part what was described in the literature for the glycosylation of membrane proteins in Aedes cells.

The transferrin receptor: identification in the human intestinal mucosa

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The mechanisms underlying iron uptake by the human small intestinal mucosa are not yet established. However, mucosal transferrin (Tf) has been found in intestinal cells suggesting that transferrin-receptor (TfR) might be expressed on these cells. We investigated this possibility by labelling intestinal biopsies for 6 h with ^{35}S-methionine and immunoprecipitation of cell lysates with anti-TfR (Okt 9). SDS-PAGE of the immunoprecipitates revealed a band of Mr similar to that of TfR found on lymphocytes. This band was absent in biopsies from patients with damaged mucosa. Tf-binding to the brush border membranes (BBM) was studied by incubating iodinated Tf with highly purified BBM, chemical cross-linking and immunoprecipitation with Okt 9. A high molecular weight band was revealed by SDS-PAGE of the precipitate presumably representing Tf-TfR complexes. We conclude that small intestinal epithelial cells express TfR. Further investigations are in progress to characterize intestinal TfR and to precisely establish its role in mucosal iron uptake.

Reactivity of monoclonal IgM autoantibodies towards glycolipids in patients with peripheral neuropathy

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Sera from patients with monoclonal IgM gammopathy and peripheral neuropathy were screened for reactivity against nerve and brain glycolipids by ELISA and by a thin layer-chromatography overlay technique. Twenty-eight sera reacted with an acidic glycolipid extract from PNS and 25 reacted with a glycolipid extract from CNS on ELISA test. The individual reactive lipid components were further identified on TLC using purified gangliosides. Several patterns of reactivity were found. One subgroup exhibited a polyreactivity against different gangliosides. An other subgroup reacted against GM 1 or GD1b but no asialo GM 1. Furthermore immunoperoxydase studies demonstrated that an IgM with specificity for GM1 and GD1b may recognize neurons, axons and granular cells in human brain. Taken together these observations demonstrate that monoclonal IgM in patients with peripheral neuropathy have an unexpected high frequency of reactivity with different gangliosides. Thus gangliosides may be common antigens in paraproteinaemic neuropathies and this may be relevant to the pathogenic mechanisms.

Structure and genomic organization of extraribosomal copies of the 28S rDNA intervening sequence of *Ascaris lumbricoides*

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About 5% of the rDNA repeats of the nematode *Ascaris lumbricoides var. suum* are interrupted within their 28S genes by a 4.5 kb long intervening sequence (IS). This IS is strikingly similar in both, structure and genomic organization, to the type I rDNA insertion elements of *Drosophila melanogaster* and represents therefore the first example of such sequences found outside of the class Insecta. As in *Drosophila*, several extraribosomal copies of the IS are present elsewhere in the genome of *Ascaris*. Recently we succeeded in isolating various clones from an *Ascaris* genomic library containing such extraribosomal IS sequences. Their structure is currently being investigated in our laboratory and will be compared to that of the ribosomal IS.

Organotypic cultivation of outer root sheath cells from human hair follicles, using a new culture device, the combi-ring-dish (CRD)

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In order to provide cultured cells with organotypic conditions we developed a simple and inexpensive culture vessel named combi-ring-dish (CRD). The CRD comprises two sets of 4 concentric teflon rings offering different culture surfaces (between 0.33 and 1.13 cm^2). It allows coculture of different cell types seeded in or on different substrata. The cells and substrata can be combined in any chronological and/or spatial sequence. Furthermore transplantation experiments are easily achievable by combining the CRD with the transplantation chamber developed by Fusenig et al. (J. Invest. Dermatol. *81*, 1983, 168s). Using the CRD we have cultured outer root sheath cells from plucked human scalp hair follicles under organotypic conditions. Under these conditions the development of morphologically defined cell layers was considerably improved when compared to conventional cultures. In addition, the CRD could be useful not only for studies on biological interactions between different cell types, but also for pharmacological and toxicological studies (mutagenicity, carcinogenicity, invasivity).

Further characterization of the mechanism involved in Semliki Forest virus induced cell-cell fusion

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To study the mechanisms involved in membrane-membrane fusion, we have been utilizing *Aedes albopictus* cells infected with Semliki Forest virus. These cells fuse upon exposure to pH 6. We have recently shown that low pH exposition leads to a conformational change of the viral proteins and thus have been able to identify the fusogenic protein of the virus. To elucidate the significance of the structural transition, we have examined the behaviour of the protein in respect to hydrophobicity considering that an increase would facilitate its penetration into the lipid bilayer of the adjacent cell and thereby trigger the fusion reac-

tion. Experimental data on structure-function relationship will be presented.

Comparison of clonal variants of the selenium induction system of extrusomes in *Pseudomicrothorax dubius*

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Sequential selection of cells from a single *P. dubius* strain produced different clones in which the formation of extrusomes (trichocysts) is either strongly, weakly or not induced when the trace element selenium is added to cells. Entire cell proteins of the clones before and after induction were separated on 1-D and 2-D gels, Western blotted and immunolabeled with Ab's directed against isolated, mature trichocysts. Following induction, proteins with MW and pI characteristic of mature trichocyst proteins were labeled in cells of strong inducer clones, cells of weak inducer clones contained quantitatively less of these proteins, and none were detected in noninducers. Higher MW proteins not present in mature trichocysts were also labeled in cells of all clones, both before and following induction. The latter may be proproteins of trichocysts which are processed into mature trichocyst proteins during induction.

The association of HLA DR 3 and 4 with diabetes is not related to special HLA class II gene sequences

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Susceptibility to many autoimmune diseases is linked genetically to particular HLA class II haplotypes. This is though to result from aberrant T cell triggering by specific HLA alleles. From RFLP genotypic studies, the closest association of diabetes (IDD) is with HLA-DQ of DR 3 and 4. We have isolated and sequenced cDNA clones for the DQ α and β chain genes from a DR 3/4 diabetic. These 4 genes were found to be identical to the DQ genes of normal individuals. By oligonucleotide hybridization, a large number of DR 3/4 diabetics also have a normal DQ α and β sequence in the polymorphic hypervariable segment of the DQ genes. Consequently, there are no IDD-specific HLA-DQ amino acid sequences. We propose an alternative model where polymorphism in the regulation of class II genes could lead to abnormal amounts of class II polypeptide chains and thus to illegitimate α/β pairing (i.e. DQ/DR). This will result in new class II phenotypes and consequently to abnormal T cell activation and autoimmune pathology.

The induction of class II gene expression by γ interferon is transcriptional and dependent on protein synthesis

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The lymphokine γ interferon (γ IFN) induces the expression of HLA class II antigens in various Ia negative cell types. We have studied the induction of class II genes in different cell lines. In 143 B cells, three conclusions are reached: 1) There is a long time lag between exposure to γ interferon and the appearance of class II mRNA (9–12 h) which contrasts with other lymphokine-mediated induction systems. The continuous presence of γ interferon is not required and short pulses of 5–10 min can induce class II mRNA 12 h later. 2) The activation of class II genes by γ interferon is a transcriptional process as shown by run-on experiments. 3) Cyclohexamide prevents the effect of γ interferon and thus induction of class II genes requires de novo protein synthesis. Class II gene induction is therefore a complex process, which involves the activation of at least one gene encoding a regulatory protein, required for transcriptional activation.

Inhibition of gene expression by antisense sequences

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Several investigators have tried in the past to interfere with eucariotic gene expression by using antisense sequences. This was done in in-vitro translation systems and in intact cells. Early studies tested the antiviral activity of antisense oligonucleotides in RSV infected cells. More recently antiviral effects of oligonucleotides could be shown in HIV-I infected cells.

Several approaches have been tested to deliver interfering sequences to cells: a) transfection or b) injection of antisense expressing vectors into cells. A third way is supplying an antisense oligonucleotide in the supernatant of cultured cells. In order to optimize the stability and uptake of oligomers, analogs of various kinds were tested.

In our experiments, we want to explore the best constructs, targets and mode of deliverance of antisense sequences using an easy quantitative test system like the CAT (chloramphenicol acetyltransferase) assay.

Action of the antimicrotubular drug benomyl on *Neurospora crassa* developmental polarity

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N. crassa wild type produces successively two types of spores during its development: multinucleate macroconidia and rare, uninucleate microconidia. Microconidiogenesis can be conveniently studied in the morphological mutant fluffy (fl) producing only microconidia. Conidiogeneses are inhibited and germination disturbed (multipolar outgrowth) by benomyl concentrations in the micromolar range. Benomyl-resistant (bml^r) mutant tub-2 as well as the double mutant fl^p; tub-2 conidiate normally in the presence of benomyl. This suggests that the b-tubulin gene tub-2 functions during vegetative growth and conidiogenesis in *N. crassa*. We have investigated the effects of the introduction by transformation of the cloned bml^r allele of the b-tubulin gene of *N. crassa* (Orbach et al., 1986) on conidial differentiation. In most fluffy bml^r transformants obtained by high frequency transformation of microconidia, microconidiogenesis is inhibited in the presence of benomyl. This inhibition is either due to the disruption of genes specifically involved in conidiation or to the expression of the extra b-tubulin sequence(s).

Deletion mutations in the broad host range plasmid RSF1010 that affect the copy number and host range

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RSF1010 is a 8685 base pair antibiotic resistance plasmid with a particularly wide host range. Its replication is initiated from a unique site (oriV) and requires the function of at least three genes, repA, RepB and repC (Scherzinger et al., PNAS *81* (1984) 654). The replication genes are transcribed from two promoters p1 and p2. We show that small deletions immediately downstream of each of these promoters increase the copy number of RSF1010 in the cell. Deletions downstream of p2 increase the copy number in both *E. coli* and *P. putida*. Deletions downstream of p1 increase the copy nomber in *E. coli* approximately

fourfold and render the plasmid incapable of replicating in *Pseudomonas*.

We postulate the existence of small regulatory factors encoded directly downstream of the promoters p1 and p2. Each factor negatively regulates the promoter thus reducing the exression of the downstream replication genes. This regulation controls the plasmid copy number and is essential for the broad host range properties.

Binding assay for the study of melanoma cell MSH receptors. Effect of 1,10-phenanthroline

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α-MSH-induced melanogenesis in malignant melanoma cells has been studied extensively with assays for adenylate cyclase, tyrosinase and melanin production but a receptor binding assay for α-MSH was not available to date. Therefore, we have established a radioreceptor assay using cultured B16 mouse melanoma cells and purified, biologically active monoiodinated α-MSH as tracer. While the degradation of the tracer and its non-sepcific binding was variable in the presence of certain inhibitors, these effects could be minimized and thus better controlled by the presence 0.3 mM 1,10-phenanthroline. The mean K_d for α-MSH of 20 competition experiments was 1.93 nM ± 0.42 nM (SD) = and B_{max} was 14920 ± 6200 (SD) sites per cell. The relative affinity of various MSH analogues paralleled their potency in the tyrosinase and melanin assay, except for ACTH (1-24) and [Nle^4]-α-MSH which were more potent in the binding assay than expected from their biological activity in the melanin assay.

In vitro fertilization and embryonic development of bovine oocytes matured in vitro

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Successful in vitro maturation and fertilization (IVM-IVF) were achieved using the modified Tyrode media described by Ball et al., Biol. Reprod. *28* (1983) 717. For capacitation of frozen thawed sperm a swim-up procedure described by Parrish et al. 1986, Theriogenology 25, 591–600, was applied. Heparin was present during coculture of 14–20 h at 39 °C. The average penetration rate was 36.2 ± 6.9% (mean ± SE).

To evaluate the developmental potential of IVM-IVF oocytes, they were washed and placed into embryo culture medium for an additional incubation period of 22–30 h at 39 °C. As a control for spontaneous development, oocytes were treated the same way as described above but without spermatozoa during coculture.

The presence of an equal number of nuclei and cells in an embryo was taken as proof for embryonic development. The experimental group showed 15.5% (23/153) development to the 2-cell stage and 5.2% (8/153) to the 4-cell stage. In the control group 11.5% (7/61) of the oocytes showed cleavage to the 2-cell stage with 2 nuclei present, indicating that spontaneous development occurs under in vitro conditions. Therefore specific parameters need to be found to allow differentiation between spontaneous and embryonic development.

The making of an enhancer

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In contrast to prokaryotes where strong transcriptional signals can be located within very short DNA sequences, typical eukaryotic enhancers are about 200 bp long. One hypothesis to explain high transcriptional activity in eukaryotes would be that a minimal length of enhancer-active DNA is required, such that even parts from a single enhancer or from different enhancers can be dimerized or combined, respectively. To this end, viral enhancer fragments of heterologous sources were combined in a recombinant SV40 virus and screened for efficiency of viral growth. The 48 combinations tested showed that the concept is basically correct. No enhancer shorter than 84 bp could promote SV40 growth, i.e., in no case we did find a short 'superstrong' enhancer segment. We therefore conclude that two subfunctional heterologous enhancer fragments may form together a functional enhancer. To test whether multimerization of a short fragment would result in a strong enhancer, we have synthesized a 50 bp enhancer segment derived from Herpesvirus saimiri. One to six copies of this oligonucleotide showed an incremental increase in enhancer activity. Thus, we propose that mammalian gene regulation is based on a redundancy of information due to a combination of multiple DNA-sequence elements (that bind transcriptional factors).

Milk fever and calcium-binding proteins

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Milk fever or 'parturient hypocalcemia' (PH), a metabolic disorder associated with parturition in dairy cows, is one of the most serious and costly produciton diseases in cattle. PH is characterized by a severe hypocalcemia and a concomitant reduction of tissue calcium-concentration and a neuromuscular disfunction. Diseased animals are unable to rapidly restore normal calcium levels. We have hypothesized that the refractory nature of the hypocalcemia is related to disfunction of specific Ca^{2+}-binding proteins, which are responsible for the regulation of a number of 'calcitonic' enzymatic systems (e.g. calmodulin) as well as for transport, mobilization, buffering and storage of calcium (e.g. parvalbumin, calbindin). As a first step in the exploration of our hypothesis, we have employed gel electrophoretic analysis to compare, in normal and diseased animals the concentration of Ca^{2+}-binding proteins (plus receptors) in sera, urine, saliva, milk and in tissue extracts as muscle, intestine, mammary glands. The most striking difference we found, thusfore, in the respective patterns of milk proteins.

Sequence of cDNAs for the La protein of *Xenopus laevis*

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Certain patients suffering from autoimmune disease produce antibodies that are directed against a normal cellular protein of 50 kD termed the La protein. This protein binds transiently to the 3' ends of nascent RNA polymerase III transcripts, suggesting that it may be involved in their termination, 3' processing, or retention within the nucleus. We have recently isolated a cDNA clone that encodes part of the La protein from the frog *X. laevis*, by screening an oocyte cDNA library in the expression vector λgt 11 with a human anti-La antibody. Several longer cDNA clones have subsequently been obtained from a second oocyte cDNA library in λgt 10. We report the sequence of the frog La protein, compare it with the human sequence, and analyse La gene expression during early *X. laevis* development.

Monoclonal antibodies specific for Ag(c/g) and Ag(al/d) polymorphism of human low density lipoprotein

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Human low density lipoprotein (LDL) shows a genetic polymorphism, the so called Ag-system. It is composed of 5 pairs of allelic epitopes x/y, al/d, c/g, t/z and h/i localized on apoprotein B. We have generated a large number of hybridomas against LDL. Two of them, namely D2E1 and H11G3, recognize epitopes related to this genetic polymorphism. Direct ELISA and ELISA inhibition experiments with different LDL of known phenotype showed that D2E1 is directed against the allelic epitope c and H11G3 against d. The 2 antibodies were used for the characterization of LDL in sera from 38 blood donors and the results compared to those obtained by passive hemagglutination inhibition using human allotypic antisera. Accordingly, sera from homo- and heterozygous donors displaying the relevant epitope could be distinguished from homozygous sera lacking the epitope.

Real-time video intensification fluorescence microscopy of the dynamics of the N-formyl chemotactic peptide receptor on human neutrophils

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The fate of reverse-phase HPLC-purified tetramethylrhodamine-conjugated N-formyl chemotactic hexapeptide (TRITC-FNLPNTL) in human neutrophils was followed by video intensificaiton fluorescence microscopy using the thermostated inverted Leitz Diavert microscope connected to the highly sensitive camera NTV-LT3/3M.

Light transmission and fluorescence images were stored on video tape. Binding of 0.5 nM TRITC-FNLPNTL was observed without washing the cells (real-time analysis). The results are consistent with initital homogeneous binding of TRITC-FNLPNTL to surface receptors and subsequent accumulation of receptors at the tail. Asymmetric redistribution of ligand-receptor complexes was observed during the process of polarization, followed by internalization.

I investigated in detail the effects of cytoskeleton-affecting reagents (cytochalasin B, colchicine, monoclonal antibody NMS-1) on neutrophil shape and receptor dynamics. Modulation of shape and receptor processing by TRITC-FNLPNTL in the presence of PMA, LTB4, and C5a will also be reported.

The cytoskeleton of *Trypanosoma brucei*

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The cytoskeleton of the parasitic hemoflagellate *Trypanosoma brucei* contains three major structural components. 1) a densely packed layer of singlet microtubules which are in tight contact with the cell membrane (subpellicular microtubules), 2) the flagellar axoneme, and 3) the paraflagellar rod, a highly ordered protein structure in the falgellum.

Considering the presence of multiple tubulin genes in the genome of trypanosomes (see presentation of Imboden et al.), we have analyzed the microheterogeneity of trypanosomal tubulins. Only two isoforms a α-tubulin have been found, one representing the primary translation product (α1), while the second is an acetylated derivative thereof (α3). Both isoforms can be post-translationally modified by a reversible tyrosinolation of their carboxy-termini. Stable microtubules, e.g. those of the flagellar axoneme, contain a high proportion of acetylated α-tubulin. Abundance of C-terminally tyrosinolated αtubulin is a marker for newly formed microtubules.

Two microtubule-associated proteins which are presumably involved in mircotubule/membrane contacts are presently under investigaiton. The gene for one of them, p60, has been isolated and is being sequenced. The two structural proteins of the paraflagellar rod (72 and 68 kDa) have been isolated and are being characterized. Their genes have been isolated and their analysis is under way.

Putative enhancer elements upstream and downstream from the pseudorabies immediate early gene

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The pseudorabies (PRV) immediate early (IE) gene encodes a protein (180 kD) that is required for the onset of early transcription and that represses its own transcription unit while acting as a potent trans-activator of other genes (about 10-fold stronger than the Ela protein of adenovirus). To gain some insight into the regulation of IE gene expression, we sonicated a plasmid harboring the PRV IE gene and cotransfected it into monkey cells, togehter with nonviable linear SV40 DNA lacking its enhancer. After several weeks, the cells lysed due to growth of recombinant SV40. Sequence analysis of two such clones (SVPR-3 and SVPR-4) showed that each of them contains a segment originating from the PRV genome, now integrated upstream of the SV40 T-antigen gene and thus replacing the missing SV40 enhancer. Surprisingly, the two segments turned out to have unrelated DNA sequences and they map 2 kb downstream and 5 kb upstream from the PRV IE gene, respectively. The influence of these sequences of gene expression is under investigation.

Genotype-phenotype relationships in heat-sensitive mammalian proliferation mutants

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Cells of a subline of the P-815-X2 mastocytoma were mutagenized with MNNG, subjected to a selection protocol resulting in cell populations containing at lest 100 heat-sensitive (hs, arrested at 39.5 °C, multiplying at 33 °C) mutant clones, and used for isolation of 40 subclones. Of these, 9 exhibited a relative number of revertants below 1.5×10^{-7}. Incubation of these hs mutants at the nonpermissive temperature resulted in an accumulation of cells with a DNA content typical of Gl phase. Complementation analysis by cell fusion indicated that these 9 hs mutants belonged to 4 different complementation groups. These differed from each other with respect to phenotypic characteristics such as kinetics of entry into and exit from the arrested state after changing the incubation temperature, relative position of the execution point within Gl phase, and morphological mast cell differentiation, 5-hydroxytryptamine content and changes in cell size after transfer to 39.5 °C.

DNA structure in recombination intermediates

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Upon binding to double- or single-stranded DNA in the presence of ATP or ATPγS, recA protein shapes DNA into a helical structure with 18 base pairs or nucleotides per turn and an average spacing between bases of 5.1 A. Such recA-DNA complexes are found with single strands during the presynaptic stage of recombination. The study of the DNA structure during the

actual pairing process has been hampered by the inability to separate the pairing stage from the subsequent stage of branch migration. The recent finding that int he presence of ATPγS the recombination reaction stops at the stage of the initial pairing without branch migration opens the possibility to study the DNA structure in joint molecules. We have isolated such recombination intermediates and we will present their properties.

Biosynthesis and processing of secretory component (SC) in human intestinal epithelial cells

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Biosynthesis and cellular processing of SC has been studied in organ culture of small intestinal mucosa by labelling with L(^{35}S)-methionine. Mucosa was homogenized, solubilized and immunoprecipitated SC analyzed by SDS polyacrylamine-gel electrophoresis and fluorography. The first detectable form of SC had an apparent molecular weight of 97 kDa. Within 30 min of chase a new, endo-H-sensitive form of 105 kDa appeared and the initial translation product disappeared. After one hour of chase an endo-H-insensitive form of 120 kDa was observed and two additional species of 85 kDa and 64 kDa became visible after 180 min. The appearence of the lower molecular weight species could be prevented by the addition to the culture medium of the proteinase inhibitor leupeptin.

In human small intestinal epithelial cells, processing of SC is therefore different to that described in other tissues in that an additional proteolytic cleavage of the precursor SC takes place.

