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Symposium 1: Neuronal regeneration and transplantation

Precursor cells of dorsal root ganglia in chick embryo: differentiation into neurons in culture

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Dissociated DRG cells from 10-day-old chick embryos were cultured into two types of culture: neuron-enriched culture and mixed DRG cell culture. In neuron-enriched cultures, the number of neurons decreases with time, while in mixed DRG cell culture the number of neurons increases with time until 7 days of culture. The incorporation of ^{3}H-thymidine into DRG ganglion cells could not account for the increased number of cultured neurons.

Microphotographs of the same area, taken every 60 min, showed that small and flat cells can differentiate into neurons which were identified by their morphological characteristic and by specific neuronal markers such as specific neuronal enolase and D_2/N-CAM. It is concluded that embryonic DRG in chicken contain progenitor cells which are able to differentiate into sensory neurons in culture.

Concanavalin A and extracellular matrix mediate rapid outgrowth of leech neurons in saline

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The outgrowth of neurites in culture by single identified neurons of the leech is markedly influenced by the substrate. On cell-free extracellular matrix, extensive sprouting occurs within a few hours. Another substrate promoting sprouting is the plant lectin, concanavalin A (con A). Neurons had to be attached directly to substrate-bound con A or extracellular matrix to grow neurites and did so in physiological saline or medium without any macromolecular substances or other cells present. Sprouting on con A was inhibited by the con A-specific hapten sugar α-methyl mannoside. These results show that the substrate can mediate the outgrowth of cultured leech neurons; soluble growth factors are not required. However, sprouting depends not simply on attachment to substrate but, critically, on its molecular composition, since other adhesive substrates did not promote sprouting.

Acute facial palsy: a virological and histopathological study

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Virological and/or histopathological investigations have been performed on 21 specimens removed from the greater petrosal nerve in the course of surgical decompression of the facial nerve (performed by Prof. U. Fisch) within 14 days from onset of Bell's Palsy. Nine specimens were examined for virus infection by a modified coculture method. The specimens were maintained in cell culture medium for 28 days at 37°C. Vero cells were inoculated every day with supernatant. Infected cell supernatants underwent two blind passages and, additionally, were inoculated into the allantois and amnion of embryonated eggs. The fluids were tested for hemagglutinins. In no case any virus could be isolated. The main morphological changes observed were nerve fiber degeneration and demyelination, and in 8 of 15 specimens endoneurial fibrosis was present. In 10 cases a minimal infiltration of lymphocytes was observed. The etiological relationship between the virological and histopathological findings in acute facial palsy is discussed.

Influence of adenohypophyseal tissue on the development of the rat fascia dentata in vitro

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In hippocampal slice cultures, the mossy fibers from the dentate granule cells were previously shown by Timm-staining to retain their normal connections to CA4 and CA3 pyramidal cells. During co-cultivation with an adenohypophyseal explant, the infrapyramidal blade of the fascia dentata largely disappeared as a cell layer; granule cell-like neurons would migrate toward the hypophyseal explant, whilst their axons still functionally innervated CA3 pyramidal cells. Axon collaterals projected in the opposite direction and presumably terminated on dendrites, thus giving rise to the intense black labeling which was observed in Timm-stained preparations as a bridge connecting the two explants.

The morphological alterations induced in the fascia dentata by co-cultured adenohypophysis were tissue-specific since co-cultured neurohypophysis, pineal gland and cerebellum failed to produce similar effects. These results suggest that cultured adenohypophyseal tissue is capable of releasing yet unidentified factors which apparently enhance neuronal migration.

Evidence for sprouting of hippocampal cholinergic and aminergic fibers following partial deafferentation in rats

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Cholinergic enzyme activities and high affinity uptake of ^{3}H-noradrenaline and ^{3}H-serotonin were measured in septotemporal regions of the hippocampus following partial or complete fornix-fimbria transection. Subsequent to an initial reduction reflecting terminal degeneration, a significant increase in these parameters was observed. Recovery of these terminal-specific markers most likely represents synapse replacement following sprouting of the remaining undamaged fibers. The extent of recovery seems to depend on the degree of deafferentation and was different for each transmitter system. Substantial recovery occurred within 8 weeks after partial lesions whereas after extensive deafferentation the magnitude of recovery was lower and was apparent only after longer survival times.

Evidence for the role of nerve growth factor (NGF) in central cholinergic neurons in rats

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Cholinergic enzyme activities and NGF levels in the septum and hippocampus were measured at various times after complete bilateral transection of the dorsal septohippocampal pathway. Within 3–7 days after transection, there was a maximal increase in NGF content of about 50% in the hippocampus and 290% in septum followed, in both regions, by a decline to control values on postlesion day 14. Cholinergic enzyme activities indicated an almost complete terminal degeneration in the hippocampus. In the septum, however, an initial decrease of 15% was followed by gradual increase to about 20% over the control levels after longer survival time. Fornix-fimbria lesion and the resulting destruction of the cholinergic system, appears to induce enhancement of NGF in the hippocampus and septum. These findings thus support the role of NGF in central cholinergic neurons.

Isolation of cDNA clones from C6 rat glioma RNA coding for a glia-derived neurite-promoting factor (GdNPF)

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GdNPF is a 43 kD glycoprotein which promotes neurite extension in neuroblastoma cells and strongly inhibits serine proteases (Günther, J., Nick, H., and Monard, D., EMBO J. *4* (1985) 1963). RNA from C6 glioma cells was isolated, translated both in vivo and in vitro and the protein immunoprecipitated with polyclonal anti-GdNPF antibodies. Sucrose gradient enriched poly(A)+ RNA was used for cDNA synthesis. The cDNA library obtained was screened by the method of hybrid selected translation. A clone with a 800 bp insert coding for GdNPF was used for the estimation of the amount of GdNPF mRNA at different stages of rat brain development.

Non-neuronal cells influence the cholinergic properties of cultured human spinal cord neurons

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Dissociated monolayer cultures of human spinal cord contain neuronal and non-neuronal cells; part of the neuronal population consists of cholinergic and GABAergic cells. In the presence of cytosine arabinoside (araC), the non-neuronal cells are virtually eliminated. Under these conditions, the number of neuronal cells remains constant over a 3-week period. During this time, however, the level of choline acetyltransferase (CAT) decreases markedly (3–4-fold) as compared to cultures containing non-neuronal cells, suggesting that cholinergic neurons require the presence of these cells. This was supported by the unexpected observation that γ-interferon (γ-IFN) could 'prevent' the effects of araC. When both agents are present, the non-neuronal cells survive and the CAT levels increase; the γ-IFN acts in a dose-dependent manner. The levels of glutamic acid decarboxylase in the same cultures increase but not to the same extent as CAT. These results suggest that 1) human cholinergic neurons are influenced by the presence of spinal cord non-neuronal cells and 2)γ-IFN can in some way counteract the effect of araC.

Carbonic anhydrase (CA) activity as a phenotypic expression of sensory neurons in vivo and in vitro

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Certain subclasses of chicken sensory neurons express a CA activity. The number of Ca^+ neurons decreases with age in vivo. To investigate the factors which could modulate CA activity in neurons, dorsal root ganglion cells of chicken embryos were grown in vitro in a defined medium or in a medium supplemented with horse serum. Factors contained in the horse serum increase the number of Ca^+ neurons and enhance the persistance of the enzymatic activity in cultured ganglion cells. In contrast, the rise of pCO_2 or the presence of non-neuronal cells did not affect the neuronal CA activity. Since in vivo and in vitro, Ca^+ neurons shared common ultrastructural characteristics, dissociated cell cultures may contribute to analyse the phenotypic expression of the enzyme under determined conditions.

Choline and acetylcholine metabolism in slice cultures of the septal area of the newborn rat

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Explants of the septal area (medial septum and diagonal band complex) of the newborn rat brain were cultured by the roller tube technique. The metabolism of choline and the synthesis of acetylcholine (ACh) were investigated, in living cultures, in presence of different radiolabeled precursors, and at various precursor concentrations, and compared with the metabolism in striatal slices of adult rat brain. Acute slice preparations of the newborn septal area and mature cultures showed different patterns of choline metabolism, indicating in vitro maturation or regeneration of cholinergic neurons and nerve terminals. Mature cultures showed many biochemical characteristics of the tissue in situ, such as enhancement of ACh synthesis by depolarization and de novo choline formation. In absence of extracellular choline, ACh was synthesized from choline derived from the methylation of phosphatidylethanolamine.

Structural and functional aspects of glia-derived neurite promoting factor (NPF)

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NPF is a protein with a single polypeptide chain of mol.wt 43,000 (SDS-PAGE). It promotes the formation of neurites in NB_2a neuroblastoma cells. NPF is a strong inhibitor of serin proteases such as urokinase, trypsin and thrombin. It forms SDS-stable complexes with each of these proteases. There is indication that the neurite promoting activity of NPF is due to its protease inhibitory activity. Reduced and carboxymethylated NPF reveals a somewhat higher mol.wt on SDS-PAGE indicating the presence of sulfhydryl groups after reduction. The CD spectrum shows extended α-helix contents. The N-terminus seems to be blocked since 200 pmoles of NPF were resistent to Edman degradation. Tryptic peptides were separated by reversed phase HPLC.

Immunocytochemical localization of myelin-associated glycoprotein in the pia mater of chicken optic nerves

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Myelin-associated glycoprotein (MAG) is a constituent of myelinating glia and myelin but not of neurons in mammalian nervous tissue. Recently it has been shown that MAG is expressed by small sensory neurons of chicken dorsal root ganglia. Other cells, which also derive from the neural crest, are positive for MAG. Vibratome sections of adult chick optic nerves were incubated in antisera to MAG, myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP), which is a specific astrocytic marker; these sections were further treated according to Sternberger's PAP technique. The surface of the nerve and the septa which both are mainly formed by cells of the pia mater were immunostained for MAG but not for MBP. Furthermore, GFAP antibodies labeled different structures than antisera to MAG or to MBP. It can be concluded and confirmed that the pia mater, which is a part of the meninges, derives from cells of the neural crest.

Transplantation and culture of dorsal root ganglia: expression of 28 K vitamine D-dependent Ca binding protein (28 K)

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Intraneuronal 28 K was revealed by immunocytochemistry in two subpopulations of chick dorsal root ganglia (DRG) cells. The expression of this protein was investigated in several experimental conditions.

In chick embryo, the first immunoreactive neurons were detected at ED11. In DRG transplanted at ED12 on the chorioallantoic membrane, the two subpopulations of 28 K immuno-

reactive neurons were present. In explant cultures of DRG at ED10, immunostaining was also found in two different types of neurons, after 7–35 days of culture.
In conclusion, the expression of 28 K in sensory neurons seems to be independent of the presence of the appropriate target tissues.

Are there multiple muscarinic receptor sites in cultivated hippocampal slices?

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Classical muscarinic antagonists bind to a homogeneous population of muscarinic acetylcholine receptors (mAChr:s). On the basis of binding experiments with muscarinic agonists three binding sites with different affinities for the agonists have been characterized. Also the antagonist pirenzepine (PZ) has been found to interact with two classes of mAChr:s.
Using ^{3}H-QNB as a ligand we have previously identified and characterized mAChr:s in homogenates from hippocampal slice cultures. We have now extended these studies to the intact culture. QNB binds to the receptors on the cultivated intact neurons with a K_d around 600 pM. A B_{max} of 60 fmol/explant was measured. The binding was more potently inhibited by classical muscarinic antagonists than by agonists or PZ. Competition studies with the drugs oxotremorine and PZ revealed Hill coefficients lower than 1. These results indicate that these drugs interact with multiple sites or affinity states of the mAChr:s labeled by QNB. Direct binding studies with ^{3}H-PZ are in progress.

Ectopic transplantation of populations of sensory neurons in *Drosophila*

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The specificity of connections between sensory neurons and their central targets can be studied by displacing the sensory elements before their axons grow out. Using a new transplantation technique, we generated supernumerary dorsal and ventral appendages at an identical abdominal site. The sensory axons originating from these grafts enter the CNS through the same peripheral nerve. In the abdominal ganglia, sensory projections from dorsal transplants (wings and halteres) resemble those of dorsal abdominal bristles, whereas projections of ventral transplants (legs and antennae) resemble those of ventral abdominal bristles. Moreover, the normal wing center in the thoracic ganglion is reached by afferents from ectopic wings and halteres only. These data suggest that sensory axons of homologous appendages on the one hand, and their central targets on the other, share serially repeated surface markers, enabling sensory fibers to recognize centers of homologous appendages.

A subtype of oligodendroglial cells represents a non-permissive substrate for neuronal attachment and fiber growth

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Dissociated cultures of rat CNS contain three subclasses of oligodendrocytes characterized by morphology (branching pattern) and antigenic properties. Of particular interest were cells with dense and anastomosing process networks. These 'highly branched oligodendrocytes' (HBO) have mature antigenic properties ($CalC^+$, MBP^+) and are present in cultures of newborn to adult rat optic nerve and spinal cord, and in adult corpus callosum and fimbria. In co-cultures with peripheral or central neurons or with fibroblasts these HBO cells represent a strictly non-permissive substrate for cell attachment and process outgrowth, in contrast to other types of oligodendrocytes. Isolated myelin from rat CNS, but not from rat sciatic nerve or frog CNS, has similar non-permissive substrate properties, suggesting that these HBO cells form myelin in vivo. This non-permissive substrate property may play a crucial role for the absence of regeneration in the differentiated mammalian CNS.

Characterization and quantification of enzymatic prostanoid formation in chicken dorsal root ganglia and sciatic nerve

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Homogenates of dorsal root ganglia and sciatic nerve converted ^{14}C-arachidonic acid into two predominant products prostaglandin E_2 (PGE_2) and prostaglandin D_2 (PGD_2) exclusively by an enzymatic process.
Subcellular fractions showed that in nervous tissue cyclooxygenase and PGE_2 isomerase are particulate enzymes while PGD_2 isomerase is mainly soluble. In dorsal root ganglia and in sciatic nerve, the ratio of newly synthetized PGD_2/PGE_2 were respectively 1.0 and 0.3, thus it is suggested that PGD_2 isomerase is predominantly localized in the perikaryon of sensory neurons.

Immunocytochemical investigation of optic nerves of female heterozygous for Jimpy

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The paucity of myelin in the central nervous system (CNS) is a characteristic feature of Jimpy (jp) mouse, which is a sex-linked recessive lethal mutation. This gene is maintained in the heterozygous female, which show no clinical symptoms. To define if glial cells are altered in these animals, we studied optic nerves of adult jp female, using polyclonal antibodies to: myelin-associated glycoprotein (MAG) and glial fibrillary acidic protein (GFAP). Vibratome sections of jp have shown a decreased number of oligodendrocytes immunostained for MAG. However, these cells present long and positive processes, which were absent in control tissue. Furthermore in jp a complex network of GFAP labeled structures is formed by astrocytes and hypertrophied processes. In conclusion: Heterozygous show immunocytochemical and morphological alterations without expression of clinical symptoms.

Lesion of septal neurons causes increase of nerve growth factor (NGF) in the hippocampus of the adult rat

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Several observations indicate that endogenous nerve growth factor (NGF) plays a physiological role for cholinergic neurons of the basal forebrain. However, site of synthesis and mechanisms regulating NGF biosynthesis in the central nervous system (CNS) are still unknown. Recent findings show that NGF production in peripheral targets is controlled by a negative regulatory feedback mechanism.
In analogy to the periphery, denervation-lesions in the CNS were performed to study whether NGF levels are controlled by afferent nerves. Electrolytical lesioning of the septum causes a significant increase of NGF content in ventral, but not in dorsal hippocampus. NGF levels reached an peak value (+70%) within four days and returned to basal levels two weeks after denervation. In contrast to cholinergic denervation, transection of the medial forebrain bundle did not influence NGF content in hippocampus, although monoamine levels were drastically reduced. These experiments suggest that a cholinergic mechanism is involved in NGF production in adult rat brain.

Symposium 2: Molecular mechanisms of genetic diseases

Splicing of β-globin premRNA: cleavage at the 3′ splice site depends on the sequence of the 5′ splice region and vice-versa

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We analyzed the effect of 20 point mutations in the 5′ and 3′ splice regions of the large intron on splicing in vivo and in vitro. 1) Conversion of the conserved 3′ AG to *T*G, *C*G, A*G* or A*A* prevented correct splicing. Even the first step, 5′ cleavage and lariat formation, was inhibited. 2) Conversion of the conserved 5′ GT to *A*T or G*A* (but not G*C*) abolished correct splicing. In vitro, RNA was found in which exons 1 and 3 were joined directly. If 1–3 splicing was prevented by using precursor lacking exon 1, lariat intermediate in which the 5′ intron end was correctly joined to the branchpoint accumulated, but no spliced product appeared. In vivo, no 1–3 spliced products, but RNAs resulting from cryptic 5′ splicing appeared; in mutant GT→G*A* the lariat intermediate accumulated. Thus, 5′ GT mutations allow cleavage at the 5′ splice site, but prevent splicing at the 3′ splice site, while 3′ AG mutations prevent cleavage at the 5′ (and 3′) splice site.

Interaction of tumor-promoters on the assembly/disassembly of porcine brain tubulin in vitro

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In the two-stage model of carcinogenesis a differentiation is made between initiators interacting with DNA and the more heterogeneous class of compounds called tumor promoters which in most cases do not directly interact with DNA. Parry et al. (Nature *294* (1981) 263) first demonstrated a relationship between tumor promoters and the induction of mitotic aneuploidy in yeast. In accordance with this finding we could show that indeed different tumor-promoters specifically induce chromosome loss in *Saccharomyces cerevisiae D61.M* without inducing any other types of genetic effects. Because compounds leading to chromosome loss may interact with the spindle apparatus (i.e. with tubulin) we studied the influence of these compounds on the GTP-promoted assembly/disassembly of porcine brain tubulin in vitro by measuring the increase in absorbancy at 350 nm. The substances either enhanced (DMSO, phenobarbital, cholic acid) or inhibited (saccharin, iso-PC, colchicine) the assembly.

First steps for the development of an optimal test protocol for testing promutagens with yeast *S. cerevisiae* D7

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Yeast cells contain cytochrome P-450 and therefore seem to be capable to endogenously activate promutagens (Callen and Philpot, Mut. Res. *45* (1977) 309) which then in turn can induce mutations of the appropriate genetic endpoints. A disadvantage of the yeast test system is its limited sensitivity. It is therefore important to optimize the treatment protocol to get reproducible results. We studied the influence of different parameters, especially treatment temperature and cell density. For aflatoxin B_1 the mutagenic response strikingly increased with increasing cell densities whereas for cyclophosphamide this tendency was much less pronounced. In case of β-naphthylamine and 2-aminoanthracene stronger mutagenic effects were found with lower cell densities. For all four test compounds the mutagenic effect was strictly temperature dependent being only detectable at 37 °C and disappeared at lower temperatures (32 °C and 28 °C).

DNA polymerase α holoenzyme can specifically initiate DNA replication on the mammalian ss porcine circo-virus DNA

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The ss DNA genome of the porcine circovirus (PCV) is a covalently closed circle (Tischer et al., Nature *295* (1982) 64). We have used this DNA template to study in vitro initiation of DNA replication. A DNA polymerase α holoenzyme form, that has been isolated by the criterion of efficient priming on ss M13 DNA, contains polypeptides 198, 140, 3, 57, 52 and 47 kD, respectively. This holoenzyme form is able to specifically prime the ss PCV DNA at one preferred site in the genome. The region of in vitro initiation contains potential to form secondary structures and sequences related to the binding site of nuclear factor I. The DNA polymerase α holoenzyme contains a polypeptide of 47 kD corresponding to the same kD as nuclear factor I. This in vitro DNA replication-initiation system can be used in analogy to small bacteriophages DNA's in *E. coli* to identify and purify factors involved in mammalian DNA replication.

Polymorphism of the HLA-DR β chain genes and disease association

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The HLA-DR molecules are transmembrane glycoproteins composed of two chains (α and β) which participate in initiating the T-cell dependent immune response. The HLA-DR β chain is highly polymorphic. The polymorphism of these chains results in the phenomenon of restriction of antigen presentation to T-cells of the same haplotype. Certain haplotypes also present certain antigens with high or low efficiency. Some HLA-DR haplotypes strongly correlate with diseases such as insulin dependent diabetes (IDDM), coeliac disease (CD) and multiple sclerosis. We have been studying the polymorphism of the HLA-DR β chain genes by isolation, mapping and sequencing of the genes. Within the haplotypes DR3, DRw6a and DRw6b, we have been able to identify the two active DR β loci and make allelic comparisons. Interestingly, HLA-DR3, which is associated with IDDM and CD, is a result of a gene conversion-like event between two DRw6a β chain loci. However, DR3 is not a homogeneous specificity and can be subdivided. We are using oligonucleotide probes capable of distinguishing one bp mismatch to test the correlation of the split of this haplotype with diseases.

Mx protein: constitutive expression in Mx cDNA transformed 3T3 cells confers selective resistance to influenza virus

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Alleles of the gene *Mx* determine susceptibility of mice to influenza virus infections. Cells of resistant Mx^+ mice (inbred strains A2G, SL/NiA and outbred wild *Mus musculus*) develop, in response to IFN, an efficient antiviral state towards many viruses including influenza viruses. In contrast, the antiviral state induced in *Mx* cells does not include protection against

influenza viruses. IFN-treated Mx^+ cells synthesize, in addition to a common set of proteins, a nuclear 75,000-da protein (protein Mx) that is not found in Mx^- cells. We have cloned and sequented a full-length cDNA corresponding to Mx mRNA. NIH 3T3 (Mx^-) cells were transfected with an appropriate expression vector containing this cDNA under the control of a constitutive promoter. Transfected cells constitutively expressed a protein which had the same M_r as protein Mx, was immunoprecipitable with 3 different monoclonal antibodies directed against protein Mx and accumulated in the cell nucleus. These cells exhibited a high degree of resistance against infection with influenza virus (but not VSV) in the absence of IFN. These results indicate that protein Mx itself is inhibitory to influenza viruses and does not require other IFN-induced proteins for its antiviral activity.

Congenital sucrase-isomaltase(SI) deficiency due to hold-up at the Golgi apparatus

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The intestinal microvillar enzyme SI is synthesized as a high M_r precursor that is cleaved into its two subunits upon arrival at the cell surface by pancreatic proteases. In search of mutant phenotypes with impaired intracellular transport (Hauri et al., PNAS, *82* (1985) 4423), we have now studied a new case of SI-deficiency. The patient's mucosa had low immunoprecipitable isomaltase but no sucrase activity. Immunoelectronmicroscopy revealed absence of SI-antigen in the brush border but accumulation in the Golgi apparatus. Detailed immunochemical analysis by monoclonal antibodies suggested that in the patient coreglycosylated SI of apparently normal M_r was synthesized but was partially degraded (probably in the Golgi) prior to complex-glycosylation. The altered SI had 2 of 4 tested epitopes in common with normal SI. We conclude that SI of the patient is defective in at least two epitopes leading to a transport block in the Golgi apparatus.

Use of restriction fragment length polymorphisms (RFLP's) in medical genetics

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Recombinant DNA techniques are increasingly becoming important diagnostic tools in clinical medicine. In medical genetics in particular, RFLP's are already used to render possible or to improve carrier detection, risk estimation and prenatal genotype/phenotype prediction for several inherited diseases. Our poster will illustrate the rationale of this approach by presenting a case study of an X-chromosome-linked disease, i.e. the Duchenne type of muscular dystrophy. Usefulness and limitations of this approach will be discussed.

Detection of promutagens with *Saccharomyces cerevisiae*: endogeneous metabolism and genotoxic activity

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Saccharomyces cerevisiae D7 contains cytochrome P-450 and is capable to endogeneously metabolize different promutagens. This activation of promutagens is possibly catalyzed by the yeast P-450 system.
We investigated the induction of mutations (convertants, revertants and chromosomal aberrants) by aflatoxin Bl (AFBl) and other promutagens without an exogeneous activation system. With up to 0.50 mM AFBl a dose related increase of mutants was observed. Mutation induction (convertants) directly correlated with the cytochrome P-450 content, the maximum amount was reached in the late log phase. Cytochrome P-450 inhibitors like ellipticine (as an inhibitor of mammalian P-450) or the yeast specific inhibitors penconazole and propiconazol completely abolished the AFB1-induced mutagenicity. These data favor the hypothesis that indeed the promutagen activation in yeast is catalyzed by a P-450 depending metabolic system.

A defect in an HLA class II regulatory gene is the molecular basis for a form of congenital immunodeficiency

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A type of severe combined immunodeficiency in man (class II-SCID), generally lethal, is characterized by an absence of Ia antigens on all cell types. We have shown that, in these patients, there is an absence of mRNA for HLA-DP, -DQ and -DR antigens, while the mRNA for the invariant chain is made in normal amounts. Various controls have shown that the class II structural genes are not affected in these patients and that the genetic defect segregates outside of the MHC. This implies the existence of a regulatory gene involved in class II gene expression and affected in the patients. The genetic defect cannot be corrected by γ interferon. Class II gene regulation might operate through a short conserved sequence 5′ upstream of all class II genes.