Regulation of nucleosome positioning in chromatin might correlate to gene activity and replication

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Functional processes on DNA such as transcription, replication, repair or recombination require accessibility of DNA sequences. Functional proteins have to compete with packaging proteins of nucleosomes, the histones. I have used recombinant yeast plasmids to study mechanisms in vivo by which nucleosomes are arranged on the DNA. 1) Protein-DNA (in particular histone-DNA) interactions play an important but not sufficient role. The determining part may be bending properties of DNA sequences. 2) I have been able to alter nucleosome positions on given DNA sequences by changing flanking structures. These flanking regions consisted of nucleosomal structures or nuclease-sensitive regions at 5' and 3' ends of genes or around a putative origin of replication. These structures therefore were dominant over protein-DNA interactions.

Furthermore, long linkers and tightly packed nucleosomes were created. This demonstrates that chromatin is much more complicated than a simple alignement of nucleosomes.

Messenger RNA binding in eukaryotic protein synthesis initiation

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We are studying the function of protein factors which mediate mRNA binding to ribosomes during initiation of eukaryotic protein synthesis. We have purified factors involved in mRNA 5' cap recognition from the yeast *Saccharomyces cerevisiae* and from mammalian cells and cloned their cDNAs and genes. Using a 'plasmid shuffling' technique we are currently trying to introduce in vitro temperature-sensitive mutations into the cloned yeast initiation factor gene (factor eIF-4E) in order to study the functions of this protein synthesis factor in vivo. In addition, we are characterizing a multigene family encoding a mammalian (mouse) mRNA binding factor (factor eIF-4A). We have identified several pseudogenes, an intron-containing gene and an unmutated intronless 'pseudogene'. We are now investigating whether this intronless gene is expressed in mouse cells together with the intron-containing gene.

Polyoma HR-T mutations affect viral and host gene expression

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Polyoma host range, transformation negative (hr-t) mutants synthesize wild-type large T-antigen and mutated small and middle T-antigens. we showed previously (J. Virol. 53: 579–586, 1985) that the onset of virus-induced S-phase is delayed in mouse cells infected with hr-t mutants as compared to wild-type infection. By measuring steady state levels of viral and some selected mouse mRNAs at different times after virus and mutant infection we found that viral early transcription is not altered by hr-t mutations but that synthesis of viral late mRNAs is delayed and less efficient even in absence of viral DNA replication. Since the increase in mouse dehydrofolate reductase mRNA is similar in wild-type and mutant infected cells and since the onset of S-phase measured by increasing histone H4 mRNA is delayed by several hours, we conclude that large T-antigen alone is able to activate enzymes required for S-phase and that small and/or middle T-antigens contribute to the actual initiation of host and viral chromatin duplication.

Plant 2',3'-cyclic nucleotide 3'-phosphodiesterases

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Enzymes hydrolyzing 2',3'-cyclic nucleotides to nucleoside 2'-phosphates (2',3'-cyclic nucleotide 3'-phosphodiesterases; CNPases) occur in plants and animals; their metabolic function is largely not understood. The only enzyme with known function is the RNA ligase-associated CNPase involved in tRNA splicing and viroid circularisation in plants; it is responsible for conversion of RNA 3'-terminal cyclic phosphate to 2'-phosphate during RNA ligation reaction (reviewed by Filipowicz and Gross, TIBS *9* (1984) 68). We have purified to homogeneity from wheat germ another protein with CNPase activity. This enzyme of M_r 24000 acts on 2',3'-cyclic mononucleotide substrates but not on cyclic phosphate-terminated oligonucleotides. This is in contrast to plant ligase-associated CNPase which preferentially uses latter substrates. The enzyme characterized is the only known CNPase active solely with 2',3'-cyclic mononucleotides.

U2 snRNA gene family of *Arabidopsis thaliana*

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U2 small nuclear (sn)RNA, together with other snRNAs, participate in pre-mRNA splicing in animal cells. Reported differences in mRNA splicing between plants and animals and peculiar transcription features of snRNA genes in animals prompted us to study U2 RNA gene family in plant *Arabidopsis thaliana*. Five different U2 RNA genes were cloned and their coding and flanking regions sequenced. 5'halves of coding regions are 100% conserved in all five genes while 3'halves differ by point mutations. 5'halves are also highly conserved between *Arabidopsis* and animal U2 RNAs, while 3' halves are not. Upstream se-

quences between cap site and position −80 show 80–90% conservation. Contrary to animal snRNA genes, TATA homology is present at position −30. Transient expression of cloned genes is being tested in electroporated plant protoplasts.

Psoralen crosslinking of DNA as tool for the analysis of chromatin in action

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The DNA in purified soluble rat liver chromatin and in Hl-depleted chromatin was photocrosslinked in the presence of psoralen at different pH and ionic strength. After purification the DNA was examined by electron microscopy under denaturing conditions. DNA from Hl-containing chromatin appears as rows of single-stranded bubbles 140–160 nucleotides in size (nucleosomal bubbles), independent of pH and ionic strength. However, DNA from Hl-depleted chromatin is fairly continuously crosslinked at pH 10 and very low ionic strength, conditions under which nucleosomes are partially unravelled, but not dissociated. The effect of unravelling is reversible by raising the ionic strength. Thus, crosslinking of DNA in chromatin at very low ionic strength and pH 10 allows to distinguish between Hl-containing and Hl-depleted chromatin by analysing the bubble pattern in the denatured spread DNA. This is of particular interest in replicating and transcribing chromatin regions.

Differences in mRNA splicing specificity between plants and animals

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We are investigating the basis of reported differences in mRNA splicing specificity between plants and animals (Barta et al., Pl. molec. Biol. *6* (1986) 347). Following experiments were performed: 1) Splicing of soybean leghemoglobin pre-mRNA (3 introns) was studied in HeLa cell system in vitro and in vivo. Only intron 2 was processed in vitro; processing was efficient and correct. In transfected cells, in addition to correct processing of intron 2, the 5' splice sites of introns 1 and 3 were cleaved efficiently, but correct processing at the 3' sites took place only rarely (intron 1) or not at all (intron 3); instead cryptic sites were used. 2) Splicing of leghemoglobin and human β-globin mRNA was asseyed in plant protoplasts transfected by electroporation. All leghemoglobin introns were correctly spliced in this system but β-globin intron 1 was not. Possible reasons for this specificity will be presented.

Effects of heparin on migration, proliferation and protein synthesis of cultured human endothelial cells (HEC), smooth muscle cells (HSMC) and dermal fibroblasts (HF)

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Effects of heparin and some glycosaminoglycans (GAG) on migration, growth and protein synthesis of HEC, HSMC from umbilical cords and HF were studied. Cultured cells maintained typical morphology. Heparin inhibited migration and growth of HSMC and HF dose-dependently (half-maximally with 1–2 and 10–20 µg/ml respectively). No effect was seen with HEC. Heparin did not influence prostacyclin release from HEC and HSMC during growth. It induced 2 proteins with molecular weights of about 35000 and 38000 in HSMC and in addition a 40000 moiety in HF. Studies with tunicamycin showed that some of the induced proteins were glycosylated. Protein patterns were different with dextran sulfates and carrageenans. Chondroitin sulfate and hyaluronic acid had no effect. No change in secreted proteins was seen with HEC. The results support the concept that heparin-like GAGs may regulate the growth of vascular smooth muscle cells in vivo.

The metallothionein gene as a model system to study the enhancer effect

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We have previously shown that the upstream region of the mouse metallothionein-1 gene contains a metal-inducible enhancer that is able to replace the enhancer of Simian virus 40 (SV40) resulting in a heavy metal-dependent virus (Serfling, Lübbe, Dorsch-Häsler and Schaffner, 1985, EMBO J. 4, 3851–3859). We have constructed a number of artificial enhancers using synthetic oligonucleotides. For example an 18 bp oligonucleotide containing one MRE (metal responsive element; Stuart, Searle and Palmiter, 1985, Nature 317, 828–831) acts as a metal-inducible enhancer when positioned in tandem copies either upstream or downstream of a globin test gene. This sequence also contains a consensus binding site for the transcription factor Spl (Kadonaga, Jones and Tjian, 1986, Trends Biochem. Sci. 11, 20–23). Analysis of mutants indicates that both MRE and Spl site are important components of this inducible enhancer. Therefore, Nature seems to achieve a given task (strong heavy metal-dependent transcription) by combining cis-acting elements of different functions.

$^{35}SO_4$-MPS-secretion from cultured mesenchymal cells stimulated by demineralized bone matrix (DBM)

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DBM when implanted under the skin or intramuscularly induces the formation of cancellous ossicles. After invasion of DBM by mesenchymal cells, chondrocytes are differentiating by the action of cytokines and cartilage specific proteoglycans are formed in vivo. We have studied the synthesis and the secretion of $^{35}SO_4$-MPS from DBM stimulated cultured human embryonic mesenchymal cells. DBM significantly increased proteins and DNA content of the culture by 30% with a lag phase of 24 h. Secretion of $^{35}SO_4$-MPS was significantly increased by up to 50% in a dose dependent manner while intracellular $^{35}SO_4$-MPS did not. Extracts from DBM were active in stimulating $^{35}SO_4$-MPS secretion into the medium but had no effect on cellular growth. This soluble effector was heat stable and pH resistant. We conclude that $^{35}SO_4$-MPS-secretion from embryonic mesenchymal cells is a rapid and reliable method for measuring the cytokine effect of DBM.

Spontaneous fluctuations of cytosolic calcium in pituitary cells monitored by dual excitation microfluorimetry

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Cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$, in GH_3 cells, when monitored with the fluorescent probe fura 2 shows spontaneous fluctuations. In regular intervalls averaging 8.4 sec, $[Ca^{2+}]_i$ rises with a very rapid onset to a peak value reached within less than 100 msec. $[Ca^{2+}]_i$ then returns exponentially to prespike levels. Peak values calculated for $[Ca^{2+}]_i$ are beyond the threshold for

$[Ca^{2+}]_i$ to trigger prolactin secretion. Spiking activity, which most likely reflects spontaneous electrical activity, can be blocked by Ca^{2+} channel blockade, and, more interestingly, by somatostatin, a hypothalamic peptide inhibiting pituitary hormone secretion. These data explain basal secretory activity of pituitary cells as well as its inhibtion by somatostatin. Furthermore these findings indicate that the regulation of pituitary cell activity has to be viewed in terms of the modulation of the mechanisms generating $[Ca^{2+}]_i$ spikes.

Tunicamycin inhibits transepithelial Na^+ transport in parallel with the cellular Na,K-ATPase pool

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The effect of the glycosylation inhibitor tunicamycin(TM) on transepithelial Na^+ transport(short-circuit current SCC) was studied in toad urinary bladder cells (TBM cells) grown on collagen-coated filters. SCC was not affected by 0.1–0.3 µg/ml of TM but decreased by 30% within 21 h with 1–5 µg/ml. The fall in SCC was accompanied by an equal 30% decrease in the cellular pool of Na,K-ATPase most likely brought about by a specific decrease by 65% in the biosynthesis rate of its catalytic alpha-subunit related to the coreglycosylation inhibition of the beta-subunit. The specific inhibitory effect of lug/ml of TM on SCC as well as on the Na,K-ATPase level could not be reversed within 24 h. However, both control and TM-treated cells responded to a medium change with a 2-fold increase in SCC indicating that TM had no general toxic effects. Thus, TM seems to be a valuable tool to modulate the Na,K-ATPase pool in order to better define its relative importance in the Na^+transport event.

Cloning of lactate dehydrogenase genes from thermophilic and mesophilic bacteria

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Amino acid sequence comparisons between homologous enzymes from thermophilic and mesophilic organisms as well as protein engineering techniques on the DNA level are being used to identify the structural features conferring thermal stability to proteins. In this context, we have cloned the genes for lactate dehydrogenase (LDH) from *B. megaterium* (mesophilic), *B. stearothermophilus* and *B. caldolyticus* (thermophilic) in *E. coli* using a pEMBL vector and synthetic oligonucleotide probes. The coding sequence of *B. stearothermophilus* LDH shows 61% homology to the one of *B. megaterium* (amino acid homology 61%) and 99% to the one of *B. caldolyticus* (amino acid homology 97%). As expected, the GC content of the 3rd base positions of the codons is much higher for the genes of the termophilic strains (31% vs 58%). The cloned genes are giving rise to considerable levels of the heterologous enzymes in *E. coli.*

Genetics

Vaccinia virus produces late mRNAs by a discontinuous mechanism

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In all vaccinia virus late genes that have so far been analyzed, the 5' end of the mRNA appeard to map within only a few nucleotides from the ATG translation initiation codon. Primer extension analysis of a major late mRNA, however, showed that its 5' end did not coincide with that defined by S1 mapping. Analysis of cDNA clones demonstrated that the extra sequences upstream from the late gene coding region differed between individual clones. Furthermore, these sequences mapped to different parts of the vaccinia virus genome and for one cDNA clone were separated from the late gene by more than 100 kb. Nucleotide sequence analysis revealed the presence of long poly(A) stretches immediately upstream from the late gene coding region.
We conclude that vaccinia virus produces late mRNAs by fusing the protein coding sequences to the 3' end of other polyadenylated RNAs.

Complexes of recA protein with DNA: the accessibility of DNA to dimethylsulfate

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Homologous recombination is proposed to be initiated by a region of heteroduplex. The recombinase of *E. coli*, recA protein, can make heteroduplexes in vitro starting from two homologous DNA molecules. The reaction can be dissected in three steps: 1) covering of one partner with recA to a presynaptic complex, 2) assimilation of the other DNA (synapsis), 3) strand exchange and branch migration.
The presynaptic complex can be formed separately (structure described in Stasiak et al. CSHSQB 47, 811 (1983)). We expect the DNA to be accessible to recognition for homologous alignment by the incoming partner. The accessibility was tested with the small reagent dimethylsulfate: N7 of guanine (major groove of DNA) was found to be more reactive than in naked DNA, N3 of adenine (minor groove) was found protected, and N3 of cytosine (hydrogen-bond in base-pairing) is accessible only in complexes with single-strands.

Validation of an efficient in vivo genotoxicity test with somatic cells of *Drosophila*

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In the *Drosophila* wing spot test larvae trans-heterozygous for the recessive markers on chromsome 3 are exposed to test compounds. Spots on the wing expressing phenotypically the markers are indicative of genotoxic effects (Würgler F. E. and E. Vogel, in F. J. de Serres (ed.) Chemical Mutagens, Vol. 10, Plenum Press, New York (1986) p. 1–72). In *Drosophila* larvae promutagens can be activated by cytochrome P-450 dependent enzyme systems. Optimizing the metabolic activities, e.g. by altering the genetic control of the P-450 systems could improve the test performance. Therefore first and second chromosomes of the test strains were substituted by those of a wild type strain with constitutively increased cytochrome P-450 activities linked to a locus on the second chromosome. Diethylnitrosamine which is activiated by cytochrome P-450 dependent alpha-C-hydroxylation show increased toxic and genotoxic effects in the new strains. Work supported by Swiss Cancer League.

Sequential expression of Pgk-1 in the preimplantation mouse embryo

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Pgk-1 is the X-chromosome linked gene that codes for the glycolytic enzyme phosphoglycerate kinae. Pgk-1 expression in the early mouse embryo is an interesting model, as it was shown

previously that the maternally (Pgk-lm) and paternally (Pgk-lp) inherited Pgk-1 loci are first activated at different stages of development[1]. We therefore propose a parental imprinting for this gene. To determine whether Pgk-lm is activated in the whole embryo at the same time or turned on asynchronously in the trophectoderm and inner cell mass (ICM); we have measured Pgk activities in whole blastocysts and in ICMs that were isolated by microsurgery with the calcium ionophore A 23187. Our results show that Pgk-lm is activated in the trophectoderm but not yet in the ICM of blastocysts at day 4 and 5, as evidenced by a Pgk activity increase in the trophectoderm and a Pgk activity decrease in the ICM.

[1] Krietsch, W., et al., Differentiation *23* (1982) 141.

Polymorphism of the *Mx* locus in mammalian species

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Mx is one of the interferon-activated genes responsible for the multiple effects of interferons (IFNs). In the mouse, the Mx^+ allele located on chromosome 16 encodes *Mx* protein which is necessary and sufficient for resistance towards influenza virus. Mx^- alleles of laboratory inbred strains are derived from Mx^+ presumably by deletions. We have studied the degree of genetic polymorphism of the *Mx* locus by genomic Southern blot analysis of DNA from various laboratory and wild mice as well as from other species using Mc cDNA probes. No RFLPs were observed with Eco RI, Hind III and Bam HI between DNA from various inbred and wild Mx^+ mice. Two distinct patterns among Mx^- mice were observed. The BALB/cJ pattern was found in 32 inbred strains and the CBA/J pattern in 3 inbred strains. Additional RFLPs were observed with DNA from wild M. musculus and other species of the genus *Mus*. Furthermore, *Mx* specific hybridization signals were obtained with genomic DNA from rat, hamster, pig, horse, cattle, and man.

Analysis of equine MHC genes for restriction fragment length polymorphism

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Class I Equine Leukocyte Antigen (ELA) loci within the Major Histocompatibility Complex of the horse were examined using the pH-2IIa mouse cDNA probe. Genomic DNA from peripheral lymphocytes was digested with various restriction enzymes, subjected to agarose gel electrophoresis and Southern blot hybridization analysis performed. Of the more than 24 restriction fragments detected, approximately half were common to different serotypes, consistent with their belonging to Qa/Tla-like genes. Among the remaining bands, which were highly polymorphic, certain were identified which could apparently be assigned to particular ELA serotypes. Southern blots were successfully rehybridized with MHC class II cDNA probes. Distinct polymorphism was exhibited amongst the smaller number of bands observed.

Structure and function of the sex-determining gene *tra* of *Drosophila*

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The primary signal for sex determination in *Drosophila* is the ratio of X-chromosomes to sets of autosomes. A few regulatory genes mediate this signal down to the sex differentiation genes which produce the sexual dimorphism. One of these genes, transformer *(tra)*, is required for female development; lack of function results in male development. The gene *tra* is involved in the regulation of doublesex *(dsx)*, the last gene in the hierarchy. To understand the genetic control of the sexual pathway, we isolated the *tra* gene and studied its structure and function.
By P-mediated transformation we could delimit the gene to 3.8 kb. We found five transcripts, only two of which are female specific; they are transcribed from a DNA region of 2 kb.

Genetic approach to identify trans-acting factors of plasminogen activator gene in LLC-PK_1 cells

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In cultured pig kidney epithelial cells. LLC-PK1, the urokinase type plasminogen activator (uPA) gene is activated by treatment with a peptide hormone, calcitonin, or a tumor promoter, phorbol ester. Activation is independent of protein synthesis, indicating that the uPA gene is a primary target of these inducers in the chromatin. To identify the components mediating the action of these inducers, we undertook a new genetic approach. A uPA-gpt hybrid gene, which combines 5 kb of the 5'flanking region of the uPA gene with the coding region of the *E. coli* XGPRT gene, was constructed and transfected into LLC-PK1 cells. A stable transformant cell line, A1, in which teh induction of uPA-gpt hybrid gene and of the resident uPA gene was under hormonal control, was obtained. Then, A1 cells were mutagenized with ethylmethane-sulfonate and screened for constitutive expression of the uPA-gpt hybrid gene. Of 25 separate clones that survived in the medium containing mycophenolic acid and xanthine, 10 clones had constitutively elevated levels of uPA mRNA. We infer that these clones have mutations affecting the activity of trans-acting factors that recognize both uPA-gpt hybrid and resident uPA genes. Further characterization of these mutant cells will be reported.

Mode of IS*21* transposition

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The broad-host-range plasmid R68.45 is widely used as a mobilization system in many gram-negative bacteria. R68.45 carries two copies of the insertion element IS*21* as a tandem repeat. This duplication allows the plasmid to form cointegrates with other replicons at high frequency whereas the parental plasmid R68, which carries a single IS*21* element, is less active in cointegrate formation. To study $(IS21)_2$-mediated transposition we subcloned this duplication into pBR325. Insertion of the Ω element into either IS*21* copy showed that the left IS*21* element (next to the kanamycin resistance gene in R68.45) was 100 × more active in cointegrate formation than was the right element. A truncated form of $(IS21)_2$ lacking the outer end (ca 50 bp) of the left element and most of the right element but retaining the junction of $(IS21)_2$ was still active in cointegrate formation. Thus, the transposase preferentially recognized the IS*21* ends at the junction of the IS*21* duplication. To explain the cointegrate structures we propose a conservative mechanism for IS*21*-promoted replicon fusion.

Pharmacology

Purification of human interferon gamma receptors by sequential affinity chromatography on immobilized monoclonal anti-receptor antibodies and human interferon gamma

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We have raised monoclonal antibodies against human interferon gamma receptors with the following properties: they inhibit the binding of ^{125}I-labelled recombinant human interferon (rhuIFN) gamma to cell surface receptors; they bind to the surface of IFN gamma receptor expressing human cells but not to heterologous cells; this binding is inhibited competitively by addition of rhuIFN gamma. A Triton X-100 extract of a membrane enriched fraction of human Raji cells was affinity purified with these monoclonal antibodies and the eluates from such columns were further purified on immobilized rhuIFN gamma. SDS-PAGE of the final eluate reveals two major protein bands with approximate M_r of 92000 and 50000 respectively. Both proteins are able to specifically bind ^{125}I-labelled rhuIFN gamma upon electroblotting to nitrocellulose and probably carry the ligand binding site of huIFN gamma receptors.

Mice interstrain differences in [8-^{14}C] caffeine metabolism

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Toxicity of xenobiotics and drugs is related to their plasma concentrations and thus dependent of the dose, the absorption and the elimination kinetics of the compound. This explanation did not seem valid for interstrain differences in caffeine toxicity reported in mice because similar plasma concentrations and pharmacokinetics parameters were observed (J. M. Carney et al., Neuroscience Let. *56* (1985) 27). As it is known that the accumulation of metabolites can contribute to toxic effects of the administered product, interstrain differences in the metabolic pathways of caffeine has been evaluated. For the complete identification of metabolites, ring labeled caffeine was synthesized and administered orally to 7 mice of CBA/J, SJL/J, A/J and SWR/J strains. Metabolic balance of radioactivity showed similar recoveries in expired CO_2 (1–2%), fecal (7–12%) and urinary (73–89%) excretion. Analysis of the metabolites in urine showed a significantly lower excretion of polar compounds in CBA/J strain when compared to the other strain (9% vs 25%). Most of the polar compounds correspond to the newly identified paraxanthine glucuronide.

These results suggest that the higher toxicity of caffeine in CBA/J strain could be due to an increase of paraxanthine plasma concentration resulting from a genetic defect of paraxanthine glucuronidation.

Modulation of catecholamine output of the rat adrenal medulla by muscarinic receptors

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Left adrenal glands of male Wistar rats were perfused from the venous side with oxygenated Krebs bicarbonate sol. at 0.2 ml/min at 36 ± 1 °C. The gland was stimulated with increasing concentrations of cholinergic agonists, during 30 sec. at 4 min. intervals · 1 min portions of perfusate were collected and catecholamines were determined in 100 µl aliquots by reversed phase HPLC-EC. At low concentrations (10^{-6} to 2×10^{-5} M) carbachol increased adrenaline, but not noradrenaline output. Stimulation with nicotine equally increased noradrenaline and adrenaline output. Omission of Ca^{++} from perfusion fluid during carbachol stimulation abolished the noradrenaline, and decreased the (85%) adrenaline output. 10^{-5} atropine abolished the prevalence of adrenaline over noradrenaline output at low carbachol concentrations. In contrast 3×10^{-5} M hexamethonium enhanced the prevalence of the adrenaline output.

The results indicate that in the rat adrenal medulla adrenaline containing chromaffine cells are selectively stimulated by muscarinic receptors.

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GC and GC-MS methods without derivatisation for routine phenotyping with dextromethorphane and mephenytoin

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The debrisoquine/spartein/dextromethorphane and mephenytoin types of oxydation by the cytochrome P-450 of the liver are two distinct genetic polymorphisms. The aim of the present study was to adapt gas chromatographic and mass spectrometric methods for the determination of dextromethorphane (D), dextrorphane, mephenytoin (M) and 4-OH-mephenytoin (4-OH-M) in urine samples after simultaneous phenotyping of subjects with 25 mg D and 100 mg M. In contrast with previous procedures, no derivatistion of M and 4-OH-M was necessary by using SE-54 fused silica capillary columns. Quantitative analysis is performed either by means of a NP-detector or EI-MS. As this technique allows also the determination of M in urine, either the hydroxylation index or the metabolic ratio can be used for the calculation of the phenotype. The method was tested by phenotyping three times seven healthy subjects: 1. dextromethorphane, 2. mephenytoin, 3. dextromethorphane+mephenytoin. The results show that there is neither a pharmacokinetic nor an analytical interference between the two test substances. The mass spectrometric procedure has been developed for phenotyping subjects who are under treatment with other psychotropic drugs which may interfere in the determination of the test compounds.

Pharmacokinetics of a vasodilator drug, pipratecol, in the dog

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Pharmacokinetic study of pipratecol (PIP) (dihydroxy-3,4 phenyl)-1 [(methoxy-2 phenyl)-4 piperazinyl-1]-2 ethanol in 4 male Beagle dogs after oral admin. of 30 mg to the fasted animal. Blood levels were determined by HPLC (RP-18, 12 cm × 4 mm ∅, 45% acetonitril phosphate buffer 0.025 M, pH 3, 1 ml/min, 120 kg/cm^2 with electrochemical detector+1,150 V, r.t. 2', 0.01 µg PIP detection limit) at 5', 15', 30', 45', 1, 2, 3, 4, 5, and 6 h after adm. Peak plasma levels measured at 45' and 1 h with 221–574 µg/ml. 6 h value about 0.05 µg/ml. One-compartment open model was used. K_a value: 4.22 h^{-1}, absorption t½: 10', K_e 0.332 h^{-1}, biological t½: 1.65 to 2.48 h; V_D 105 L, mean total clearance 0.559 L/min. Pharmacokinetic data indicates that after a rapid absorption phase PIP is eliminated with a mean t½ of 2 h. The high V_D values might be explained by vasodilator properties. The chemical structure of PIP suggests substantial glucuronic acid conjugation. Plasma level values can be related to the rapid vasodilator effect of the drug.

4-Aminopyridine (4-AP) derivatives in rat hippocampal slices

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4-AP blocks the early, transient K current IA and enhances synaptic transmission in rat hippocampal slices (J. Physiol. 326, 109). We have now tested several analogues in this preparation on the following parameters: Fibre potentials were broadened and their refractory period was markedly prolonged. Extracellular field potentials (e.p.s.ps) from the dendritic area of pyramidal neurons in the CA 1 area were increased. Paired pulse facilitation of these e.p.s.ps was reduced, indicating a presynaptic site of action. The derivatives showed the same order of potency that was previously found at peripheral synapses: 3,4-DAP > 3-Methyl-4-AP (3-Me) > 3-Methoxy-4-AP (3-MeO) > 3-N-Methyl-4-AP (3-NMe) > 3-Ethyl-4-AP (3-E). The effect of 3,4-DAP was antagonized by the weaker compounds 3-E and 3-NMe. The probability to elicit long term potentiation, which is possibly related to memory processes, was not reduced in the presence of the 4-AP analogues. This makes the involvement of A current modulation in LTP unlikely.

Basis of the differences in transport of N^1-methyl-nicotinamide (NMN) and tetraethylammonium (TEA) by the rabbit renal proximal (PT) S_2 segment

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In the rabbit in vivo, the PT active transport of NMN is known to be less efficient than that of other organic cations, like TEA. When investigated in vitro, the saturable intracellular accumulation of NMN across the basolateral membrane in isolated non-perfused PT showed an uptake maximum of 2094 fmoles/nl tissue water (TEA: 10000) and an apparent K_m of 69 µM (TEA: 80 µM), with a maximum tubule/medium concentration ratio of 16 (TEA: 160). In the perfused PT, the saturable transcellular net secretory transport showed an overall apparent V_{max} of 58 fmoles/mm.min, with an apparent K_m of 17 µM (TEA: 2480 and 67, respectively). No net reabsorption of NMN was observed. Therefore, both the basolateral and the luminal steps of NMN PT secretion appear less efficient than those of TEA, suggesting a smaller amount of cation transporters accessible to NMN, and/or a higher basolateral passive NMN backleak after intracellular accumulation.

Effect of acute renal failure (ARF) on ciclosporin (Ci) kinetics in dogs

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Although Ci is metabolized by the liver, its disposition may be altered by renal failure, as suggested by the inverse relationship between plasma creatinine (Cr) and Ci through blood concentrations observed in renal transplanted patients (Proc. EDTA 1984, 21: 1002). We investigated the effect of ARF on Ci kionetics in 8 previously uninephrectomized dogs. Ci (10 mg/kg) was infused i.v. before, during and following recovery from acute tubular necrosis induced by a 1-h warm ischemia. At the time of the study, Cr levels ranged from 175 to 730 µmol/l ($\bar{x}$ 479). Blood levels were monitored over 30 h and Ci measured in whole blood by RIA. The systemic clearance (Cl) of Ci increased from 3.9 ± 0.5 ml/kg·min before to 4.4 ± 0.4 during ARF and returned to 4.1 ± 0.6 after recovery. T½ did not appreciably change (12.1 vs 11.5 vs 12.0 h). The volume of distribution was slightly lower during ARF (2.6 ± 0.1) than before (2.8 ± 0.2) and after (2.9 ± 0.3 l/kg).

In conclusion, Cl of Ci is altered in ARF, probably by a stimulation of the hepatic oxidative metabolism.

Biochemical and pharmacological properties of molindone and buspirone, two presynaptic dopamine (DA) receptor antagonists

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Molindone and buspirone are preferential blockers of presynaptic DA receptors. In clinic, molindone displayed antipsychotic and antianxiety properties. Buspirone main's indication is anti-anxiety, whereas antipsychotic effectiveness has not been really investigated. Both compounds were studied in a large test battery, in vitro and in vivo, assessing biochemical and behavioral parameters, and receptor-interaction. Molindone as well as buspirone interacted potently in vitro with striatal D_2 receptors and in a release model on striatal slices. They displayed a preference for presynaptic DA receptors with similar potency. Both compounds inhibited in vivo (^{3}H)spiperone binding to D_2 sites in the same dose-range. However, whereas buspirone showed a marked preference for hippocampal DA receptors, molindone did not, but this drug shared a double sigmoidal dose-response curve. In cluclusion, both compounds displayed very similar effects on central DA receptors, arising the question of whether anti-anxiety properties are associated with this property, or, to other effects such as interaction with 5-HT_{1A} receptors.

Noradrenaline (NA) turnover in hippocampus (HIP); effect of vincamine (V) and hydergine (H)

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Idazoxan (I), haloperidol (Hal) V and H all increase the firing of locus coeruleus (LC) Naergic neurons. This should increase the NA turnover in Hip. The dopamine (DA) metabolite DOPAC, variations of which reflect NA turnover in Naergic areas was determined in Hip. The DA-β-hydroxylase inhibitor FLA63 (40 mg/kg i.p.) increased DOPAC to 237 ± 16%, the DOPAC/DA ratio (D/D) being reduced to 89 ± 10%. I, 10 mg/kg i.p., increased DOPAC to 241 ± 24%, D/D being unaffected. The effects of FLA63 and I were additive. Associated with FLA63, I (1 mg/kg i.p.) increased DOPAC to 171 ± 14% of FLA63, Prazosin (P) (5 mg/kg i.p.) and Hal (1 mg/kg i.p.) being ineffective; D/D was increased by P and Hal but not by I. Instead of the minor DA input to Hip, alterations in Hip DOPAC indeed reflect changes in NA turnover. The data demonstrate that V (10 mg/kg i.p.) and H (10 mg/kg i.p.) do not affect NA turnover although they increase LC firing, in contrast to I.

Halogenated hydrocarbons and halogenated ethers increase thiopental binding to human serum albumin

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It has been shown already that halothane (I) increases thiopental binding to human serum albumin (Büch et al., Acta pharmac. toxic., Suppl. V, No 547, 1986). Question arose whether other inhalational anesthetics as enflurane (II) and isoflurane (III) influence thiopental binding analogously; with regard to the structure activity relationship also experiments with chloroform (IV), carbon tetrachloride (V), methoxyflurane (VI), diethylether (VII) and ethanol (VIII) were from interest. Equilibrium dialysis in 1/15 M phosphate buffer solution (pH 7.4) was car-

ried out as described elsewhere (Büch et al., J. Pharmac. exp. Ther. *175* (1970) 709). $0.04 \cdot 10^{-3}$ M thiopental and 1% human serum albumin were added in one of the dialysis chambers, whereas I ($4.7 \cdot 10^{-3}$ M), II ($4.1 \cdot 10^{-3}$ M), III ($4.1 \cdot 10^{-3}$ M), IV ($6.3 \cdot 10^{-3}$ M), V ($5.2 \cdot 10^{-3}$ M), VI ($4.3 \cdot 10^{-3}$ M), VII ($4.9 \cdot 10^{-2}$ M) or VIII ($8.9 \cdot 10^{-2}$ M) were given in both chambers. Thiopental concentration was assayed by HPLC in samples of both dialysis chambers after equilibrium was reached. Results are presented in the table.

$\bar{x} \pm$ S.D.	contr.	I	II	III
% bound	41 ± 3	55 ± 2*	45 ± 3*	50 ± 4*
n	79	13	8	8
IV	V	VI	VII	VIII
53 ± 3*	61 ± 1*	47 ± 1*	42 ± 2	38 ± 3
8	8	8	8	8

*vs contr. $p < 0.0001$

Dextromethorphan O-demethylation in human microsomes: A selective probe for the debrisoquine-type of oxidation polymorphism

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Dextromethorphan (DEM) O-demethylation is controlled, in vivo, by the debrisoquine-type of oxidation phenotype. We developped an in vitro assay of DEM oxidation to characterize the polymorphic monooxygenase and factors influencing its activity.

Human liver microsomes are prepared by a micro-scale technique from small liver samples or biopsies (phenotyped patients). DEM demethylation (Km 1–5 μM) is monitored in incubation medium by direct injection of supernatant onto a LC column (DP, 2 cm) with fluorescence detection (198 nm/band filter). Quinidine selectively inhibits the high affinity reaction (Ki 10–20 nM). Other substances also block this reaction: neuroleptics, dihydropyridines, known substrates of this isozyme, and non selective P-450 inhibitors. Levomethorphan O-demethylation kinetics and inhibitions are superimposable on that of its dextroenantiomer. These results agree with in vivo data. The assay is selective for the polymorphic isozyme and shows predictive value (e.g. drug interactions).

Pharmaco-electroencephalographic (EEG) differences in RHA/Verh and RLA/Verh rats

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Previous EEG studies have shown that RLA/Verh rats show more wakefulness than do RHA/Verh rats, in addition to fewer paradoxical sleep (PS) phases and shorter total duration of PS. The present experiments were based on EEG spectral analysis in rats immobilized with alcuronium. Eserine (0.3 to 1.0 mg/kg ip) caused a pointed, dominant peak at 6 Hz in the RHA/Verh rats, and a broader peak over 6–8 Hz in the RLA/Verh rats. The subsequent administration of 10 mg/kg ip atropine normalized the amplitude of the 6 Hz peak in both rat lines, but the 8 Hz peak in the RLA/Verh rats remained. Further studies confirmed that RLA/Verh rats are more sensitive to pentobarbital (PB) anesthesia than are RHA/Verh rats. Following 20, 30 or 40 mg/kg ip PB, the anesthesia was less rapidly antagonized by 1.0 and 3.0 mg/kg ip amphetamine (AMP) in RLA/Verh rats. After AMP, the waking phase was characterized by a dominant peak at 6 Hz in RHA/Verh, and at 8 Hz in RLA/Verh, rats.

Josamycin (JOS) and troleandomycin (TRO), increase hepatic glutathione (GSH) turnover in rats

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A reactive metabolite is thought to be responsible for the inhibition of drug metabolism and the depletion of hepatic GSH by TRO. Yet, no GSH-TRO adduct has been described, and other mechanisms could lead to a loss of GSH. Therefore, we studied the effect of TRO and JOS which does not form reactive metabolites, on hepatic GSH turnover. 24 h after 4 mmol/kg b.wt p.o. of TRO and JOS hepatic GSH had decreased to 27 and 70%. The fractional rate of GSH turnover in fasted rats was significantly higher following JOS ($0.60 \pm 0.14\ h^{-1}$) and TRO ($0.56 \pm 0.05\ h^{-1}$ than in controls ($0.22 \pm 0.08\ h^{-1}$), and the estimated hepatic GSH synthesis had increased from 0.55 ± 0.17 μmol/g.h in controls to 1.05 ± 0.42 and 1.32 ± 0.21 μmol/g.h in the JOS and TRO groups, respectively. The fact that JOS which does not form a reactive metabolite markedly stimulates hepatic GSH turnover, and the magnitude of the increase in hepatic GSH synthesis suggest that the increased turnover of GSH may result from a loss of free GSH from the liver rather than from a loss in the form of GSH adducts of the antibiotics.

Metabolic effects of quinidine on the isolated perfused rat heart

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1. Rat hearts were perfused according to Langendorff with KH-buffer (Krebs-Henseleit, pH 7.4 with 10 mM glucose, equilibrated with 95% O_2/5% CO_2) to which quinidine was added at 0.5 μg/ml and 5 μg/ml after 15 min perfusion (T = 0). Heart rate and coronary flow were measured during perfusion. At 60 min (T = 1) the reaction was stopped and the tissue analyzed for glycogen, pyruvate, lactate, creatine, glucose-6-phosphate, ATP, ADP, and AMP. Only the glycogen content decreased in 60 min to 50–55% of control at both drug concentraitons. 2. Rats were given 50 mg/kg quinidine per os 5 days a week for 2 weeks. 24 h after the last dose the hearts were perfused with drug-free KHb and the same analyses performed at T = 0 and T = 1. Already at T = 0 the glycogen content of guinidine exposed hearts was decreased to 57% of controls, to fall further at T = 1. The results suggest that quinidine as membrane active compound might interfere with glucose uptake in the heart and thus force the tissue to utilize its glycogen reserve.

Transmission of a single nerve impulse coincides with ultrastructural changes in the pre- and postsynaptic membranes

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Ultra-rapid freezing was used to cryofix small pieces of *Torpedo* electric organ at 1 ms time intervals before, during and after the passage of a single nerve impulse. Then, freezefracture replicas were made in the most superficial layers of the frozen specimens. In the plasma membrane of presynaptic nerve terminals, transmitter release was found to coincide with a brief (2–3 ms) increase in the number of large intramembrane particles. In contrast there was no change in the number of vesicle openings at these times. The action of transmitter on the postsynaptic membrane was also accompanied by transient ultrastrucutral alterations.

In vitro binding studies with amitriptyline to the isolated S- and F-forms of variants of human alpha-1-acid glycoprotein

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Antidepressants like amitriptyline (AT) are bound to several plasma proteins, but mainly to alpha-1-acid glycoprotein (AAG), which presents two forms of variants, i.e., the F- and S-form. In an earlier clinical study free plasma AT and nortriptyline correlated significantly with the concentrations of the S- but not the F-form of AAG in the plasma of depressed patients. In the present study a method was developed to prepare both forms from commercially available AAG (Sigma) for binding experiments. Therefore, AAG was desialated and submitted to isoelectric focusing on immobiline flat gels. Binding studies were performed by equilibrium dialysis by means of a Dianorm-apparatus with AT in the concentration range of 200–90000 ng/ml. Free and bound AT levels were assayed by gaschromatography. Preliminary results confirm the previous findings, in that the S-form of AAG has a higher affinity for AT than the F-form. This finding could suggest a genetic factor contributing to the interindividual differences in the binding of drugs to AAG.

Response force titration for the assessment of neuromuscular toxicity in rats

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Rats were subjected daily to a force titration schedule in an operant chamber equipped with a non-moving glas-lever. Strain-gauges mounted on the lever measured the vertical force exerted during a response. In a discrete trial titration schedule, the threshold force, above which a water reinforcer was delivered, was adapted stepwise by a bisection algorithm to the rat's capacity. At the end of each session, a stable force requirement was reached. Since, in addition to the force measure, also the number of responded trials and response latencies are registered, this technique is able to distinguish between sedation or motivational effects and force decrements, a distinction which can not be made by grip-strength measures. This technique was used to measure the neuromuscular performance decrement due to repeated 2,5-hexanedione treatment (250 or 500 mg/kg/day). The results were compared with fore- and hindpaw grip-strength measures on the same animals.

Influence of acetylcholinesterase inhibitors (AChE-I) on ACh levels and turnover in rat brain

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Existing AChE-I have effects in the treatment of memory impairment in patients with senile dementia, but are poor as regards CNS selectivity and duration of action. Present experiments describe the influence of AChE-I physostigmine (Py), tacrine (Tc) and the miotine derivative RA7, on ACh levels and turnover (TO) in rat brain regions. All compounds increased ACh and reduced TO in striatum, cortex and hippocampus. These in vivo results were compared with AChE-activity ex vivo in the same regions. RA7 could be distinguished from Py and Tc in that it induced significantly greater increases in ACh and AChE inhibition in cortex and hippocampus than in other regions. At comparable doses (for CNS activity), Py and Tc, but not RA7, led to signs of peripheral ACh overactivity. A good correlation between the ACh-TO reduction and the remaining AChE-activity after RA7 was observed in the different brain areas. The selective AChE inhibition by RA7 in cortex and hippocampus may explain its relative lower peripheral side-effects compared to the other drugs.

Synthesis of cadmium-metallothioneins in LLC-PK1 cells

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Cadmium (Cd), a nephrotoxic metal ion, is known to accumulate in cells of renal proximal tubules. In order to characterize this Cd uptake, cells of an established line (LLC-PK1) with proximal tubule properties were cultured in vitro and when confluent, were exposed to Cd (as $CdCl_2$ 5 µM, in a serum free medium) at the apical side for 1, 3, 6 or 24 h. The cells were then scraped, sonicated and the homogenate ultracentrifuged (110000 × g, 75 min). At the end of the various incubation times, Cd was measured in the incubation medium, in the cell pellet and in the supernatant. 70% of the initial amount of Cd in the medium was taken up by the cells at 6 h, and 83% at 24 h. After 1 h, Cd was mostly bound to the cell pellet fraction, while at 3 h most of the Cd taken up by the cells was in the supernatant, cytosolic fraction (0.64 ± 0.08 ng Cd/µg protein, $n = 16$). Starting at 3 h of incubation, the occurence of a low molecular weight Cd binding protein could be detected, by separation on a Sephadex G75 SF column, in the cytosolic fraction. By immunoblotting technique, using a rabbit antiserum against rat metallothionein (Mt), the low molecular weight protein could be identified as Cd-Mt. The amount of Cd-Mt in the cytosolic fraction increased steadily from 3 to 24 h of incubation with inorganic Cd. Thus, LLC-PK1 cells constitute a further line of renal origin where increased synthesis of Mt can be directly demonstrated in vitro.