A novel method for the estimation of the efficiency of DNA repair using monoclonal antibodies

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Monoclonal antibodies specific to UV-C induced thymidine-dimers are used to study the DNA repair capacity in patients with xeroderma pigmentosum, melanoma and basalioma. Fibroblasts and/or lymphocytes from patients and controls were exposed to varying UV-irradiation doses and subsequent recovery periods. DNA extracted from these cells by phenol/ether extraction method was further treated and the binding of thymidine-dimer specific antibodies was measured by ELISA.
In comparison with the conventional methods this method has the advantage of being non-dependent on the cell cycle stage and other photoreactive products. It is an easier and more direct method for the study of DNA repair capacity.
This method can be useful for detection of persons at risk for some malignancies, such as breast- and colorectal cancer.

Low plasma alkaline phosphatase activity in a case of osteogenesis imperfecta (OI) type II (lethal perinatal)

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We report a case of OI type II with biochemical characteristics of hypophosphatasia, born to healthy, unrelated parents. Analysis by SDS-PAGE of pepsin-extracted collagen from skin and of collagen synthesized by cultured fibroblasts, indicated that in addition to normal α1(I) and α2(I) chains, chains of slightly higher molecular weight, as a result of excessive post-translational modification, were synthesized. This suggests a structural mutation causing delayed triple helix formation. All CNBr-derived peptides were overmodified, indicating that the mutation was situated in α1(I)-CB6 or α2(I)-CB5. Since the parents'

collagen was normal, the case represents a new dominant mutation. Plasma alkaline phosphatase activity was considerably reduced and plasma phosphoethanolamine was elevated. Since the inheritance of hypophosphatasia is autosomal recessive, these latter findings are presumably secondary to the disturbances in bone matrix formation engendered by OI.

Symposium 3: Intracellular signals – postreceptor events

Structural organization of the parvalbumin gene

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Parvalbumin (PV) is a Ca^{2+}-binding protein which belongs to the troponin-C (TNC) superfamily. These proteins have sequence homology especially within the Ca^{2+}-binding domains. It has been speculated that these proteins evolved from a common one Ca^{2+}-binding domain containing ancestor protein by gene duplication events. To investigate this hypothesis the gene encoding PV was cloned and compared to the known genes for the two other Ca^{2+}-binding proteins myosin alkali light chain and calmodulin. PV cDNA clone 9d which contains 94% of the coding and 65 bp 5' noncoding sequence was used to screen a λ Charon 4A rat genomic library. Nine overlapping PV clones were purified and their DNA analyzed by 'Southern' blotting. They represent five independent recombinants and cover about 25 kb of chromosomal DNA. Exons were localized by hybridizing cDNA fragments to cloned genomic DNA digested with several restriction enzymes. Intron/exon junctions and 700 bp upstream from the transcription initiation site were sequenced by the M13 dideoxy sequencing method. Several common structural features of the genes encoding Ca^{2+}-binding proteins were found indicating divergent evolution of these proteins.

Mutations affecting the catalytic subunit of cAMP-dependent protein kinase

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Using forskolin (in the presence of isobutylmethylxanthine) as selective agent, a number of mutants, resistant to the cytotoxic effects of cAMP, were isolated from the porcine epithelial cell line LLC-PK_1. Two of the cAMP-resistant mutants, FIB4 and FIB6, had altered cAMP-dependent protein kinase (cAMP-PK), resulting in a 90% reduction of activity compared to wild type. The reduction of kinase activity was found to be due to the total absence of type I kinase in both mutants. Further analyses of C subunit levels using a polyclonal antibody revealed that the mutants produced between 60 and 90% immunoreactive protein when compared to wild type. These results suggest that the genetic lesion for low kinase levels was due to a mutation in either the structural gene for the catalytic subunit, or in an enzyme involved in posttranslational modification of the catalytic subunit. Somatic cell hybrids formed between wild type and mutant cells expressed the genetic lesion codominantly.

Cyclic AMP responses in murine T- and B-cell lines

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The cAMP rise in response to isoproterenol, PGE_2 and histamin was measured in twenty murine lymphoid cell lines. The following quantitative and qualitative differences were found: in seven cell lines carrying neither Ly_3T_4 nor Ly_2 or the cytotoxic T-cell marker Ly_2 only, the isoproterenol response was higher or only slightly lower than the PGE_2 response. In contrast, none of the five cell lines carrying Ly_3T_4 (a helper T-cell marker) showed any isoproterenol response although three showed a marked response with PGE_2. Forskolin at low concentrations potentiated the hormone response in 14/16 T-cell lines but in none of the four B-cell lines. Treatment with hormone alone achieved saturation of protein kinase with cAMP in only two cell lines while in six other cell lines an elevation of protein bound cAMP was seen when the hormone was added in the presence of a phosphodiesterase inhibitor.

MSH induces phosphorylation of a membrane protein in Cloudman S-91 mouse melanoma cells

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We have investigated a possible role of protein phosphorylation in the melanogenic action of α-MSH on Cloudman S-91 mouse melanoma cells. Incubation of the cells with ^{32}P-phosphate results in the incorporation of the label into a large number of phosphoproteins. In the presence of α-MSH a significant increase of ^{32}P incorporation was observed into two phosphoproteins with apparent mol.wt of 43 kD and 34 kD, respectively. This increase was concentration-dependent, reversible and could be induced by melanotropic peptides only. Subcellular fractionation of labeled melanoma cells and analysis of the proteins by PAGE revealed that the 34 kD protein is a membrane component whereas the 43 kD protein is of mitochondrial/melanosomal origin. Phosphorylation of the 34 kD protein is rapid which points to a participation in the receptor/adenylate cyclase-mediated signal transduction.

Characterization of the $[Ca^{2+}]_i$ response induced in adrenal glomerulosa cells by angiotensin II and potassium

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The mechanisms of the cytosolic free calcium ($[Ca^{2+}]_i$) responses induced by angiotensin II (AII) and potassium (K^+) were studied in isolated adrenal glomerulosa cells loaded with the fluorescent probe quin 2. Ca^{2+} release from intracellular stores was assessed in Ca^{2+}-free medium, and Ca^{2+} influx across the plasma membrane by the restoration of extracellular Ca^{2+} to Ca^{2+}-depleted cells, quenching of quin 2 by influx of Mn^{2-} and the use of voltage-dependent channel (VDC) blockers. Non-stimulated cells maintained $[Ca^{2+}]_i$ by continuous Ca^{2+} influx through VDC. The $[Ca^{2+}]_i$ rise induced by AII was transient, dose-dependent, specific and due to Ca^{2+} mobilization from intracellular stores. The $[Ca^{2+}]_i$ rise induced by K^+ was due only to Ca^{2+} influx through VDC, sustained (< 15 min), and was inhibited by activation of protein kinase C. These data demonstrate that the mechanisms of the $[Ca^{2+}]_i$ response to AII and K^+ in adrenal glomerulosa cells are entirely different.

Endocytosis of a yeast pheromone

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Certain analogies exist between pheromone action in yeast and peptide hormone action in higher eucaryotes. We have begun to investigate whether the yeast pheromone, α-factor, is internalized by endocytosis as are peptide hormones in vertebrates. To this end, we labeled α-factor biosynthetically with $(^{35}S)O_4^{2-}$ and purified it. We have shown that this $(^{35}S)\alpha$-factor binds to a cells, not to α cells at 0 °C, that binding is reversible and completed out by unlabeled synthetic α-factor. Once bound to a cells at 0 °C, $(^{35}S)\alpha$-factor can be dissociated by washing the cells with pH 2 buffer. Taking advantage of the fact that a wash at pH 2 removes all of the external bound α-factor, we have studied the

kinetics of (^{35}S)α-factor internalization by measuring the radioactivity which is resistant to the wash at pH 2 as a function of time. Internalization begins after a short lag at 30 °C and is temperature- and energy-dependent. This could represent uptake of α-factor by endocytosis. We have tested the internalization of α-factor by temperature-sensitive, endocytosis-defective mutants. These mutants are less sensitive to α-factor than their wild type counterparts. These results will be presented.

Adaptation of phosphate (P_i) transport across small intestinal brush border membrane vesicles

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Dietary P_i restriction enhances net P_i absorption in small intestine. We have investigated the mechanism of P_i transport adaptation in brush border membranes (BBMV) isolated from rat jejuna. The adaptative response was studied after long- (8 d) and short- (3 h) term exposures to a low (0.2%) P_i diet (LPD) and normal (0.8%) P_i diet (NPD). In long-term experiments, Na-dependent P_i uptake was in NPD 51 ± 20 and in LPD 173 ± 44 pmoles/mg protein · 20 s. No difference in P_i transport was observed in absence of Na. Under initial rate conditions V_{max} was 87 ± 13 in NPD and 193 ± 26 pmoles/mg · 10 s in LPD; K_m was 0.17 ± 0.03 in NPD and 0.05 ± 0.02 mM in LPD. In two short-term experiments, 3 h after low P_i feeding the Na-dependent P_i transport in BBMV was 162% and 226% as compared to NPD. Thus P_i transport adaptation in rat BBMV results from an increase in the capacity and in the apparent affinity of the Na-P_i cotransport system. The short-term experiments suggest that small intestine can adapt rapidly to a low P_i meal.

Influence of sex and age on pituitary T3 and T3 nuclear receptors: correlation with TSH secretion

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In view of previous observations (Mol. cell. Endocr. *33* (1983) 281) that T3 modulates its own nuclear receptors (T3nR) in the anterior pituitary gland (AP) in hypothyroid male rats with an inverse correlation between the density of pituitary T3nR and plasma TSH, the influence of sex and age on these parameters was studied. Long-Evans female rats of 3–5 months of age were compared to males of the same age and to females of 22 months of age. The concentrations of AP T3 and T3nR are lower in the males than in the females (4.6 ± 1.8 vs 6.3 ± 2.9 pg T3/mg AP, and 1.4 ± 0.2 vs 2.2 ± 0.2 fmoles/mg AP, respectively, $p < 0.05$) whereas TSH AP content as well as plasma TSH are greater, with no differences in plasma T4 and T3 between sexes. The concentrations of T3nR (2.6 ± 0.3 fmoles/mg AP) and plasma T3 in old females remain within the range of the young ones whereas plasma TSH is increased ($p < 0.01$) and TSH and T3 AP concentrations are decreased.

In conclusion, the inverse correlation of both AP T3 and T3nR concentrations with plasma TSH is confirmed in both sexes whereas with age plasma TSH correlates only with AP T3 and not with T3nR concentrations. This is suggestive of a reduced T3nR turnover with age.

Effects of amiloride on intracellular pH (pHi) in the mouse soleus muscle

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To clarify the relationship between the rate of Na^+/H^+ exchange across the sarcolemma and the rate of other energy dissipating processes involved in pHi homeostasis, the effect of amiloride on pHi was observed under four different conditions by continuous measurement with double-barreled microelectrodes. During physiological steady state and 'respiratory acidosis' (PCO_2 14 kPa), pHi was not altered by exposure of the muscle to amiloride 10^{-4} M (inhibitor of Na^+/H^+ exchange). Upon intracellular acidification by exposure of the muscle to benzoate (20 mM), amiloride induced but a small additional acidification (circa −0.02 pH unit). During the slow intracellular acidification following reduction of $[HCO_3^-]o$ to 5 mM, amiloride accelerated the drop of pHi by 50%. We conclude that, whereas Na^+/H^+ exchange is quasi inactive under the first two conditions examined, and hardly active during intracellular acidification induced by benzoate, it is strongly stimulated upon lowering of $[HCO_3^-]o$.

Membrane parameters and noradrenaline sensitivity of hippocampal CA 1 pyramidal cells in the totterer (tg) mouse

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We have compared membrane parameters, the response to noradrenaline (NA), isoproterenol, phenylephrine, histamine (HA) and to electrical stimulation of afferent inputs in transverse slices from the hippocampi of tg/tg (which are atactic and epileptic and display an exaggerated NA projection from the locus coeruleus) and phenotypically healthy tg/+ mice. Resting, action- and after-potentials, membrane impedances and time constants were not significantly different in 11 cells from each group. NA and HA caused long lasting increases in the extracellularly recorded population spike and a block of the calcium dependent after-hyperpolarization. Tetanization of the afferent fibers evoked post-tetanic potentiation and long-term potentiation. In none of these paradigms could we find a significant difference between tg/tg and tg/+ mice.

Quantitative kinetic measurement of the oxidative burst in bovine polymorphonuclear leucocytes (PMNLs) using a computer-interfaced fluorometric microtiter plate reader

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Using various stimuli, the kinetics of hydrogen peroxide formation by bovine peripheral blood PMNLs was studied by means of a coupled fluorescent assay (horseradish peroxidase and 7-hydroxy-6-methoxy-coumarin). With a simple timer and software, simultaneous automatic measurement of all 96 wells of the microtiter plate at 1-min intervals and storage on disc was possible. Response kinetics of four animals (duplicates + controls) could be studied at eight different stimuli concentrations on a single plate. Significant differences were found between calf PMNLs and those from mature animals. The system and our storage/evaluation software could also be used for initial rate enzymatic assays.

Recombinant interferons inhibit the mitogenic activity of platelet-derived growth factor

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The ability of highly purified, *E. coli*-derived human interferons (rIFNs) to inhibit platelet-derived growth factor (PDGF)-induced DNA synthesis in human dermal fibroblasts (HF) was examined. rIFN-γ was the most potent, since its inhibitory con-

centration 50% was 10 U/ml, while those of rIFN-αA and rIFN-β were 4000 U/ml and 600 U/ml, resp. There was a close parallelism between PDGF-blocking and growth inhibitory activities of these rIFNs. The mode of action of rIFN-γ was analyzed further. rIFN-γ affected neither ^{125}I-PDGF binding to nor ^{3}H-thymidine uptake into HF. However, it shifted the time point of initiation of DNA synthesis from 14 h after PDGF addition to about 21 h and lowered the DNA synthesis rate. rIFN-γ was still fully active when added 6 h after PDGF. rIFN-γ inhibited also the mitogenic activity of EGF and basic fibroblast growth factor. These results suggest that rIFN-γ interferes with a step in the PDGF signalling cascade which occurs relatively late and onto which the activity pathways of other mitogens converge.

Evidence for the existence of dopamine receptors on cultured astrocytes of rat striatum and spinal cord

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Electrophysiological and binding studies indicate that glial cells possess receptors for noradrenaline and histamine (Hösli and Hösli, Neuroscience *7* (1982) 2873; Hösli et al. Neurosci. Lett. *48* (1984) 287). We were therefore interested whether glial cells also have receptors for dopamine, another biogenic amine. By means of autoradiography we have observed binding sites for ^{3}H-dopamine, the D_1-antagonist ^{3}H-cis-flupenthixol and the D_2-antagonists ^{3}H-domperidone and ^{3}H-spiperone on astrocytes in cultures of rat striatum and spinal cord. Binding of the radio-ligands was inhibited by the unlabeled compounds suggesting 'specific binding'. Preliminary electrophysiological investigations from our laboratory demonstrate that dopamine causes changes in the membrane potential of astrocytes. From these findings it is suggested that glial cells possess receptors for dopamine.

Changes in latency to onset of birefringence signal and tension in skeletal muscle after prolonged activity

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Prior tetanic stimulation of frog single muscle fibers increases the delay to onset of the early large birefringence signal (thought to reflect myoplasmic [Ca^{++}], Oetliker, J. Physiol. *318* (1981) 11P) and the subsequent rise of tension in single twitches. An increased delay for both signals, up to 3 ms at 22 °C, is obtained as well by increasing extracellular [Ca^{++}] or [K^{+}] from 1 to 10 mM. To test if the posttetanic increase of these delays can be due to increased t-tubular [Ca^{++}] fibers were stimulated (40 Hz) with [Ca^{++}] buffered to 1 mM by 15 mM citrate. Neither activation delays nor the time course for restitution to normal delays were affected by keeping [Ca^{++}] at 1 mM. This indicates that increased activation delays after tetani are not caused by increased t-tubular [Ca^{++}], while an increase in [K^{+}] remains a possibility.

5-HT (serotonin) stimulated adenylate cyclase activity is mediated by 5-HT_{1A}-receptors in the rat hippocampus

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5-HT stimulated adenylate cyclase activity in the rat hippocampus was pharmacologically characterized with different agonists and antagonists. These data were compared to data obtained with selective radioligands for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 receptors.

The rank order of potency of 13 agonists was typical of a 5-HT_{1A} receptor mediated effect and very similar to that observed in 5-HT_{1A} binding. The use of selective antagonists allowed to exclude a role for 5-HT_2, 5-HT_{1C}, 5-HT-M or D_1 receptors in the stimulation of adenylate cyclase by 5-HT. Only metitepin was a potent antagonist of this effect, while ketanserin, mesulergine, ICS 205-930 and SCH 23390 were very weak antagonists.

There was a very good correlation between 5-HT stimulated cyclase activity and 5-HT_{1A} binding for the tested agonists: $r = 0.9$, $p = 0.001$. In contrast, there was no correlation with 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 binding. The data suggests strongly that 5-HT stimulated adenylate cyclase activity in the rat hippocampus is mediated by a 5-HT_{1A} receptor.

Partial purification of an S6-kinase from *Xenopus laevis* oocytes

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Stimulation of quiescent cells in culture with serum or growth factors leads to multiple phosphorylation of 40 S ribosomal protein S6 (Thomas, Cell *19* (1980) 1015). Dose response studies with mitogens or inhibitors indicate that the activation of protein synthesis requires S6 phosphorylation (Thomas, Cell *30* (1982) 235). The kinase responsible for S6 phosphorylation can be extracted in a stably activated form from EGF or serum-stimulated 3T3 fibroblasts (Novak-Hofer, JBC *259* (1984) 5995). Recently, it has been shown that during maturation of Xenopus oocytes, S6 also becomes phosphorylated and that an extract from unfertilized eggs, prepared in the identical manner as in cultured cells, is able to phosporylate S6 in vitro (Nielsen, PNAS *79* (1982) 2937). Affinity chromatography of the extract on Affigel-Blue, followed by phenyl-Sepharose, HPLC-ion exchange, HPLC-hydrophobic chromatography and gel filtration leads to a highly enriched S6 kinase, which can be labelled with tritiated FSBA, an irreversible inactivator of the ATP-binding site.

Ca^{2+}-binding parvalbumin in the Leydig cells of rat testis

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Maximum luteinizing hormone stimulation of testosterone production and secretion in Leydig cells of rat testis can only be obtained in the presence of Ca^{2+} (Biochem. J. *160* (1976) 433). The effects of Ca^{2+} in cells are mediated by a group of Ca^{2+}-binding ('EF'-hand) proteins, e.g. parvalbumin (PV). This protein was isolated from rat testis by various methods and shown to be identical to PV of muscle and brain. RNA blot hybridization with PV clone 9f showed the presence of two mRNA species of 1100 and 700 nucleotides in length demonstrating that PV is synthesized in testis. PV is localized specifically in the Leydig cell of rat testis and a developmental study (using immunohistochemistry, RIA and RNA blot hybridization) is in progress to look for a possible correlation between Leydig cell activity and appearence of PV.

Inhibition of purified phospholipase A_2 from sheep erythrocyte membranes by various drugs

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Inhibitors of soluble phospholipase A_2 (PLA_2) are used in cell stimulation experiments to demonstrate an involvement of membrane bound PLA_2 without knowing their direct influence on the enzyme activity. In this work the common PLA_2 inhibitors mepacrine, chlorpromazine and p-bromophenacyl bromide (pBPB) were compared in their potency against isolated mem-

brane bound and soluble PLA_2. We found 50% inhibition of the membrane bound PLA_2 isolated from sheep erythrocytes with 1.8 mM mepacrine, 24 µM chlorpromazine or 13 µM pBPB. The corresponding results for soluble PLA_2 from porcine pancreas were 1.0 mM mepacrine, 250 µM chlorpromazine or 25 µM pBPB. The inhibition by pBPB was dose and time dependent and was reversed to a certain extent by the addition of calcium-ions during preincubation. Our experiments thus proved that the tested compounds are able to inhibit membrane bound PLA_2 in a direct assay system, which confirms that these inhibitors can block the generation fo eicosanoids.

How are NMDA receptors of central vestibular neurons activated?

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Synaptic excitation of central vestibular neurons of the frog was investigated. EPSPs were recorded in vitro after electrical stimulation of either the ipsi N.VIII or the contra N.VIII/nuc. Using a Mg^{++} free saline, the cEPSP was reported to be mediated via NMDA receptors. In the presence of 1 mM Mg^{++} the initial part of the cEPSP remained unaffected during bath application D-APV (25 µM). However, D-APV blocked reversibly a later component of the cEPSP. During wash out of Mg^{++} this later component increased and spontaneous and EPSP-triggered jumps (1–2 mV) in membrane potential were observed. Similar jumps were evoked by application of NMDA.

The D-APV sensitive component of the cEPSP could be due to a *direct* synaptic activation of NMDA receptors. However, the occurrence of jumps in drug-free solution suggests a rather continuous activation of NMDA receptors. Since the NMDA-ionophore exhibits a range of negative slope conductance the shape of the evoked cEPSP could *indirectly* depend on the degree of postsynaptic NMDA receptor activation.

Atrial natriuretic peptide (ANP) inhibits renin release from juxtaglomerular cells

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Atrial natriuretic peptide (ANP) has been found to impair renin release (RR) from the kidney and to lower plasma renin activity. Since the intrarenal mechanism by which ANP could inhibit RR is as yet unknown, we tested whether or not ANP affects RR by a direct action on juxtaglomerular cells (JGC). Using cell cultures containing 80–90% JGC, as described previously we found that ANP (10^{-13} M–10^{-9} M) strongly inhibited RR from the cells to about 10% of control in a dose dependent fashion ($K_1 \sim 10^{-11}$ M). Inhibition of RR by ANP was paralleled by a rise in cellular cGMP levels. In presence of the cGMP-phosphodiesterase specific inhibitor M&B 22948 (1 mM) tenfold lower concentrations of ANP were required to obtain the same effects on RR and cGMP levels as in absence of M&B 22948. The guanylate cyclase inhibitor methylene blue (10 µM) on the other hand led to a shift of the dose response curves for ANP on RR and cGMP to hundredfold higher concentrations of ANP. From the entirety of the results we conclude that: ANP inhibits RR by a direct action on JGC; the inhibitory effect of ANP is mediated by cGMP.

Insulin alters the hepatic response to exogenous Ca^{2+}

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α_1-Adrenergic agonists activate glycogenolysis and K^+ uptake in perfused livers from fed rats. Both effects can be elicited under certain experimental conditions by increasing the extracellular Ca^{2+} concentration in the absence of hormones. Insulin is known to counteract the stimulation of glycogenolysis by α_1-adrenergic agents and enhances itself K^+ uptake by the liver cell. We have studied the question whether the hepatic responses to exogenous Ca^{2+} are influenced by insulin. Livers were perfused with Krebs-bicarbonate medium containing different amounts of Mg^{2+} and were pretreated with EGTA in some experiments. Insulin (10 nM) alone caused an ouabain-sensitive K^+ uptake. In EGTA-pretreated livers insulin significantly decreased the effect of exogenous Ca^{2+} on glycogenolysis and K^+ uptake. Perfusion with high concentrations of Mg^{2+} abolished insulin-induced K^+ uptake as well as the response to Ca^{2+}. Mg^{2+} appears to interfere with insulin action and with Ca^{2+} stimulation.

Angiotensin II induces redistribution of protein kinase C in bovine adrenal glomerulosa cells

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The distribution of calcium-activated, phospholipid-dependent protein kinase (protein kinase C, PK C) between cytosol and membrane fractions was examined in bovine adrenal glomerulosa cells during treatment with angiotensin II and potassium. PK C was isolated from cytosol and detergent-solubilized particulate fractions by DEAE cellulose chromatography. About 95% of the total enzyme activity was found in the cytosol. Exposure of isolated cells to 10^{-8} M angiotensin II resulted in a rapid decrease in cytosolic PK C activity to about 30% of control values, and in a corresponding increase in PK C activity associated with the particulate fraction. This hormone-induced redistribution was found to be dose-dependent. Preliminary experiments indicate that extracellular potassium does not alter the subcellular distribution of PK C. These observations suggest that translocation of PK C to the membrane is an intermediate step in the hormonal stimulation of adrenal glomerulosa cells by angiotensin II.

Ecdysteroid binding in cultured *Chironomus tentans* cells

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We have cloned an ecdysterone-inducible gene from salivary gland chromosomes of *Chironomus tentans* larvae. According to the classical scheme of steroid hormone action, in the target cell ecdysterone binds to a receptor which then alters gene activities. As a first step towards an understanding of the receptor-DNA interaction we are characterizing the receptors in an ecdysterone-sensitive epithelial cell line. Incubation of cells with active tritiated ecdysteroids shows a ligand specificity of the binding of ecdysteroids. Using cytosol the receptor was characterized as follows: The K_D-value for ponasterone A is 3.5×10^{-10} M. The affinity of the receptor toward 20-OH-ecdysone and ecdysone is about 50 and 1000 fold lower than towards ponasterone A as is shown by competition curves.