Toxicological screening: urine cell counts as marker of renal toxicity

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Toxicological screening tests reduce the use of experimental animals and help in selecting the most promising chemical compounds. Groups of 6 female rats (SIV) were treated for 5 days. If no evidence of renal toxicity was observed, higher doses were given in the following weeks. Rats were housed for 15 h in metabolism cages once ore twice per week. Total cell counts were determined in a Neubauer chamber. Mean values in untreated rats were: leucocytes (L)(4726 ± 6891, $n = 862$), epithelial cells (E) (14555 ± 9961, $n = 850$), erythrocytes (Ec) (280 ± 896, $n = 862$) and unspecified cells (C) (7101 ± 7798, $n = 331$). For validation, phenacetin (P), furosemide (F), Na oxalate (O), ethylene glycol (G) and hexachlorbutadiene (H) were tested. P, F and O caused increase in L, E, and Ec excretion. With G only Ec were elevated. H caused increase in E and C excretion. Cylinders were found with P, G and H. Elevated urine cell excretion was usually the most sensitive indicator of nephrotoxicity.

Ciclophilin, a ciclosporin binding protein in human erythrocytes

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In blood the immunosuppressant Ciclosporin (CSA) accumulates within erythrocytes. As possible binding proteins of erythrocytes hemoglobin, calmodulin and ciclophilin were suggested.

In using a ^{3}H-CSA binding assay, exogenous hemoglobin did not inhibit ^{3}H-CSA uptake into erythrocytes. However, added erythrocyte lysate prevented ^{3}H-CSA uptake into erythrocytes. Thus, a protein other than hemoglobin was responsible for CSA accumulation within erythrocytes. SDS electrophoresis of erythrocyte lysate showed the presence of a 17 kD protein. This protein was identical to ciclophilin (Handschumacher et al., Science 226: 544, 1984): 1) the 17 kD protein cross-reacted with

rat antibody against ciclophilin as demonstrated by immunoblot and ELISA; 2) the protein could be depleted by CSA affinity chromatography; 3) the protein had no cross-reactivity with polyspecific antiserum against calmodulin. Based on these experiments, it is concluded that CSA accumulation in erythrocytes is due to the presence of ciclophilin.

Competition for sucrose-pellets in triads of Wistar rats: manipulation of the individual's performance by α_2-adrenergic drugs

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Non-deprived, male Wistar rats, which have been accustomed to consume consecutively presented sucrose-pellets when exposed individually, show in their familiar surrounding (presence of familiar cage-mates, homecage) strikingly divergent (but stable) performances. By using a score (taking into account 3 different behavioral parameters) rats showing a high competition-rate and rats almost completely avoiding any competition have been discriminated within many of the triads tested so far. Previous pharmacological studies indicated that the situation-dependent inhibition to partake in competition for sucrose-pellets, as present in poor performing animals, can, temporary, be overcome by reducing the activity of the 5-HT-system (Gentsch et al., Psychopharmacology 89: S15 (1986)). As an extension, we here report that yohimbine, an α_2-antagonist, similarly improves the competition-rate in the poor performing rat. On the other hand, after the administration of clonidine (0.01–0.1 mg/kg) to high performing animals, competition-rates are attenuated in a dose-dependent manner.

Such data suggest that interindividual differences in some α2-adrenergic mechanisms may equally account for the rank-orders in triads of rats, as observed under the present experimental conditions.

Effects of 2-(4-phenylpiperidine)cyclohexanol (AH5183) on cholinergic synaptic transmission

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AH5183 is supposed to depress cholinergic synaptic transmission by blocking ACh uptake into synaptic vesicles. By using the *Torpedo* electric organ, we found that AH5183 inhibits the uptake of labelled ACh into the vesicles, and also reduces the content in ACh of the vesicular compartment. On the other hand, the drug blocks transmission after 6–10 impulses in a 100 ms train of repetitive stimulations at 100 Hz. Transmission recovers 1–2 s after such a train. This electrophysiological effect of AH5183 was attributed to dynamic depression of transmitter release, and was readily reversible after 1 h washout. In contrast, the ACh compartments remained altered for several hours. These results suggest that AH5183 affects cholinergic synaptic transmission by another mechanism than its inhibition of ACh uptake into synaptic vesicles.

Isolation and characterization of two forms of arylamine N-acetyltransferase from human liver

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To investigate the molecular basis of the clinically important genetic polymorphism of arylamine N-acetyltransferase (NAT), we undertook its isolation from human liver cytosol. Sequential chromatographic separations resulted in a 1200-fold purification with essentially complete recovery of NAT activity, based upon acetyl CoA-dependent N-acetylation of sulfamethazine (SMZ), a prototype substrate for this polymorphism. Two peaks of SMZ-NAT activity were observed on ion exchange columns, and these could later be completely resolved by affinity chromatography on CoA-Sepharose. The two activities displayed markedly different kinetic behavior towards SMZ and a variety of other sulfonamide and arylamine substrates. Of note were observations that p-aminobenzoic acid (PABA) and procainamide, substrates for NAT in various animal model systems, were *not* metabolized to a significant degree by either of the SMZ-NAT activity pools. These data suggest that at least two molecular species of NAT coexist in individual human livers. We are presently investigating if and how these forms contribute to polymorphic drug acetylation in human populations.

Role of adenosine in hypoxemia- induced renal vasoconstriction

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Acute hypoxemia induces intense renal vasoconstriction. This effect could be mediated by endogeneous renal adenosine. To test this hypothesis, low-dose theophylline (0.5 mg/kg), acting as an antagonist of adenosine receptors, was administered to anesthetized mechanically-ventilated newborn and adult rabbits submitted to an acute hypoxemic stress. Hypoxemia induced a significant fall in glomerular filtration rate (GFR) ($-27 \pm 6\%$) and U/P inulin ratio ($-23 \pm 5\%$) in 8 newborn rabbits and a significant decline in GFR ($-27 \pm 6\%$) and renal blood flow ($-28 \pm 6\%$) in 8 adult rabbits. Intravenous low-dose theophylline completely prevented the hypoxemia-induced decline in GFR in newborn rabbits, whereas the same dose of enprofylline, a xanthine with poor adenosine antagonistic properties, was ineffective. In adult rabbits the unilateral intrarenal infusion of theophylline (23 µg/min) protected the infused kidney from the hypoxemia-induced vasoconstriction. These data suggest that adenosine could mediate the hypoxemia-induced vasoconstriction by increasing renal vascular resistance and/or altering the ultrafiltration coefficient.

Renal hemodynamic changes induced by hypoxemia in newborn and adult rabbits

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Hypopxemia-induced changes in urine flow rate (V), glomerular filtration rate (GFR), renal blood flow (RBF) and renal vascular resistance (RVR) were studied in anesthetized mechanically-ventilated newborn and adult rabbits. Inulin and PAH were used as markers of GFR and RBF, respectively. Arterial $PaCO_2$ and pH, hematocrit and protein levels remained stable throughout the experiments. PAH extraction during the control period amounted to $55 \pm 4\%$ in newborn and $92 \pm 2\%$ in adult animals. It was not significantly modified by hypoxemia. In 8 newborn rabbits, acute hypoxemia ($PaO_2 = 38 \pm 2$ mmHg) induced a significant ($p < 0.01$) fall in GFR (-27%) and filtration fraction (-20%), without significantly changing diuresis, RVR and RBF. By contrast, in 8 adult rabbits, acute hypoxemia (41 ± 2 mmHg) induced a significant ($p < 0.01$) decrease in GFR (-30%) and RBF (-29%), an increase in RVR ($+44\%$) and a decrease in V (-24%). Filtration fraction remained stable. These data indicate that acute hypoxemia induces intense renal vasoconstriction in the rabbit, and that this vasoconstriction is not potentiated by renal immaturity.

Epidermal growth factor levels and ulceration in the gastrointestinal system of the rat

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Historically epidermal growth factor (EGF) production has been localized to the submandibular and Brunner's glands in rats and humans. EGF enhances proliferation of epithelial tissues, inhibits spontanous gastric acid and pepsin secretion in the stomach and stimulates DNA synthesis in the gastric mucosa. This suggests that EGF acts as a cytoprotective agent.

In order to investigate EGF's role in ulceration, we devised a sensitive (10 pg/ml), standard solid-phase immunoassay of the sandwich type, using polyclonal antibodies. EGF levels were determined in the submandibular gland, antrum, fundus, duodenum, jejunum, illeum and colon of controls and rats with ulceration induced by stress, ethanol and indemethacin. This experimental approach provides new leads for the investigation of the gastric ulcer disease.

Evidence for a unitary vascular alpha-adrenoceptor coupled to different signal transduction mechanisms

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The rabbit main pulmonary artery (RMPA) contains a homogeneous population of α-adrenoceptors. However, contractions of strips of RMPA in response to selective α_1- and α_2-agonists differ in several aspects. These include a high susceptibility of α_2-agonist-, but not α_1-agonist-induced contractions, to inhibition by calcium withdrawal, calcium antagonists, to reduction of bath temperature, changes in membrane potential and to inactivation of guanine nucleotide binding regulatory protein (G_i and/or G_o) by pertussis toxin. By contrast, rat resistance vessels contain different populations of both α-adrenoceptor subtypes. In pithed rats, phenoxybenzamine inhibited the pressor effects of only α_1-agonists, while in the RMPA responses to both α_1- and α_2-agonists were impaired. Apparently, the pulmonary vascular α-adrenoceptor, although characterized by a unitary recognition site that is alkylated by phenoxybenzamine, is able to trigger different post-receptor events depending on the type of α-agonist employed.

Ultrastructural evidence of drug induced phospholipidosis in cultured rat yolk sac tissue and primary hepatocytes

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Cultured rat yolk sac tissue or primary hepatocytes were used to study ultrastructural changes induced by compound F, which was developed to treat heart insufficiency and which had caused marked phospholipidosis in various dog tissues in vivo.

10-day-old rat embryos within the visceral yolk sac or primary hepatocytes were cultured in the presence or absence of the compound (10 µg/ml). Yolk sac tissue and hepatocytes were taken for electron microscopy after various time intervals and at the end of the culture period.

Yolk sac tissue already showed myelin figures after 6 h incubation. After 24 and 48 h, increased lysosomal sizes and numbers were seen in yolk sac epithelium. Hepatocyte changes were similar but less marked. Yolk sac tissue after 24 h drug exposure showed decreased lysosomal size and number after 24 h recovery; the myelin figures were unchanged.

In conclusion, compound F induced alterations were observed in vitro within 48 h, corroborating in vivo results and giving new information on the mechanism of cellular injury.

Ontogeny of the glycogenolytic action of K^+ and noradrenaline (NA) in the cerebral cortex (CC) of the epileptic mouse mutants quaking (qk) and tottering (tg) and VIP levels in the CC of tg

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The qk mutant is characterized by an impaired differentiation of oligodendrocytes. We have observed an age-dependent increase in the potency of K^+ to elicit glycogenolysis in qk older than 8 weeks when compared to control littermates (c). Differences between qk and c were 11% at 8 w, 17% at 15 w and 25% at 30 w for 11 mM K^+. The tg mutant is characterized by an increase in NA axons and levels in the CC. In tg older than 6 w we have recently reported a decrease in the glycogenolytic action of NA (Brain Res., in press). Preliminary results indicate that the decreased potency of NA is already present at 1 w, a period where the epileptic symptomatology is not yet expressed. The VIP content assessed by RIA was similar in the CC of tg and c with the exception of the right occipital pole: tg = 456.2 ± 16.4, c = 386 ± 27 pmol/g prot ($p < 0.02$, $n = 8-11$).

Behavioral studies in rats treated during different periods of gestation with methylmercury

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Differences in behavior between two control groups and six methylmercury treated groups (either from day 6–9 of gestation with 0.025, 0.05, 0.5 and 5 mg/kg b.wt/day or from day 9–12 and 12–15 with 5 mg/kg b.wt/day) were examined in a wheel shaped activity monitor and in an operant conditioning paradigm (discrete trial spatial alternation schedule). In the activity monitor the path iteration frequency (relative number of reiterated paths during the whole session) showed a clear and reproducible increase for all treatment periods at 5 mg/kg b.wt methylmercury. In the activity, no effects due to methylmercury were noted. The operant conditioning experiments showed marginal effects. Only the passiveness (percent of non-responded trials) pointed out weak methylmercury-induced effects in the males.

Age and organ specific induction of chromosome aberrations in vivo by heterocyclic amines

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Heterocyclic amines found in heated meat, fish and meat extracts were reported to act as strong mutagens in vitro, to cause moderate carcinogenic effects in rodents but not to cause mutagenicity in vivo.

The absence of induced chromosomal aberrations of 2-amino-3,4-methylimidazo (4,5-f) quinoline (IQ) in vivo was confirmed in our laboratory using the classical bone marrow micronucleus test. However when IQ (at doses up to 300 mg/kg b.wt) or bacterial grade meat extract (up to 16 g/kg b.wt) was given by gavage to mice a significant and dose dependant induction of colonic micronuclei were observed. This effect was enhanced in old mice as well as in a specific mutagen sensitive strain of mice. Since the colonic micronucleus test is considered as an adequate predictor of colon carcinogens it can be concluded that age and individual susceptibility might be involved in mutagen (and possibly also carcinogen) dependant risks.

CQA 206–291, an ergoline derivative with biphasic dopaminergic (DA) action

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CQA 206–291 ([5R(5β, 8α, 10α)]-N, N-diethyl-N'-(1-ethyl-6-methyl-ergoline-8-yl)sulfamide is an ergoline derivative which exhibits an unusual DA profile of action. Initially, 206–291 enhances rat striatal DA metabolism, action consistent with an acceleration of DA synthesis and release. Furthermore, immediate DA antagonistic effects at D_2 receptors are shown in animal models designed to identify actions at presynaptic DA receptors. Delayed DA-agonistic effects of 206-291 are demonstrated in vivo by induction of contralateral circling in 6-OHDA-lesioned rats, antiakinetic action and by a moderate but long-lasting reduction in the concentrations of the DA metabolites. These results suggest an interesting profile of action of CQA 206–291 as an antiparkinsonian agent, since the initial acceleration of DA synthesis and release could maintain DAergic transmission before the agonistic action on the DA receptors is exerted.

Effects of age, nucleus basalis lesions and scopolamine on spatial learning in the Morris water maze

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The effects of age, ibotenic acid-induced lesions of the nucleus basalis and scopolamine on place learning in the Morris water maze were investigated. Learning was severely impaired in rats receiving scopolamine (1 mg/kg) before each session, in animals with bilateral ibotenic acid-induced lesions (5 µg in 1 µl of phosphate-buffered saline) of the nucleus basalis and in a subgroup of the old (30-month) animals. In a learning set paradigm involving visually cued trials followed by un-cued trials scopolamine produced no significant impairment on the cued trials but a marked impairment on the subsequent trials. This impairment was partially reversed by pilocarpine. The results provide further evidence for the importance of cholinergic mechanisms in spatial learning.

The effect of phenylpentenyl-khatamines on the release of radioactivity from physiological catecholamine stores

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In certain areas of East Africa and Yemen, leaves of the khat shrub are used as a stimulant. The CNS effects of this drug are known to be due mainly to cathinone, a phenylpropylamine alkaloid that has been shown to have an amphetamine-like releasing effect on physiological catecholamine stores.

Recently, three phenylpentenylamine alkaloids have been identified in khat leaves. In the present study, these have been evaluated with regard to their ability to induce the release of radioactivity from ^{3}H-dopamine-prelabelled rat striatum, as well as from ^{3}H-noradrenaline-prelabelled rabbit atrium, and their effect was compared to that of the corresponding phenylpropylamines. In both series of experiments, the phenylpentenylamines were found to have a rather weak effect. These observations suggest that the khat alkaloids of the phenylpentenylamine type do not contribute to any important extent to the symptoms caused by khat chewing, and that these can be generally accounted for by the phenylpropylamines present in the leaves.

Pharmacokinetics of isoniazid (INH) and acetylhydrazine (AcHz) in man using stable isotopes and capillary GLC – ammonia CI – MS

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Therapy of tuberculosis with INH is associated with a high incidence of hepatotoxicity which may be related to the formation of the toxic metabolite AcHz. A potentially critical step in determining toxicity is the detoxification of AcHz via acetylation which may be inhibited by competing substrates such as INH itself. To study the effect of INH on the disposition of AcHz in man we synthesized $^{15}N_2$-AcHz and followed the time course of labelled AcHz (100 µmol i.v.) in the absence and presence (300 mg p.o.) of INH in 4 male healthy volunteers. INH and its hydrazino metabolites were analyzed in plasma as benzaldehyde hydrazones by GLC-CI-MS (OV1701vi). Selected ion monitoring was carried out at m/z 163, 165, 169, 209, 211, 221, 226, 232 (M+1). In the absence of INH, AcHz was eliminated at the same rate as INH suggesting that both compounds are acetylated by the same enzyme. In the presence of therapeutic concentrations of INH, however, the acetylation of the toxic AcHz to the non-toxic diacetylhydrazine was inhibited, particularly so in slow acetylators. This delayed detoxificaiton of AcHz may contribute to the hepatotoxicity of INH in slow acetylators.

Pain management in laboratory animals

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The present study evaluated possibilities and side-effects of pharmacological pain reduction in laboratory animals. Five centrally acting analgesics have been studied: D 9998, D 16949, buprenorphine, nalbuphine, and tramadol. Analgesic efficacy tests included 11 current pain-testing procedures. All agents were first tested in acute trials. Thereafter, three of them were examined in subchronical studies (2–4 weeks) involving both implantation of osmotic minipumps (rats) and treatment through drinking water (mice). All three compounds produced significant analgesia in subchronical treatment, thought neither in all tests nor in all sessions. Some compounds were also studied in a wheel-shaped activity monitor. Some had sedative effects on total activity; tramadol also produced stereotyped locomotor activity. Neither in rats nor in mice did any of the selected agents produce addiction symptoms or a withdrawal syndrome on naloxone challenge.

Polymorphism of midazolam metabolism in man (?): studies in human liver microsomes show no evidence for involvement of S-mephenytoin-4-hydroxylase

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The basis of the markedly prolonged half life of midazolam in 6% of the population is unknown (J.W. Dundee et al., Br. J. Clin. Pharmac. *21* (1986) 425–429). To investigate a possible correlation with the genetic S-mephenytoin 4-hydroxylation polymorphism we developed an HPLC based assay for microsomal formation of 1'-hydroxy-midazolam and 4-hydroxy-midazolam. Reactions were linear with protein and time and dependent on the cytochrome P450 concentration. Involvement of S-mephenytoin-4-hydroxylase was disproved by 1) absence of inhibtion of midazolam metabolism by mephenytoin, 2) lack of correlation between midazolam and S-mephenytoin-4-hydroxylase in vitro in 15 different human liver samples, 3) lack of immunoinhibition of midazolam metabolism with antibodies which, under identical conditions, potently inhibit S-mephenytoin-4-hydroxylase.

In vitro test of embryotoxic drugs: a standardized method using chick embryo

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Chick embryos at the stage of gastrulation were explanted into transparent silicone chambers and their development studied in presence of methotrexate, cadmium chloride, caffeine, phenobarbital, diphenylhydantoin, aspirin and saccharin. Survival scores, growth perturbations and early anomalies of the nervous, skeletomotor and cardiovascular systems were analyzed with respect to the used concentrations. Dose-response curves were obtained with a good precision and allowed us to compare the toxic potency of the used drugs. The anomalies produced were dependent on the drugs and corresponded well to data obtained in other species including man. Evaluation of one drug took about 3 weeks to one technician and costed about 150 eggs. The method is proposed as a routine teratogenicity and embryotoxiciy test which allows primary screening of many compounds and which can thus substantially reduce the number of ultimate experiments using pregnant mammalian females.

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Cryopreserved rat hepatocytes with metabolic function used for in vitro hepatotoxicity screening

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Adult rat hepatocytes were isolated by perfusion and slowly (1.5°C/min) frozen in serum (10–50%) and DMSO (10–20%) containing medium. Up to two years later, quick thawing was performed to remove the cryoprotectant DMSO and the osmotic shock was smoothened by adding 1 M glucose to the thawing medium. The cells were monitored for viability and metabolic function. For each cell batch, enzyme leakage and cellular enzyme content (LDH, GOT) was determined throughout a short (up to 3 d) culture period. The freezing and thawing media were optimized as well as the culture dish surface coating. Besides morphological criteria (cell attachment, ultrastructure) functional parameters were assayed before and after cryopreservation (protein synthesis and cytochrome P-450). When plated the cryopreserved hepatocytes lost their metabolic function faster than the freshly isolated control.

By treating such freeze-stored hepatocytes with various hepatotoxins, we evaluate their usefulness as screening systems for hepatotoxicity and drug metabolism, with the goal in mind to reduce and replice the corresponding animal tests with frozen hepatocytes form larger species including man. (Supported by the 'Schweizer Tierschutz' and the 'Fonds für versuchstierfreie Forschung, Zürich').

Release of vasoactive intestinal peptide (VIP) from mouse cerebral cortical slices

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A sensitive RIA (detection limit 3 pg, Martin et al., J. biol. Chem. *261* (1986) 5320) has been used to examine the regulation of VIP release (VIP-R) from mouse cerebral cortical slices. Experiments were performed under static conditions by assessing VIP-R into the incubation medium. VIP-R is very sensitive to increases in extracellular K^+ concentrations. Thus at 10 mM, 15 mM, 20 mM and 25 mM K^+, VIP-R increases 134.3 ± 9.2%, 251.6 ± 26%, 380.9 ± 30% and 677.7 ± 59% respectively (n = 8–12) over basal level (basal level = 100% = 20 ± 1.62 pg/mg prot/6 min, n = 26). The K^+-evoked VIP-R is Ca^{++} sensitive. Ouabain 100 µM increases VIP-R by 356 ± 41% (n = 6) over basal level (= 100%). Other neuroactive agents are curently being examined for their effect on VIP-R.

Buspirone fails to exhibit antipunishment effects in drug-naive mice

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Buspirone has been reported to be much more effective in patients not previously treated with benzodiazepines. In view of frequent failures to show any clear activity of this compound in various animal models predictive of anxiolytic action in man, this study attempted to optimize the detection of its antipunishment effects by using drug-naive mice and extensive testing. Female mice maintained at reduced body weight were trained during daily sessions in which lever pressing produced food reinforcement on a CRF schedule throughout the 20-min test or produced only food during the first 5 min and thereafter each response produced both food and footshock. Each dose condition for buspirone HCl (B; 1, 3, 10, 30 & 60 mg/kg, p.o. N = 8) and diazepam (DZ; 10, 15, 20, 25–30 & 60 mg/kg, p.o. N = 8) was given 3 times at weekly intervals 25 min prior to a test involving footshock. Vehicle sessions were given intermittently. All DZ doses, but none of the B doses, significantly increased punished responding. In a crossover, 5 of 6 mice which had previously received B (2 had died after 30 mg/kg B) exhibited clear antipunishment effects to 10 and 15 mg/kg DZ; whereas, only 1 to 2 of 8 mice which had previously received DZ exhibited antipunishment effects (which were weak) to 1 and 3 mg/kg B.

Organic anion transport by pig and rabbit renal brush border membranes (BBM)

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BBM of species exhibiting net renal reabsorption of urate, like dogs and rats, possess a mechanism allowing the exchange of luminal anions like urate and p-aminohippurate (PAH) against intracellular anions or OH^-. We investigated if such a mechanism exists also in rabbits and pigs, two species exhibiting net urate secretion. In rabbit and pig BBM vesicles, an outwardly-directed OH^- gradient (pHi = 7.4), pHo = 6.0) did not stimulate ^{14}C-urate uptake (50 µM), as compared to controls (pHi = pHo = 7.4): in both conditions, 15 s uptakes were (in pmoles/mg prot.) about 10 in rabbits and 20 in pigs. In the same conditions, ^{3}H-PAH (1 µM) uptake was not stimulated in both species, but ^{14}C-lactate (15 µM) uptake was stimulated 1.5 and 1.8-fold in rabbits and pigs respectively. The BBM urate-OH^- and PAH-OH^- exchanger appears therefore to be absent in species secreting urate, while, and as expected, a lactate-OH^- exchanger (or H^+-cotransporter) is present in rabbit and pig BBM.

Qualitative and organspecific phospholipid changes in rats after chronic antidepressant administration

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The mode of action of antidepressant drugs is still unknown. A common phenomenon resulting of chronic treatment with these drugs is a downregulation of β-adrenoceptors in brain but also in peripheral organs. This drug effect on neurotransmitter receptors coincides with the onset of the clinical effectiveness. A desensitization of β-adrenoceptors could also be shown in cultured human cells following chronic exposure to tricyclic antide-

pressants. Cultured cells also revealed profound drug effects on the cellular phospholipid-(PL)-metabolism. An accumulation of PL was accompanied by a change in the PL-composition. In order to verify these in vitro findings rats were chronically treated with desipramine. Beside of the expected β-adrenoceptor desensitization (except in heart) changes were observed in the cellular PL-compositions (except in heart and total brain). Since there is a positive correlation between changes in PL-composition and β-adrenoceptor desensitization a causal relationship between the two phenomena might be considered.

Transport of organic anions across the basolateral membrane of the non-perfused rabbit renal proximal (PT) S_2 segment

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The PT basolateral membrane transport of the anions p-amino hippurate (PAH) and pyrazinoate (PZA) was investigated by preloading the tubular cells during 30 min with ^{3}H-PAH or ^{14}C-PZA, and measuring the half-life of basolateral radioisotope rate of efflux ($T_{½eff}$) in control medium or in media containing 1 mM of various organic anions. Control ^{3}H-PAH $T_{½eff}$ was 66.9 ± 4.4 s (n = 39), and was decreased significantly in PAH (32.8 ± 7.0 s), probenecid (40.4 ± 4.0 s), urate (50.6 ± 5.8 s), or salicylate (37.4 ± 2.8 s) containing media (n = 3–8). Replacement of all inorganic anions by gluconate and HEPES in the control medium increased the $T_{½eff}$ of ^{3}H-PAH. Control ^{14}C-PZA $T_{½eff}$ was 43.3 ± 2.8 s, and decreased significantly in PAH (29.1 ± 1.4 s) but not in probenecid (38.4 ± 6.2 s) containing media (n = 8–14). The data demonstrate the existence of a basolateral organic anion exchanger for which various organic anions may show affinity.

I.c.v. captopril, but not i.c.v. (Sar^1, Ile^8)angiotensin-II, antagonises the salt appetite induced by oral captopril in the rat

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Forty-four hours following the onset of an oral treatment with the angiotensin-I converting enzyme (ACE) inhibitor, captopril (SQ 14,225) at 100/l to water and 2.7% NaCl solution, offered as drinking fluids in a choice situation, male adult Wistar rats tripled their intake of hyperosmolar saline (from 3 to 10 ml/rat·day), with a slight increase of water intake (from 22 to 26 ml/rat·day). Subsequently, the animals under chronic oral captopril were continuously infused i.c.v. for 7 days with captopril (15 µg/h) or with the ANG-II receptor blocker (Sar^1, Ile^8) ANG-II (3, 10 and 30 pmoles/h), by osmotic minipumps. I.c.v. captopril abolished the increase of 2.7% NaCl due to oral captopril. I.c.v. (Sar^1 Ile^8) ANG-II not only failed the effect of oral captopril on 2.7% NaCl consumption, but paradoxically elicted an immediate increase water intake and a delayed (after the 3rd day) increase of 2.7% NaCl intake. In contrast, the ANG-III inhibitor (Ile^7) ANG-III infused icv at 3 pmoles/h, blocked the oral captopril-induced increase of 2.7% NaCl intake, but had no effect on water intake. These preliminary results suggest that brain angiotensin receptors may be of different types or/and sites for water and salt intake, while supporting the hypothesis that salt appetite produced by oral captopril may be due to diffusion into the brain of elevated ANG-I concentrations achieved by blockade of peripheral ACE. (Supported by Swiss National Scientific Fund, Grant No 3.168.0.85)

Psychophysiological effects of pre- and postsmoking rapid information processing

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The psychophysiological effects of subject paced rapid information processing performance (RIP) were compared between consistently low (L, n = 12) and high (H, n = 12) CO absorbing smokers before and after smoking. The pre- to postsmoking increase in RIP was similar in the two groups. RIP produced decreased pulse transit times (correlated to blood pressure) increased peripheral vasoconstriction, respiratory and skin conductance (SCR) frequency and a continuous increase in frontal EMG. Smoking increased heart rate more in the H than L group while vasoconstriction and SCR were similarly increased in the two groups. Additive effects of RIP and smoking on cardiovascular measures were less pronounced than those described for other active coping demands and SCR and EMG responses seemed to be diminished by smoking.

Substance P depresses neuronal activity in the rat olfactory bulb in vitro and in vivo: possible mediation via GABA-release

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The rat olfactory bulb displays a particularly high density of substance P receptors in the glomerular cell layer. Bath applied and microiontophoretically administered substance P depressed the activity of glomerular neurons in coronal sections of the olfactory bulb. This inhibitory effect was abolished in medium containing zero Ca^{2+}/high Mg^{2+}. Since the substance P mediated depression was blocked also by bicuculline (10 µM) and picrotoxin (100 µM), we propose that the peptide acts via the release of GABA. In vivo, microiontophoretically applied substance P also depressed the firing activity of most unidentified spontaneously active cells. Similar results have been reported in rat spinal cord (Ryall and Pini, Proc. XXX Congress of Physiological Sciences, Montreal, 1986).

Effect of Ca on changes in NADH fluorescence produced by electrical activity in nerve fibres

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The effect of electrical activity on the NADH fluorescent signal (excitation 366 nm, emission 450 nm) was recorded from non-myelinated nerve fibres of the rabbit vagus. Base-line drift was reduced by addition of heavy metal chelator DTPA (100 µM). Stimulation (30 Hz, 30 s) produced a diphasic response: during activity a decrease in fluorescence, which was abolished by ouabain and partially reduced by omission of external Ca, and a subsequent increase in fluorescence, which was abolished by ouabain or Ca-free solution. When the stimulation was applied in the absence of Ca, re-introduction of Ca produced a transient increase in fluorescence. The amplitude of this transient peak was found to depend on the glucose concentration that was present before and during the period of activity. These results suggest a role of the Ca entry during activity (see Pralong & Straub, J. Physiol. *371* (1985) 266P) on the control of glycogenolysis in this tissue.

Allosteric effects of verapamil and diltiazem on dihydropyridine binding to cardiac Ca channels are voltage-dependent

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Verapamil (VER), d-cis-diltiazem (DIL) and 1,4-dihydropyridines (DHP) block voltage-dependent Ca channels by binding to different, but interacting sites on the channel protein. We have used intact tissue-cultured rat cardiac cells to study the allosteric interactions between the 3 channel ligands at two different membrane potentials. At −40 mV, 3 μM DIL maximally enhanced specific binding of the DHP 3H-(+)-PN 200–110 (PN) by decreasing the Kd value for PN from 1.9 to 0.5 nM. In depolarized cells at 0 mV, DIL reduced PN binding by a slight effect on Bmax. In cell homogenates 100 μM DIL enhanced PN binding but the relative effect was 10fold lower than in polarized cells. (±) VER (1 μM) by an effect on Kd, stimulated the binding of PN in polarized cells but inhibited its binding in depolarized cells. These results suggest that cooperative interactions between the 3 ligands depend on the voltage-modulated conformational state of the Ca channel.

Validation of five alternative methods (4 cell tests+hen's egg test) for predictive eye irritation testing (Draize Test)

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51 different cosmetic ingredients (identical batches) were tested in five alternative methods to animal experimentation and compared with in vivo guinea pig eye irritation data. By diluting these chemicals in culture medium, a concentration which induced a 25% cell detachment of BHK 21–C13 cells (CD_{25}) and 50% growth inhibition (GI_{50}) was determined (Reinhardt et al., Cell Biol. Toxicol. *1*, 33, 1985). Membrane permeability was quantified by measuring dye retention (fluorescein) and dye exclusion (ethidium bromide) of rat thymocytes in 3-parameter flow cytometry (FS_{25}; VR_{25}) (Aeschbacher et al., Cell Biol. Toxicol. *2*, 247, 1986). The hen's egg test was performed on the chorionallantoic membrane of 10-day-old chick embryos according to Lüpke (Fd. Chem. Toxicol. *23*, 287, 1985). Eye irritation was scored according to Draize after application of diluted chemicals (≤ 300 mM) to guinea pigs. 24 of the chemicals (anionic tensides and tenside mixtures) were blind tested part of which underwent an additional test on human skin. Physicochemical parameters such as the critical micelle concentration were included in the estimation of the irritation potential.

A good correlation between the 4 alternative in vitro tests and the in vivo irritation data was obtained which allowed a prediction of a strong and/or moderate irritation potential of unknown test chemicals based on cell toxicity. The hen's egg test was shown to be of limited value as long as the original scoring scheme was used. (Supported by the 'Fonds für versuchstierfreie Forschung', Zürich and the 'Schweizer Tierschutz').

Determinants of hepatic dextromethorphan (DM) extraction (E) in the rat

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Hepatic first pass E of a drug could be determined by uptake and/or metabolism. To dissect these effects, DM, a compound subject to the debrisoquine type polymorphism, was administered to intact (iv and intraportally) and liver perfused male SD rats. *Results:* In vivo, systemic Cl (D/AUC) of DM was 42 ml/min.kg, E 74% and Vd 8.6 l/kg. Single pass perfusion showed an E of 97.5% during the initial 2 min, a mean hepatic transit time of 8.6 min and a hepatic Vd of 17.5 ml/g, suggesting extensive hepatic uptake peaking at 2.5 min. Thereafter, unmetabolized DM was slowly released from the liver with a release constant of -0.11 min^{-1}. Consequently recirculating liver perfusion showed a decrease of hepatic E from 92 ± 0.8% at 5 min to 46 ± 26% at 60 min (n = 4). *Conclusions:* Hepatic uptake and distribution are main determinants of initial DM first pass extraction, whereas back-diffusion of unmetabolized DM decreases initial E by 50%. Distribution phenomena, therefore, are major determinants of DM pharmacokinetics in the rat.

Experimental ciclosporin nephropathy: new findings

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Experiments in rats indicated that experimental CSA nephrotoxicity has some resemblance to human clinical toxicity. Renal functional changes consisted of a dose dependent reduction of the glomerular filtration rate as measured with isotope clearance methods. Functional nephrotoxicity was not associated with morphologic changes; prolonged treatment, however, resulted in tubular and arteriolar lesions.

Vasoconstriction most likely plays a major role in functional toxicity. Vasoconstriction may be due to a direct effect on small vessels or due to indirect mechanisms, e.g. stimulation of nerve activity, increased renin release and/or altered prostaglandin metabolism. Tubular and vascular toxicity may be caused by accumulation and cytotoxic effect of CSA.

Microangiopathy with intravascular coagulation may progress in interstitial fibrosis. Based on the present view on the pathogenesis, strategies to reduce or minimize CSA nephrotoxicity are currently in progress.

Reduction of high energy phosphate consumption in brain by calcium-antagonists

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We have shown by in vivo 31-P NMR spectroscopy (P-MRS) that the disappearance of high energy phosphates (HEP) in rat brain, following global ischemia induced by cardiac arrest, is slowed down by dihydropyridine calcium-antagonists (DCA) (J. Magn. Res. Med., in press (1986)). Two mechanisms might cause such effects: an increased production and/or a decreased consumption of HEP. Under anoxic conditions prevailing in brain after global ischemia, on-going HEP production causes an accumulation of lactate and a fall of intracellular pH. Using P-MRS, we find that injection of glucose before cardiac arrest slows down the disappearance of HEP, similar to DCA, while hypoglycemia causes the opposite. However, in contrast to DCA, high glucose causes a fall of intracellular pH to values lower than in controls, presumably due to accumulation of lactate, suggesting an increased HEP production. Since DCA do not influence the pH, we conclude that the slow down of HEP disappearance observed with DCA is not due to an increased HEP production, but probably to a decreased HEP consumption.

In vivo evidence for direct dopaminergic modulation of Leydig cell function, in the rat

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Rodent Leydig cells are subject to direct dopaminergic modulation in vitro. The present studies were designed to explore the

possibility that such regulatory systems are relevant in vivo. Acute experiments were performed on ♂Wistar rats. Dopamine (D-2) agonists of different chemical structure (Sandoz compounds CU32-085: CV 205–502: 209–002) led to dose-dependent reductions in serum testosterone (T) levels, independent of effects on LH. Various D-2 antagonists, including sulpiride at a dose which does not cross the blood-brain barrier, blocked the I-inhibiting actions of the D-2 agonists, while the serotonin (5-HT-2) antagonist ketanserine was ineffective in this regard. D-2 agonists also suppressed HCG- and oPRL-stimulated T secretion. These results demonstrate that in the rat, Leydig cell function is subject to direct dopamine D-2 regulation, and suggest that endogenous dopamine may interact with endocrine and paracrine factors within the testis. On the other hand, human studies with D-2 agonists suggest that there are no comparable regulatory mechanisms in the human male.

Prostanoids mediate the α-1 adrenergic potentiation of cAMP increase elicited by VIP in mouse cerebral cortex

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We have previously described a synergistic interaction between VIP and noradrenaline (NA) in stimulating cAMP formation in mouse cerebral cortical slices (Nature *308*, 280–282, 1984). This synergism results from a potentiation by NA, via α-1 adrenergic receptors, of the VIP-stimulated cAMP formation (J. Neurosci. *5*, 362–368, 1985). The mechanisms of this α-1mediated action of NA were examined. We have observed that the synergistic interaction between VIP and NA is antagonized by indomethacin (EC_{50} 2 μM), an inhibitor of cyclooxygenase, the enzyme responsible for the conversion of arachidonic acid (AA) to prostaglandin G2. This indicates that AA metabolites mediate the synergism between VIP and NA. Among various prostanoids tested only PGF2α and PGE2 were able to mimick the action of NA in potentiating the stimulatory effect of VIP on cAMP formation. Furthermore, mepacrine, an inhibitor of phospholipase A2, one of the enzymes responsible for AA formation, also antagonized the synergism between VIP and NA.

Caffeine breath test: effect of acute and chronic loss of liver mass and cholestasis on 3-demethylation of caffeine

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We investigated 3-demethylation, the major pathway of 1,3,7-trimethylxanthine metabolism, in unanesthetized male Sprague Dawley rats 1 h following ⅓ or ⅔ hepatectomy (⅓ H, ⅔ H), 48 h after bile duct ligation (BDL), 2 weeks after portocaval anastomosis (PCA) and in respective sham operated controls (SOC). 5 mg/kg b.wt caffeine (together with 0.2 μCi 3-methyl-14C-caffeine) was injected i.p. and all exhaled air collected during 4 h. From specific activity of exhaled $^{14}CO_2$ fractional dose (FD) and peak exhalation rate (PER) were calculated.
Results:

		SOC	⅓H	⅔H	BOL	PCA
n		27	6	6	8	6
liver weight	g	7.8 ± 1.2	4.8 ± 6.4	3.1 ± 0.3	10.4 ± 1.8	4.8 ± 0.8
	%b.w.	3.50 ± 0.31	2.68 ± 0.21	1.33 ± 0.06	4.58 ± 0.30	2.11 ± 0.12
FD	%dose	17.79 ± 1.5	17.1 ± 2.2	9.6 ± 2.3**	8.1 ± 2.4***	6.3 ± 0.8***
PER	%dose/min	0.12 ± 0.02	0.11 ± 0.01*	0.05 ± 0.01***	0.04 ± 0.01***	0.03 ± 0.05***

*p < 0.05, **p < 0.001, **p < 0.0001, results: x ± SD. Conclusions: Changes in caffeine kinetics are proportional to the loss of functioning liver mass, both in the acute (⅓ or ⅔ H) and chronic (PCA) models. In addition, in BDL the functional changes due to cholestasis are clearly measurable. These results therefore document the usefulness of caffeine as a quantitative indicator of hepatic function.

Characterization of rabbit retinal dopamine sensitive-adenylate cyclase with new types of D_1-agonists (SKF-38393-A) or -antagonists (SCH-23390)

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Homogenates of rabbit retina were used to study the effect of new dopamine D_1-agonists and antagonists on the generation of cAMP in absence or presence of dopamine (DA) or of various agents acting on the Ns regulatory and/or on the catalytic site of adenylate cyclase (NaF, GTP, forskolin). SKF-38393-A was found to stimulate cAMP generation in a dose-dependent manner and to be more potent than DA (ED50 = 0.1 μM compared to 1 μM for DA). Maximal effects were already obtained at 1 μM. However, SKF-38393-A was less efficacious than DA (respectively 160% and 210% stimulation over controls). The SKF-28393-A-induced accumulation of cAMP was blocked by (+)-butaclamol, and not by (−)-butaclamol, although the active concentration of the antagonist was larger than that used for DA blockade, reflecting a higher affinity of SKF-28393-A for D_1-receptors. Furthermore, the agonist actions of DA or SKF-38393-A were inhibited by a new D_1-receptor antagonist, SCH-23390, in a dose-dependent manner. The specificity of the retinal preparation for studying D_1-receptors was also confirmed by the lack of activity of new D_2-drugs, such as LY-171555 and RU-24926.

Liposomes as antibody targeted carriers of lipophilic cytostatic drugs

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The cytostatic effect of drugs like cytosine arabinoside (ara-C) or 5-fluoro-2'-deoxyuridine (FUdR) can be improved by their chemical modification to lipophilic prodrugs and by subsequent incorporation into unilamellar liposomes. Ara-C prodrug-liposomes exert in vivo an antitumor activity against L1210 lymphoid leukemia which is superior to free ara-C by factors of 2–8 and drug concentrations which are 2–4 times lower yield equal or even higher tumor inhibitory effects. These favourable effects may be improved by the coupling of tumor cell specific antibodies to the prodrug-liposome surface. Various lipophilic spacer molecules have been prepared and incorporated into prodrug-liposomes providing the liposome surface with functional groups for covalent of high affinity binding of tumor cell specific antibodies.
The cell specific targeting is demonstrated in vitro with the biotinylated MHC class I antibody B 8-24-3 linked via avidin to biotinylated ara-C prodrug-liposomes. Specific binding to H-$2k^b$ carrying EL4 cells is shown by FACS analysis.

Cardiovascular effects of obidoxime, atropine and sarin in rats

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Obidoxime (Toxogonine^R 50 mg/kg), atropine (5 mg/kg) and sarin (50 μg/g) were injected intravenously to anesthetized (urethane, 1 g/kg i.p.) and artificially ventilated rats. Blood pressure was recorded from the cannulated right artery. Heart frequency was determined via ECG. – Sarin induced a rise in the mean

arterial blood pressure (MAP) of 55%. The onset of the pressor response began within seconds of drug administration and lasted 3 min. A transient bradycardia was observed; heart frequency, however, returned to control levels well before normalization of the pressor response. – Obidoxime and atropine, both lowered MAP rapidly and increased heart frequency. Obidoxime caused a 40%-fall in MAP. Administered after sarin-intoxication it effected a 27%-fall. Atropine, in contrast, lowered MAP by 23%, but administered after sarin, a 41%-decrease was recorded. – It is concluded that obidoxime and atropine exert a synergistic effect on MAP which can be accounted to the mainly centrally active atropine and to the peripheral effect of obidoxime as ganglionic blocking agent.