^{3}H-Glycogenolysis in the cerebral cortex of spontaneously epileptic mouse mutants: subsensitive glycogenolytic response to norepinephrine (NE) in the *tottering* (tg/tg) mutant and age-dependent supersensitive response to K^+ in the *quaking* (qk/qk) mutant

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The tg/tg mutant is characterized by an increased noradrenergic innervation of the cerebral cortex. We have observed in this

mutant, when compared to the wild type (+/+), a 50-fold increase in the threshold concentration of NE necessary to elicit glycogenolysis (tg/tg = 500 nM; +/+ = 10 nM) and in the EC_{50} of NE (tg/tg = 5 μM; +/+ = 100 nM; N = 9). The qk/qk mutant is characterized by a myelin deficiency due to an impaired differentiation of oligodendrocytes. In this mutant we have observed an increased potency of K^+ to elicit glycogenolysis. Results were: (^{3}H-glycogen hydrolysis in % basal levels ± SEM; N = 6); K^+ 5 mM: qk/qk = 35.1 ± 2.2, +/+ = 14.,1 ± 2.4; K^+ 7 mM: qk/qk = 51.5 ± 1.7, +/+ = 30.7 ± 3.2; K^+ 10 mM: qk/qk = 66.9 ± 1.9, +/+ = 48.1 ± 2.2. This supersensitive response to K^+ was only observed in mice older than 7 weeks.

Phosphate transport in OK cells is inhibited by PTH, TPA and A23187

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Parathyroid hormone (PTH, 10^{-10} M) and increased cellular cAMP inhibits Na/P_i-cotransport in OK-cells (derived from an American opossum kidney), by decreasing the V_{max} in a time-dependent way (70% decrease after 4 h exposure). The change of the transport capacity can be observed at the membrane level using membrane vesicles. The PTH effects are reversible and only the recovery phase involves protein synthesis.
Other agents which directly effect regulatory pathways also affect Na/P_i transport. 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and phorbol 12,13-didecanoate inhibit Na/P_i transport in a concentration dependent way (> 10^{-10} M). The inactive phorbol ester α-phorbol 12,13-didecanoate does not alter Na/P_i-transport. The TPA induced inhibition is reversible. A23187 also inhibits the Na/P_i-transport in a concentration dependent way (1–5 μM) but the process is not reversible. At present it is not known whether PTH, cAMP, A23187 and TPA affect Na/P_i-transport via a common regulatory pathway.

Possible role of chromatin-bound poly (ADP-ribose) in establishing an altered chromatin conformation in DNA excision repair

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Irradiation of primary cultures of rat hepatocytes with UV-light (254 nm, 45 J/m^2) leads to the formation of pyrimidine dimers and DNA strand breaks. The repair of this DNA damage is accompanied by a conversion of NAD into poly (ADP-ribose), which results in increased chromatin-bound poly (ADP-ribose) levels as determined by a boronate affinity chromatography-HPLC technique. The earliest consequence of suppressed chromatin-associated poly ADP-ribosylation was a marked disturbance in the coordinate development of the repair conformation of chromatin as probed by 8-methoxypsoralen and micrococcal nuclease accessibility (Mathis and Althaus, J. biol. Chem. 1986, in press). Under these conditions, the rearrangement of newly synthesized repair patches relative to the nucleosomal array was almost completely blocked. This suggests that the primary function of poly (ADP-ribose) in DNA repair is related to the transient establishment of an altered chromatin conformation.

IgE-receptor-coupled Ca^{2+}-influx and serotonin release are IL-3-dependent in mast cells: involvement of PKC

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Mast cell lines (e.g. PB-3c) require interleukin-3 (IL-3) for proliferation. Upon immunological stimulation and after a transient increase in cytosolic free Ca^{2+}, they liberate serotonin and other allergic mediators. These processes require the presence of IL-3 and are blocked by the phorbol ester TPA, suggesting a role for protein kinase C (PKC), the TPA receptor, in the regulation of exocytosis and Ca^{2+} fluxes. In malignant mast cells (PB-1 line), which proliferate without IL-3, serotonin release and the increase in free Ca^{2+} cannot be induced. These cells exhibit only 30% of the PKC activity found in PB-3c cells, but the enzyme is largely associated with membranes, a distribution normally observed in IL-3-dependent cells only when treated with TPA. We propose that a constitutive activation of PKC (TPA-like) might be responsible for both the malignant phenotype of PB-1 cells and their inability to release serotonin.

Erythrocyte-mediated microinjection of Semliki forest virus (SFV) core protein into vertebrate cells

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Several functions have been attributed to the core(C)-protein in SFV infected vertebrate cells, suggesting that it may act in a fashion similar to a 'pleiotropic' regulator protein: a) a core associated autoprotease and phosphokinase activity; b) a transient interaction with binding sites on the 60S ribosomal subunit during core assembly and disassembly; c) an interaction with the viral polymerase complex leading to a regulation of viral RNA transcription; and d) a postulated role in the shut-off of host protein synthesis. The study of these presumed regulatory functions is difficult, since many of the different virus-controlled processes appear to be intimately connected and are not separable. Our goal is to study some of these processes in intact cells in the absence of other viral functions. We have compared several microinjection techniques and found that optimal incorporation of macromolecules into erythrocyte ghosts occurs if octylglucoside is present during resealing. The efficiency of C-protein incorporation into ghosts and its delivery into the cytoplasm of recipient cells are presented.

Intracellular pH regulation of LLC-PK_1 cells by amiloride sensitive Na^+ transport

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We have evaluated the capability of a continuous renal epithelial cell line (LLC-PK_1) to maintain intracellular pH (pH_i) near neutrality by Na^+-dependent processes. Four hours after removal from a monolayer, the cells in suspension restore normal (compared to monolayer) gradients of Na^+ and K^+. Experiments using FCCP and valinomycin indicate low intrinsic H^+ conductance and maintenance of pH away from electrochemical equilibrium. Neither replacement of medium Na^+ with TMA^+, nor addition of 100 μm amiloride affects resting pH_i (7.0–7.1) within 5 min. This implies that when the pH_i is at steady state, any Na^+/H^+ exchange reaction present in the plasma membrane is 1) not reversible and 2) not catalyzing a net H flux. However, the recovery from an acid load is strongly dependent on medium Na^+ and is amiloride sensitive. Between pH_i 6.8–5.9, it is possible to observe a marked (10×) stimulation of (amiloride sensitive) 20 mM Na^+ influx into the cells.

Isolation of endocytosis mutants in yeast

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We have begun to isolate mutants defective in endocytosis. Starting with the assumption that a block in endocytosis would

be lethal for the cell, a bank of temperature sensitive lethal mutants has been made and mutants defective in the uptake of lucifer yellow at non permissive temperature have been isolated. We tested those mutants for normal protein synthesis and normal invertase secretion. This screening of 750 temperature sensitive lethal mutants permitted the isolation of two endocytosis mutants. These mutants are less sensitive to alpha factor than the parent strain. This suggested to us that endocytosis may be involved in pheromone response. So one possible enrichment procedure developed was to mutagenise a cells and plate them out on plates at 24 °C containing alpha factor. After growth on alpha factor plates the clones are replica-plated to 37 °C and thermosensitive clones are screened for endocytosis, protein synthesis and invertase secretion. We found 300 thermosensitive mutants and among them 70 are endocytic mutants. Surprisingly all 70 endocytic mutants are also secretory mutants. But they are different from those isolated by Novick, Schekman. Complementation analysis has been done and seven different groups of mutant have been found.

Activation of protein kinase C stimulates parathyroid hormone secretion and lowers cytosolic calcium

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Stimulation of parathyroid hormone (PTH) secretion by low extracellular calcium (Ca_e) is associated with a decrease of cytosolic calcium (Ca_i) implying that Ca_i mediates PTH secretory responses. We have investigated the role of protein kinase C on PTH secretion and Ca_i. TPA (100 nM) stimulated PTH secretion twofold at high Ca_e, but TPA was ineffective at low Ca_e, indicating that protein kinase C was fully active at low Ca_e. At high Ca_e TPA caused reduction of Ca_i from 639 ± 36 nM (SEM) to 335 ± 21 nM ($P^e < 0.001$); at low Ca_e TPA was ineffective. Moreover, TPA suppressed the rise of Ca_i evoked by high Ca_e. Thus TPA presumably stimulates PTH secretion via activation of protein kinase C, and the lowering of Ca_i is a secondary event related to diglyceride availability. To this end, an inverse relationship between diglyceride levels and PTH secretion has been described. Moreover, La^{3+}, not permeating the plasma membrane inhibited PTH secretion in a dose dependent manner (ID_{50}: 10 μM) probably through calcium binding sites at the plasma membrane. In conclusion, activation of protein kinace C rather then changes in Ca_i appear to mediate PTH secretory responses.

Translocation of the catalytic subunit of cAMP-dependent protein kinase from the Golgi complex to the nucleus

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In bovine epithelial (MDBK) cells, the regulatory (R II) and the catalytic (C) subunits of the type II enzyme of cAMP-dependent protein kinase are associated with the Golgi complex (Nigg et al. Cell *41* (1985) 1039). However, after stimulation of cellular adenylate cyclase with forskolin, or treatment of cells with dibutyryl-cAMP, C subunits dissociate from the Golgi complex and accumulate in the nucleus, while R II subunits remain associated with the Golgi complex. C subunit translocations are rapid and reversible. Our results suggest that nuclear translocation of activated protein kinase subunits (and phosphorylation of key nuclear substrates) may represent an important link between hormonal stimulation and physiological responses.

Stimulation of growth of ZR-75 cells by estradiol and epidermal growth factor: different effects on S6-kinase and protein synthesis

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Stimulation of 3T3 cells with epidermal growth factor (EGF) leads to rapid activation of a protein kinase specific for phosphorylation of ribosomal protein S6 (Novak-Hofer and Thomas, J. biol. Chem. *260* (1985) 10134). We asked, if the enzyme can also be activated by steroids. For this purpose we chose the human mammary tumor cell ZR-75 which can be stimulated to proliferate by EGF and estradiol (E_2). In this cell line EGF leads to a rapid and transient activation, whereas E_2 does not lead to a significant increase in S6-kinase activity. The effect of EGF and E_2 on protein synthesis during the first 6 h of stimulation was compared, in order to investigate if a rapid increase in protein synthesis depends on activation of S6 kinase and phosphorylation of ribosomal protein S6.

Guanine nucleotide regulatory protein couples angiotensin II receptors to phospholipase C in rat renal mesangial cells

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Incubation of rat mesangial cells with angiotensin II (10^{-7} M) resulted in transient breakdown of phosphatidylinositol 4,5-bisphosphate and simultaneous increase in 1,2-diacylglycerol and inositoltrisphosphate, indicating an activation of phospholipase C. All of these processes were abolished by prior exposure of cells to pertussis toxin. Incubation of membrane fraction of mesangial cells with pertussis toxin resulted in a ADP-ribosylation of ~ 42 kDa protein. The entirety of these results makes it likely that a G-protein is involved in angiotensin II receptor mediated signal transductions in renal mesangial cells.

Intracellular free calcium and number of neurotubules in rabbit vagus nerve fibers

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The intracellular concentration of free Ca ions (Ca_i) was measured in vitro by the use of quin2 (Pralong and Straub, J. Physiol., in press); for the determination of the number of neurotubules (NT), the preparations were transferred to a fixative (glutaraldehyde 1%, p-formaldehyde 1% in 0.1 M phosphate buffer) and then prepared for e.m. examination. At 37 °C and 1.5 mM external Ca (Ca_e), the free Ca_i was 74 ± 14 nM, lowering the temperature for 30 min to 20 °C produced a rise in Ca_i to 107 ± 12 nM and a small decrease in the number of NT. Increasing Ca_e to 5 mM caused a significant loss of NT compared to controls at both 20 (Ca_i 145 ± 10 nM) and 37 °C, the effect being larger at 20 °C. Omission of Ca_e (Ca_i 52 ± 5 nM) prevented the effects of lowering the temperature. The experiments show that the disappearance of NT is correlated to an increase in free Ca_i.

Signal transmission: lipolytic enzyme activities in plasma membranes of chromaffin cells from adrenal medulla

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The importance of lipolytic enzymes for the mechanism of signal transmission is well known. We investigated corresponding en-

zyme activities in isolated plasma membranes of chromaffin cell from bovine adrenal medulla. Only a minute activity of phospholipase A_2 could be detected using externally added phosphatidylcholine (PC) and phosphatidylethanolamine (PE) as substrates. Treatment of the membranes with exogenous phospholipase C resulted in a liberation of fatty acids from the sn-2 position of PC. The enzyme responsible for this effect is a newly found diglyceride lipase localized in the plasmamembrane. Using phosphatidylinositol (PI) as a substrate we found the endogenous phospholipase C, which co-purifies with the membrane preparation. The produced diglyceride is subsequently hydrolyzed by the diglyceride lipase. The two enzymes were characterized. Phospholipase C is calcium-dependent and PI-specific, whereas the diglyceride lipase was calcium-independent.

Control of cytosolic free Ca^{++} by organelles in adrenal glomerulosa cells

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The steroidogenic response to angiotensin II in glomerulosa cells is mediated by a transient rise in cytosolic free Ca^{++}, due to Ca^{++} mobilization from intracellular pools. The regulation of Ca^{++} transport by intracellular compartments was studied in digitonin-permeabilized cells, using a Ca^{++} selective electrode. Two pools pumped Ca^{++}, maintaining it at fixed set points; a) mitochondria (set point at 700 nM $[Ca^{++}]$), requiring respiratory substrates and whose efflux was activated by Na^+ ($ED_{50} = 5$ mM); b) a non-mitochondrial vesicular pool (set point at 200 nM), requiring ATP and whose efflux was activated by inositol-1,4,5-trisphosphate ($ED_{50} = 0.75$ µM), with a marked desensitization of this response. Thus two compartments, mitochondria and a non-mitochondrial pool, each differently regulated and with characteristic set points, are able to maintain intracellular Ca^{++} homeostasis in glomerulosa cells.

Calcium-binding proteins in rat skin

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Two Ca^{2+}-binding proteins are described in the epidermis. S-100 proteins are localized in melanocytes and Langerhans cells and calmodulin in all keratinocytes. There is, however, a controversy about a third skin Ca^{2+}-binding protein (SCaBP), reported to be present in the proliferative cells of the epidermis. On the one hand (Biochemistry *21* (1982) 4805), SCaBP was reported to be immunologically and biochemically distinguishable from the Ca^{2+}-binding parvalbumin (PV), on the other hand (Biochem. J. *229* (1985) 39), the complete amino acid sequence of rat SCaBP showed identity to PV. Therefore, we prepared epidermis (free of dermis and underlying muscle layer the latter containing PV) from adult and newborn rats. From these extracts two proteins were purified and characterized. One was found to be indistinguishable from PV (M_r 12,000), and a second (M_r 12,000), is different from PV by its immunological and biochemical properties. Its detailed localization in rat skin will now be investigated.

Evidence for a modulating effect of H_2-receptors in the metabolic response of brown adipose tissue

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Published data show that, in isolated brown adipocytes, stimulation of α_2-receptors induces an inhibition of β_1-stimulated lipolysis. To our surprise, and α_2-agonist clonidine potentiated the metabolic effect of isoproterenol (ISO) in brown adipose tissue (BAT) fragments. To elucidate this potentiating effect 1) measurements of cAMP in tissue incubated with clonidine (10^{-7} M) and/or ISO (10^{-6} M) show that the cAMP content was increased when both were added and no inhibitory effect of clonidine was obtained when a maximal accumulation was induced. 2) Since clonidine has been reported to increase, via H_2-receptors the ISO-stimulated lipolysis in white fat, cimetidine was tested and found to patially inhibit the potentiating effect of clonidine on BAT respiration. Furthermore, histamine was found to produce a similar potentiating effect as clonidine when added with ISO.

Separation of phosphoinositide cycle intermediates in mammalian nerve fibers by HPLC

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To our knowledge, it has not been possible to separate the majority of intact phosphoinositides (PIdes) by HPLC. Based on the method of Yandrazitz et al. (J. Chromat. *225* (1981) 319) and using a gradient of two solvents containing hexane, isopropanol, water and H_2SO_4 for ion suppression, we have found conditions that allow the utilisation of HPLC with a silica 5 µm column (30 cm length, 4 mm int. diameter) for the study of PIdes turnover in nerve. By this method it is possible to resolve phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylinositol-1-4-biP (PIP), and phosphatidylinositol-1-4-5triP (PIP_2) from the total phospholipid extract. Bidimensional thin layer chromatography on silica plates confirms the results obtained by HPLC. The results show that the application of HPLC methods to intact (non deacylated) phospholipids gives sufficient resolution to separate, if not all phospholipids, at least the phosphorylated intermediates of the PIdes cycle.

Tumor-associated Ca^{2+}-binding proteins in chemically transformed and in growth stimulated rat fibroblasts

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Two tumor-associated Ca^{2+}-binding proteins are present in some cancer cells: a protein with M_r 12,000; pI 4.8 (PNAS *81* (1984) 6632) and oncomodulin, M_r 12,000; pI 3.9 (Oncodev. Biol. Med. *3* (1982) 79). Extracts of chemically transformed fibroblasts were analyzed by 2-D-PAGE and HPLC for the presence of these two proteins. Fibroblasts from a rapidly dividing granulation tissue ('granuloma pouch') of 50-day-old, male S/D rats were treated in vivo/in vitro with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; 0.6 mg/rat and 0.5 µg/ml) or in vitro with aristolochic acid (20 µg/ml). The mutagen exposed cells were screened for colony growth in soft agar. The obtained clones produced solid tumors in nude mice within two weeks. An early expression of the two proteins was found in the transformed cells, and also in the granulation tissue by day 3 in vivo, but not in normal, untreated fibroblasts.

Acidification of the cytosol accompanies human neutrophil activation

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Changes in cytosolic calcium (Ca_i) and pH (pH_i) which are rapid in onset and transient in nature occur upon stimulation of neutrophils. Using the intracellular indicator 5-carboxy-4',5'-di-

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methylfluorescein, we have studied pH_i in human neutrophils stimulated with the chemotactic peptide fMLP or the bioactive lipid PAF. Both stimuli caused a concentration dependent, transient acidification of the cytosol. The initial drop in pH_i was compensated within a few minutes. At high stimulus concentrations, the pH_i recovery was somewhat slower. In all cases, re-alcalinisation was sensitive to amiloride and thus possibly dependent on a Na^+/H^+ exchange. PAF and fMLP also induced a rapid rise of Ca_i. The onset time and the extent of the Ca_i changes were virtually independent on the stimulus concentration. Not so the pH_i changes which had a longer lag time at low concentrations of either fMLP or PAF. This suggests a large cytosolic pH buffering capacity. Like other responses, the acidification was prevented by *B. pertussis* toxin, which blocks GTP-binding proteins. In addition, pH_i changes depend on the availability of releasable intracellular calcium. Our results suggest that cytosol acidification results from stimulus-dependent metabolic processes and that the protons generated have no role in signal transduction.

Influence of neurotransmitters on the electron transfer rates of plasma membrane dehydrogenases in rat brain synaptosomes

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Purified rat brain synaptic membranes exhibit high levels of NAD-dehydrogenase activity with various redox agents as acceptors. These activities – which in many tissues usually participate to energy-linked membrane processes and control the redox state of components in plasma membranes – are highly influenced in synaptic membranes by neuroactive amino-acids such as Glu and Asp (both highly activating), catecholamines (inhibiting) and some Ipsp effectors (activating), but not by cholinergic or -receptor agents, adenosine, amphetamines or ion-channel effectors. The effect of a number of Dopa-analogs and Phe-metabolites or related substances are presented. These redox activities are selectively modulated by either dopamine, Glu or Asp with respect to acceptors, reflecting the presence of several different enzymes in synaptic membrane preparations. These NAD-dehydrogenases could be partially solubilized by detergents.

Stimulus-induced increase of mitochondrial respiration in a single neuron

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In isolated mitochondria an increase of [ADP] in the medium causes an increase of O_2 consumption (ΔQO_2) (Chance and Williams, J. biol. Chem. *217* (1955) 409). In *Limulus* ventral photoreceptor, light stimulation causes a flux of Na into the cell; the pumping out of this Na hyperpolarizes the membrane by 5–10 mV and is a major use of cellular ATP, which is converted to ADP. With microelectrodes we measured PO_2 and membrane potential. ΔQO_2 was calculated from ΔPO_2 using the diffusion equation. A light flash induced a rapid transient ΔQO_2. However, when we pulse injected Na into the same cell no ΔQO_2 was detected, although the marked hyperpolarization (10–34 mV, $N = 18$) indicated that the Na pump was more active than after light. We conclude that in this cell an increase in [ADP] (or $[Na^+]_i$) is not the main stimulus of mitochondrial respiration after a light flash. A role for cytosolic Ca cannot be excluded.

Tumor-promoting phorbol esters increase basal and inhibit insulin-stimulated glucose transport in isolated perfused heart from lean but not from obese rats

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Glucose transport and its stimulation by insulin are impaired in hearts from genetically obese (fa/fa) rats (Zaninetti et al., Diabetol. *25* (1983) 525). The nature and origin of this defect are undefined. We decided to study the effects of phorbol esters on glucose transport because these have been shown to modulate insulin action in several tissues. We found that in heart from lean animals phorbol myristate acetate (PMA) produced a dose-dependent stimulation of basal glucose transport (threefold increase at an optimal concentration of 50 ng/ml) but inhibited insulin-stimulated transport. In contrast, hearts from obese rats were completely unresponsibe to PMA. Tumor-promoting phorbol esters stimulate protein kinase C because of their structural analogy with the endogenous activator diacylglycerol. Possible relationships between defective protein kinase C and insulin resistance in obese rat hearts are currently investigated.

Analysis of bovine leukocyte phagocytosis and intracellular bacteria killing monitored by acridine orange fluorescence staining

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Phagocytosis and intracellular killing of bacteria by polymorphonuclear leukocytes (PMNLs) and monocytes is considered as a major defense mechanism against invading microorganisms in man and animals. We used the acridine orange/cristal violet staining technique which was developed for the human cell system, to monitor simultaneously phagocytosis and intracellular killing of bacteria by single bovine phagocytic cells. Using this technique we quantitatively analyzed the kinetics of uptake and intracellular killing of serum-opsonized *Escherichia coli* K12 and *Staphylococcus aureus* SG511 by adherent bovine PMNLs and cultured monocytes (macrophages). Data obtained for phagocytic cells of 22 bulls of four different Swiss dairy breeds, which were analyzed with aid of the SAS-computer program, will be presented.

Symposium 4: Transport and secretion of macromolecules

Biosynthesis of the influenza virus hemagglutinin: characterization of the monomeric and trimeric stage

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The hemagglutinin (HA) of influenza virus is produced by the infected host cell as a membrane glycoprotein. We have generated and characterized monoclonal anti-HA antibodies which are able to react with the HA at distinct stages of its biosynthesis. These include a monomeric form of the protein which is associated with the endoplasmic reticulum, and the mature trimeric form which is detectable in the Golgi region and on the plasma membrane. The transport of the HA in the cell and its precise localization was studied by the infection of human fibroblasts (MRC-5) under single-cycle conditions and was visualized by a computer-assisted video-immunofluorescence microscopy.

Hepatoprotector properties of a dibenzo (a, c) cyclooctene derivative from *Fructus schizandrae chinensis* evaluated in rat with the ^{14}C-aminopyrine/demethylation model

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In a previous study (Benakis et al., Int. Cong. Pharmac. Tokyo, 1981) we have used the model of ^{14}C-aminopyrine (^{14}C-AMPY) demethylation giving $^{14}CO_2$, in order to assess the effectiveness of hepatoprotector agents after CCl_4 and D-galactosamine (d-GAL) intoxication. The same model has been used in the present study to evaluate the hepatoprotector properties of dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxy-biphenyl-2,2′-dicarboxylate (DDB), a synthetic derivative of a compound isolated from *Fructus schizandrae chinensis*. Pharmacological properties of schizandrae derivatives and DDB, particularly with regard to hepatic function, have been extensively studied (Pao Tien-tung et al., Chin. med. J. *3* (1977) 173, and K.T. Liu et al., Chem. biol. Inter. *39* (1982) 301; *39* (1982) 315; *41* (1982) 39).
Results of the present study in rats (6 male Sprague-Dawley, 220 g) showed that DDB pretreatment (3 × 200 mg/kg oral) reduced the demethylation value of ^{14}C-AMPY (25 mg/kg) after CCl_4 intoxication (0.2 ml/kg) from 33% to 23%. When d-GAL (500 mg/kg) was used as the intoxication agent in DDB pretreated rats the reduction in demethylation value was from 32% to 19%. Although present results show DDB to be an effective hepatoprotector agent, further studies are needed to determine the dose required for optimal effect.