Formation of ion channels by the nematocyst toxins of *Hydra attenuata*

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The nematocyst extracts from *Hydra* – similar to contact formation of living polyps – causes muscle contraction in larvae of *Drosophila melanogaster*, followed by partial or total paralysation*. In addition, these extracts are highly hemolytic to human erythrocytes. After addition of the extract into the aqueous phase (100 ng/ml) in contact with a planar lipid bilayer, formation of ion channels is observed. Whereas the single channel conductivity is about the same for KCl, NaCl and NH_4Cl (120 pS at 300 mM) it is reduced to half for Tris-Cl solutions. Typically cooperative opening and closing of up to 15 channels was found as well as fast flickering between two conductivity states. Whereas incubation of the extract with divalent ions or alcohol prevents lysis of cells it had no effect on the ability of pore formation. Therefore it is suggested that other (probably enzymatic) processes are effected which preceed pore incorporation into the membrane of native cells.

* J. Weber, M. Klug, P. Tardent, 1984. Proc. 6th Europ. Symp. of animal, plant and microbial toxins.

Debrisoquine/sparteine-type genetic polymorphism (DSP) of drug oxidation in man: the deficient cytochrome P450 is characterized by a new enzyme assay and by specific human autoantibodies

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To elucidate the molecular mechanism of the clinically important DSP we have investigated bufuralol (buf) 1'-hydroxylation as a prototype reaction mediated by NADPH/O_2 or cumene hydroperoxide (CHP) in liver microsomes of extensive (EM) and poor metabolizer (PM) subjects. We have observed that the CHP-mediated reaction is highly specific for P450 bufI, one of two isozymes catalyzing NADPH/O_2-dependent bufoxidation (J. biol. Chem. 261, 11734). In PM-microsomes the CHP-mediated activity was drastically reduced. In EM-microsomes it was specifically inhibited by autoantibodies (LKM-1) derived from sera of children with chronic hepatitis. Immunopurification and charaterization of the LKM-1 antigen from EM-livers suggest that it is identical to P450 bufI. No protein could be immunoisolated from PM-livers. These preliminary results suggest the absence of P450 bufI as cause of the DSP.

Excretion of dextromethorphan (DM) and its metabolites in urine of SD and DA rats

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Metabolism of DM, recently shown to be subject to the debrisoquine hydroxylation phenotype in man, was evaluated in female SD (n = 7) and DA (n = 8) rats by HPLC. After a DM dose of 50 mg/kg p.o. total urinary excretion in SD and DA rats was 56 ± 12% and 61 ± 12% of the dose/72 h. Dextrorphan (DO, O-demethylation) was the main metabolite: 40 ± 9% in SD vs 25 ± 6% in DA ($p < 0.002$). N-demethylation (methoxymorphinan, MEM) was 0.9 ± 0.7% in SD and 8 ± 3% in DA ($p < 0.001$) whereas OH-morphinan (OHM) was 14 ± 6 and 24 ± 4%, respectively ($p < 0.001$). Metabolic ratios of DM (DM/DOR) and of debrisoquine similarly discriminated between SD and DA. Microsomal incubation of DOR and MEM showed OHM to be primarily formed by demethylation of MEM. Conclusions: 1. Similar to debrisoquine, the DA rat is an animal model for poor DM O-demethylation. 2. The DA rat switches from O to N-demethylation, thus resulting in increased MEM excretion. 3. As MEM is the main substrate for OHM formation, this metabolite is increased in DA.

Physiology

On the motor function of substantia nigra

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Motor performances of 11 patients with Parkinson's disease were studied in an 'off' state without medication as well as in an 'on' state with optimal compensation of Parkinsonian deficits. Responses to a step stimulus in the 'on' and 'off' states were averaged and used to identify a linear transfer function G(p) for the tracking oparator. If

$G(p) = e^{-T}d*p/(p*(T_s+K_d)+K_p+1+K_f+e^{-T}d*p)$

is interpreted as a dynamic system with the dopaminergic neurons of the substantia nigra performing a feedback control function as part of a circuitry with a cortical-basal ganglia-cortical loop, then the role of dopaminergic substitution with levodopa can be explained as a controller action with a tonic and phasic effect on step tracking performances. With dopaminergic substitution significant changes of 78% were found for the differential (phasic) feedback (k_d'off' 0.12 ± 0.05 K_d'on' 0.28 ± 0.12) and of 69% for the proportional (tonic) feedback (K_p'off' 0.16 ± 0.05 K_p'on' 0.05 ± 0.03).

Distribution of blood flow to right and left brain after unilateral ligature of the carotid artery in the rat

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The use of microspheres for the study of blood flow in the rat makes necessary the ligation of one carotid artery. An immediate, and possibly long term, consequence could be an inhomogenous distribution of blood flow between right and left sides of the brain. A study of a series of experiments dealing with blood flow in rats shows that there is no difference between flows in right and left side of brain, when the carotid artery was ligated one week prior to the experiment (brain side homolateral to the ligature: 1.0% blood flow than in the contralateral side, n = 67, $p_\alpha = 0.80$, $p_\beta < 0.01$). By contrast, experiments performed one hour after ligation show a significant, although small, decrease in blood flow in the homolateral brain side (–9.1%, n = 25, $p\alpha < 0.05$). It is concluded that distribution of blood to both

sides of the brain is only minimally affected by ligation of one carotid artery in the rat, thus proving the physiological efficiency of the circle of Willis.

Presence of substance P-like immunoreactivity (SPLI) in quail trigeminal ganglion neurons in situ and in culture

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Studies with cultured sensory neurons from quail trigeminal ganglia indicated that a calcium-activated chloride current was present in nearly 80% of these neurons. We were interested to see whether this current was associated with a particular population of sensory neurons. For that purpose we first examined the localization of SPLI in sections of quail trigeminal ganglia. Most labelled neurons were localized in the proximal part of the ganglion (cf Fontaine-Perus et al., Dev. Biol. *107* (1986) 227). Microdissections of the proximal and distal part of the ganglion were then performed and the neurons cultured after dissociation. SPLI was present in 79 ± 5% (SD) of the proximal and in only 30 ± 3% of the distal trigeminal neurons. The sizes of proximal, SPLI-positive neurons follow a normal distribution suggesting that the cells constitute a population with a mean diameter of 13.8 ± 1.8 µm. We are currently examining these proximal neurons to see what fraction of these cells will exhibit a calcium-activated chloride current.

The role of red blood cell volume on oxygen diffusing capacity

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In vitro studies with rapid reaction technique show that O_2 uptake and release velocities are higher in small than in large red blood cells. In order to demonstrate this effect in the lung microcirculation, cat lungs were isolated and perfused alternately with suspensions of sheep red blood cells (30 µm3), and oxygen lung diffusing capacity (DLO_2) measured. The experiments were carried out at 27 °C; ventilation was 1.0 l/min, perfusion 110 ml/min and hemoglobin concentration 70 g/l. DLO_2 with sheep red cells was 0.57 ± 0.10 ml/min/torr, not different from DLO_2 with human red blood cells (0.58 ± 0.11). A large surface-volume ratio of the red blood cell thus confers no advantage for O_2 transfer in the lung. This could be explained either by the fact that the cells have a negligible share of the overall lung resistance to O_2 transfer or that the presumed higher diffusing resistance at the surface of large red cells is offset by hemodynamic effects.

Extracellular phosphate regulates Na/P_i cotransport activity in opossum kidney (OK) cells

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The activity of Na/P_i cotransport of OK cell monolayers was studied as a function of extracellular phosphate (P_i). Incubation of the cells in a P_i-free medium leads to an approx. 2-fold increased transport rate of P_i, which was maximal already after 2 h and was stable for the next 5 h. Increased transport of P_i was expressed at 200 µM (and lower) extracellular P_i and was manifested in a change of the V_{max} but not of the app. K_m for phosphate. It is suggested that low extracellular P_i selectively affects the P_i transport rate since other sodium-dependent transport systems were not altered. The described response of the OK cells to extracellular P_i could be blocked by protein synthesis but not by transcription inhibitors. By 3'-deoxyadenosine however, complete inhibition of the increase of the Na/P_i transport activity was achieved. The results suggest that low extracellular P_i might lead to an increased synthesis of P_i transporter molecules in OK cells by influencing the processing of hnRNA in a yet unknown way. (Supported by SNF 3.881.085).

Duality of ACH- and SP- effects on mechanical tension and transmembrane potential of rabbit aorta smooth muscle: role of endothelium

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Rabbit aorta (thoracic part) is commonly used to study arterial smooth muscle. The mechanical tension of a transversal strip and the transmembrane potential of smooth mnuscle cells are recorded. Acetylcholine (ACh) as well as substance P (SP) induce relaxations accompanied by hyperpolarisations (transient for SP and continuous for ACh). These effects are dependent on the presence of intact endothelium. If the strip is desendothelialized, ACh and SP contract and depolarize the cells. A 'cascade' experiment where the upstream intact aorta is perfused before the downstream desendothelialized strip, has demonstrated that the endothelium stimulated by ACh and by SP releases a humoral factor relaxing smooth muscle.

Similar characteristics of the Na-dependent Pi transport (NaPiT) responses to variations in extracellular (EC) Pi and Ca in opossum kidney (OK) epithelium

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In vivo, lowering either [Pi]ec or [Ca]ec stimulates renal Pi transport by a parathyroid hormone (PTH)-cAMP independent mechanism. These two stimulatory effects can be reproduced in vitro using renal epithelium culture technique. In OK epitheloium the response of NaPiT to lowering either [Pi]ec or [Ca]ec share several similar characteristics: 1) increased V_{max} with no change in Km, 2) transport specificity, i.e. no change in other Na-dependent solute transports, 3) no change in cellular cAMP, 4) lag-time for induction and dependence on the de novo protein synthesis, 5) marked attenuation by PTH. The only difference was the magnitude of the response, low [Pi]ec being a more powerful stimulus than low [Ca]ec. In conclusion, these results suggest that [Pi]ec and [Ca]ec may affect NaPiT via some common regulatory pathway(s).

The postpotential hyperpolarization (PPH) in rabbit vagus nerve is mediated by a calcium activated potassium permeability

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PPH was recorded by the sucrose-gap method, enregistred and analysed by computer. The results of a multiexponential analysis showed that the time course of the PPH can be decomposed in two distinct components. The first one, with an amplitude of 0.22 mV and a rate constant of 35 μs^{-1} was almost independent of the external ionic composition. On the other hand, the second, much slower one, with a rate constant of 3.9 μs^{-1} was dependent on the gradient of K, increasing from 0.17 mV (5.6 mMK) to 1.64 mV (OK). A large increase was observed with increasing $Ca^{2+}{}_o$. In presence of Ca entry blockers Cd^{2+}, La^{3+} and D600, the PPH was almost completely abolished. The PPH was also inhibited by external TEA, known to block both the delayed rectifier and the Ca-dependent K permeability ($P_K(Ca)$, and enhanced by 4-AP which blocks only the delayed rectifier. The amplitude of

PPH was also decreased by Apamine and Dendrotoxine. These results show that the PPH is mediated by an increase in the $P_K(Ca)$.

Direct inhibition induced by opioid peptides of guinea pig ventromedial hypothalamic neurones

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The action of selective agonists for μ-, δ- and K-type opioid receptors was examined using intracellular recordings from guineapig hypothalamic slices. In 58 of 68 cells, the μ agonist DAGO at 1 μM caused a membrane hyperpolarisation and/or an inhibition of firing. In 6 out of 9 cells, the effects were exclusively linked to a μ receptor, while in the remainder the δ agonist Tyr-DPen-Gly-Phe-Pen was also active. U50,488 had no effect. Characterization of the impaled neurones was performed using pipettes filled with Lucifer Yellow. Out of 15 responsive neurones, 12 were located in the ventromedial nucleus of the hypothalamus (VMH), the others in the cell-poor zone ventral to VMH. The action of DAGO was direct since it persisted in a low calcium-high magnesium medium or in a medium containing 1 μM tetrodotoxin. The hyperpolarization caused by DAGO resulted from an increase in membrane conductance. Experiments are in progress to determine which conductance(s) is involved.

Coding of intensity in the peripheral auditory system

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Numerous physiological and psychophysical studies have been done on the coding of intensity but few have tried to couple these two sets of experimental data. Using a digital model, our aim was to explain the psychophysical data based on the physiological behavior of the cochlear transducer.

The digital model of the peripheral auditory system developed by Dolmazon and Bastet (1978 and 1980) is made of 128 cells simulating both the mechanical and the transduction behavior of the basilar membrane, with an hypothesized interaction between inner and outer hair cells.

Loudness curves show 'inversion points' at very high intensities: low frequencies grow rapidly and catch up to the growth rate of higher frequencies. Such a behavior may be explained by the spread of frequencies along the basilar membrane: the excitation area for low frequencies can extend much more than for high frequencies. The intensity difference limen, as long as obeying Weber's law, is due to the basilar membrance behavior, whereas the 'near miss' to Weber's law is explained by the threshold and saturation limitations.

The activity of 5'deiodinase in the rat pituitary gland is sex-dependent

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The pituitary concentration of T3 (T3 AP) and density of T3 nuclear receptors (T3nR) have been reported to be determinant parameters involved in the negative control of TSH secretion rather than the plasma levels of thyroid hormones. As we have previously shown that the concentrations of T3 AP and T3nR were greater in young females than in males, the purpose of this study was to investigate whether this sex difference was due to a greater activity of the pituitary 5'deiodinases (5'D) in females since T3 AP proceeds mainly from local deiodination of T4.

The activity of 5'D was determined in the pituitary gland of male and female Wistar rats, 4 months of age. Pituitary homogenates were incubated with ^{125}I-reverse T3 as substrate in the presence of 20 mM DTT and with or without PTU. The iodide formed after 60 min at 37 °C was separated by ion exchange chromatography. The activity of either total or type II 5'D was greater in females than in males (5135 ± 129 vs 2916 ± 334 fmol rT3/h × mg prot, 506 ± 52 vs 206 ± 32 fmol rT3/h × mg prot, respectively).

These data demonstrate that the sex differences in the regulation of the thyroid axis are secondary to a different modulation of the activity of 5'D in the pituitary gland of male and female rats.

Vasopressin in the auditory system of the guinea pig brain

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The distribution of the C-terminal glycopeptide of the vasopressin prohormone was mapped in the brain of guinea pigs using immunocytochemical methods. In the male, immunoreactivity was detected in the same structures than in the rat (Dubois-Dauphin and Zakarian, Experientia *41:* 774, 1985), except that immunoreactive neurones were seen in the bed nucleus of the stria terminalis and the medial amygdaloid nucleus even without pretreatment of the animals with colchicine. In addition to these structures found in the male, we observed a consistent and specific labelling along auditory pathways in the female. Immunoreactive neurones were found in these animals in the medial trapezoid body and the central nucleus of the inferior colliculus; and immunoreactive fibres in the lateral trapezoid body, the medial superior olive and the dorsal cochlear nucleus. The presence of neurones containing the vasopressin-related glycopeptide along auditory pathways of the guinea pig brain indicates a possible action either of this peptide or of vasopressin or neurophysin, which are the other products of the vasopressin prohormone, in a sensory system.

A method for classification of muscle afferents in man

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Classification of muscle afferents in recordings from human subjects is fraught with uncertainty, since decisive, but invasive, tests (such as measurement of axonal conduction velocity) which are routine in acute animal experiments cannot be applied due to ethical constraints. Conventionally in man muscle afferents are classified indirectly, relying on sensitivity to the velocity of imposed stretches. Yet this is not sufficiently discriminative.

In experiments on anesthetized cats a classification test has therefore been designed which is based on a more comprehensive estimate of dynamic sensitivity. The responses of muscle afferents to ramp stretches were compared with those to sigmoid stretches (with triangular velocity and rectangular acceleration components). It emerged that a simple index of dynamic (velocity and acceleration) responsiveness was more effective in distinguishing between muscle spindle primary and secondary afferents than the conventional 'dynamic index' derived from responses to ramp and hold stretches.

Is fusimotor control different in flexor and extensor muscles?

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Recordings from extensor Ia afferents have suggested that fusimotor action during movement in alert cats is largely tonic, and

that it switches from static during routine movements to dynamic during unfamiliar movements. The evidence was derived from acute simulation experiments, in which chronically recorded Ia responses could be reconstructed, when γ-efferents were stimulated with these patterns during reproduction of the original movements.
Simulations of PBSt Ia responses during the same movements suggest that γ-action in flexor muscles is very similar: Ia discharge during stepping was best matched by tonic static (with little dynamic) drive, whilst imposed movements featured increased levels of dynamic and reduced levels of static drive.
This adds to the reservations about extrapolations from studies in reduced (e.g. decorticated) preparations, since for these flexor γ-action was suggested to be static and extensor action dynamic (Exp. Brain Res. *18* (1973) 178).

Calcium transport in plasma membrane subfractions of rat liver

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ATP-driven Ca^{2+} transport was investigated in basolateral (bLPM) and canalicular (cLPM) rat liver plasma membrane vesicles. In bLPM ATP increased Ca^{2+} uptake 5–10 times at pH 7.0, but minimally at pH 8.0. The apparent K_m and V_{max} for Ca^{2+} were 0.33 µM and 1.19 $\mu mol \cdot mg^{-1} \cdot min^{-1}$, respectively. Ca^{2+} uptake was unaffected by Calmodulin. In contrast to bLPM to ATP-driven Ca^{2+} uptake was found in cLPM. In addition, incubation of the LPM subfractions with (γ-32-P) ATP revealed the exclusively presence of a 135 kD phosphorylated intermediate in bLPM. Its formation demonstrated the typical characteristics of a Ca-ATPase and exhibited the same pH sensitivity as ATP-driven bLPM Ca^{2+} uptake. These findings indicate that the liver plasma membrane Ca^{2+} pump is selectively localized at the basolateral surface of heaptocytes, and corresponds to a Ca-ATPase with an apparent molecular weight of 135000. (Supported by SNF 3.881.085).

Unstirred layer an O_2 transfer in the lung

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The thirtyfold increase of O_2 uptake and release velocities in hemoglobin solutions as compared to red blood cell suspensions in vitro (rapid reaction apparatus) is currently explained by the existence of an unstirred layer around red blood cells. In this study we wanted to find out whether such a difference also exists in the microcirculation of the lung. Isolated rabbit lungs were perfused with either human hemoglobin solutions or red blood cell suspension of identical hemoglobin concentration and viscosity. Oxygen lung diffusion capacity (DLO_2) was measured. The experimental conditions were: temperature 29 °C, hemoglobin 50 g/l, ventilation 1.8 l/min, perfusion 125 ml/min. DLO_2 with hemoglobin solution was 0.68 ± 0.12 ml/min/torr and the ratio DLO_2 hemoglobin solution/DLO_2 cell suspension was 1.39 ± 0.29.- Among several hypotheses attempting to explain this result, only the theory of an unstirred layer around the red blood cell could not be ruled out.

Calcium transport in isolated renal plasma membranes from Milan strain of genetically hypertensive rats

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Ca transport systems were investigated in kidney plasma membranes isolated from Milan hypertensive rats (MHS) and their normotensive controls. The V_{max} of ATP-dependent Ca transport was found to be 16% lower in MHS than in MNS. In MHS, in contrast to MNS, the Ca-ATPase was found to exist predominantly and a high-affinity form. The extraction with salt-EDTA removed the activator(s) from MHS membranes which then showed 'normal' Ca transport kinetics. Higher activation of Ca-ATPase was obtained by cytosolic extracts of MHS than with either MNS extracts or pure calmodulin. These data suggest that in MHS plasma membranes a higher proportion of Ca-ATPase molecules exist in an activated, high-affinity form. This might possibly result in a decrease of cell Ca, increased Na reabsorption and Na retention. (Supported by SNF 3.881.085).

Evidence of a transglial channel system (TGCS) and of tight junctions in the schwann cell (SC) sheath of the squid giant axon

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Electron microscopy reveals a 3-dimensional anastomosing tubular system in the SC sheath surrounding the squid giant axon, representing an alternative transglial pathway aside from the well known intercellular cleft system. An analogous system described in crayfish and lobster was previously shown to be continuous with the periaxonal space, the cleft system and the extracellular space (Holtzman et al., J. Cell Biol. *44*, 438–45, 1970; Shrager et al., J. gen. Physiol. *82*, 221–44, 1983). In freeze-fracture, the scattered tubular openings (density: $3.3/\mu m^2$) constitute 3.25‰ of the SC surface facing the axon, as compared with a mere 1.5‰ of the cleft system. Furthermore, a circumferential single-stranded tight junction separates the SC clefts from the periaxonal space. These findings suggest that the TGCS, with its ubiquitous access, provides a preferential route for ion fluxes underlying impulse propagation.

Quercetin modifies the stimulus-hydrosmotic response coupling

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We have reported that quercetin (Q), high K and depolarizing agents (ouabain, veratridine) enhance the hydrosmotic action of vasopressin (VP). Since Q had the unique property of increasing the action of a supramaximal concentration of VP (50 mU/ml), we carried out a more detailed study of the effects of this flavonoid on water transport across the urinary bladder of toads B. marinus. The following observations were made: 1) Q (1 to 100 µM) increased the action of VP (1 mU/ml) in a dose-dependent manner; 2) Q enhanced VP whether the drug was given 2 h before VP or added simultaneously with the hormone; 3) Q (100 µM) also increased the hydrosmotic action of exogenous cAMP, theophylline and forskolin; 4) Q (100 µM) markedly increased the magnitude, and accelerated the time-course, of the action of serosal hypertonicity; 5) except for theophylline, Q was still effective when the response to a given hydrosmotic agent was fully developed. In summary, Q modifies the stimulus-hydrosmotic response coupling but the mechanisms involved in this effect are yet to be defined.

Liver specific gene expression in aggregate culture of fetal rat liver cells

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Cell differentiation and function in aggregate cultures of fetal liver cells was studied by determining the mRNA steady state levels of albumin, α-fetoprotein (AFP) and ferritin and the regulation of tyrosine aminotransferase (TAT) by dexamethasone and/or glucagon. Aggregate cultures of fetal rat liver cells showed a histotypic reorganisation with parenchymal cells organised in small acini with a central lumen. Between 1 and 14 days of culture, ferritin and albumin mRNA levels were maintained, while AFP expression was greatly reduced and TATmRNA basal level increased. At 1 day of culture, there was no response to glucocorticoid, but after 21 days TATmRNA were increased by dexamethasone and/or glucagon (additive responses) as in adult hepatocytes. These results present evidence of differentiation and maintenance of liver specific functions up to at least 3 weeks in aggregate culture of fetal rat liver cells.

Cardiovascular, electrocortical and behavioral effects of nicotine

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The effects of orally administrated nicotine on visual event-related brain potentials (ERP), heart rate and rapid information processing were assessed in deprived female smokers. In a pre-post treatment design, 10 subjects received a 4-mg nicotine chewing gum, 10 subjects a placebo.

The mental task required the subjects to detect sequences of three odd or even digits in a pseudorandom series of single digits. Thereby event related potentials of the correctly responded triplets were analysed, yielding a CNV after the second digit and a P300 after the third digit. By using a variable subject-paced interstimulus interval, performance was analysed continuously in terms of the achieved picture rate.

Nicotine did not affect mental performance in this task. However heart rate and the CNV magnitude increased, suggesting a general arousal augmentation. The results indicate that while the effect of nicotine upon arousal is similar to that following cigarette smoking, nicotine does not affect mental performance as has been reported after smoking.

Hypoxia in brain cell cultures

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Dissociated brain cell cultures were kept for 14 days in vitro (DIV) and then exposed to hypoxia. The effect of hypoxia on protein, DNA, the oligodendroglia enriched enzymes, cerebroside sulfotransferase (CST) and cyclic nucleotide phosphohydrolase were investigated. In addition the direct cellular effects of oligodendrocytes, characterized by specific markers, were studied. After hypoxia CST was 22% of that of controls and the number of oligodendrocytes reduced to 50%. Astrocytes characterised with the specific marker glia fibrillary acidic protein (GFAP) showed no effect.

In order to investigate possible regeneration, cells were kept after hypoxia at 14 DIV for an additional 7 DIV under normoxic conditions. This resulted in a clear recovery of the CST activity but not of the number of oligodendrocytes. The results indicate that under the experimental conditions the oligodenrrocytes are more vulnerable to hypoxia than astrocytes and that there is a recovery of the enzyme activity but not of the number of cells.

Effects of muscle temperature on maximal instantaneous power in man

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The maximal anaerobic power of man ($\hat{w}$) determined during an all-out vertical jump on a force platform may reflect the maximal ATP splitting rate in the muscles. Temperature, affecting the latter, may influence also $\hat{w}$. This hypothesis was tested in a group of differently trained subjects whose vastus lateralis temperature (Tm) was monitored by a thermocouple inserted 3 cm deep in the muscle. $\hat{w}$ was measured before (T_{air} = 22 °C) and after 1 hour immersion in a thermoregulated water bath at 20 ± 0.2 °C. The control Tm and $\hat{w}$ values ranged from 33.7 to 36.6 °C and from 48.7 to 65.5 $W.kg^{-1}$, respectively. Following cold exposure, Tm decreased by 8.0 ± 1.0 °C and $\hat{w}$ by 31% ± 8 (n = 6), i.e. less than could be expected on the basis of the usually assumed Q_{10} of 2 to 3. This seems to indicate that either the actual Q_{10} is less than 2 and/or that the efficiency of contraction increases with decreasing Tm. (Supported by the Swiss National Funds for Scientific Research, Grant No. 3.364-0.82)

Increased plasma clearance rate of thyroxine despite decreased 5'-monodeiodination: study with a peroxisome proliferator in the rat

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Nafenopin, a hypolipid agent and peroxisome proliferator, decreased serum total and free T_4 concentrations to 32 ± 2% and 62 ± 8%, respectively without change in serum T_3 and TSH concentrations. Plsama clearance rate (PCR) of T_4 was decreased 2-fold, while T_3 PCR was decreased by 30%. Liver, kidney, cerebral cortex and interscapular brown adipose tissue (IBAT) [^{125}I]T_4 contents were also decreased to 71%–84%. Hepatic and renal 5'-deiodinase type I activities were strongly inhibited by nafenopin treatment, whereas total and 5'-deiodinase type II activities in cerebral cortex were increased. In IBAT and anterior pituitary, however, total and 5'-deiodinase type II activities were not modified.

In conclusion, the modification in the thyroid hormone levels under nafenopin treatment was mainly due to a marked increased in T_4 PCR and decreased T_3 PCR and not to T_4 to T_3 conversion. Yet, the mechanisms of the altered thyroid hormone metabolism remain to be investigated.

Location of pulmonary stretch receptors in the guinea pig

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The increased and tonic discharges of pulmonary stretch receptors (SR) during bronchial asthma-attacks has been considered an indirect evidence for a quite peripheral location of SR in the guinea pig (Koller and Ferrer, Respir. Physiol. 17: 113–126, 1973). To verify this suggestion, the site of SR was searched for in anesthetized, thoracotomized guinea pigs by analyzing the changes of SR discharge pattern in response a) to occlusion of the airways, b) to local probing, and c) to microinjection of the local, non-diffusible anesthetic cinchocaine into the presumed receptor site. The great majority (92%) of SR was found to lie in small airways or in lung parenchyma ('peripheral SR'), whereas only 8% were located in large airways, i.e., in the trachea, main bronchi, lobar bronchi ('central SR'). The discharge responses to lung inflation and to ammonia-inhalation slightly differed between these two SR groups. With the pronounced prevalence of peripheral SR, the guinea pig seems to take a unique position among the species examined hitherto.

Serotonin is the mediator of a slow excitatory potential in mammalian sympathetic ganglia

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Repetitive nerve stimulation elicited in celiac cells, in addition to the nicotinic fast excitatory postsynaptic potential (EPSP), a slow non-cholinergic EPSP. Neuropharmacological, neurochemical and histochemical evidence presented her indicate that in a portion of the cells, serotonin is the mediator of the slow-EPSP. First, the electrophysiological action of 5-HT on celiac neurons is similar to that of the transmitter released synaptically. Second, perfusion with 5-HT reversibly abolishes the non-cholinergic EPSP; this observation is compatible with the phenomenon of receptor desensitization that can be obtained in tissues endowed with serotoninergic receptors. Third, the slow depolarization whether evoked presynaptically or by 5-HT were affected by a number of pharmacological agents in a manner consistent with the hypothesis that 5-HT is the mediator of the non-cholinergic EPSP. Finally, 5-HT-like immunoreactivity is localized in the ganglia, primarily in nerve fibers surrounding the ganglionic neurons.

The respiratory and cardiovascular responses to high altitude during autonomic blockade

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In previous papers we demonstrated the significance of the sympathoadrenal system in mediating the short-term adjustments to hypoxic hypoxia. In this communication the effects of autonomic blockade on the acute respiratory, circulatory, and in particular the ECG responses to high altitude were studied in 11 male volunteers during standardized ascent to 6000 m in a low pressure chamber 1) without (control), 2) with propranolol, and 3) with propranolol and atropine in a randomized manner. Results: Autonomic blockade does not influence the hypoxic hyperventilation and alveolar gases, but betareceptor stimulation and vagal withdrawal account for the increase in heart rate and in systolic blood pressure, for the P pulmonale, and in part for the lengthening of Q–T and the T wave depression during hypoxia. The fall in diastolic blood pressure, the lengthening of P–Q, and the minor but still significant T-flattening after autonomic blockade are thought to be direct hypoxic effects.

'Medial hypothalamus-syndrome' as a model of atypical symptoms in seasonal affective disorders (SAD)

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SAD are characterized not only by depressive mood, decreased activity, libido and social contact, but also by a number of atypical symptoms[1]: 1. Recurring periodic depression in autumn and winter. 2. Increased sleep need in winter. 3. Carbohydrate craving. 4. Body weight increase. 5. Increased frequency and altered timing of meals (recent results). In animals, seasonal rhythms (e.g. hibernation, metabolism, melatonin secretion) are mediated by the photoperiods acting via the medial hypothalamus, especially the paraventricular nucleus (PVN). Carbohydrate craving, body weight increase and circadian rhythm disturbances (e.g. of food intake, sleep parameters, melatonin secretion) are induced by lesions of nuclei in the medial hypothalamus. Alpha2-noradrenergic and serotoninergic mechanisms are known to affect neural activity in the PVN and the behavioral and physiological parameters described above[2]: these neurotransmitters are therefore putative canditates for induction of the atypical symptoms in SAD. [1]N. E. Rosenthal, etal., Arch. gen. Psych. 41, 72–80 (1984); [2]S. F. Leibowitz, Psychopharmacol. Bull., 21, 412–418 (1985)

Rat median eminence: a possible source of cerebrospinal fluid vasopressin

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There are two separate pools of vasopressin (VP) in the median eminence (ME). In the external layer fibers originating in the paraventricular nuclei terminate on to the portal capillaries which drain to the adenohypophysis. The internal layer contains fibers on their way to the neural lobe originating in the supraoptic nuclei. It has been shown that these fibers could also be a source of VP release at the level of the ME. Moreover, by slicing the median eminence so that the two layers are crudly separated, we found that 70% of the ME-VP are in the internal side. Is a release of VP from the ME into the third ventricle possible? And what would be the source of this VP? To answer these questions we used a double compartimented chamber to test on which side a peptide is released. Results obtained with this new technical approach concerning VP release are presented and compared with LHRH release in the same conditions.

Representation of the rat's forelimb digits in the cuneate nucleus

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Digit representation was studied by means of anatomical and physiological techniques. The anatomical approach consisted of injecting individual digits intracutaneously with small portions (2 µl) of 5% HRP-WGA. Injections placed laterally and medially into glabrous skin of individual digits resulted in the pattern of discrete patches of the reaction product. For each digit one or more dorsal and ventral patches could be distinguished. The caudorostral extension of these patches seems to be slightly different for digits II–V. In the mediolateral dimension, representations of neighboring digits partly overlap.

Microelectrophysiological mapping was achieved by electrical stimulation of digits with fine insect pin needles and by gentle natural stimuli of the skin. The site of field potentials and of units displaying small receptive fields (e.g. finger tip) are marked with Alcian blue for histological reconstruction. Preliminary results indicate that it is possible to establish a detailed micromap of the digit representation in the cuneate which extends from the obex about 2 mm caudally.

Heptanol impairs the electrical and mechanical properties of isolated guinea pig ventricular cells

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Heart cells isolated from adult guinea pig ventricles were used to perform electrical and mechanical measurements by means of the wholecell, tight-seal recording technique and a laser diffraction method. Experiments carried out on single cells yielded a dose-dependent decrease in calcium inward current (I_{si}) upon exposure to 1-heptanol (0.5 to 3 mM). This was inferred from single voltage-clamp data. The decrease in I_{si} was accompanied by a reduction in mechanical tension both during systole and diastole. This was documented by monitoring the sarcomere spacing in a separate study. Experiments carried out on isolated pairs of cells demonstrated that the same treatment leads to a

variable electrical uncoupling. This was assessed by determining the nexal membrane resistance by means of a double voltage-clamp approach. The long-chain alcohol exhibited reversible effects on the three parameters investigated. Supported by S.N.S.F. (3.253-1.85).

Polarity of Na/H exchange in cultured kidney cells (LLC-PK$_1$)

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LLC-PK$_1$ is a continuous cell line with renal epithelial characteristics. LLC-PK$_1$ cells were seeded onto a Nucleopore filter. One to three days after the formation of a confluent monolayer, cells were mounted in a miniature Ussing-type chamber and intracellular pH (pH$_i$) of single cells was examined by microspectrofluorometry (BCECF). Cells were acidified by ammonium pulsing and examined for the mechanism of recovery from the acid load. The pH$_i$ recovery is Na-dependent (versus TMA) and is sensitive to 100 nM ethylisopropyl amiloride. The pH$_i$ recovery requires Na at the basolateral membrane (the surface attached to the filters). Seeding density was varied over a 30-fold range and filters were tested both with and without collagen treatment. Neither variable affected the results. It is concluded that LLC-PK$_1$ cells recover from acid loads via Na/H exchange in the basolateral membrane. (Suppported by SNF 3.881.085).

The effect of calcitonin on nociceptive transmission to the ventrobasal thalamus of the rat

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Calcitonin, the well known hypercalcemic peptide, has profound physiological functions mediated by the central nervous system. Recent reports have shown that calcitonin influences pain perception through antinociceptive activity. In the present study the effect of salmon calcitonin on the transmission of noxious messages to the thalamic ventrobasal complex was studied in rats anesthetized with pentothal. Calcitonin injected into the femoral vein consistently inhibited the discharge of thalamic neurones induced by noxious mechanical, electrical or thermal stimuli. This inhibition began within 5 min of application and attained its maximum effect 20 min after the injection. Full recovery was seen 30 min after the maximum analgesic action. Control application of a calcitonin placebo produced no significant change in the discharges evoked.

Parvalbumin in rat skeletal muscles of different thyroid states

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In hypothyroidism isometric twitch contraction time and half relaxation time are increased, while the contrary is the case in hyperthyroidism. These changes are largely paralleled by alterations in the histochemical fiber type composition. Parvalbumin (PV) is thought to be involved in the relaxation process of fast-twitch muscle fibers. To investigate whether the content of PV varies according to or independently of these muscular changes, EDL, SOL, and gastrocnemius muscles of rats were analysed by histo-, immunohistochemistry, and 2D-PAGE, while PV was quantified by HPLC and RIA. PV distribution and PV levels were largely found unaffected in all thyroid states. If PV levels (as is generally assumed) are neuronally controlled, our finding support the view that the occurring changes are generated by a direct action of thyroid hormone on muscle fibers, and not via their nervous input.

Continuous versus single bolus enteral nutrition: comparison of energy metabolism in man

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Continuous respiratory exchange measurements were performed on 5 women and 5 men for one hour before and six hours following: 1) the ingestion of a milkshake (53% CHO, 30% lipid, 17% protein energy) given as a single bolus does or: 2) continuous nutrient administration via a nasogastric tube for 3 hours. The energy administered corresponded to 2.3 times the post-absorptive resting energy expenditure (REE) calculated for 3 hours. REE, respiratory quotient (RQ), plasma glucose and insulin concentrations rose sooner and steeper and plasma FFA levels decreased earlier with 1 than with 2. Nutrient induced thermogenesis was greater ($p < 0.01$) with 1 ($\bar{X} \pm$ SEM, $10.1 \pm 0.6\%$) than with 2 ($8.3 \pm 0.5\%$). It is concluded that the mode of enteral nutrient administration does influence the thermogenic response as well as the pattern of changes of RQ, glycemia and insulinemia. The energy efficiency of enteral nutrition by continuous nutrient administration is greater than that of oral bolus feeding.

CRF-activity of deamino-8-D-arginine vasopressin (DDAVP) in sheep and its possible relationship to hemostasis

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Two different mechanisms are likely to be involved in the release of ACTH by neurohypophysial peptides and their analogs in vivo: an indirect action (via endogenous CRF release) mediated by V1 receptors and a direct effect on the pituitary initiated on receptors different from V1 and/or V2. DDAVP and some other non-vasopressor analogs seem to act by the latter mechanism. In sheep, time change of plasma ACTH following DDAVP injection (1 µg/kg body weight; iv) displays two peaks at approximately 30 and 100 min. The control (saline injection) showed no effect.

Intravenous administration of DDAVP also increases the plasma concentration of the coagulation factor VIII and the plasminogen activator. The shape of the time-response profiles ressemble those of ACTH response. The question arises whether these effects are causally connected.

Does the initial part of the early birefringence signal reflect T-tubular conduction delays in isolated single muscle fibres?

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The early large birefringence signal (BS) [Baylor and Oetliker, J. Physiol., 264, 141 ff], assumed to reflect changes in free myoplasmic Ca during the initial phase of EC-coupling, departs from its horizontal baseline about 1 ms after stimulation. The slope becomes progressively more negative before reaching a steady value. This raises the question whether the delay in reaching the linear part of the decline in light intensity is related to the fact that more myofibrils are activated as excitation spreads inward or to the intrinsic time course of the signal itself. Relating the delay to fibre diameter yields conduction velocities of inward

spread of activation of 7 to 10 cm/s. These values compare well with 8.2 cm/s found by Gonzales-Serratons (J. Physiol. 212, 763 ff) using high speed cinegraphy. Increasing Ca to 10 mM reduces inward conduction veolocity by about 20%. The above findings suggest that the initial part of the BS contains information about inward conduction.

Light-induced changes of O_2 consumption and intracellular sodium in the photoreceptor cells of the barnacle, *Balanus eburneus*

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In the photoreceptor cells of the honey bee drone, the recovery of O_2 consumption (QO_2) after a flash of light is faster than that of intracellular sodium (Na_i^+). This led to the surprising conclusion that the light-induced increase of QO_2 may not be caused by an increased working of the Na^+ pump (Tsacopoulos et al., 1983, Nature *301*: 604). We have measured QO_2 and Na_i^+ in the photoreceptor cells of the lateral eye of the barnacle, a crustacean. In sea-water, the light-induced increased of QO_2 (ΔQO_2) and Na_i^+ (ΔNa_i^+) recovered with the same time course. Removal of Na^+ or Ca^{2+} from the bathing solution almost suppressed ΔNa_i^+ and ΔQO_2. Removal of K^+ from the bathing solution strongly reduced Na^+ pumping and greatly shortened ΔQO_2. Since some K^+ may leak out of the cell after stimulation and shortly activate the Na^+ pump, we conclude that so far, and unlike in the drone retina, we have not observed any clear dissociation between ΔQO_2 and the working of the Na^+ pump in the lateral eye of the barnacle.

Contribution of the mitochondria-rich cells to the oxygen uptake of the toad skin epithelium

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The chloride conductance pathway in amphibian skin was localized recently to the mitochondria-rich cells (MRC). The density of the MRC increases with the decrease in the environmental chlorinity. Toads *(Bufo viridis)* have been acclimated to either NaCl or $NaNO_3$ solutions (100 mmol/l for over two weeks) to affect the density of the MRC in the epithelium of their skin. The oxygen consumption of the split epithelia was measured in vitro at 24 °C by the spectrophotometric oxyhemoglobin method. The O_2 uptake of the epithelia from NaCl acclimated toads was 0.74 nmol/mm^2 · h and that from $NaNO_3$ acclimated toads was 1.25 nmol/mm^2 · h. Theophylline (1 mmol/l) applied to the serosal side did not have any significant effect on these values. Ouabain (100 µmol/l; serosal side) decreased the O_2 uptake of the epithelia from the $NaNO_3$ acclimated toads by 17%, but did not affect the rate of O_2 uptake of the epithelia from the NaCl acclimated toads. These measurements suggest that under the acclimation conditions where sodium transport across the skin is greatly suppressed, the O_2 uptake of the whole epithelium is linked to the density of the MRC.

An excitatory action of neurohypophysial peptides on septal neurones mediated by vasopressin V_1 receptors

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The effect of vasopressin and of oxytocin on the firing of neurones in the dorsal septum of rats was tested by extracellular recording from coronal slices. Forty-two out of 83 neurones responded to vasopressin at 2–100 nM by a reversible, concentration-dependent increase in firing. Usually, at peptide concentrations greater than 200 nM the neuronal membrane was so strongly depolarized that action potentials inactivated and that the neurones stopped firing transiently. The action of vasopressin could be mimicked by a vasopressor agonist, [Phe^2, Orn^8]VT, and was reversibly blocked by the antagonists d(CH$_2$)$_5$Tyr(Me)AVP and dEt$_2$Tyr(Me)DAVP. By contrast, dDAVP – an antidiuretic agonist – was without effect. Oxytocin was 20–100 times less potent than vasopressin and the selective oxytocin agonist HO[Thr^4, Gly^7]OT was devoid of any activity. These results indicate that in the rat, neurohypophysial peptides excite a subpopulation of dorsal septal neurones by acting onto V_1 (smooth muscle-type) vasopressin receptors.

Functional organization of the ventral and dorsal divisions of the medial geniculate body (MGB) of cat

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The functional organization of the MGB was studied by correlating the location of 2500 single units with their response properties to click, noise and tones. In the ventral division, significant changes of response occured along the rostrocaudal axis, perpendicular to the best frequency gradient. Going from rostral to caudal, the response latencies became progressively longer and more variable, the proportion of inhibitory response patterns increased, the responsiveness to broad band stimuli decreased, the tonotopic arrangement was less strict and units became more broadly tuned. In the dorsal division, the distribution of some response properties could be related to its anatomical parcellation: the dorsal nucleus (D) differed from the suprageniculate nucleus (SG) by longer response latencies and a greater proportion of inhibitory responses, whereas the deep dorsal nucleus had response properties intermediate between D and SG.