Molecular characterization of two genes important in endocytosis by yeast

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Two temperature sensitive lethal and endocytosis defective mutants, which secrete normally, have been isolated and characterized. They display several features which may provide interesting information regarding the possible functions of the putative END gene products. Beside deficiency in endocytosis, they are considerably defective in pheromone response and have morphologically abnormal vacuoles. One of them (END1) is defective for vacuolar carboxypeptidase Y biogenesis, while the other (END2) accumulates an interesting novel membrane-bound organelle.
END1 and END2 genes have been cloned by complementation of temperature sensitive phenotype, with yeast genomic library in the CEN vector, using yeast DNA transformation techniques. The restriction maps of both genes and their sequence analysis will be presented.

Gene and protein structure of *Xenopus laevis* vitellogenin

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Vitellogenin, the precursor of the yolk proteins, is synthesized under the control of estrogen in the liver of oviparous vertebrate females. Vitellogenin is then secreted into the bloodstream and transported to the oocytes where it is taken up and cleaved into the yolk proteins. A prerequisite for complete understanding of this developmentally important storage protein and its regulation is an extensive knowledge of its molecular structure. For this purpose we sequenced the *Xenopus laevis* A2 vitellogenin and the corresponding cDNA. The detailed analysis of the nucleotide sequence allowed us to determine gene structure, splicing sites, open reading frames, codon usage and the primary structure of the vitellogenin protein.

Physiology and immunolocalization of trypsin during a digestive cycle in the mosquito *Aedes aegypti*

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Mosquito trypsin was followed electrophoretically during a digestive cycle. A few hours after the blood meal the isozyme bands developed a complex pattern towards the peak of digestive activity. Decapitated females however, lacking an intact endocrine system and thus being non-oogenic, had a specifically reduced pattern in certain bands. To follow the synthesis of trypsin in more detail it was purified from midguts in order to produce a polyclonal antibody in rabbits. With light microscopical immunocytochemistry we first detected trypsin immunoreactivity at the periphery of the blood bolus around 8 h after blood meal. Gradually the immunoreactivity in the bolus increased until its defecation began. With electron microscopical immunocytochemistry we could follow the secretory process from the formation of immunopositive secretory granules in the Golgi complex until their release at the base of the microvilli into the midgut lumen by exocytosis.

Glucose transporters in brown adipose tissue (BAT)

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The substrate requirement of BAT increases during cold adaptation. Glucose is an important fuel to this tissue and its transport is the limiting step for its utilization. Due to this, the effect of cold adaptation on the number of glucose transporters in purified BAT plasma and microsomal membranes from lean and obese Zucker rats has been studied. Cytochalasin B has been used to quantitate the glucose transporters (in pmoles/mg protein) of membranes prepared from BAT of rats kept at 22 °C and rats which were cold-adapted (4 °C). BAT plasma membranes from lean rats at 4 °C have 5 times more glucose transporters than controls. At 4 °C obese plasma membrane glucose transporters are half those of lean. In normal and obese, the translocation of glucose carriers from intracellular pool to plasma membranes occurs normally. In addition, in membranes from cold-adapted rats, has been found an increase in the Hill coefficient for cytochalasin B that may indicate a change in the properties of glucose for the transporters.

Introduction of antibodies into permeabilized exocytosis-competent chromaffin cells

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Bovine adrenal chromaffin cells store catecholamines in vesicles which upon stimulation and subsequent Ca^{2+} influx fuse with the plasma membrane, thereby releasing their content. ^{3}H-norepinephrine preloaded cells, rendered permeable by digitonin, still show Ca^{2+} dependent release. By electron microscopical studies we could show that upon stimulation with 10 μM free Ca^{2+} vesicle antigens appear on the cell surface of permeabilized cells proving that release is due to vesicle fusion and not to leakiness of the vesicles. These cells, still competent for exocytosis, are permeable for molecules of the size of antibodies as shown by microfilament staining with anti-actin antibodies in such cells. Thus, by digitonin treatment specific antibodies can be introduced into chromaffin cells as a tool to investigate the molecular mechanisms of exocytosis.

Intracellular sorting of membrane proteins: Nuclear envelope versus plasma membrane glycoproteins

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The pathway by which an integral membrane protein of the plasma membrane is synthesized, processed and transported to the cell surface has been well worked out. Unfortunately the basic cellular problem of how such proteins are distinguished from integral proteins of the ER, or Golgi, or lysosomes or the nuclear membrane remains without any answer. In fact the exact pathway leading to these organelles are undefined. The sorting problem is critical for the cell for it is only by sorting that the organelles are differentiated and defined. We have developed a system whereby we can study the pathways by which membrane glycoproteins are sorted into the nucleus. We compared the gD envelope protein of Herpes simplex virus I, who's final destination is the nuclear envelope with the G-protein of vesicular stomatis virus, a plasma membrane protein. It was found that these two proteins were synthesized at mechanistically distinct locations, namely the gD at the outer nuclear membrane and the G-protein at the ER, which is resolved in microsomes upon subcellular fractionation.

Extracellular lignin degrading peroxidases from white-rot fungi

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We have studied lignin degradation by several wood-rotting fungi and isolated extracellular hemoproteins from their culture filtrates. The induction and production of these lignin degrading peroxidases has been studied in detail using a basiomycete, *Phanerochaete chrysosporium*. The production of the ligninolytic enzymes is a secondary metabolic phenomenon and occurs under nutrient limitation. Eight major hemoproteins have been purified and characterized. All of these proteins are able in the presence of an H_2O_2 producing system to oxidize lignin and several aromatic compounds through a one electron transfer mechanism. As model reactions we have studied the oxidation of benzo(a)pyrene and different types of lignins.

In vitro release of vasoactive intestinal peptide (VIP) from mouse cerebral cortical slices

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A sensitive RIA for the detection of small quantities of VIP (lower limit 3 pg) has been used to examine the regulation of VIP release from mouse cerebral cortical slices. These experiments were performed either by superfusing the slices with Krebs Ringer bicarbonate buffer and by assessing VIP in the collected fractions or, in static conditions, by measuring VIP released into the incubation medium. Basal levels of VIP release are 4–8 pg/800 µl/2 min (superfusion) and 5–7 pg/mg prot (static). VIP release is dependent upon the concentration of extracellular K^+ (15–55 mM) with a maximal release at 55 mM corresponding to a 6-fold increase over basal levels. We are currently examining the potential stimulatory or inhibitory effect of cortical neurotransmitters on VIP release.

High level expression of a proteinase inhibitor in yeast

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The coding sequence of the proteinase inhibitor eglin c from the leech *Hirudo medicinalis* has been synthesized by the phosphotriester solid-phase method. The mature coding sequence has been expressed in yeast *(S. cerevisiae)* under the control of the inducible *PHO5* promoter. Proteins from a yeast cell extract are precipitated by acetic acid. Recombinant yeast eglin c stays in the supernatant. It inhibits human leucocyte elastase by 97%. HPLC analysis shows that yeast produces a mixture of natural eglin c (free NH_2-terminal threonine) and N^{α}-acetyl-eglin c at a ratio of 2:1. The ratio of both expression products varies upon fermentation conditions. Expression in *E. coli* mainly leads to N-acetyl-eglin c (Rink, H., Liersch, M., Sieber, P., Meyer, F., NAR *12* (1984) 6369).
Similar constructions with the coding sequence of a thrombin inhibitor, hirudin, from the leech *H. medicinalis* gave only minor expression in yeast using the same promoter system.

Structural features of the human intestinal lactase-phlorizin hydrolase (LPH)

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The monoclonal antibody HBB/1/303/909 (Hauri et al., JCB 1985) reacts specifically with the human LPH molecule and has been used to isolate and characterize this disaccharidase. LPH purified from the brush border membrane fraction of biosynthetically labeled biopsies consisted of a polypeptide of $M_r = 160{,}000$. In the intracellular membranes two polypeptides were identified of $M_r = 215{,}000$ and 160,000 which are not linked by disulphide bridges. The 215-kD component was the only intermediate identified in short pulse labeled biopsies and was endo H sensitive implying that it corresponds to the high mannose precursor of LPH. The 160-kD species possesses mainly complex oligosaccharides and due to its partial endo H sensitivity at least one chain of the high mannose type. It is generated from the precursor molecule by intracellular cleavage. This is supported by the inhibition by leupeptin, a protease inhibitor of bacterial origin, of the appearance of the 160-kD.

Cloning of the rabbit pIg receptor gene and its expression in an immortalized rabbit mammary cell line

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In rat liver, the polymeric immunoglobulin receptor (pIg-R) is synthesized on the ER as a core-glycosylated glycoprotein, terminally glycosylated and phosphorylated in the Golgi. The pIg-R is directed to the sinusoidal membrane, where it binds IgA and transport it into bile. Absence of phosphorylation in the Golgi has been correlated with mis-sorting of the receptor. To test whether phosphorylation is the signal for directing the receptor to the basolateral membrane of epithelial cells, the pIg-R was cloned and an immortalized cell line was established. The cloned receptor cDNA was inserted into the pSV_2 expression vector and stable transformants were selected by cotransfecting the hygromycin resistance gene. The expression and the routing of the receptor, as well as its phosphorylation are currently analyzed in such a system.

phol-mutants of *Schizosaccharomyces pombe* that are blocked in glycosylation and secretion of acid phosphatase

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What are the signals in the structural gene *phol* of acid phosphatase (aPase) of *S. pombe* that control glycosylation and secretion of this cell surface enzyme? 68 *phol*-mutants which have no *phol*-aPase activity were tested with the immunoblot technique.

59 of these mutants give no signal on the blots. One mutant exhibits the same aPase pattern as the wild type. Two mutants have an aberrant core-glycosylated form and reduced amounts of highly glycosylated aPase. Six mutants reveal no highly glycosylated aPase, but accumulate the membrane-bound core form. Pulse-chase experiments confirm that accumulation of the core form occurs since outer chain carbohydrates can not be attached to the protein. We conclude that the mutation in these mutants prevents aPase activity and simultaneously blocks glycosylation and secretion of the enzyme. Sequencing of the *phol*-mutations is in progress.

Measurement of exocytotic activity from single cells

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PC 12 cells are a widely used model to study the release of neurotransmitter. Investigation of this process is hampered by the inaccessability of the intracellular compartment for molecules affecting exocytotic steps. This difficulty can be overcome by the technique of intracellular injection, but so far it has been impossible to measure the release of catecholamines from single cells. As stimulated exocytosis seems to be strictly coupled to subsequent endocytosis, we have developed an assay for quantitating endocytosis of a fluorescent marker. Lucifer Yellow added to PC 12 during stimulation is internalized by pinocytosis. The fluorescence intensity of single cells, measured by a microspot photometer, correlates with the release of catecholamines from cell populations and can be used as a parameter for exocytotic activity of single cells.

Asynchronous protein transport to the cell surface in intestinal epithelial cells

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Previous studies have shown an asynchronous transport of newly synthesized peptidases and disaccharidases from the endoplasmic reticulum (ER) to the trans-side of the Golgi apparatus in $CaCo_2$ cells (Hauri et al., J. Cell Biol. *101* (1985) 838). In order to address the question of whether a similar asynchronism also exists for the Golgi-to-cell surface pathway we isolated with high purity a brush border membrane fraction of these cells. This preparation was used to study the appearance of sucrase-isomaltase and dipeptidyl-peptidase IV at the cell surface. It was found that these two enzymes arrived at the brush border with different rates. However, this asynchronism entirely reflected the ER-to-trans-Golgi pathway. Therefore, the results suggest that the transport from the Golgi to the cell surface of the two enzymes is synchronous. Present efforts concentrate on the dissection of the ER-to-Golgi pathway.

Symposium 5: Functional compartmentation in cells and organs

The creatine kinase (CK) puzzle in chicken: More complex than expected?

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In addition to M-CK and B-CK, which we have already cloned, there is the mitochondrial CK. A cDNA library made from chicken leg muscle RNA in the expression vector λgt11 was screened with anti chicken mitochondrial CK antibody (kindly provided by Dr. T. Wallimann) and several positive clones were sequenced. The active center peptide appears to be highly conserved also in these clones, while the nucleotide sequences diverge substantially. We present evidence that there are further CK like proteins in chicken tissues that have not been described so far. The clone λgt11–18c, isolated by screening a gizzard cDNA library with antibody against B-CK, contains a 3′ portion almost identical with the B-CK amino acid sequence and 431 nucleotides are exactly conserved. However at the 5′ end the amino acid sequence diverges completely and extends beyond the corresponding B-CK start codon. S1 protection experiments showed that RNA protecting 18c cDNA is tissue specifically expressed.

Carrier mediated uptake of α-amanitin in rat liver

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The mushroom poison α-amanitin (αA) exerts its most dramatic toxic effects in the liver. In order to characterize uptake of α-amanitin into hepatocytes we studied transport of {[6′–0,1′–N–di[^{3}H]methyl)trp^4}-αA in isolated sinusoidal rat liver plasma membrane vesicles. An out to in Na^+gradient (100 mM) stimulated αA uptake ~ 2 fold compared to a K^+gradient or sucrose and ~ 2.5 fold compared to αA equilibration (overshoot). Both Na gradient dependent as well as Na^+independent uptake were temperature sensitive, exhibited saturability (K_m ~ 190 and 220 μM; V_{max} ~ 10.5 and 5.7 $nmol \cdot min^{-1} \cdot mg^{-1}$, resp.) and could be inhibited by cholate, taurocholate, prednisolon and silybin, but not by penicillin G and thioctic acid. The data indicate that uptake of αA into liver is mediated by the same membrane transport systems that also transport bile acids. Furthermore, the data prove that the therapeutically recommended drug silybin (Legalon®) exerts its effects by blocking hepatic uptake of αA.

Functional heterogeneity of the nephron

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Due to different vascular supplies the kidney is subdivided into the cortex with the compartments of the labyrinth and the medullary rays, and the outer medulla with the outer stripe, the inner stripe with the vascular bundle and interbundle compartments, and the inner medulla. The nephron is a succession of different compartments, traversing the various vascular compartments. The functional qualities of the nephron segments are determined by 1) the intrinsic properties of their lining epithelial cells, 2) the peritubular compartment, and 3) the luminal environment of the cells. The areas of basolateral cell membranes (BLM) in the nephron cells, the site of the Na-K-ATPase, correlate with the activity of the enzyme and the transcellular transport capacity of the segment under control conditions and after adaptation to altered electrolyte metabolism. Changes in BLM area are associated with specific changes in the peritubular environment of the segment, e.g. plasma levels of mineralocorticoids, but also with specific changes of the luminal environment, the tubular solute load. The latter may account for intrasegmental axial heterogeneity of BLM area in some segments which are exposed to the same peritubular environment along their length.

Male copulatory apparatus and formation of spermatophores in a marine mollusc

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Many invertebrates pack their sperm into spermatophores. These are placed into the female genital tract during copulation.

Runcina, a small primitive opisthobranch, living in intertidal rockpools, produces spermatophores of about 400 µm in length. The formation of sperm cells takes place in the hermaphroditic gland. This autosperm then leaves by the common genital aperture and travels through a ciliated seminal groove along the external right surface to the head. The sperm then enters the male copulatory tract which lies to the right of the mouth and is separated from the rest of the genital system. Through penial sac, penis and prostate the gametes reach the spermatic bulb, where they are stored. Prior to copulation these autosperms are packed into spermatophores during their transport back. The study focusses on the correlation between the structure of the spermatophore and the type of cells within the copulatory system taking part in the formation of the different layers of the spermatophore.

Calcium induces fusion of small unilamellar vesicles containing cholesterylsulfate to form planar lamellae

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When cholesterylsulfate was incorporated into phospholipid liposomes, the presence of Ca^{++} caused fusion of vesicles and formation of large multilamellar sheets. This change in lipid phase morphology involves the formation of interliposomal bridges with the divalent cation linking the sulfate esters. The epidermal permeability barrier which separates the 'milieu intérieur' from the environment consists of similar planar bilayers obstructing the intercellular space of the lower horny layer. These cutaneous lipid sheets are formed by fusion of small lipid vesicles that are extruded from the lamellar granules of the outermost viable epidermal cells. Since the barrier lipids contain high amounts of cholesterylsulfate the relatively high intercellular Ca^{++} concentration may induce the formation of barrier sheets.

Accessibility of *Trypanosoma brucei* glycosomal enzymes to labeling agents of various sizes

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Trypanosoma brucei brucei, the parasite causative of the African cattle sickness nagana, has a particle grouping most of the glycolytic enzymes called glycosome. Since glycolysis is the main energy source for the mammal infective form of the parasite, glycosomes are interesting therapeutic targets. Because of the enormous glycolytic rate, the exchange of metabolites through the particle membrane is critical. Exposition of intact glycosomes to radioactive labeling agents of various polarity and sizes, and to proteolytic enzymes, shows that such membrane is permeant for small molecules regardless of their polarity. However it presents exclusion properties for larger molecules of the size of lactoperoxidase.

Purification and structural characterization of intact and fragmented nidogen

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The genuine form of the matrix protein nidogen (M_r = 150 kD) and two fragments (M_r = 130 kD and 100 kD) could be isolated from EHS-tumor by extraction with 6M GuHCl in the presence of protease inhibitors. Less stringent control of endogenous proteolysis led to smaller fragments (M_r = 80 kD and 40 kD). Intact nidogen and the larger fragments were similar in amino acid and carbohydrate composition, the presence of a single polypeptide chain, conformation and all shared the epitopes located on the 80 kD fragment. Step-wise degradation was indicated by different N-terminal sequences. Hydrodynamic and electronmicroscopical studies of the 80 kD fragment demonstrated a structure consisting of a globular head connected to a thin tail. Elastase digestion of the 80 kD fragment led to a globular 50 kD fragment and a 25 kD presumedly representing the head and the thin tail.

Identification and isolation of the putative canalicular bile acid carrier from rat liver

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Kinetic transport studies with canalicular rat liver plasma membrane vesicles have indicated that excretion of bile acids into bile canaliculi is a carrier mediated process. In order to identify the canalicular bile acid transport protein we incubated cLPM vesicles with the photolabile bile acid derivative (7,7-azo-3α,12α-dihydroxy-5β-cholan-24-oyl)-2-amino [2-^{3}H]ethanesulfonic acid. Subsequent photolysis resulted in the predominant labeling of an integral membrane protein with an apparent MW of 100,000. This canalicular specific 100 kD bile acid binding protein was isolated by sequential DEAE-cellulose and wheat germ agglutinin chromatography. Furthermore, monospecific antibodies against the purified 100 kD protein inhibited taurocholate transport in cLPM vesicles. These data indicate that the isolated 100 kD protein is involved in the canalicular excretion of bile acids.

Metabolic zonation of the liver

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The functional unit of mammalian liver parenchyma is the liver acinus with its different zones. Originally this unit had been defined on the basis of microcirculation; in the last years, however, it was possible to describe the intraacinar heterotopy of substances and enzyme activities by means of histochemical and microbiochemical techniques. Thus, the periportal area was found to be the gluco(neo)genic zone, whereas the perivenous area acts as a glycolytic and lipogenic zone. To understand the functioning of metabolic zonation it is important to regard the zones not being static but dynamic areas which are modified by nutritive, hormonal and nervous influences. Examples for such modifications will be presented: the effects of triiodothyronine on the activity and distribution pattern of the lipogenic enzymes G6PDH, malic enzyme and ATP citrate lyase, and the changes of ADH activity after castration and testosterone treatment.

Cytoarchitecture probed by cellular engineering

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In skeletal muscle it has been shown that part of the muscle specific MM-creatine kinase (MM-CK) isoenzyme can be localized in the M-band of the myofibrils. BB-CK, the ubiquitous and embryonic isoenzyme, having 82% amino acid homology with M-CK, however is not found in the M-band. In chicken heart muscle, no MM-CK is expressed although other M-band proteins such as myomesin or M-protein are present. Therefore the conditions for MM-CK association should in principle exist. The normally not expressed MM-CK was introduced into heart

cells by microinjection of: a) purified MM-CK, b) muscle polyA$^+$RNA and c) 'in vitro' transcribed M-CK mRNA from M-CK cDNA cloned in the vector SP6. Association of the newly introduced M-CK with the M-band region was shown by immunofluorescence. M-CK cDNA was altered by introduction of mutants or chimeric CK cDNA was constructed by combination of M-CK and B-CK cDNA fragments. Such experiments will lead to a better understanding of the significance of protein specificity for cytoarchitecture.

Immunofluorescense study of the localization of type VI collagen and of the basement membrane components laminin, nidogen, type IV collagen and low density proteoglycan in the mouse eye

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Unfixed 8-µm cryosections were labeled with affinity purified antibodies against the various components, stained with FITC labeled second anti rabbit IgG antibody and viewed by fluorescence. Laminin, nidogen (respectively entactin), type IV collagen and low density proteoglycan were only observed in the basement membranes (BM's) and in small spots in the corneal stroma. Since all five BM-components were located in these spots, they presumably represent BM-fragments. Type VI collagen appeared uniformly distributed in the stroma of the cornea and the sclera. It was recently proposed on the basis of electron microscopical observations, that the lattice like structure seen in Descement's membrane might be composed of type IV collagen. Furthermore it was often discussed, that type VI might be this collagen. The present study does not lend support to either of these two suggestions.

Biochemical and ultrastructural changes in chronically stimulated cat gracilis muscle

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Skeletal muscle adapts to chronic activity with a change in structural and functional features, among them an increase in mitochondrial volume density. We have studied physiological, structural and biochemical changes in cat m. gracilis upon chronical electrical stimulation for up to 28 days. The increase in maximal O_2 uptake measured on the isolated in situ perfused muscle after stimulation was matched by a proportional increase in mitochondrial volume density determined in these muscles by ultrastructural morphometry. Isolation of mitochondria from stimulated and non-stimulated muscles showed that the specific activity of oxidative phosphorylation with various substrates was not altered through stimulation. Similarly, a combined biochemical and morphometric analysis of the isolated mitochondria showed no differences in the ultrastructural and molecular architecture of the inner membrane in either mitochondria.

Electron microscopy of yeast endocytosis mutants

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Yeast cells endocytose a fluid phase marker Lucifer Yellow in a time-, temperature- and energy-dependent way and accumulate it in the vacuole. Many of the yeast secretory mutants are also defective in endocytosis (Novick et al., Cell *21* (1980)). In summary, these results suggest that the endocytic pathway does not depend upon membrane movement from the rough endoplasmic reticulum to the Golgi. The endocytic pathway seems to constitute a circuit which may overlap only with the post-Golgi stages of secretion (Riezman, H., Cell *40* (1985)). We would like to test this further. We are using two techniques to embed yeast cells in resin for electron microscopy. 1) Embedding of spheroplasts in Epon after pre-embedding staining with reduced osmium-thiocarbohydrazide-reduced osmium. 2) Embedding in Lowicryl HM-20 after progressive lowering of temperature technique (Carlemalm et al., J. Microsc. *126* (1982) 123). As a marker for endocytosis we are going to use a derivative of Lucifer Yellow which is photo-activable (prepared by Dr. J. Brunner, ETH Zürich) and which can be detected in EM by a specific antibody and protein A gold on ultrathin sections. The secretory pathway will be followed in the same way by an antibody against deglycosylated invertase. Some of our preliminary results will be presented here.

In situ localization of creatine kinase (CK) in skeletal muscle

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Indirect immunofluorescence experiments on cryosectioned muscle with anti-M-CK antibodies showed strong staining of the M-band as described earlier for myofibrils and in addition strong staining of the whole I-band sparing the Z-line. Lack of A-band staining was not due to inaccessability to antibodies since anti-C-protein antibody stained the A-band. Glycerinization of the tissue prior to fixation led to complete loss of I-band CK, but the M-band signal remained. I-band CK, however, was not removed if glycerinization followed the fixation indicating that the 'soluble' CK is fixed in place at the I-band by our fixation.

These data demonstrate the intracellular compartmentation of strongly bound MM-CK at the M-band and of 'soluble' MM-CK within the I-band. 'soluble' I-band CK representing the bulk of this enzyme in muscle may be loosly associated within the network of thin filaments or form functionally coupled compartments with glycolytic enzymes also located in the I-band. These findings are discussed with respect to the CP-shuttle model.

Structural maturation of Na, K-ATPase requires glycosylation of its β-subunit

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Active Na,K-ATPase is composed of a catalytic α-subunit (Sα) and a glycosylated β-subunit (Sβ) of unknown function. We have studied whether glycosylation influences the membrane organization of Sα and Sβ during intracellular routing by using controlled trypsinolysis of microsomes of toad bladder (TBM) cells labeled for various periods with ^{35}S-methionine. Sβ occurs in three forms: a) unglycosylated and highly trypsin-sensitive in the presence of tunicamycin (5 µg/ml, 18 h), b) core-glycosylated and less trypsin-sensitive (up to 30 min pulse) and c) fully glycosylated and trypsin-resistant. While tunicamycin had no effect on the synthesis rate of total proteins and of an unglycosylated 30 kD membrane polypeptide, it decreased the synthesis and trypsin resistance of Sα. In conclusion, glycosylation of Sβ seems to influence the membrane disposition of both Sα and Sβ and might thus be important for the correct assembly of the two subunits into the functionally active enzyme.

Determination of the structural parameters of reverse micelles after uptake of proteins

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A method for the determination of the structural parameters of protein-containing reverse micelles is presented. The method is

based on analytical ultracentrifugation and utilizes two dyes which are monitored with the UV-scanner device. The first one is water soluble and the second one is a cosurfactant. In each case, the population of both filled and unfilled micelles can be measured and the partition of water and surfactant (AOT) in filled and unfilled micelles can be determined. The data allow the conclusion that the protein uptake in the reverse micelles is attended by an increase of the micelle water content, which causes an increase of the dimensions of the filled micelle. The protein needs at least 1500–3000 water molecules in order to be hosted in the AOT reverse micelles. As a consequence of the redistribution of material, the unfilled micelles become smaller than initially.

Symposium 6: Mechanisms in morphogenesis

Postimplantation whole rat embryo culture: application to in vitro teratogenicity testing

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Whole embryo culture represents a valuable tool for studying basic teratogenic mechanisms. However, it has not yet been fully evaluated for systemic teratogenicity testing. Using essentially New's technique (New, DAT, Biol. Rev. *53* (1978) 81), but modifying the culture conditions (Cicurel, L., and Schmid, B. P., Meth. Find. exp. clin. Pharmac. (1986) in press), we have tested 20 chemicals, including known or suspected in vivo teratogens. Our system not only affords an excellent correlation between in vitro observed dysmorphogenic effects and in vivo reported abnormalities, it also allows a distinction between overall embryotoxicity and teratogenicity. The advantages of this method (accurate control of exposure conditions, assessment of the direct effect of a substance, reduced animal number and time consumption) over conventional in vivo teratogenicity testing, as well as its reliability and sensitivity, point towards the great value of the embryo culture as testing procedure for potential teratogens.

Non-radial migration of the isthmic complex in chick embryos – a Golgi study

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The various isthmic nuclei arise in the alar plate of the anterior rhombencephalon and migrate ventrally (Clarke, J. comp. Neurol. *207* (1982) 208). The present Golgi studies on 4–8-day-old chick embryos show that soon after an isthmic neuron has left the cell cycle, its external radial process detaches from the pia, withdraws slightly, turns lateroventrally, and regrows in a latero-ventral or ventral direction, forming a long, axon-like, leading process tipped by a prominent growth cone. The nucleus subsequently translocates down the leading process, and the trailing process is gradually withdrawn. This migration is not radial; it is oblique, and can be almost tangential to the surface of the brain. Some of the growth cones and leading processes are mingled among the translocating nuclei, but many others are segregated in a well defined region, unstained by Nissl-methods, and situated lateral and ventral to the mass of translocating nuclei. The subsequent fate of the leading processes is poorly understood.

Synaptogenesis in the visual cortex of *Tupaia*

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Most of the optic input coming from the lateral geniculate nucleus is projected to layer IV of the visual cortex (Area 17). There, synapses are formed chiefly with stellate cells. We have followed the development of synapses in layer IV during postnatal ontogeny to see when and how the geniculo-cortical terminals make their connections. Several morphological changes were noted for the synaptic development between the neonate and the adult animal. At birth synapses are sparse (10/1000 μm^2) and rather small in profile (< 1 µm). At day 8 the density of synapses is already three times higher, at day 14 five times and in the adult about ten times (104/1000 μm^2). Concomitantly, the number of synaptic vesicles/terminal and terminal size gradually increase. At the present we are investigating whether or not the density distribution of synapses within layer IV corresponds to the sublaminae found in transneuronal transport (Rager and Nowakowski, 1983).

The *paired* gene family of *Drosophila*

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A family of twelve *Drosophila* genes that share homologous sequences with the carboxyl terminal of the *prd* protein is described. The *paired* gene is required for proper segmentation early in development. Assuming that the homologous sequences reflect a common function, we have started to analyze individual members of the gene family with respect to their temporal and spatial expression in *Drosophila* embryos. Four genes, including the *paired* gene, have been examined. Transcripts of all of them predominate during early development or in oocytes and disappear during later stages of embryogenesis. In addition, two genes exhibit a specific distribution of transcripts. First, the *paired* gene itself changes its initial pattern of expression from a two-segment to a one-segment periodicity during syncytial blastoderm. Transcripts of the other gene accumulate at the anterior pole of the oocyte. They persist during cleavage stages of the embryo but disappear during syncytial blastoderm.

Formative cells

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Cells of the amphibian blastoderm have been filmed during their changing into mobile cells and their entering the germ (IWF, 1967). These cells later display inductive potencies. It is supposed those mobile cells of being also responsible for all formative powers which hitherto are attributed to the socalled formative chemicals. Several circumstantial proofs, by early experimental embryology otherwise interpreted, exist for this supposition. According to this new understanding the injected substances themselves do not organize the cells, e.g., for the second embryo in an urodele germ (Spemann and Mangold, 1924). Their inducing power, on the contrary, only consists in attracting formative cells. The first example, where cells could be observed during their formative work concerned the early cardiomyogenesis in vitro. Here, as the time lapse shows, wandering fibroblasts are the promotors in the development of isolated heart muscle cells into synchronously pulsating muscle cell units.

Topography of the retino-tectal pathway in the chicken

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It is known that the retinotectal projection in the chicken is organized retinotopically. It is, however, not known how the fibers are arranged in this pathway. Since in a visual projection several topographies may be intermingled (Torrealba et al., 1982), we had to select those fibers which exclusively project to the optic tectum. For this purpose we injected HRP at various

sites of the tectum and reconstructed the fiber bundles stained by HRP back to the retina. The topography of these bundles was determined in cross-sections at several well defined sites of the pathway. We found: 1) There is a high degree of order in the whole pathway. 2) Already in the optic nerve the dorsal and the ventral retinae are mirror imaged. The whole retina is represented topologically which means that neighborhood relationships are maintained. 3) The topological image of the optic nerve is transformed to a flattened band in the optic tract which transformation can be explained by ontogenesis. 4) The transition from the optic tract to the tectal surface is straight forward. The medially located fibers terminate rostrally, the superficially located ones terminate caudally.

The control region of the *Drosophila* heat shock gene hsp22

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The four genes encoding the small heat shock (hs) proteins are induced by stress but also during development. To determine the sequence requirement for the two kinds of induction we truncated the flanking region of the hsp22 gene, and reintroduced that gene into a fly strain which synthesizes a variant of hsp22. The product of the endogenous and the transformed genes could thus be distinguished. We localized a 33 bp long sequence 176 bp upstream of the cap site whose deletion results in 5-fold lower promoter strength during heat shock. Within that region there is a 14 bp element (HSE), present in most hs genes, and required for heat induction of at least the hsp70 gene. The two other HSEs near the TATA box of the hsp22 gene are apparently not sufficient for efficient heat induction. A similar region is required for expression during development. Removal of the distal HSE results in strongly reduced gene expression. The heat shock transcription factor which binds to the HSE is therefore most likely also involved in gene activation during development.

In vitro mutagenesis of Rous sarcoma virus protein P12

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In the course of the Rous sarcoma virus replication, viral RNA must be selectively packaged into the budding particles. From what we know RSV Gag protein P12 might perform this selection. It is a basic nucleic acid binding protein which is closely associated to the 70S RNA in the virion. Amino-acid comparisons between numerous retroviral nucleic acid binding proteins equivalent to RSV P12 have shown a strong conservation of a Cys-His box. In order to better understand the role of this protein, we have used a molecular genetic approach and constructed mutants by linker insertion mutagenesis. Results obtained with four mutants will be discussed.

Structure and spatial expression of the maternal homeo box gene *caudal* in *Drosophila melanogaster*

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The gene *caudal* was isolated on the basis of its cross-hybridization to other homeo box genes of *D. melanogaster*. It maps on the 2nd chromosome at position 38E. On Northern blots, it shows a maternal (2.4 kb) as well as a zygotic (2.6 kb) transcript. The difference in the two transcripts lies at the 5′ end of the mRNAs, they are presumably activated by different promoters. The maternal transcript is expressed during oogenesis in the nurse cells and becomes evenly distributed in the egg. At the syncytial blastoderm stage transcripts disappear from the anterior pole of the egg, resulting in an antero-posterior gradient. The zygotic transcript can first be detected during the cellular blastoderm and is localized in precursor cells of the hindgut, the Malpighian tubules and posterior midgut at the posterior end of the egg. *Caudal* is expressed in those organs until the end of embryogenesis and also in posterior epidermal cells. In the 3rd instar larva the transcripts are localized in the genital disc and in the germ cells in male and female gonads. Injections of sense and anti-sense RNA to determine the function of this gene are in progress.

Postnatal development of the dorsal lateral geniculate nucleus (dLGN) of the tree shrew (*Tupaia belangeri*): a Golgi study

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The formation of the laminated pattern in the terminal arbors of the retino-geniculate axons was examined in rapid Golgi preparations of the tree shrew dLGN during its early postnatal life. Before laminae appear, which starts at postnatal day (P) 4, the retino-geniculate axons develop extensive terminal arbors. These arbors spread across the borders of their prospective laminae, but as the laminae and interlaminar zones grow in width, the terminal arbors undergo a process of reshaping and pruning. At P 18 the laminar and interlaminar zones have essentially reached their adult width. The initially wide terminal fields of most retino-geniculate axons become in general confined to one single lamina. The present study shows that there are at least two partially overlapping processes which may be responsible for the formation of the structural basis for a precise information channeling in the adult animal: 1) Overgrowth and reshaping of retino-geniculate terminal arbors and 2) the appearance of interlaminar zones and their maturation.

Structural organization and sequence of the homeotic gene *Antennapedia (Antp)* of *Drosophila melanogaster*

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Homeotic genes are involved in the control of spatial organization during development. Molecular analysis and sequencing of one of these genes, *Antp*, shows a complex and unusual gene structure. The gene is split into eight exons, spans more than 100 kb and gives rise to four different polyadenylated transcripts including very long leader and trailer sequences in the range of 1–2 kb. Two of the transcripts start in the middle of the gene, where a second promoter is located. The open reading frame is the same in all transcripts despite of the complexity of the transcriptional organization. The putative gene product has a rather unusual amino acid composition (10% Pro and 18% Gln) and includes the homeobox, a common domain in different homeotic genes, which is supposed to be DNA-binding. Dominant mutants at the *Antp* locus show a transformation of antennae into legs. The protein product in these mutants is not altered, but the controlling region of the *Antp* gene has been exchanged due to an inversion and the new controlling sequences probably activate the gene product in the wrong place leading to the mutant phenotype.

Pattern formation in vertebrate limbs

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Models of morphogenesis have been proposed now for almost a century, but few were amenable to a systematic biochemical study since the inducer molecules were undefined. Our approach to the intriguing problem of pattern formation is based on the

observation that local application of all-trans-retinoic acid (RA) to the developing chick limb bud results in a well defined duplication of the digit pattern. RA is released from a 200-μm diameter bead implanted into a limb bud. Our experiments suggest that RA acts like a morphogen. E.g. locally applied RA is most effective in causing duplications if present in form of an anteroposterior gradient, the biologically effective RA concentration in the bud ranges between 1 and 10 nM. RA is quickly metabolized, and must be applied continuously to the bud for about 15 h in order to induce and maintain the yet unknown biochemical processes that form additional digits. We have chemically identified endogenous RA in early limb buds. The amount present matches that required to induce duplications when exogenously applied from a bead.

Isolation of homeo box sequences from honeybees

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To study genes that control early embryonic development of the honeybee, we have isolated five homeo box-containing genes using the *Antennapedia* and *engrailed* homeo box sequences of *Drosophila melanogaster* as probes. Sequence analysis of all homeo boxes was performed. One clone, E60, has strong homology to *engrailed* within its homeo box as well as in a region of 87 bp immediately 3′ to the homeo box. The protein sequence, encoded by this region, includes 89 amino acids of which 90% are identical to those of the putative translation product of the corresponding *Drosophila engrailed* sequence. A second clone, H28, has strong homology to the *Drosophila* gene *sex combs reduced*. A region of 79 amino acids including the homeo box is 97% homologous to the corresponding *Drosophila* gene sequence. Studies are in progress to determine the expression of all the genes during embryonic development using Northern blot analysis and in situ hybridization to sections.

Symposium 7: Cellular environment and cell differentiation

Distribution of smooth muscle and nonmuscle myosin in tissue sections as seen by immunofluorescence

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Myosins from bovine aorta and human platelets were purified (Analyt. Biochem. *60* (1974) 258) and antisera against the two proteins were raised into rabbits. No cross reactivity by immunoblotting was observed between the two antibodies. Smooth muscle myosin was detected in smooth muscle layers and muscle fibers located in the core of microvilli of rat intestine, in the media of large arteries in mammals (but not in birds) and in myoepithelial cells of rat mammary and salivary glands. Nonmuscle myosin was detected in the endothelium of capillaries and in the endothelium (but not in the media) of large mammal arteries, in rat granulation tissue myofibroblasts and in stress fibers of cultured fibroblasts. Immunoblotting with an extract of cultured fibroblasts gave a positive reaction only for the antibody against nonmuscle myosin. These antibodies may be useful for the study of myosin differentiation and for the analysis of the function of contractile structures in various cells.

Myotendinous antigen: a novel extracellular matrix protein steps out of fibronectin's shadow

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Myotendinous antigen (MA) is a disulfide-linked extracellular matrix protein distinct from fibronectin (FN) consisting of subunits comigrating with FN upon SDS-PAGE (JCB *98* (1984) 1237). It can be detected in primary embryonic fibroblast cultures and can be purified from their conditioned medium as well as from so-called cell surface FN preparations. During embryonic mammary gland development MA is present only in the dense mesenchyme surrounding the developing epithelium, whereas FN, laminin (LA) and type I and IV collagen are present throughout the mesenchymal tissue. The extracellular matrix in the adult gland can be stained for FN, LA, type I and IV collagen and not for MA. In carcinogen-induced mammary tumors, however, MA can again be detected in the fibrous tissue closely surrounding the malignant epithelium. Investigating the role of MA in the cell's interaction with its environment in vitro and in vivo will be the aim of our future studies.

Articular cartilage morphometry

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A stereologic study of the articular cartilage tissue from the medial chondyles of six NZW rabbits knee joints was undertaken on the light- and electron microscopic level. The morphometric estimators determined were analyzed by a comparison between weight bearing to less weight bearing zones. Optimal cartilage preservation by chemical fixation was obtained by processing the distal femurs in a buffered glutaraldehyde solution containing the cationic dye RHT. After postfixation the undecalcified material was dehydrated and embedded in Epon. For the establishment of a sampling scheme fulfilling the criteria of systematic randomness, the condyles were sawn in four slices and each slice subdivided in ten sectors. One block was chosen from each slice for the production of thick and thin sections. A comparison between the morphometric estimators obtained for the different weight bearing zones showed the existence of significant differences concerning mean cell volume, mean organellar contents and mean matrix volume per cell.

Proliferation and differentiation of granulocyte-macrophage progenitors in serum-free culture

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The activities of three purified factors that stimulate colony formation (colony stimulating factors, CSF's) by murine granulocyte (G)-macrophage (M) progenitors have been characterized in a serum-free clonal assay system. Comparison of the CSF dose-response curves, cell number titration curves and analyses of cellular composition of generated clones indicates that there are only minor differences between results obtained in the presence or absence of serum for two of these factors: 1) FGM-CSF which stimulates formation of clones with G and M in combination as well as alone and 2) CSF-1 which induces predominantly pure M clones. The third factor, G-CSF, induces significantly fewer clones in the absence of serum, but they are virtually all pure G compared to up to 65% M-containing clones in cultures with serum. These results show that CSF's are the sole factors required for GM colony development. Insulin and platelet-derived growth factor do not induce clonal growth but enhance the activities of GM-CSF and CSF-1.

Basic fibroblast growth factor (bFGF) – like activity in human amniotic fluid

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Using an antiserum generated against the synthetic replicate of the N-terminal sequence of bFGF we have found immunoreac-

tive (ir) FGF in amniotic fluid. Unlike bFGF, amniotic fluid ir-FGF is not bound by heparin-sepharose. ir-FGF concentrations are high: 5–20 nmol/l for amniotic fluid from 15–19 weeks gestation. Like bFGF, amniotic fluid stimulates the proliferation of vascular endothelial cells in vitro but bioactivity is considerably lower than ir-FGF levels. These data suggest that amniotic fluid contains bFGF-like substance(s) that differ from but are structurally related (parallel displacement in highly specific RIA) to the characterized growth factor (high mol.wt or bound forms).

Developmental genetics of Pgk-1 in the early mouse embryo

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We have recently found that the maternally and paternally inherited alleles of the X-chromosome linked enzyme phosphoglycerate kinase (Pgk-1) are activated at different periods during early embryonic development of the mouse. Furthermore, the rate of synthesis of the two allozymes was shown to be different during oogenesis, resulting in a skewed allozyme ratio in oocytes, fertilized eggs and cleavage embryos. In adult tissues of heterozygous females to allozyme ratio is also skewed. In the present communication the influence of the Xce locus on the skewed allozyme pattern in adult tissues is discussed. Data are presented on Pgk-1 expression in early embryos which may be regulated by the Xce locus or an oocyte specific Pgk-1 regulatory gene. Attempts are made to correlate Pgk-1 expression with morphogenesis.

Molecular and biological characterization of two forms of acidic fibroblast growth factor

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Two forms of acidic fibroblast growth factor (aFGF) have been isolated from bovine brain. Structural characterization by amino acid analysis, N-terminal sequence analysis and SDS gel-electrophoresis revealed that the two aFGFs are closely related in structure. aFGF-1 (des 1–6 aFGF, MW: 15,500) is an amino-terminally truncated form of aFGF-2 (MW: 16,000). aFGFs are structurally distinct from basic FGF. However, peptide mapping and sequence analysis of aFGF fragments show high homology between aFGF and bFGF. Both aFGFs possess the same intrinsic activity with a slightly different potency to stimulate the proliferation of endothelial cells in vitro.

Analysis of the maturation of transcripts of the gene for the ribosomal protein L1 of *Xenopus laevis*

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After injection of the L1 gene into the nucleus of *X. laevis* oocytes it was observed that most of the L1 mRNA is present in a precursor form containing two out of nine introns of the gene. By Northern analysis we identified other splicing intermediates accumulated in low amounts. In order to elucidate the composition of the different splicing intermediates as well as the order of intron removal we have prepared specific intron probes. The results show that sequences hybridizing to the the different intron probes are not only visible in the respective precursors but are also *accumulated* in stable low mol.wt forms which are present also in the poly A^+ fraction. The quantitative representation of the accumulated introns is not explained by the distribution of the splicing intermediates but rather suggests different stabilities of the spliced liberated introns.

Oxygen tension in vitro alters the ploidy and protein content of cultured rat hepatocytes

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Protein and DNA content of freshly isolated and cultured rat hepatocytes were determined by 2-parameter flow cytometry. This approach allows the dynamics of the 2N, 4N and 8N cells in culture to be monitored. The influence of the oxygen tension (pO_2) in vitro was investigated. Oxygen tensions chosen corresponded to 5%, 13%, 19% and 31% O_2 in the incubators. Protein and DNA content of hepatocytes varied between individual animals and were not strictly related to age or weight. The contribution of 2N, 4N and 8N hepatocytes is maintained during culture (1–7 days). Karyorrhexis was observed within 48 h. At low pO_2 the 2N population decreased and cellular protein content increased, preferentially within the 2N cell population. High oxygen tension reduced karyorrhexis and stabilized the phenotype of the hepatocytes in culture.

Tumor promoters enhance the differentiation of astrocytes in aggregating brain cell cultures

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We have reported previously (Honegger, P., and Guentert-Lauber, B., Dev. Brain Res. *11* (1983) 245) that in serum-free aggregating brain cell cultures subnanomolar concentrations of epidermal growth factor cause a decrease in DNA synthesis and an enhanced expression of glutamine synthetase. Now we can show that these responses, reflecting the stimulation of astroglial differentiation, are elicited also by the potent tumor promoters phorbol 12-myristate 13-acetate and mezerein, but not by the non-promoting phorbol ester 4 α-phorbol 12,13-didecanoate. Since these tumor promoters are potent and specific activators of C-kinase, the present findings suggest that C-kinase plays a regulatory role in the growth and differentation of astrocytes.

An in vitro model for bleomycin-induced pulmonary fibrosis

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Pulmonary fibrosis is a serious side effect of bleomycin treatment for cancer therapy and is associated with an elevated collagen content in the affected lung tissue. By studying the effect of bleomycin on logarithmically growing human skin fibroblasts in vitro we show that a stimulation of collagen synthesis occurs without other cell types present. Collagen and noncollagen protein (NCP) synthesis was measured by using ^{14}C-proline incorporation (5 h) and purified bacterial collagenase (Kirchhofer et al., Experientia *41* (1985) 821). Cell number and volume were determined with a Coulter Counter. Bleomycin stimulated collagen relative to NCP synthesis at cytostatic concentrations (10^{-2} to 10^{-3} U/ml, for 30 h) to about 150% of controls. In addition, cell volume and total protein content per cell doubled at the higher bleomycin concentration (10^{-2} U/ml). These results will be compared with effects of bleomycin on already growth-inhibited cells such as dense monolayers of human fibroblasts.

Naturally occurring anti-band 3 antibodies and complement mediate phagocytosis of diamide-treated human red blood cells (RBC)

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Naturally occurring antibodies against the major integral membrane protein, band 3 (anion transport protein) of RBC have

recently been purified (J. Immun. *133* (1984) 2610). These antibodies, in the presence of complement-inactivated serum, mediated phagocytosis of diamide-treated RBC (20 μM) by adherent monocytes. This Fc receptor dependent phagocytosis required opsonization at anti-band 3 antibody concentrations (up to 10 μg/ml) exceeding the normal one (200 ng/ml). Thus, anti-band 3 antibodies, unlike erythrophilic IgG, bound and acted specifically in the presence of an excess of serum IgG. Whole serum was similarly effective in mediating phagocytosis in the absence of exogenously added anti-band 3. Hence, an efficient phagocytosis in serum at normal anti-band 3 concentrations requires complement.

Primary cilia in the follicular epithelium of the human thyroid: analysis of motility and ultrastructure suggests their sensory function

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During evolution the thyroid transformed from an exocrine gland with many motile secondary cilia into an endocrine organ with primary cilia (one per cell) within the follicular epithelium. In order to clarify contradictory reports about ciliary structure and function, thyroid tissue from 26 patients was processed immediately after surgery for parallel video-analysis with Nomarski optics and transmission electron microscopy. Cilia of living cells were immotile. EM-analysis revealed 9 + 0 pattern at the ciliary base changing towards the distal end of the shaft to diminishing numbers of microtubular doublets. Dynein arms, radial spokes and central microtubules were absent. The Golgi apparatus was regularly detectable near the centriole pair. Because of their immotility and structure cilia of the follicular epithelium are thought to be of sensory function.

Nucleo-cytoplasmic ratio versus chromosomal replication: regulation of stage-specific gene activity during mouse preimplantation development

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Nucleo-cytoplasmic ratio in fertilized mouse eggs was changed by microsurgical bisection of denuded eggs, leaving both pronuclei in one egg half. These half eggs were capable to cleave as controls at least twice or even to reach the blastocyst stage. When fertilized eggs were treated with cytochalasin B, however, cleavage was prevented. The eggs remained at the one cell stage but chromosomal replication proceeded normally, generating highly polyploid eggs. In both types of eggs and embryos protein synthesis, appearance of stage-specific surface antigens and activation of the paternal genome was analyzed in comparison to controls. Stage-specific gene activity seems to proceed in parallel to chromosomal replication, independent whether cleavage was prevented or the nucleo-cytoplasmic ratio was changed.

Measurements of total and regional CO_2 productions in chick blastoderms cultured in vitro

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A conductometric micromethod has been developed allowing to determine the CO_2 production of 'two-dimensional' tissues such as chick blastoderms. The blastoderms were explanted into an airtight chamber separated in two compartments by a thin silicone membrane permeable to gases. The lower compartment contained the preparation. The upper compartment, and a conductivity measuring capillary connected in series, were perfused to-and-fro with a solution of $Ba(OH)_2$. As the decrease of electrical conductivity was proportional to the rate of $BaCO_3$ formation, it was possible to determine the total CO_2 production of the embryos (from 0.5 μl/h for the blastula to 6 μl/h for the neurula). In addition, as the $BaCO_3$ crystallized upon the silicone membrane, a pattern of CO_2 production was directly observable. Taking into account the total production, the local variations of CO_2 production were estimated by a TV image processor.

Analysis of hemoglobin transition and correlation of globin polypeptides with cDNA clones of *Xenopus laevis*

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In amphibians a transition from larval to adult hemoglobin synthesis takes place during metamorphosis and is characterized by the complete replacement of all larval globin subunits by adult types. Analysis of the globin subunits by high-resolution PAG-electrophoresis in acid area-Triton X-100 revealed 4–6 slowly migrating components which predominate in larvae and at least four faster components appearing during metamorphosis and persisting in the adult stage.