Discordance between salivary and calculated free testosterone levels in the assessment of androgenicity

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The close correlation usually observed between salivary and free plasma testosterone levels led to admit the occurrence of passive mechanisms governing the flux of testosterone (T) from blood to saliva.

In contrast, the present results obtained in 8 normal males in basal conditions (NMB) and 24 h after a T injection (NMT), 8 impotent men (IM), 15 normal females (NF), and 14 hirsute women (HW) suggest the intervention of concomitant active mechanisms. Saliva T levels (sT) were measured and plasma free T values (fcT) were calculated (Sodergard et al., 1982). The sT-fcT differences were evaluated (dfT). According to the concept of passive flux, we had to observe identical values (dfT = O). On the contrary significant differences in dfT were registered between the following groups: NMB < NMT (-23.3 ± 41.5 pmol/l vs 405.0 ± 120.6, mean $\pm$ SEM), IM < NMB ($-164.,6 \pm 55.1$ vs -23.3 ± 41.5), NF < HW (31.0 ± 6.5 vs 78.6 ± 8.3).

We propose that dfT will allow to quantify the active role of salivary gland in T flux and furthermore to determine the factors implicated in regulation of this function.

Profiles of extracellular currents in the cardiac region of the chick embryo

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The detection of small electrical currents in biological preparations by means of a vibrating probe has become a widely used method (Jaffe, TINS 1985: 517–521). In order to study in situ the electrical activity of embryonic tissues we have developed such a probe and used the lockin detection of signals with adjustable integration time. This technique allowed us to obtain the best signal to noise ratio. The cardiac region of 10–16 somites chick embryos explanted in vtiro was scanned by using this vibrating electrode. Steady-state currents of the order of 1–100 $\mu A/cm^2$ were detected. The source was located at the right contour of the bulboventricular region, the sink was diffusely distributed over the sino-atrial regions. Alternative currents synchronous with heart beats were superimposed onto the steady-state component and represented about 10% of its amplitude. This method allows non-invasive in situ studies of the differentiation of electrical properties of the cells within the heart primordia.

Lack of responsiveness of the TIDA neurons to prolactin in aged female rats

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Aging in rats is characterized by a sustained reduction of the secretory activity of the hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons ($p < 0.005$) which is associated with a chronic hyperprolactinemia ($p < 0.001$). This observation appears paradoxical in view of the reported regulatory effect of PRL on TIDA neurons and it is suggestive of an impairment of the neurons responsiveness to PRL with age. In the present study we have evaluated DA synthesis in TIDA neurons (estimated by DOPA accumulation in the median eminence after inhibition of DOPA decarboxylase) and DA release into hypophysial portal blood in young (4–5 months) and old (25–26 months) female rats treated with oPRL (4 × 5 mg/kg b.wt s.c.) or vehicle. In young rats oPRL induced an increased synthesis of DA in TIDA neurons ($p < 0.01$) and an enhanced release of DA into hypophysial portal blood ($p < 0.01$) whereas in old animals oPRL had no effects on either synthesis or release of DA. The data are illustrative of an impairment of the TIDA neurons responsiveness to PRL with age in rats and they are supportive of an age-related loss of the short loop feedback of PRL on its own secretion through regulation of the TIDA neurons.

Cable analysis during early hypoxia and ischemia in rabbit myocardium

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Conduction velocity (v), extra- and intracellular longitudinal resistances (r_o and r_i) were determined in blood perfused papillary muscles from extra- and intracellular recordings during hypoxic perfusion and after interruption of flow (ischemia). Hypoxia for 10 min produced a decrease of r_o ($-28 \pm 5\%$; S.E.; $n = 10$) after 10 min and an increase of v by $8 \pm 1\%$. In contrast, ischemia increased r_o after 10 min by $57 \pm 7\%$ ($n = 8$) and decreased v by $-16 \pm 3\%$, r_j did not change under both, hypoxic and ischemic conditions. We conclude that during the early hypoxic and ischemic phase changes of extracellular volume and associated changes in r_o influence impulse propagation. Hypoxia induces relaxation of vascular muscle cells and most likely endothelial macromolecular leakage with an increase of extracellular space and a decrease of r_o. Ischemia leads to interstitial shrinkage consequent to osmotic cell swelling with an increase of r_o. The results underline the importance of the restricted extracellular space of ventricular muscle during the early phase of O_2-withdrawal.

Continuous measurement of beat frequency of monolayer myocyte cultures under incubation conditions

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An optical device has been built for continuous registration of the spontaneous beat frequency of cultured myocyte monolayers in the incubator. Contractions are registered through changes in scattering of light directed towards the monolayer. Beating rates (1/min to far beyond physiological range) are displayed and can be recorded continuously on a chart recorder. This device avoids drawbacks of conventional long term observations of the beat frequency of cultured myocyte monolayers: the cultures do not have to be removed from the stable environment of the incubator (temperature/pH shift) and the observations are continuous, allowing a precise measurement of the time course of the beating rate. Ongoing experiments supply long term frequency spectra of serum supplemented and of serum free cultures under control conditions, as well as under the influence of chronotropically acting substances.

Oxygen tension studies in experimental peripheral arterial ischemia in rats

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We have earlier reported on the hemodynamic aspects in experimental unilateral periopheral arterial ischemia in rats. A significant decrease in blood flow to flexor digitorum muscle (−41%, $p < 0.001$) and hind paw (−55%, $p < 0.001$) on the ligated side was observed after 2 days of ligature. Presently, we have made an attempt to study oxygen tension at muscular level in gastrocnemius muscle after unilateral (left side), ligature of femoral and ileofemoral arteries. Measurement of oxygen tension (PO_2) at the surface of gastrocnemius muscles of left and right legs were made with two multichannel (8 channels each) electrodes of Kessler and Lubbers. These measurements were made in anesthetized rats with unilateral peripheral arterial ischemia induced two days earlier. Reproducible and uniform PO_2 values were obtained after numerous electrode rotations for a minimum period of 10–15 min. In the control series of animals ($n = 70$), the PO_2 difference between left and right side was 6% ($p < 0.001$). In experimental group (unilaterally ligated animals, $n = 48$), a marked decrease in PO_2 (−51%, $p < 0.001$) was observed after 2 days of ligation. These results support the earlier obtained findings on hemodynamic aspects.

From the motor command to the movement

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In order to study the transition from a motor command to the ensueing movement two motor commands were transmitted simultanously to a motor pool and the interaction of the corresponding movements was investigated.

Subjects were required to maintain a steady force in both legs at the onset of a warning signal. At the onset of a light, they had to superimpose a ballistic contraction on the steady torque. If both contractions were in the same direction the size of the ballistic contraction was independant of the steady force. Since we had given reasons to believe that the motor command for the ballistic

contraction was independant of the steady flexion force, we concluded that the output of the motor pool is linearly related to its input.

In a second experimental paradigm, the superposition of H reflexes on steady contractions was studied. Similarly as in the preceding experiment, the amplitude of the H reflexes did not depend on the level of voluntary drive.

Assuming 1) a linear input-output relationship of the motor, 2) a regular recruitment of motor units according to the size principle, and 3) a constant threshold for ll motor units, we computed the size of EPSPs of motor units in relation to their contraction force. The relation agreed with published experimental data on the size of EPSPs of motor units in the cat.

EMG activation patterns during precision grip

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In each of two monkeys *(M.fascicularis)* we recorded intramuscularly from finger, arm and shoulder muscles while the animals generated and maintained two successive low-force levels between thumb and index finger. All intrinsic and extrinsic finger muscles and some wrist muscles were coactivated. Their EMG activity was modulated with force and increased shortly before onset of force increase from low to high force level. Arm and shoulder muscles had other characteristics: irregular coactivation, little modulation with force and late increase of activity. One monkey was trained to change force levels either in a slow, controlled fashion or in a fast 'ballistic' mode. Under this last condition several of the arm muscles, which were irregularly coactivated in the controlled mode, showed clear and regular activity increase with force. In conclusion many muscles are coactivated in low-force precision grip. Intrinsic and extrinsic finger muscles are always tightly coupled with force whereas arm and shoulder muscles participate in precision grip in specific motor strategies only.

Does a tonic inhibition on brown fat heat production exist in the central nervous system of the rat?

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Temperature of interscapular brown fat (Tibf), colon (Tc) and skin (Ts) were monitored after μ-wire-knife cuts at the post-mammillary and pre-pontine level in urethane-anesthetized rats. While post-mammillary cuts produced no temperature change, pre-pontine cuts were followed by an increase of Tibf and Tc of 3–4 °C. Tibf rose faster than Tc and Ts rose only 0.8 °C. Procaine injection into the pre-pontine area caused a reversible increase in Tibf and Tc. Propranolol or hexamethonium injected during the hyperthermic plateau produced a decrease of Tibs followed by a decrease of Tc whereas tubocurarine injection was without effect. Cardiac output distribution showed a 10-fold increase of fractional output in brown adipose tissue with little or no change in other organs, particularly squelettal muscle, skin and extremities. It is concluded that hyperthermia following knife-cut in the pre-pontine level is probably due to the release of a tonic inhibition of the sympathetic afference to brown adipose tissue.

Analysis of S1-nucleotide binding to regulated thin filaments

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Theoretical analyses of the interactions among thin filament proteins which are responsible for the Ca activation of contraction in vertebrate striated muscle have led to varying results. Hill et al. in an analysis of S1-nucleotide binding to regulated thin filaments (PNAS 77: 3186, 1980 and 80: 60, 1983) found an attractive tropomyosin-tropomyosin interaction and assumed no interaction between actin-myosin complexes, whereas I (Shiner, Biophys J. 50: 601, 1986) found a repulsive tropomyosin-tropomyosin interaction and an attractive interaction between actin-myosin complexes from an analysis of Ca-binding to troponin incorporated into thin filaments. Furthermore, my results predict hysteresis in the Ca-activation of contraction, whereas the models of Hill et al. cannot predict hysteresis. The S1-nucleotide binding data treated by Hill et al. are analyzed here on the basis of my theoretical considerations. Preliminary results are consistent wiht my previous results; the values calculated for S1-ADP binding also predict hysteresis.

Exposure to novelty induces naltrexone-reversible analgesia in rats

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In a first series of experiments, the exposure of rats for 2 min to an open field, or to a small box, or to inhibitory avoidance training was followed by a mild analgesia measured by the tail-flick method. The analgesia was observed as soon as 10 sec after exposure and lasted between 10 and 30 min. It was not observed in animals familiar with the test situation and it was reversed by the administration of naltrexone (0.1 mg/kg, i.p.). In a second experiment, additonal stressful stimuli (0.5 mA footshocks of 1.5 sec, light flashes, or tones) presented during the 2 min exposure to the small box did not significantly alter novelty-induced analgesia. Post-novelty application of light flashes or tones lasting 10 sec or 2 min, while the animals were alone in a waiting cage, prevented the analgesic response. The data suggest that novelty per se is a sufficient stimulus to activate an opioid-mediated analgesic system, and that the expression of this response can be impaired by treatments that follow novelty.

Use of a relational database system for the analysis of the functional organization of a brain structure

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Recordings of multiple spike trains in thalamic and cortical structures can provide very large amounts of data. In order to store the results in a suitable way for various types of analysis, we chose the relational Database management system 'ORACLE'. We subdivided the data set into several tables, each one concerning a particular topic, such as unit location, characteristics of spontaneous activity obtained by renewal density functions, response characteristics to various stimuli and properties of unit interactions. This system allows a totally independent update of each table, and permits a step to step study of the data obtained on a structure. Moreover, we have realized an interface for a three dimensional representation of the units in a stereotaxic referential (Villa et al., Int. J. Bio-Medical Comp. 19, 1986).

Neural regulation of insulin secretion: fine abdominal vagal pathways

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The vagus nerve is known to carry impulses modulating insulin secretion. Its gastric, coeliac and hepatic branches have been indirectly shown to play a role in conveying these modulatory impulses. The coeliac branch divides in at least three distinct bundles, the mesenteric (m), splenic (s) and pancreatico-duodenal (pd) ones. The m-bundle consistently displays an action potential upon cervical vagal stimulation. The s-bundle does so only occasionally, and the pd-one fails to do so. When electrically stimulated, the m-bundle induces an increased insulin secretion, the s-bundle a trend towards an increase, and the pd-one is ineffective. It appears that the m-bundle is a main vagal pathway, and a major supplier of parasympathetic fibers to the endocrine pancreas. Our view is that when dealing with a particular abdominal branch, one has to keep in mind its possible further divisions and to avoid ascribing too specific functions to it.

A new method to assess deficiency of brain performance in the aged

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The therapeutic effects of nootropic drugs are difficult to evaluate because few noninvasive, reliable and reproducible methods for the assessment of brain performance are available. Visual information processing in humans may be a promising marker function. We therefore studied performance in visual backward masking in a group of young ($\bar{x} = 23 \pm SD$ 3 years) and a group of elderly ($\bar{x} = 73 \pm SD$ 5 years) healthy, community residing volunteers. In backward masking, stimulus letters are presented followed by a mask composed of chopped letter fragments. At temporal intervals between onset of stimulus and mask (SOA) < 100 msec the mask interferes with the stimulus, thus reducing readability of the letters. At a SOA of 80 msec the mean rate of errors in the aged group was twice as high than in the young group. ANOVA indicated a strong main effect of age $F(1.7 = 39.0, p < 0.001$. In addition, the elderly subjects were retested 2–4 weeks later revealing a high stability of performance over time.

Visual masking may be a useful paradigm to assess brain performance deficiency in the aged and to evaluate possible effects of nootropic drugs.

The circadian concentration of hormones and neurotransmitters is individually determined and stable with time in normal subjects

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In the course of a study on depression, 5 normal males (control group) were studied during two 24 hours periods, once during usual activities, and once during fasting, bed rest and dim light. Activity increased heart rate, temperature and TSH but decreased melatonin; cortisol increased in some subjects. Melatonin and cortisol secretions tended to occur earlier at night during rest. Prolactin and GABA were minimally changed. In one subject studied twice, the patterns were highly reproducible. Rest versus activity influenced the secretion of melatonin, cortisol and TSH (which, when abnormal, are considered biological markers of depression). The simultaneous study of different rhythms and their reactivity to the environment leads to defining patterns that characterize each individual. Grant 3.9680.85 from the Fonds national suisse.

Dopamine neurons of the monkey midbrain discharge in response to behaviorally significant visual, auditory and somatosensory stimuli

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Monkeys were seated in a primate chair and trained to keep their hand relaxed on a holding key. They reached for food reward into a small box ahead of them when its door opened visibly or audibly. They were also able to emit self-initiated movements, in which they released the holding key in order to reach into the covered food box without an external trigger. Discharge activity was recorded from single dopamine (DA) neurons in substantia nigra and adjoining midbrain areas. Most DA neurons responded with a short burst of impulses to visual or auditory opening of the food box as behavioral trigger stimulus, but not to identical control stimuli. A similar response occurred during self-initiated movements when the animal's fingers touched the morsel of food inside the covered box, but not when touching other objects. These data show a direct implication of primate DA neurons in mechanisms of behavioral responsiveness.

Equality of CO affinity in foetal and maternal blood

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The relative O_2/CO affinity of foetal blood is known to be smaller in foetal than in maternal blood although the relative role of the O_2 and CO affinities of foetal hemoglobin (HbF) is not known. In the present study we have compared the CO equilibrium curves (COEC) in maternal blood and in blood sampled from the umbilical cord immediately after delivery.

Blood samples of both origins werer tonometered in a special open bubble tonometer allowing equilibration within 5 hours. Tonometry gas mixtures contained various amounts of CO, no O_2, and 6% CO_2 in N_2. After tonometry, blood was analysed chromatographically for its CO and O_2 contents, for its 2,3-DPG molar ration and for its HbF.

It was found that P_{50} Co and Hill's n were the same in maternal blood and in blood from the umbilical cord containing on the average 70% HbF. Thus our results suggest that the low value of M of foetal blood is entirely due to the left shift of foetal blood O_2EC. In this respect, the possible accumulation of CO by the foetus in a smoking mother is not explainable by a higher CO affinity.

Extracellular K^+ and membrane potential affect hippocampal IPSPs in vitro

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Cl^--dependent IPSPs in hippocampus are reduced by repetitive stimulation. Possible mechanisms include reduction of the evoked conductance increase (g_{IPSP}) and reduction of Cl^- driving force. Under voltage clamp, stimulation of mossy fibers at 3 hz for 1 min reduced g_{IPSP} 25–50% in CA3 neurons of cultured hippocampal explants and decreased E_{IPSP} 5–10 mV. The shift in E_{IPSP} resulted from an increase in Cl^-_i because it was not observed when cells were held more negative than E_{IPSP}. The involvement of activity-dependent increases in K^+_o in the shift in Cl^-_i was examined by altering K^+ in the bathing media. Reducing

K^+_o from 5.8 to 1 mM increased E_{IPSP} 8–12 mV, even when V_m was held constant. Blocking K^+ channels with intracellular Cs^+ did not block this effect of K^+_o. Increases in K^+_o from 1 to 5.8 mM decreased E_{IPSP}. The decrease was smaller when V_m was held at more negative potentials. Polarization of V_m away from E_{IPSP} could alter E_{IPSP} when spontaneous IPSPs were prominent. When the frequency of spontaneous IPSPs was reduced, changing V_m had no effect on E_{IPSP}, suggesting that these IPSPs contribute a significant resting Cl^- conductance.

Castration reduces the density of oxytocin but not of vasopressin binding sites in the rat brain

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The effect of castration and of gonadal steroid treatment on the distribution and density of oxytocin and vasopressin binding sites have been studied in the rat brain by in vitro autoradiography. Vasopressin binding was found to be unaffected by gonadectomy. In contrast, castration caused a marked reduction of oxytocin binding sites in the Calleja islands, the hypothalamic ventromedial nucleus and a partial reduction in the central amygdaloid nucleus, while their density remained unchanged in the anterior olfactory nucleus and the ventral subiculum. Treatment of castrated animals with either testosterone or oestradiol prevented the disappearance of oxytocin binding sites, but did not affect the density of vasopressin binding. These results indicate that the synthesis of oxytocin but not of vasopressin receptors is dependent upon gonadal steroid hormones. Those oxytocin binding sites which disappear after castration may be part of a neural circuitry involved in reproductive function.

An extracellular signal modifies glial metabolism during light stimulation in honeybee drone retina and it is not K^+

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Drone retina is composed of two types of cells: photoreceptors, which respond to light, and glia, which store glycogen. Freshly dissected retinas contain about 45 mg glycogen (ml tissue)$^{-1}$. During washout in substrate free Ringer, photoreceptor O_2 consumption was increased (by 300%) either by DNP or by light stimulation. Light, but not DNP caused extracellular $[K^+]$ to increase. Increase of $[K^+]$ in the bath from 10 to 50 mM induced ΔQO_2 in the dark by less than 10%. We conclude that with photostimulation the photoreceptors send a signal to the glia and that the signal is not K_o.

Tachykinin-biosynthesis and -release by primary sensory neurons in culture

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Peptides including the tachykinins substance P (SP) and substance K (SK) from primary sensory neurons are involved in transmission and mediation of nociceptive and inflammatory stimuli. Two mRNA's, derived from one preprotachykinin-gene encode for either SP or both SP and SK.

We have used a well-characterized long-term cell culture system for primary sensory neurons of the dorsal root and trigeminal ganglia of neonatal rats as well as specific radioimmunoassays in combination with HPLC to characterize tachykinin-release and -synthesis mechanisms.

Our results show that peptide content in cultured sensory neurons closely parallels the in vivo development of tachykinin biosynthesis. Moreover, mediator substances such as bradykinin and serotonin as well as capsaicin, a substance selectively stimulating nociceptors, release SP and SK in a dose-dependent manner.

Functional characterization of 4 classes of units in the auditory part of the nucleus reticularis thalami (RE) of the cat

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Spike trains were recorded from 153 single units in the auditory part of the RE of 7 nitrous oxide anesthetized cats. The units were grouped in 4 classes according to their spontaneous discharge pattern. Two classes (I: 19%, II: 55%) were distributed throughout the RE whereas the other classes (III: 15%, IV: 11%) were found in mutally exclusive places. Out of 73 pairs of cells 34 (47%) showed interactions, evaluated by cross-correlograms. This proportion was higher than that found in the medial geniculate body (27%) and depended on which classes the units belonged to. The correlograms were computed both from single electrode pairs (49: 55% interactions) and separate electrode pairs (24: 33%). The diversity of the repsonse patterns to noise and tone bursts in these 4 groups of units suggests the possibility of a fine time-dependent modulation of the acoustically evoked activity in the MGB by the RE.

Turning behavior in the rat after unilateral intranigral injection of MPTP

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Unilateral injection of MPTP (50 µg) into the pars compacta of the substantia nigra in rats induces strong contraversive turning (i.e. away from the site of injection) immediately after the application of the drug, whereas a control injection of saline induces a short initial period of ipsiversive turning. MPTP-induced contraversive turning slowly decreases and, after about half an hour, reverses its direction. Spontaneous ipsiversive turning is still present 24 h after the injection of MPTP. Rats in the control group do not turn preferentially in either direction when observed 24 h after the intranigral injection. Systemic injection of the MAO inhibitor pargyline (15 mg/kg) does not prevent the contraversive turning induced by subseqeunt intranigral injection of MPTP. The results suggest that MPTP has an initial stimulatory effect on the activity of dopaminergic neurons in the substantia nigra followed by a longer lasting depressive effect on these neurons. The finding that pargyline is not effective in inhibiting contraversive turning induced by MPTP suggests that the acute effects of MPTP exposure can be dissociated from its neurotoxic effects on the substantia nigra.