To determine the relationship between the globin subunits and the eight types of the previously described cDNA clones (H.J. Widmer et al., Dev. Biol. *88* (1981) 325) derived from larval and adult globin mRNAs, individual mRNAs were hybrid-selected and translated in a wheat germ system. The products were identified by comparison with the globin subunits of hemolysates. The selected mRNA molecules were further characterized by electron microscopy of the hybrids formed with the corresponding cDNA clones. Our results demonstrate that each of our cDNA represents a specific globin subunit.

Monoclonal antibodies to human neuroblastomas

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Monoclonal antibodies (Mab) were derived from mice immunized with cells of the human neuroblastoma line IMR-32. Five hybridomas were selected according to their selective binding to human cell lines, tumors and normal tissues. One of them, CE7, reacts with all sympatho-adreno-medullary cells (neuroblastoma, ganglioneuroblastoma, ganglioneuroma, pheochromocytoma, adrenal medulla, sympathetic ganglion cells). Weak cross-reactivities were observed with melanocytes and with some human melanoma and glioma cell lines. The antigen recognized by CE7 is expressed to high degrees on neuroblastoma tumors of all histological grades independently of the adrenergic or cholinergic nature of these cells. Mab derived from clones AD2, BC1, BC4 and CB10 bind to various degrees to some, but not to all neuroblastoma cells. By using these Mab, three phenotypes of neuroblastoma lines and tissues could be distinguished. Such typing might be of relevance for diagnosis, prognosis and therapy of this tumor.

Enhancer effect can be transmitted over large distances through mammalian DNA but not prokaryotic DNA

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Transcriptional enhancers dramatically stimulate transcription of linked genes, in either orientation and over large distances of many 1000 bp. However, some laboratories have also reported a much shorter range of efficient enhancer action. We have resolved this paradox, at least in part, by showing that remote activation depends on the nature of the intervening DNA se-

quences which are separating the enhancer from the responsive promoter. We have properly analyzed preliminary findings of a 'poisoning' of the enhancer effect by prokaryotic DNA (Banerji, Rusconi and Schaffner, Cell *27* (1981) 299). Using β-globin and SV40 T-antigen test genes, we show that the effect of an SV40 enhancer can be transmitted, essentially without loss of efficiency, through some mammalian DNA segments derived from β-globin or immunoglobulin gene regions. By contrast, a strong reduction of enhancer activity is seen when defined segments of prokaryotic DNA, from plasmid pBR322 or from phage λ, are inserted between enhancer and promoter. Experiments to determine the structural peculiarities of the 'poisonous' DNA are in progress.

The metallothionein gene enhancer stimulates transcription in a cell-free extract

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Within the upstream region of the mouse metallothionein-I gene we have identified a transcriptional enhancer which is inducible by heavy metal ions (Serfling, E., Lübbe, A., Dorsch-Häsler, K., and Schaffner, W., EMBO J. *4*, No.13B (1985), in press). This enhancer region contains several 'metal-responsive' elements (MREs) previously identified by others, as well as 'constitutive' elements. To further elucidate the sequence requirements and the factor(s) involved in heavy metal induction, we have performed transcription experiments with a nuclear extract from human HeLa cells, using a truncated β-globin gene template and heterologous enhancers. We find that both the metallothionein gene enhancer as well as the SV40 enhancer, which was used as positive control, stimulate transcription of the linked β-globin test gene in vitro.

Developmental potential of a mouse neuro-teratocarcinoma

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The teratocarcinoma derived tumor line TDN2283 used in these studies is subcutaneously passaged in adult syngeneic mice and shows only a homogeneous morphology of immature neural differentiation, containing blast-like cells with occasional neural rosette formation. To examine the developmental capacity of this undifferentiated teratocarcinoma, cells from the tumor were cultured in vitro or injected in small numbers into the brain of newborn allogeneic mice. To document the presence of the injected cell progeny, these mice carried GPI-1C that is different from GPI-1A of the teratocarcinoma. In both instances, the tumor cells were able to differentiate into a variety of different cell types of the neural lineage. Histological, biochemical, histochemical and immunohistochemical data demonstrate the multiple developmental capacity of these tumor cells for both the peripheral and central nervous system. We propose that this model can be applied to study pathogenesis and histogenesis of neuronal-like tumors as well as the normal ontogeny of the mammalian nervous system.

A new low mol.wt adrenergic neurotrophic factor

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Cultured chick superior cervical ganglion neurons synthesize and accumulate both catecholamine (CA) and acetylcholine (ACh) but the amount of CA produced is several fold higher than the amount of ACh. A new low mol.wt factor has been found that specifically stimulates the growth and development of these neurons. It is present in medium condition by chick liver cells in culture (LCM). LCM does not allow neuronal survival in the absence of nerve growth factor nor does it increase survival in its presence. It does, however, increase the neuronal growth and adrenergic differentiation of these cells. CA production is increased several fold whereas ACh production is decreased. Both the growth and differentiation promoting activity are of very low mol.wt (lower than 1000 daltons). The effect appears to be specific for sympathetic neurons since LCM does not increase neuronal growth in other types of cultured neurons, nor does it induce CA production in these cells.

Symposium 8: Biochemical basis of differentiation in plants

Homologous DNA recombination during plant protoplast transformation

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In order to optimize conditions of plant protoplast transformation to maximize the rate of homologous recombination, we have constructed plasmids containing a set of deletions in a selectable hybrid gene [APH(3')II]. Regions of homology of between 0 and 400 bp which allow restoration of a full size functional gene by recombination were provided. The interaction between the size of homology and the physiological conditions for protoplast transformation were studied.

New bacterial nitrogen fixation genes involved in the *Bradyrhizobium japonicum*-soybean symbiosis

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Symbiotic nitrogen fixation genes *(fix)* determine essential functions in bacteroid development or, more directly, they code for components involved in the formation of an active nitrogenase complex, in which case they are usually called *nif* genes (e.g. the nitrogenase structural genes *nifH, nifD,* and *nifK*). Here we report the identification and characterization of several new *nif* and *fix* genes in *Bradyrhizobium japonicum,* the soybean symbiont, and present data on the phenotypic properties of strains with mutations in those genes. Mutations in *nifE, nifB, fixA, fixB,* and *fixC* were obtained by site-directed insertion and/or deletion mutagenesis of corresponding cloned DNA in *E. coli.* Further Fix^- mutations were found to be located adjacent to *nifE* and *nifB.* In addition, mutants were obtained by random Tn*5* mutagenesis which are Nif^+ in free-living microaerobic culture, but which are Fix^- in symbiosis. Hence, the insertions may be in genes which code for specific functions in bacteroid development rather than being determinants of nitrogenase activity.

Studies of the regulation of betalain biosynthesis in red beet cells in culture: competence for light induction

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Pigment accumulation in plants is under the regulation of physical and chemical environment. Nevertheless not all cells in a culture have the same competence to respond to a trigger signal.

Betalain biosynthesis in red beet tissue culture is controlled by light. After a number of transfers under non-inductive condition

(dim light), pieces of an unpigmented callus transferred to light show the same number of variegated spots as those incubated under light (19 mW/m^2). We can demonstrate that the appearance of competent cells is not conditioned by the inducing factor. Moreover the pigment composition is more complex in cells maintained in the light than in the induced cells which let us to suppose that this factor acts at multiple steps in the pigment synthesis.
The distribution of the variation rate is correlated to the growth rate of the calli. Subsequent culture of the high- and low-variegated calli show that this is an heritable property. The isolation of a 'constitutive-like' culture not regulated by light is therefore possible. Our results indicate that the changes in the cell ability to accumulate pigments involve no permanent change in the genetic information since it is reversible.

Structure and differential expression of the two genes for the small subunit of ribulose bisphosphate carboxylase-oxygenase in *Chlamydomonas reinhardii*

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The two closely linked *rbcS* genes that encode the small subunit of ribulose bisphosphate carboxylase-oxygenase (RuBPCO) in the unicellular green alga *C. reinhardii* have been isolated. The comparison of the genomic and cDNA nucleotide sequences with those of other plants shows that they have an unusual distribution of introns. It may be of evolutionary significance that in some cases the positions of introns coincide with sequence insertions or deletions. The proteins encoded by the two *C. reinhardii* genes differ by a few amino-acid residues. The relative levels of RNA transcripts from the two genes are influenced by the growth conditions (light and carbon source). This differential expression of the variant genes provides a mechanism whereby the composition and thus possibly the properties of the multimeric RuBPCO holoenzyme could change in response to the environment.

Metabolism of carotenoids in senescent leaves of meadow fescue wild type and nonyellowing genotype

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Senescence is an integral part of leaf development and therefore subject to direct genetic control. The nonyellowing phenotype of meadow fescue, Bf993, is characterized by its inability to degrade the intrinsic thylakoidal chlorophyll-proteolipid complexes (Thomas, H., Lüthy, B., Matile, Ph., Planta *164* (1985) 400). The product of the recessive nuclear coded gene has so far not been identified. Using HPLC the analysis of carotenoids in senescent leaf segments has yielded another conspicuous difference between the two phenotypes. In the wild type carotenoids are readily degraded, whereas they are retained in the nonyellowing mutant. In addition lutein fatty acid esters appear only in the wild type reflecting the high retention of thylakoid lipids in the nonyellowing mutant.

Evidence for a cellular gene with potential oncogenic function in plants

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The autonomous growth of plant tumor cells is believed to result from the inappropriate production of growth-promoting substances, the most important being cytokinin and auxin. In crown-gall disease, the production of cytokinin depends, at least in part, on expression of the *tmr*-gene of the T-DNA. Non-transformed cells of *Nicotiana tabacum* homozygous for the *Habituated leaf* gene (Hl/Hl) differ from wild type hl/hl cells in that they do not require cytokinin for proliferation in culture. We transformed Hl/Hl and hl/hl cells with *Agrobacterium tumefaciens* strains carrying a wild type (*tmr*$^+$) plasmid and with a defective plasmid with an inactive *tmr* region (*tmr*$^-$). Cloned lines of hl/hl*tmr*$^+$, Hl/Hl*tmr*$^+$, and Hl/Hl*tmr*$^-$ grew in culture without cytokinin and auxin, did not regenerate into plants and formed tumors by grafting. hl/hl*tmr*$^-$ clones did not show these tumor phenotypes, indicating an oncogenic function of the Hl-gene, similar to the tmr of the T-DNA.

Isolation of auxin mutants in vitro

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Cell clones arising from mutagen-treated haploid leaf protoplasts were tested for an induced auxin requirement. One temperature-sensitive auxin auxotroph was found amongst 60,000 clones of *Hyoscyamus muticus*. Regenerated shoots have the same phenotype. The trait is recessive in fusion hybrids with other ts and amino-acid auxotrophs. This variant is rescued by IAA but analysis with HPLC/RIA has shown that variant cells can synthesis and accumulate IAA to wildtype levels. Five nonts auxin auxotrophs found by testing 56,000 clones of *Nicotiana plumbaginifolia* are stable, have an absolute requirement for auxin and are not cross-fed by wildtype cells. Regenerated shoots are abnormal. Complementation studies by protoplast fusion are in progress.

Petunia as a model species for chromosomal localization of foreign genes in transgenic plants

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The well established genetics of *Petunia hybrida*, its low DNA content and the fact that all seven haploid chromosomes can be distinguished cytologically facilitate the analysis of the fate of foreign genes. We have introduced a selectable foreign marker gene into isolated protoplasts of a complex heterozygot *P. hybrida* line and regenerated plants for subsequent genetic crossing analysis. In an alternative approach we applied in situ hybridization to metaphase chromosomes, as established for a single copy gene in tobacco by one of us (A. Mouras).

Disease and aging induce different molecular forms of antifungal hydrolases in pea tissue

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Plants possess hydrolases that can attack the cell walls of fungal pathogens. Using immature pea pods as a model system, we found that two of these enzymes, chitinase and β-1,3-glucanase, were strongly induced by fungal infections, biotic elicitors, aging, and by the plant hormone, ethylene. We identified and purified three molecular forms of chitinase and two of β-1,3-glucanase. Collectively, these proteins constituted more than 50% of the soluble basic proteins in the tissue. The different molecular forms of chitinase and β-1,3-glucanase were differentially induced in the course of aging and in response to fungal infection.
In vitro, chitinase and β-1,3-glucanase strongly inhibited growth of fungi, indicating that they play a role in the defense of higher plants against pathogens.

Adventitious rooting in *Populus tremula:* characterization of the synergy between vitamin D_3 and indolyl butyric acid

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Vitamin D_3 promotes adventitious rooting in *Populus tremula*, but not in the same way as indolyl butyric acid. The synergy between vitamin D_3 and indolyl butyric acid is statistically significant, the age of the cuttings, the timing of the effector application and the effector concentration modify the synergy. The results obtained in the timing application experiment can be taken to suggest that the synergy is due to an interaction of vitamin D_3 with a system which is activated when the plants are treated with indolyl butyric acid during the first two days of culture.

Cotton fibers: a model system to study differentiation in plant cells

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Cotton fibers are well suited as a model system for the study of differentiation of plant cells for several reasons: a) Cell elongation and vacuolation, followed by secondary wall thickening, are typical steps occurring during the differentiation of many plant cells. In cotton fibres these steps occur in reasonable synchrony. b) The orientation of cellulose microfibrils, and the monosaccharide composition of primary and secondary cell walls, are known, as well as the ultrastructure of the protoplast and the organization of cytoskeletal elements. c) Cotton ovules can be cultured in vitro and they produce fibers similar to those produced in vivo. d) There exist color mutants in which specific biochemical constituents of seed epidermal cells are also synthesized in the fibers, e.g. suberin and associated 'waxes' in the green lint mutant. (For a review see: Ryser, U., Eur. J. Cell Biol. 39, in press.)

Amplification of introduced foreign genes in plants

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Selection of protoplasts from previously transformed plants at high levels of the selective agent (kanamycin), produced resistant colonies at a level normally inhibitory for these transformed protoplasts. Analysis of DNA extracted from these colonies (which appeared at a frequency of approx. 10^{-3}) showed evidence for independant amplification events involving part but not all of the introduced foreign DNA, although the foreign DNA is known to be present at one position in the plant genome.

Rapid and simple isolation of nuclei from barley endosperm

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A number of studies of the control of gene expression have used seed storage protein genes as their subject. The high starch content of endosperm tissue is a problem in the isolation of nuclei which are required for certain experiments. We have developed a rapid and simple method for the isolation of intact nuclei from this tissue. A polytron is used to homogenize endosperm in a buffer modified from Willmitzer and Wagner (Exp. Cell Red. *135* [1981]). The chelating agent which is used to inhibit endogenous nucleases is ortho-phenanthroline rather than EDTA. The homogenate is centrifuged for 8 min at 1000 × g in a Beckman J-6B centrifuge. The nuclei are resuspended in a few ml of the same buffer using a paintbrush, leaving the starch pellet behind. This step is repeated four times with centrifugation for 4 min. This method allows the preparation of intact nuclei containing 45 µg of HMW DNA per gram of endosperm in less than one hour. There is very little degradation ot the DNA. Restriction patterns and hybridization to a b_1 hordein gene are identical to CsCl purified DNA.

Attempts to obtain auxin mutants by positive selection

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Hormone mutants would provide a useful means for gaining more insight in the auxin system which is still far from being well understood. The most straightforward strategy for the isolation of mutants is a positive selection. Presently tree groups of substances are being tested for their suitability as selective agents: a) auxins: 1-NAA, 2,4-D; b) auxin analogues: 2-NAA, 2,6-D; c) transport inhibitors: NPA, TIBA.
All these compounds interfere with the binding of auxin to its cellular receptors and/or with the auxin transport system and are toxic at high concentrations. Currently the dose response tests and selections are performed using *Nicotiana plumbaginifolia* because haploid protoplasts can be obtained and plants can be regenerated after selection. No clear cut resistant mutants have been isolated up so far mainly because wildtype calli show high tolerance to the selective agents – thus the resistant phenotype cannot be demonstrated unequivocally at the callus level.

Regulation of fructan metabolism in barley

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Many grasses of temperate origin synthesize fructan as their main carbohydrate storage compound and accumulate it in the vacuoles of their mesophyll cells. We observed rapid synthesis of large quantities of fructan in excised primary leaves or barley (*Hordeum vulgare* L. cv Gerbel) upon illumination. In the dark fructan was rapidly degraded again. In the course of a light/dark cycle we measured the activities of sucrose-sucrose-fructosyltransferase (SST) fructan hydrolase and invertase. SST activity increased 20-fold within 24 h in the light and decreased again upon transfer to the dark. Invertase and fructan hydrolase showed only minor changes of their activities. The variation of SST activity therefore seems to play a key role in the regulation of fructan metabolism. The increase of SST activity in the light was inhibited by cycloheximide or by inhibition of photosynthesis. Interestingly SST activity was induced even in the dark when leaves were immersed in a solution of sucrose, trehalose or several other sugars. We hypothesize that, in whole leaves, vacuolar fructan metabolism is controlled by the endogenous supply of sugars.

Symposium 9: Molecular mechanisms of photosynthesis

Regulation of protein synthesis in chloroplasts of *Chlamydomonas reinhardii*

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Chloroplasts of *Chlamydomonas reinhardii* contain free and membrane-bound ribosomes and polysomes. In vitro translation, in a reticulocyte lysate, of RNA extracted from the soluble

and the membrane fractions, demonstrated the presence of mRNA for a soluble (LS RuBCase) and a membrane polypeptide (rapidly labeled 32 kD protein) in both chloroplast fractions. Hybridization with radioactively labeled DNA from cloned genes indicates that the ratio of the amounts of mRNA for the two proteins are the same in these fractions. We conclude that no compartimentation of mRNA for soluble and membrane proteins exists between stromal and thylakoid-bound polysomes. However, the amount of each mRNA in the membrane fraction is higher than in the soluble fraction.
ATP-driven, homologous translation of the endogenous mRNA in the soluble and membrane fractions of the chloroplasts resulted in different product patterns compared to those of light-driven protein synthesis in intact chloroplasts.

Structural principles of membrane-bound antenna polypeptides from purple and green bacteria

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In purple as well as in green bacteria bacteriochlorophyll a is associated noncovalently to small and hydrophobic antenna polypeptides. When bound to these polypeptides, BChl a exhibits absorption maxima at either 800, 830, 850 or 880 nm, whereas in vitro the absorbance is blue-shifted to 770 nm. Based on this the following questions arise: a) are there principal binding regions (contact regions) of BChl a in the antenna polypeptides from purple and green bacteria, b) what is the origin of the in vivo absorbance of BChl a antenna pigment-protein complexes? We isolated, purified and sequenced a serie of light-harvesting polypeptides from purple and green bacteria. They all show the same tripartite structure with a central hydrophobic domain bearing a conserved His residue. The most homologous, conserved amino acid residues appear in the vicinity of this His residue. This gives evidence for possible ligand formation between the His residue and BChl a.

Enrichment of the chlorophyll-binding proteins of the chloroplast by selective extractions and characterization of a 21 kD antenna polypeptide of PS 1

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Chlorophyll a/b-protein complexes, designated LHC-1, have recently been isolated from PS 1 complex by different authors. They all are composed of 2–4 polypeptides of 20–24 kD molecular mass. A 21 kD polypeptide was shown to contain chlorophyll a and b. In order to isolate a PS 1 antenna protein without contaminating polypeptides we selectively extracted the chloroplast membrane with protein perturbants. The released polypeptides are not associated with chlorophyll. The extracted membrane contains the polypeptides of RC 1, RC 11, LHC 11, polypeptides of 24 and 21 kD and minor bands at 16, 15 and 10 kD. The 77 K fluorescence emission spectrum still shows a maximum at 730 nm, the characteristic emission of PS 1. The 21 kD polypeptide was isolated and characterized.

Fine structure mapping of chloroplast photosystem I mutants in *Chlamydomonas reinhardii*

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Several uniparental mutants of *Chlamydomonas reinhardii* have been isolated that lack photosystem I (PSI) activity. The polypeptides associated with PSI are undetectable when thylakoid polypeptides of these mutants are fractionated by polacrylamide gel electrophoresis and stained with Coomassie blue. However when cells are pulse-labeled with ^{14}C acetate in the presence of cycloheximide, a truncated unstable form of the P700 apoprotein is synthesized in two of these mutants. The genes of the apoprotein P700 have been mapped on the chloroplast EcoRI fragments R15 (psIA2) and R17 (psIA1) which are located on opposite sides of the chloroplast genome of *C. reinhardii*. Since recombination studies indicate linkage between rbcL mutations on R15 (affecting the large subunit of ribulose-bisphosphate carboxylase) and the two PSI mutations, the psIA2 gene on R15 was cloned from both mutants and its sequence was compared to wild-type. In both cases nonsense mutations were detected in agreement with the pulse-labeling experiments.

The role of the two 32 kD core proteins of photosystem II in electron transport, herbicide resistance and stability of PSII particles

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We have used uniparental mutants of the green alga *C. reinhardii* to study the function of two core proteins of photosystem II (PSII), D1 and D2. Single point mutations in the D1 gene which alter amino acid residues 219, 255, 264 or 275 are correlated with different patterns of herbicide resistance and electron transport, and have implicated the D1 protein in quinone binding. Photosynthetic mutants which lack the D1 gene do not accumulate PSII particles. Analysis of a mutant with a truncated, unstable D2 protein suggests that D2 is crucial for the synthesis of D1 and the stability of the PSII complex. Experiments are in progress to further examine the role of D1 and D2 in translational regulation, PSII assembly and stability, electron transfer and herbicide resistance. (Erickson et al., Science *228* (1985) 204; Bennoun et al., Pl. Molec. Biol. (1985) in press.)

Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus:* amino acid sequences and relationships

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Three linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus* were isolated and sequenced: 1) A 8.9-kD polypeptide, which is the terminator of the phycobilisomal rods, was completely sequenced. 2) The N-terminal 44 residues and the 114 C-terminal residues of a 34.5-kD polypeptide associated to phycoerythrocyanin (PEC) were determined. 3) The N-terminal 44 residues of a 34.5-kD polypeptide, which forms a complex with phycocyanin, were elucidated. The N-termini of the two 34.5-kD polypeptides are homologous. The C-terminal sequence of the 34.5-kD protein associated to PEC is related to the 8.9kD polypeptide and to a 8.9-kD protein associated to allophycocyanin. The N-terminal sequence of the 34.5-kD polypeptide associated to PEC exhibits 22% homology to β-PEC while the C-terminal part is related to α-PEC. Hence, all phycobilisomal proteins are derived from a common ancestral phycobiliprotein subunit.

Oligomeric structure of light-harvesting complexes from the carotenoidless mutant *Rhodospirillum rubrum* G9

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Light-harvesting complexes (LHC) from the carotenoidless mutant *Rhodospirillum rubrum* G9 + were isolated using selective

LDAO treatment, followed by DEAE-cellulose chromatography in octylglucoside. The absorption maximum (873 nm) of the LHC dialyzed against detergent-free buffer was abolished by the addition of 0.1% LDAO to be replaced by an absorption peak at 780 nm. This transition was completely reversible upon dialysis or dilution against detergent-free buffer, UV-CD showed that the LHC structure is not unfolded in the presence of LDAO. Analytical ultracentrifugation studies suggest that the dialysed LHC consists of aggregates and possibly a hexameric 37 K form whereas the 780 nm form consists of a mixture of 24 K, 13 K and 6 K components, corresponding to tetrameric (α_4, β_4), dimeric and monomeric forms of the component polypeptide chains α and β (mol.wt approx. 6.5 K, resp.).

Transmembrane distribution of galactolipids in inside-out structures derived from spinach grana thylakoids

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Inside-out structures enriched in PS-II were obtained by Triton X-100 treatment (BBY particles) and by the two phase partition method. BBY were fully active as judged by oxygen evolution capacity and depleted in PS-I electron flow activity. Inside-out vesicles displayed reversible proton extrusion and a high 77°K 685/740 nm fluorescence ratio. Treatment of both types of membranes with lipase from *Rhizopus arrhizus* showed that galactolipids had an asymmetric distribution. However, the partial conversion of monogalactosyldiacylglycerol (MGDG) into its acylated derivative allowed only a semiquantitative estimation. The outer monolayer contained 35% of total MGDG and 80% of DGDG. This distribution was roughly the reverse of that found in whole right-side-out thylakoids. These results confirm our previous proposal that galactolipids are asymmetrically distributed within thylakoid membranes.

Further characterization of ferredoxin/thioredoxin reductase from spinach chloroplasts

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The ferredoxin/thioredoxin reductase catalyzes the light dependent reduction of thioredoxins in chloroplasts. Its relative molecular mass, estimated by gel filtration through Sephadex G-75S and by FPLC using Superose 12, is 38,000 resp. 36,000. Upon SDS gel electrophoresis according to Weber and Osborn, the protein splits into two dissimilar subunits of 11 kD and 13.5 kD. Scannings of the stained protein bands and the relative molecular masses suggest that the native enzyme is composed of two small and one large subunits. The subunits have been separated and their amino acid compositions determined. In isoelectric focusing gels the native protein shows a main band at pH 4.9 and a minor band at 4.75, after dissociation bands at pH 6 (large subunit) and 5.75 (small subunit) appear. The enzyme seems to contain 4 atoms of each Fe and S per molecule.

The accumulation of photosystem II polypeptides and their mRNAs in PSII mutants of *Chlamydomonas reinhardii*

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We have monitored the accumulation of photosystem II (PSII) polypeptides and their mRNAs in *Chlamydomonas reinhardii* mutants which lack PSII activity. We find that a mutation, either nuclear or chloroplastic, which results in the loss of an integral PSII component leads to the failure of an PSII polypeptide accumulation. However, the loss of a peripheral PSII polypeptide, like those associated with oxygen evolution, does not affect the accumulation of the PSII particle core. In both nuclear and chloroplastic mutants lacking the PSII complex, the mRNAs encoding PSII polypeptides other than the affected gene product are found to be at wild type levels. These results suggest that the synthesis and assembly of photosynthetic complexes is dependent on the concomitant expression of nuclear and chloroplastic genes and that at least one level of regulation occurs post-transcriptionally.

Is access of CO_2 to C_3-plants purely by diffusion?

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The ratio of the two activities of the enzyme ribulose bisphosphate carboxylate oxygenase as determined in vitro was compared with the ratio of photosynthesis to photorespiration in leaves as determined from differential $^{14}CO_2$ $^{12}CO_2$ uptake and from CO_2 compensation concentration. Discrepancies between measurements made in vitro and in vivo were attributed to effects of CO_2 translocation within the leaf cells on stromal concentration of CO_2. The results are suggesting a CO_2 concentrating mechanism which is more efficient at low than at high temperature. The efficiency decreases as CO_2 concentration is increased.

On the force ratio at static head for acid-base transition driven ATP formation in spinach thylakoid membranes

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The aim of the work is to determine the lower limit for n near static head conditions; n being the number of protons transduced by the ATP-synthase in order to make an ATP. On assuming that ATP synthesis is driven by the free energy change ($\Delta\mu_{H+}$) of n protons upon outflow from the space surrounded by the thylakoid membrane into the reaction medium, then net ATP synthesis will only take place if $n \cdot \Delta\mu_{H+} > \Delta G'$ (ATP); $\Delta G'$ (ATP) being the free energy needed for ATP synthesis. On setting $\Delta\mu_{H+}$ with the acid-base transition and defining $\Delta G'$ (ATP) by giving known amounts of ATP, ADP and PI into the reaction medium both parameters are known and can be varied. On keeping $\Delta G'$ (ATP) fixed while lowering $\Delta\mu_{H+}$ one will arrive at the static head state, where ATP synthesis is no longer found and where $q \cdot n \cdot \Delta\mu_{H+} = \Delta G'$ (ATP), with $0 \leq q \leq 1$.

Streptomycin-resistance of *Euglena gracilis* chloroplasts: identification of a point mutation in the 16S rDNA

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Mutations which confer antibiotic resistance provide useful genetic markers. Streptomycin interacts with the small subunit of the ribosome and abolishes normal protein synthesis. In *E. coli*, streptomycin-resistance is linked with mutations in the gene for ribosomal protein S12. Streptomycin also interacts with chloroplast ribosomes and, as a result, leads to bleached cells. To test whether the gene for ribosomal protein S12 is involved in streptomycin-resistance of *Euglena* chloroplast, we sequenced it in both wild type and mutant strains, but no sequence differences were found. However, analysis of the 16S rDNA of two mutants reveals one base pair change (C to T) at position 876. This

position is equivalent to the invariant position 912 of the *E. coli* 16S rDNA and is conserved in all chloroplast 16S rDNA. Light dependent protein synthesis with purified chloroplasts from streptomycin-resistant cells is not inhibited by streptomycin. Based on the results, we postulate linkage between the observed point mutation in the 16S rDNA and streptomycin-resistance of chloroplast 70S ribosomes.

pH changes induced by uncharged galactolipids in aqueous media

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Pure monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) from spinach or lettuce leaves or from wheat flour have been transferred from ethanol solutions (10 µl) into CO_2-free 10 mM NaCl/KCl solution (2 ml) under N_2 atmosphere according to Batzri and Korn (Biochim. biophys. Acta *298* (1973) 1015). Initial pHs were adjusted with HCl/NaOH from 4.6 to 8.5. DGDG and wheat flour MGDG (bilayer-forming lipids) decreased the pH only when the initial pH was > 7. In contrast, MGDG from leaves (non-bilayer) increased the pH only when the initial pH was < 7. The rate and extent of the lipid-induced pH changes depended on initial pHs, amount of lipid added, and the presence of salts or sugars in the medium. Back titration allowed the estimation of the equivalent 'acidic' or 'alkaline' capacity of these lipids. In contrast, di-16:0-phosphatidylcholine had no effect at all. The results point to a possible alteration of the properties of water (e.g. ionization constant) by galactolipids.

Hydrophobic labeling of chromatophores from *Rhodospirillum rubrum*

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Chromatophores from the photosynthetic bacterium *Rhodospirillum rubrum* were labeled with hydrophobic unspecific markers. These markers are highly reactive, photogenerated carbenes which react with amino acid residues in contact with the lipid bilayer. Labeled light-harvesting polypeptides B-870-α and B-870-β were isolated (Brunisholz, R.A., Suter, F., Zuber, H., Hoppe-Seyler's Z. physiol. Chem. *365* (1984) 675). Edman degradation of the isolated B-870-α chain and thin layer chromatographic and autoradiographic identification of the labeled PTH-amino acids gave information on the contact of this light-harvesting polypeptide with the phospholipids of the bilayer (Meister, H.P., Bachofen, R., Semenza, G., Brunner, J., J. biol. Chem. (1985) in press).

The primary structure of phycocyanin-645 from the cryptomonad *Chroomonas* sp.

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Cryptomonad biliproteins are located in the intrathylakoidal lumen of the chloroplast. The subunit structure is $(\alpha_1\beta)$ $(\alpha_2\beta)$. PC-645 absorbs at λ 550–650 nm. It contains eight chromophores: on each β-subunit two blue cyanobilins and one red cryptoviolin and on each of the two different α-subunits one green mesobiliverdin-like chromophore. In comparison to cyanobacterial biliproteins, the first 60 amino acid residues of PC-645 α_1 and α_2 subunits are deleted. The green chromophores are bound to cys-18, homologous to the bilin binding site at cys-84 of other biliproteins. The β-subunit is most related to the erythrins (61% homology to β-C-PE). The two cyanobilins are at homologous positions (cys-84 and cys-155). The cryptoviolin is double bound to cys-50 and cys-61 similar to the double bound erythrobilin in β-C-PE. An insertion of five amino acid residues was found homologous to the insertion if β-C-PE. The structure is compared with the x-ray structure of C-phycocyanin.

Effects of monogalactolipid (MGDG) depletion on photosynthetic activities in oat thylakoids

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Special conditions were used allowing lipase from *Rhizopus arrhizus* to selectively hydrolyze MGDG. It was found that both PS-I and PS-II electron flow rates were inhibited and that cyt f photooxydation decreased both in rate and amplitude. However, the PS-I activity could be restored by adding back Triton X-100, but not plastocyanin. Thus, it is proposed that the inhibition site of the lipase is at the level of the cyt b/f complex. Indeed, without TX-100, artificial electron donors reduced mostly cyt f, whereas an addition of detergent allowed a shunt around cyt f. The inhibition of PS-II was much more complex. Electron flow inhibition was observed mainly at high light intensities, and both fluorescence at 20°C and 77°K F685/F740 ratio decreased. Thus, it is proposed that MGDG removal inhibited the electron flow at two different sites: one at the level of the cyt b_6/f complex, and the other at the level of PS-II, by favoring spillover from PS-II to PS-I and inhibiting the basal electron flow.

Characterization of a c-DNA clone encoding the chlorophyl A/B binding protein

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A c-DNA clone, LHC2, encoding the light harvesting protein (LHCP) has been isolated from a *C. reinhardii* λgt10 c-DNA library, using as probe a short LHCP c-DNA clone, obtained from S.H. Howell (Shepard et al., 1983) which contains 300 bp from the 3′ untranslated region. The 1.2 kb c-DNA insert carrying the LHCP sequences was subcloned into pUC18 (pSY1) and a restriction map was established. Work is in progress to determine the nucleotide sequence of the 5′ end of this c-DNA in order to determine the sequence of its transit peptide. Genomic clones are being isolated from a genomic library of *C. reinhardii* by using pSY1 as a probe in order to obtain LHCP promoters. (Sheperd, H.S., Ledoigt, G., and Howell, S.M., Cell *32* (1983) 99.)

Analysis of the psbC gene coding for the 44 kD chlorophyll a apoprotein of *Euglena gracilis*

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It is known (Keller and Stutz, FEBS Lett. *175* (1984) 173) that the *E. gracilis* chloroplast genome codes for the 44 kD protein of the PSII reaction center. This gene extends over at least 6.5 kb and contains several introns of variable length. The five exons identified sofar represent about 70% of the total length of the corresponding spinach chloroplast protein (472 amino acid residues) (Holschuh et al., NAR *12* (1984) 8819). In particular we located by nucleotide sequencing an intron between codon position 50 and 51 (codon numbering according to the spinach protein) which is 1604 nucleotides long, containing an ORF with 458 codons. This represents the largest identified intron in a

protein coding chloroplast gene and the first one shown to contain an ORF. The 5′ and 3′ intron boundary sequences are according to class II introns and in 'Northern' hybridization experiments a 1.7 kb mRNA (iRNA?) interacts with an intron DNA probe. The decoded ORF 458 protein has no significant sequence homology with a known chloroplast protein nor could we detect resemblance to a protein of the NBRF database (release May 1985).

Profile of electric potential for isolated thylakoid stacks

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The distances between the approximately plain membranes of thylakoids are of the same order of magnitude as the Debye-Hückel length, particularly for solutions of monovalent salts in the millimolar range. Accordingly, the electric potentials which arise from the charges of membrane bound proteins extend over the whole inner and interthylakoidal spaces. The partitioning of marker substances used to determine the difference in electrochemical potential of protons between inner space of thylakoids and suspending medium therefore includes substantial contributions from the interthylakoidal space and the surface region of the stacks. An experimental procedure is presented which allows to determine the average potentials in all spaces and hence yields correct data for the electrochemical potential difference. Computer simulations provide the corresponding profiles for the electric potential.

Structure and function of the photosynthetic antenna systems and reaction centers

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The light reactions of photosynthesis are characterized by a coordinated action of light-harvesting antenna systems and the photochemical reaction center. The antenna systems which are found in a great variety in photosynthetic bacteria, cyanobacteria, algae and plants, are composed of defined pigment-protein-complexes. These complexes, as well as the reaction center, are aggregates of polypeptides of various sizes binding specifically pigment molecules. In these highly organized systems energy transfer (antennae) or charge separation (energy-transduction by the special pair) takes place. Primary- and three-dimensional structure analysis provided extensive data on the molecular structure of these complexes and on possible mechanisms of energy transfer and energy transduction.

Symposium 10: Membrane proteins, ion channels

Hydrophilic and amphiphilic forms of choline acetyltransferase

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The enzyme choline acetyltransferase (ChAT) catalyzes the biosynthesis of the neurotransmitter acetylcholine (ACh) from choline and acetylcoenzyme A (AcCoA). Triton X-114 solubilization of synaptosomes isolated from *Torpedo* electric organ or rat brain followed by phase partition of the detergent, reveal the existence of hydrophilic and amphiphilic ChAT activity. Properties of these two forms of the *Torpedo* enzyme are analyzed: they have similar affinities for choline (~ 0.9 mM), for AcCoA (~ 12 μM), similar app MW as estimated on immunoblots using a monoclonal antibody which inhibits both hydrophilic and amphiphilic activities; they differ in their sensitivity to pH variations and to inhibition by physiological concentrations of ACh and coenzyme A. Amphiphilic ChAT corresponds to activity which is non-ionically bound to the plasma membrane of the purely cholinergic nerve endings of *Torpedo*. This form may be an integral protein or bound to a 'receptor' molecule of the plasma membrane.

Is alkaline phosphatase (AP) an genuine integral membrane protein?

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After non conclusive results from AP labeling by NBD-Cl (Biol. Chem. Hoppe-Seyler *366* (1985) 763) we tried to use another hydrophobic reagent, 3-(trifluoromethyl)-3-(m-[^{125}I]iodophenyl) diazirine (TID) which produces high reactive carbenes after photolysis. The purified watersoluble AP (Portmann et al., Helv. chim. Acta *65* (1982) 2668) as well as crude deoxycholate extract of microsomes from calf intestinal mucosa were reacted with this reagent. Results by autoradiography after PAGE, by affinity chromatography and by immunoprecipitation agree to demonstrate that TID is not bound to either AP. At least the deoxycholate solubilized AP which can be reintegrated in liposomes should react with TID, since previous published observations indicate that all integral membrane proteins, yet tested, react with this labeling reagent.

Glycoprotein V is not the thrombin-trigger on human blood platelets

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Thrombin activation of platelets involves two receptors, glycoprotein (GP)Ib and a strong candidate for the second receptor is GPV, a hydrophobic, 82 kD glycoprotein with pI 5.85–6.55. Whole platelets were treated with endogenous platelet calcium-activated proteases, yielding a major fragment (79 kD) of GPV, which was purified by affinity-chromatography on wheat germ agglutinin followed by chromatography on DEAE-Sephacel. A rabbit was immunized with the purified fragment. Crossed immunelectrophoresis and 2-D PAGE electrophoretic blotting with the phases of a Triton X-114 phase partition of human platelets as antigen showed the characteristic pattern of GPV in the hydrophobic phase. During thrombin-induced platelet aggregation GPV is hydrolyzed, releasing a fragment, GPV_{fl}, to the supernatant. Anti-GPV-antibodies blocked GPV proteolysis, but did not inhibit platelet activation induced by thrombin. We conclude that proteolysis of GPV by thrombin is not essential for platelet activation.

Purification of protein kinase C from pig brain

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Phospholipid/Ca^{2+}-dependent protein kinase (PKC) plays a central role in mitogenic signal transduction, and represents the receptor for tumor-promoting agents such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA). To better investigate the interactions of such phorbol esters with PKC, the enzyme was purified from pig brain by a simple 2-step procedure including DEAE-cellulose chromatography followed by phosphatidylserine-affinity chromatography on polyacrylamide gels (PS-Affi). – After PS-Affi, three major protein bands were detected on SDS gels (Coomassie-stained) with MWs of 80 kD, 50 kD and 30 kD respectively. We could show that the 80 kD represents

the PKC holoenzyme by several criteria. Furthermore, after electroelution the 80 kD protein was injected into rabbits to start antibody production.

Effects of three H_1-antagonists on ion transport in tracheal epithelium

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The effects on transepithelial ion transport of three H_1-antagonists, chloropyramine, dimetindene and diphenhydramine, were examined in bovine tracheal epithelium. In this tissue, the short-circuit current I_o is the sum of active Cl^- secretion and Na^+ absorption. All three drugs induced a reversible dose-related inhibition of I_o, up to 100%. The concentration giving 50% of maximal effect was 1.4 10^{-4} M for chloropyramine, 2.0 10^{-4} M for dimetindene and 2.5 10^{-4} M for diphenhydramine. The effect was unrelated to the agonist binding site of H_1-receptor, since it was not altered by 10^{-3} M histamine. No appreciable change in the O_2 consumption was seen following inhibition of I_o by dimetindene, a finding in contrast to the well-defined relation that exists between active ion transport and oxidative metabolism.

The α subunit of the type I IGF receptor is an extrinsic membrane protein

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Type I IGF receptor is a disulfide-linked heterotetramer $(\alpha\beta)_2$. A strong homology between the type I IGF receptor and the insulin receptor is now accepted. The sequence of the insulin receptor does exhibit only one hydrophobic part long enough to span the membrane as an α-helix in its β subunit. No physical or chemical evidence has been presented for the extrinsic localization of the α subunit.

To establish the extrinsic character of the type I IGF receptor α subunit, we labeled the type I receptor in cell membranes isolated from human placentas by affinity cross-linking with ^{125}I-IGF I. The labeled membranes were treated with different salt solutions in the presence of disulfide reducing agents (2-ME, DTT). This treatment liberates the α subunit from the transmembrane β subunit whereas in controls (without reducing agents) the α subunit is not liberated.

The glucose-permease of the bacterial phosphotransferase system is a dimer which is stabilized by an intersubunit disulfide bridge

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The high affinity glucose-permease (IIGlc) of *E. coli* mediates active transport of glucose by a mechanism which couples sugar translocation with sugar phosphorylation. Purified glucose permease is found both as a dimer and as a monomer. The dimer can be stabilized by air-oxidation. It then can be dissociated only in the presence of disulfide reducing reagents. When monomer and dimer are separated by sucrose gradient centrifugation, enzymatic activity is found with the dimer. Reaction of the reduced permease with iodoacetamide completely inactivates the protein, while the oxidized permease is protected against inactivation. It is concluded that an intersubunit disulfide bridge is essential for the function of the permease.

Comparison of two related glucose-specific membrane permease of *E. coli*

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The high- and low-affinity glucose (glc) permeases of the bacterial phosphotransferase system catalyse sugar transport and phosphorylation. No common antigenic determinants could be detected with polyclonal antibodies and 13 monocolonal antibodies. However, there is overlapping substrate specificity which is characterized as follows: Substrates of the high affinity permease (IIGlc) have a free hydroxyl at position two of the pyranose ring, while their glycosidic hydroxyl can be alkylated. In contrast substrates of the low affinity permease (IIMan/IIIMan) have a free glycosidic hydroxyl, while structural alterations at carbon two of the pyranose are tolerated. 6-fluoroglc does not inhibit either of the two permeases while 2-fluoroglc weakly inhibits IIGlc and strongly inhibits IIMan/IIIMan. 5-thioglc is toxic for bacteria overproducing IIGlc. Similarly 2-deoxyglc is toxic for bacteria overproducing IIMan/IIIMan.

Glycophospholipid membrane anchoring of the major surface protein of *Leishmania* promastigotes

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Promastigotes of the protozoan parasite *Leishmania major* were biosynthetically labeled with myristic acid. Phase separation in the nonionic detergent Triton X-114 and analysis by SDS-PAGE showed that the protein most intensely labeled with the fatty acid was identical to the major surface protein of the cell. Phospholipase C digestion of the amphiphilic protein found in the membrane removed the labeled fatty acid and generated a water-soluble form of the protein, concomitantly uncovering the complex carbohydrate known as the cross-reacting determinant (CRD) found on the hydrophilic forms of all variant surface glycoproteins (VSG) of *Trypanosoma brucei*. These data indicate that the membrane anchor of the major surface protein of *Leishmania* consists of a glycophospholipid moiety similar in structure to the membrane anchor of *T. brucei* VSG.

Mapping of functional sites of the nicotinic acetylcholine receptor using antibodies to synthetic peptides

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The structure of the nicotinic acetylcholine receptor (nAChR) is of interest not only because of its role as an important neurotransmitter receptor but also as the major autoantigen in the human disease, myasthenia gravis. Both the ligand-binding site and the main immunogenic region (MIR) are carried on the α-chain of the nAChR. As one means of mapping these regions, we have treated the nAChR of *Torpedo marmorata* with proteolytic enzymes and characterized the products by electrophoresis and blotting using labeled toxins, and antibodies to the MIR and to synthetic peptides. Digestion with papain produces a 19 kD N-terminal fragment containing the MIR and sugars linked to Asn 141 and a 27 kD C-terminal fragment reacting with antibodies to synthetic peptides P1 (151–169) and P3 (C-terminus) and with α-bungarotoxin. The MIR is thus contained in the N-terminal 169 residues and the ligand-binding site in the C-terminal 286 residues.

Cell-free synthesis, membrane integration and glycosylation of pro-sucrase-isomaltase

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Cell-free translation of total RNA from rabbit intestinal mucosa in a rabbit reticulocyte lysate, after immunoprecipitation with antibodies directed against sucrase-isomaltase, yielded a polypeptide of M_r 200 kD, which was identified as pro-sucrase-isomaltase. In the presence of pancreatic microsomal vesicles an additional 220 kD polypeptide was synthesized. The latter was associated with the membranes in a way that made it inaccessible to proteolysis; this protection was abolished by lytic detergent concentrations. The 220 kD polypeptide was glycosylated as evidenced by it being bound to concanavalin A-Sepharose and eluted with α-methyl-D-mannopyranoside; treatment with Endo-H increased its electrophoretic mobility to that of the 220 kD polypeptide which was obtained in the absence of membranes. Partial N-terminal amino acid sequence of a translation product labeled with [^{3}H]Leu in the absence of membranes revealed that Leu was incorporated into identical positions as in the final (pro)-sucrase-isomaltase thus indicating the lack of a transient signal peptide.

Improvements in recording of gating currents at the squid giant axon

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Based on an analysis of instrument noise a low noise voltage clamp was constructed to obtain high resolution gating currents without long and fatiguing signal averaging. The main noise sources and methods to reduce them were as follows: the voltage noise of the potential electrode was substantially lowered at high frequencies by combining the conventional pipette electrode with a parallel platinum wire extending 2–3 mm from a separate pipette and capacitively coupled (1–10 μF) to the input of the differential amplifier DA (Levis and Bezanilla, Biophys. J., *41* (1983) 101a). A DA input voltage noise of 2 $nV/\sqrt{Hz}$ was achieved using FETs. The IV-converter had similar specifications. Partial separation between central and guard electrodes of the chamber brought the shunt resistance above the series resistance Rs. Using Rs compensation fast and stable gating currents were recorded with eight times better S/N-ratio than with a previously used clamp of conventional design.

Phosphate transport across the basolateral membrane from rat kidney cortex: sodium-dependence?

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Basolateral membrane vesicles were isolated from rat renal proximal tubules by a Percoll-centrifugation method. Transport of phosphate was stimulated by a sodium-gradient but transport of D-glucose was not affected by the sodium gradient. Phosphate uptake could be trans-stimulated by phosphate in the absence of sodium. For further purification the Percoll-basolateral membrane fraction was applied to free-flow electrophoresis. It was possible to separate basolateral from brush border marker enzymes. Net sodium-dependent phosphate transport could be correlated with the brush border marker enzyme activity.

We conclude that sodium-dependent phosphate transport in the Percoll-basolateral fraction from rat kidney cortex is due to cross-contamination with brush border membranes but that the translocation of phosphate across the contraluminal membrane is carrier-mediated and sodium-independent.

Association of the Thy-1 glycoprotein to the interleukin-2 receptor in the plasma membrane of a murine lymphoma cell

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A minor fraction of the Thy-1 cell-surface molecules are associated to a surface component of 55 kD in detergent-resistant complexes that can be isolated from purified plasma membranes of whole cells after non-ionic detergent extraction. By 2-D electrophoresis, the 55 kD molecule consists of a heterogeneous series of components, the acidic end of which comigrates with the cell-surface molecules immunoprecipitated from soluble cell lysates with the PC 61 mab specific for the interleukin-2 receptor. Partial removal of sialic acid residues from the immunoprecipitated, soluble receptor by neuraminidase generates a 2-D pattern which is superimposable to that of the detergent-resistant and Thy-1 associated 55 kD glycoprotein. An incompletely sialylated interleukin-2 receptor is therefore associated to Thy-1 in a detergent-resistant plasma membrane domain of P1798 murine lymphoma cells.

cDNA cloning of pro-sucrase-isomaltase

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The complete primary structure of rabbit intestinal pro-sucrase-isomaltase was deduced from the sequence of a nearly full-length 6 kb cDNA. Pro-sucrase-isomaltase is anchored in the membrane by a single 20 amino acid segment spanning the bilayer once in an N-in/C-out orientation. The amino terminal, cytoplasmic domain consists of only 11 amino acids and is not preceded by a cleaved leader sequence. This suggests a dual role for the amino terminal segment, as an (uncleaved) signal for membrane insertion and to anchor the protein in the bilayer. The transmembrane sequence is followed by a 22 residue Ser/Thr rich glycosylated stretch, presumably forming the stalk on which the globular, catalytic domains are directed into the intestinal lumen. Starting just beyond this segment there is a high degree of homology between the isomaltase and sucrase portions (41% amino acid identity), indicating that pro-sucrase-isomaltase evolved by partial gene duplication.

Effect of Na_o on Ca efflux from mammalian nonmyelinated nerve fibers

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The efflux of ^{45}Ca from rabbit vagus nerve was measured and submitted to a computer multiexponential analysis. The results show three components of efflux, one corresponding to the washing of the extracellular space and to the desaturation of the membrane bound Ca, the two other reflecting the efflux from the internal Ca pools. In physiological conditions the Ca efflux amounts to 20, 42 and 72 fmoles/cm^2·s at 0.9, 1.8 and 3.6 mM Ca_o respectively. Depolarisation by increasing, or hyperpolarisation by decreasing K_o, has no significant effect on the efflux. On the other hand, absence of Na_o decreased, in a Ca_o-dependent manner, both the rate constants of efflux and the amounts of exchangeable Ca. These results suggest that in rabbit vagus nerve part of Ca efflux is mediated by a Na_o-Ca_{in} exchange mechanism. They indicate further that in the absence of Na_o part

of the intracellular Ca pool is moved into a stable nearly unexchangeable form.

Semliki Forest virus induced polykaryocyte formation of *Aedes albopictus* cells is ATP dependent

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Aedes albopictus cells infected with Semliki Forest virus (SFV) produce polykaryocytes at low pH. This syncytium formation can be inhibited by a wide variety of chemicals. Some of these drugs are known to deplete the cells of ATP. We therefore investigated whether there is a correlation between the ATP level and polykaryocyte formation. A decrease in cellular ATP was observed by lowering the pH of the medium of infected cells to 6 at 16 hpi. Drugs which interfere with the oxidative phosphorylation were also found to be inhibitors of syncytium formation under the conditions mentioned above. Addition of these drugs led to a rapid, total depletion of ATP in infected cells at pH 6. However, when the cells were exposed for only a few minutes to pH 6 in the presence of the inhibitors and then kept at pH 7.2, the ATP level was regenerated to > 50% of the initial value, which was sufficient for the cells to fuse. In general, cells only formed polykaryocytes at an ATP level above 10–20% of the initial value. Thus it can be concluded that SFV induced syncytium formation is an ATP dependent event.

Identification and partial characterization of vitellogenin receptors in oocyte membranes of the cockroach, *Nauphoeta cinerea*

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In insects, vitellogenins (vg) are synthesized in the fat body and, after extensive processing, secreted into the hemolymph from where they are sequestered by the maturing oocytes by receptor-mediated endocytosis to serve as the main source of nutrient for the developing embryos. We have recently demonstrated the existence of vg receptors in oocyte membranes of the cockroach, *Nauphoeta cinerea* (Insect Biochem. *15* (1985) 735). To study the hormonal regulation of vg uptake on the molecular level, the receptor molecule has to be identified first. We present data on the identification of the vg receptor using SDS electrophoresis in combination with Western blotting. To identify the vg receptor, the blots were incubated with labeled vg followed by autoradiography. Also, vg was cross-linked to its receptor using a radioiodinated photoactivable heterobifunctional reagent. The labeled proteins were analyzed by electrophoretic techniques combined with autoradiography.

Proliferation of human mammary carcinoma cell line in defined media: hormone and growth-factor dependency

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For the study of hormone and growth factor long-term effects on human mammary tumor cell proliferation, a serum-free culture system was developed. The inpact of estradiol, epidermal growth factor (EGF), somatomedin-C (SM-C) and combinations thereof on the growth of three cell lines with (MCF-7, ZR-75-1 and T-47-D) and three cell lines without detectable estradiol receptors (MDA-MB-231, BT-20 and HBL-100) was studied. It has been found that the estradiol receptor-containing cells were stimulated not only by estradiol but also by EGF and SM-C. As expected, the cell lines without estradiol receptors did not respond to estradiol, but they also did not (except BT-20 line) react to EGF and SM-C. The possible mechanisms of growth-factor dependency or independency are discussed.

Some properties of the Na^+-induced Mg^{++}-efflux from human red blood cells

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A Na^+-induced Mg^{++}-efflux has been described in chicken erythrocytes (Günther, T. et al., Biochem. biophys. Res. Commun. *119* (1984) 124) as well as in human red blood cells (Feray and Garay, Biochim. biophys. Acta (1986) in the press).
This efflux is small at physiological Mg^{++} concentrations within the cells (4.6 µMol/l cells · h), but is increased after loading the cells with Mg^{++} using either PCMBS or the ionophore A 23187, which can be removed with cystein or BSA. Efflux rates of 125 µMol/l cells · h are obtained at free Mg^{++} concentrations of > 1–2 mM within the cells. The Na^+_{out} concentration, which half maximally stimulates the Mg^{++}-efflux, is ~ 180 mM. If the cells are loaded with Na^+ and then diluted in a Na^+-medium no Na^+-induced Mg^++-efflux was observed, if compared with the efflux obtained after dilution into a K^+-medium. This indicates that the Na^+-gradient is the driving force for the Mg^{++}-efflux. Furthermore, the Mg^{++}-efflux is more than 50% decreased, if the Ca^{++}-concentration outside the cells is raised from 0–5 mM.

A ^{2}H-NMR study of calcium binding to mixed POPC/POPG model membranes

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Calcium binding to negatively-charged phospholipid model membranes – consisting of various proportions of POPG plus – was studied by employing deuterium nuclear magnetic resonance (^{2}H-NMR) spectroscopy.
The ^{2}H-NMR spectra were characteristic of lipids in a fluid bilayer state at all proportions of POPC/POPG investigated and over most of the range of Ca^{++} concentrations, employed (0–5.0 M). Furthermore, there was no evidence of any phase separation of POPG even upon extensive Ca^{++} binding. The Ca^{++} binding isotherms were most adequately described in terms of the Gouy-Chapman electrical double layer theory with a Langmuir Adsorption isotherm to extract binding constant. These investigations indicate that negatively-charged model membranes maintain a fluid-like bilayer state despite the increase in Ca^{++} binding and that the increased binding of Ca^{++} to model membranes containing negatively-charged phospholipids can be related to their effect upon the membrane surface potential as predicted from the Gouy-Chapman theory.

Molecular dissection of the acetylcholine receptor using expression cloning in *E. coli*

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The α-subunit of the nicotinic acetylcholine receptor bears the acetylcholine-binding site, to which α-bungarotoxin binds irreversibly. The α-subunit also contains the main immunogenic region (MIR). We mapped these two important structural features on the known sequence of the mouse α-chain by constructing a family of expression plasmids: different restriction fragments of the mouse α-chain cDNA were cloned into the β-galac-

tosidase gene of plasmid pUC8 and the fusion proteins obtained in *E. coli* were characterized as to their ability to bind α-bungarotoxin and anti-MIR antibodies. The toxin-binding site was mapped to the interval between amino-acids 158–216, whereas the MIR lies within positions 6–85. More precise map positions are being determined using Ba131 mutagenesis.

The 'Fluorescent molecule counter', a new method to measure mol.wt and association and diffusion of macromolecules in monolayers or in solution

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The method consists of a laser spot, rotating on a diluted sample of fluorescently labeled molecules and a sensitive fluorescence detection system. The noise in the fluorescence signal is proportional to the square root of the number of molecules in the spot volume. By applying the autocorrelation function to the fluorescence signal, one can directly obtain the number of independent particles in the defined spot volume. Given a known weight concentration of a purified protein, the method allows the determination of mol.wt within 10%. Association of molecules can be observed as a reduction in the mean number of independent particles. First results will be presented.

Binding of cytochrome *c* to monomeric and dimeric cytochrome *c* oxidase

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Binding of cytochrome *c* to cytochrome *c* oxidase causes a difference spectrum with a maximum at 414 nm for beef heart oxidase, and at 410 nm for *Paracoccus denitrificans* oxidase. The difference spectra are used to titrate the number of binding sites. For all molecular forms of the oxidase a ratio of one cytochrome *c* bound per heme aa_3 is found. The Scatchard plot of the binding data with dimeric beef heart enzyme indicates cooperativity whereas the monomeric beef and *P. denitrificans* oxidase show no cooperative binding.
Kinetic measurements were performed in addition to the binding studies. The aggregation state of the oxidase was measured by gel filtration and on an analytical ultracentrifuge.

Polymerization of a diene lipid in vesicles

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Bilayer forming lipids containing polymerizable functional groups are of broad interest with respect to practical application and theoretical considerations. By introducing polymerizable entities into lipid phases the stability and permeability of membranes can be moderated by the degree of polymerization.
In order to investigate the physico-chemical properties of stabilized membranes the amphipathic dihexadecadienoyl ammonium bromide (DHDIAB) has been synthesized (Gaub et al., Biophys. J. *45* (1984) 725). The positively charged lipid analog contains photoactivatable diene functions in its constituting fatty acids. DHDIAB forms vesicles in aqueous media as shown by glucose entrappment. Tracer permeabilities are investigated in both unpolymerized and polymerized vesicles. Membrane structures are preserved upon photopolymerization, diene polymerization occurs concomitantly. Light dependent proton translocation is detected in liposomes containing reconstituted bacteriorhodopsin and DHDIAB/phospholipid mixtures.

Morphology of proteoliposomes containing fluorescein-phosphatidylethanolamine reconstituted with native and subunit III-depleted cytochrome *c* oxidase

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Beef heart cytochrome *c* oxidase was reconstituted in asolectin liposomes containing the pH indicator fluorescein-phosphatidylethanolamine (FPE) by the cholate-dialysis procedure. The influence of FPE on the asolectin liposome size and of the removal of subunit III from the complex on its incorporation into liposomes was analyzed by freeze-fracture electron microscopy. The vesicle size distribution of native enzyme reconstituted into asolectin liposomes was homogeneous, 84% of the population having a diameter of 14–37 ± 7.5 nm. The preparation containing FPE had a similar vesicle size distribution, but with bigger diameter range (20–50 nm). The majority of particles containing vesicles was found to have one particle (42–81%). The absence of subunit III did not influence the incorporation of the enzyme into the liposomes. The suppression of the H^+-pump activity was due to the intrinsic properties of subunit III and not to defective incorporation into artificial membrane systems.

Partial purification of the mitochondrial pyruvate carrier and its reconstitution into phospholipid vesicles

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The monocarboxylate transporting protein was solubilized from bovine heart submitochondrial particles and purified 120-fold by a single hydroxylapatite chromatography step. Pyruvate transporting activity in a reconstituted system could only be recovered, when cardiolipin was present during the isolation procedure. The measured pyruvate/pyruvate exchange reaction was sensitive to 2-cyano-4-hydroxycinnamate, a specific inhibitor of the pyruvate transport in mitochondria. Also the thiol reagents p-chloromercuribenzoate, p-chloromercuriphenylsulphonate and mersalyl but not N-ethylmaleimide were shown to inhibit transport activity. These results, together with the demonstrated temperature and protein concentration dependences of the transport as well as the competitive reactivities of other 2-ketoacids, gave evidence, that the measured substrate exchange reaction was due to partially purified and properly reconstituted carrier molecules.

Interaction of the cytoskeletal component vinculin with bilayer structures analyzed with a photoactivatable phospholipid

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Vinculin, a cytoskeletal protein component, has been postulated to function as an actin-plasma membrane linker. We have investigated a possible direct interaction of vinculin with bilayers using a photoactivatable analogue of a lecithin, which contains a carbene generating diazirine group attached to a fatty acid. This highly reactive phospholipid has been shown to label selectively membrane embedded domains of membrane proteins. Vinculin is significantly labeled upon incubation and photolysis with liposomes containing trace amounts of this photoactivatable phospholipid, but only when the liposomes also contain acidic phospholipids. Our results provide evidence, that vinculin inserts into the hydrophobic part of the bilayer by interacting with acidic phospholipids.

Towards elucidation of the mechanism of cell-cell fusion induced by Semliki Forest virus

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Several viral and bacterial proteins undergo under acidic conditions a conformational change. Some of the bacterial toxins thus acquire the capacity to penetrate the lipid bilayer forming ion channels in the cell membrane. The viral proteins are considered to follow a similar mechanism eventuating in infection or fusion of the cells. In several viruses the fusogenic peptide appears to be located at the N-terminus of the fusogenic protein. Although the identity of the fusogenic protein of Semliki Forest virus (SFV) had not been established it was suggested on the basis of hydrophobicity plots that the fusogenic peptide may be in the N-terminal region and not at the N-terminus. We have previously show that the proteins of SFV undergo a conformational change at low pH and have been able to identify the fusogenic protein. Further analysis by applying the virions to phenyl sepharose clearly revealed a prominent increment in hydrophobicity of virions exposed to low pH as reflected in the elution profile compared to untreated virions. Results obtained using various conditions are presented.

Topographic labeling of the lactose permease from *E. coli*

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The lactose permease, which catalyses proton-galactoside symport across the cytoplasmic membrane of *E. coli*, has been studied in membrane vesicles with native or inverted orientation and after purification and reconstitution into proteoliposomes. The labeling techniques used were either selective modification of nucleophilic residues (arginyl, lysyl, carboxyl and tyrosyl groups) using reagents of different size and polarity or nonselective modification using photo-activated, membrane-bound probes. The protein was found to have several membrane-exposed sites on the polypeptide chain and also a considerable number of buried nucleophilic groups. Tyrosyl residues were conspicuously unreactive and, in general, the N-terminal 147 residues had a high density of unreactive nucleophilic groups.

Allosteric interactions of dihydropyridine (DHP) Ca-channel ligands in living cardiac cells

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We have studied the effects of two stereoisomeric DHP compounds, (+) and (−) 202-791 (Sandoz) on the specific binding of the Ca-channel blocking DHP 3H(+)PN 200-110(PN). The (+)-isomer of 202-791 is an activator, the (−) isomer a blocker of myocardial Ca-channels. In depolarized cells, both isomers competed at a 1:1 ratio with PN for the same high affinity binding site. At a membrane potential of −40 mV competitive displacement curves showed Hill coefficients of one and three for the blocking and the activating isomer, respectively. At non-displacing concentrations the activator decreased the kD value of PN from 0.9–0.2 nM. Ca blocked this effect. In the presence of the activator, Ca transformed the hyperbolic binding curve for PN into a strongly sigmoid curve. Together these cooperative effects suggest that more than one specific channel binding site must exist for channel activators. Ca itself seems to interact allosterically with DHP binding sites.

Characterization of the binding site of DPI 201-106 at the fast sodium channel

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DPI 201-106 increases cardiac force by prolonging the life-time of the open state of the cardiac sodium channel thus increasing myoplasmic [Ca^{2+}]. Binding studies at neural Na^+ channels and measurements of the action potential duration in guinea pig papillary muscles show that DPI binds to a site of the channel which is different from that of the scorpion- and sea anemone toxins and which is allosterically coupled to the batrachotoxin/veratridine site.

Radio-flux assay at single turnover resolution of the pure sodium pump molecule with exposed receptor

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On the average, two purified sodium pump molecules are incorporated into an artificial 100 nm lecithin-vesicle (Anner, B.M., Robertson, J.D. and Ting-Beall, H.P., BBA *773* (1984) 253). By preparing vesicles containing an internal Na^+ and ATP reservoir, the right-side-out oriented pump starts to turn over upon addition of external ^{42}K [KCl] or ^{86}Rb [RbCl]. The rate is adjusted by the K^+ or Rb^+ concentration and can be reduced to a single turnover detectable on a minute-scale.
While the right-side-out oriented pump is actively transporting, ligands, e.g. cardioactive steroids, can be added to the external receptor site and the change in the turnover-rate recorded. With this precise radio-flux assay of the pure sodium pump, the effect of ligand-receptor interaction on the flux component can be analyzed on a molecular level.

Membrane incorporation and aggregation of alamethicin, a pore forming peptide

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The interaction of alamethicin (a fungal antibiotic and pore former) with unilamellar lipid vesicles has been followed by means of the accompanying change in circular dichroism. Apparent lack of saturation in the isotherm even at lipid/alamethicin ratios as low as five is taken as evidence for membrane incorporation rather than binding of the amphipathic peptide. Partition coefficients have been determined as a function of temperature, salt and lipid type. At high peptide/lipid ratios there is evidence for non-ideality which can be described satisfactorily using a simple model approach. Careful analysis of the data as a function of the aqueous alamethicin concentration indicates aggregation of the peptide in the membrane phase above a 'critical concentration'. This appears to have interesting implications for the still much disputed gating mechanism of alamethicin pores.

The detergent-resistant skeleton of T-lymphoma cell membranes

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A plasma membrane fraction of light density has been isolated from P1798 murine lymphoma cells which is considerably enriched in the two characteristic groups of detergent-resistant membrane components. One group consists of the surface glycoproteins Thy-1 and interleukin-2 receptor, the other of membrane proteins giving a highly reproducible 2-D pattern of five

families of acidic proteins clustered between 50–70 kD. These proteins form a hydrophobic complex which is stable in high salt, but dissociates in low salt-8M urea and reassociates in isotonic buffer upon removal of urea. It thus behaves similarly to intermediate filament proteins. The polypeptides of one distinct family resemble vimentin in their V8 digestion pattern. Such complexes could be involved in linking intermediate filaments to the plasma membrane.

Reconstitution of the organic anion transport mechanism from rabbit renal brush border membranes (BBM) into liposomes

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Proteins from renal BBM vesicles, prepared by Mg^{++} precipitation and differential centrifugation, were solubilized with Zwittergent 3–12 (protein to detergent ratio 1:2, detergent concentration 0.3%). The solubilized proteins (84.9 ± 2.5% of the total membrane proteins, n = 7) were reconstituted by a freeze-thaw method into liposomes made of 80% purified egg yolk phosphatidylcholine and 20% cholesterol. Transport of the organic anion p-aminohippurate (PAH) in liposomes was measured by rapid filtration. Initial uptake rates of 0.1 mM ^{3}H-PAH, measured at 15 s, were inhibited by 80% by probenecid (1 mM). Preloading the proteoliposomes with 2 mM PAH, resulted in a eight fold stimulation of the initial uptake rates (15 s) of ^{3}H-PAH demonstrating trans-stimulation. This effect was abolished when 1 mM probenecid was present in the medium. Such reconstitution might represent a useful tool for monitoring the progress in purification of the organic anion carrier from the renal BBM.

A fast and efficient purification of mitochondrial creatine kinase yielding more than 90% octameric $(MiMi)_4$-CK

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Mitochondrial creatine kinase (mt-CK) was purified to homogeneity from a phosphate extract of purified, washed mitoplasts from chicken heart muscle. Material obtained from a 35–55% ammonium sulfate cut was loaded onto a Sepharose-blue column at pH 6.0, the pH increased to pH 8.0 and $(MiMi)_4$-CK specifically eluted by the addition of 10 mM ADP to the pH 8.0 buffer. An additional purification step on a cation exchange resin (Mono S) by FPLC (Pharmacia) yielded more than 99.5% pure mt-CK as judged by SDS-PAGE and silverstaining with a specific activity of 130 EU · min^{-1} · mg $protein^{-1}$. Gelfiltration on FPLC using Superose 12 resin confirmed the octameric nature of mt-CK as described earlier by Wallimann et al. (J. Muscle Res. and Cell Motility, in press). However, with this improved method the yield of octameric $(MiMi)_4$-CK was more than 95% with approximately 5% stable dimeric (MiMi)-CK.

Ca-activated chloride current in cultured quail neurones

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Cultured ganglionic neurones from quail embryos were studied in voltage clamp with the whole-cell recording technique. The voltage-dependent Na current was blocked by adding TTX to the external medium. Voltage-dependent and Ca-activated K currents were blocked with external 4AP and TEA and by substituting part of the internal K with Cs ions. Under these conditions, a step depolarization from −100 to +20 mV triggered an early inward Ca current followed by an outward current. This delayed current could be monitored as a long lasting tail current at the end of the voltage step and was identified as a Ca-dependent Cl current for the following reasons: 1) replacing external Ca by Co suppressed both inward and outward currents; 2) the reversal potential for the delayed current was close to the chloride equilibrium potential; 3) Cl substitution was the only extracellular ion substitution that shifted the reversal potential of the delayed current. This Ca-activated Cl current was present in 80% of sensory ganglionic neurones (trigeminal and DRG) and in only 10% of the autonomic ganglionic neurones (ciliary).

Surface accessibility of outward-facing tyrosine residues in human erythrocytes

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Human erythrocytes modified by approximately 6×10^6 copies of diazotized arsanilic acid retain their structure and cellular functions. The extent of label binding is controlled by atomic absorption spectroscopy and chromophore absorption. Surface exposure of derivatized residues is monitored immunologically using anti-p-azobenzene-arsonate antibodies on immobilized cells (ELISA). The anion transport protein (band 3) is modified by the surface label. p-Azobenzene arsonate can be detected on transmembrane segments derived from band 3. The chemical modification combined with selective immunological detection provides a useful tool for topological investigations and probing membrane protein insertion and folding.

Thermodynamic analysis of incorporation and aggregation in a membrane

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We note that agents which incorporate in a lipid bilayer have to be treated as a solute rather than a bound ligand. The interaction with an external (aqueous) phase is accordingly considered as a partition process. Deviations from the ideal linear relationship must be expected at higher solute/lipid ratios where appreciable solute-solute interactions arise. We have derived a simple generally applicable expression for an appropriate activity coefficient. This has been used to develop theoretical expressions for the incorporation and aggregation in a membrane which very well describe experimental data obtained with the pore forming peptide alamethicin.

Expression of functional Na channels in *Xenopus* oocytes after injection with chick muscle mRNA

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Oocytes from *Xenopus laevis* were injected with isolated mRNA from chick muscle. After a 36-h lag phase functionally active Na channels appeared in the oocyte plasmamembrane. They carried a fast transient inward current which increased over a period of several days and reached up to 5 μA. In control oocytes injected with water no Na channels were seen. Actinomycin D did not suppress channel formation. The induced Na current was similar to the one in the original tissue with respect to activation, inactivation, modulation by veratridine and inhibition by TTX. A single fraction of mRNA migrating at 30 s position in sucrose density gradients was responsible for the channel formation. Thus, the *Xenopus* oocyte is able to translate foreign mRNA, to correctly modify, assemble and insert into the plasmamembrane the translation product, to form an active Na channel. Due to its

easy access this system may proof useful in the study of channel regulation.

Selective proteolytic fragmentation of bacteriorhodopsin at glutamic acid 166

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Selective cleavage of bacteriorhodopsin is attained by treatment of purple membrane with the staphylococcal protease V8. In presence of sodium dodecylsulfate the V8 protease cleaves preferentially the Glu 166–Val 167 peptide bond. The two fragments produced are separated by Sephadex LH 60 chromatography in organic solvents. The peptides are identified by N-terminal sequence analysis and characterized by selective label binding studies. The fragment V-1 consists of amino acids 1–166 and includes the arylisothiocyanate binding site (Lys 41) whereas the fragment V-2 comprises the two C-terminal rods of bacteriorhodopsin (amino acids 167–248). With reference to the structural arrangement of the seven transmembrane segments, the site-directed proteolytic cleavage provides – in conjunction with the chymotryptic fragmentation – information required for a 2–3–2 segmental overlap analysis.

Post-translational membrane anchoring of acetylcholinesterase

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The amphiphilic membrane bound acetylcholinesterase (AChE) from *Torpedo* can be transformed into a hydrophilic form by digestion with phospholipases C from *Trypanosoma brucei* or from *Bacillus cereus*. Further lipase digestion uncovers the complex carbohydrate, known as crossreacting determinant (CRD), which is present in the hydrophilic form of all variant surface glycoproteins (VSG) of *T. brucei*. The CRD is also detected on human erythrocyte AChE after digestion with *T. brucei* lipase. These data indicate that the glycophospholipid, anchoring the protozoan variant surface glycoproteins and the two vertebrate acetylcholinesterases, appears to have a common structure, suggesting that this novel type of membrane anchoring by post-translational modification is more widespread than previously assumed.

Aldosterone induces accumulation of mRNAs coding for Na,K-ATPase in amphibian kidney cells (A6)

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Aldosterone promotes sodium reabsorption in epithelia such as A6 cells from *Xenopus laevis* kidney. The physiological action of mineralocorticoid hormones appears to be mediated by the control of the expression of a number of proteins. Part of the response is mediated by an increase in Na,ATPase synthesis. To test whether the aldosterone effect was transcriptional and/or post-transcriptional, we identified cDNAs for the α subunit of the Na,K-ATPase of *Xenopus laevis* by screening an expression library with anti-α subunit antibodies. These probes have been used to measure the accumulation of specific mRNAs in response to aldosterone. The relative increase of specific messengers and its time course paralleled that determined at the protein level. These results indicate that there is no major translational control but that aldosterone acts on the transcriptional and/or post-transcriptional modification of the Na,K-ATPase gene.

Symposium 11: The biochemistry of cell-mediated immunity

Monoclonal antibodies against human interferon gamma receptors

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Interferon (IFN) gamma is a product of activated lymphocytes which differs structurally and functionally from IFNs alpha/beta and binds to distinct receptors. The binding properties of IFN gamma receptors have been investigated on various lymphoid cells, however, the structural elements of these receptors have not been characterized as yet. We have purified cell lysates on a human IFN gamma column and immunized Balb/c mice with the eluates. Monoclonal antibodies were selected according to the following criteria: competitive inhibition of receptor binding of labeled human IFN gamma, and recognition of cell surface antigens which are down-regulated much as IFN gamma receptors when cells are cultivated in the presence of low doses of human IFN gamma. These antibodies are currently used for immunofluorescence, immunoprecipitation and purification of receptor protein.

The formation of platelet activating factor (PAF) and leukotrienes (LT) in eosinophils: are these correlated phenomena?

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Since the synthesis of PAF results in the parallel generation of free arachidonic acid, we have tested how far the formation of PAF and LT are correlated phenomena in horse eosinophils. The cells stimulated by A23187 (pH 7.3) generated predominantly the sulfidopeptide LT (LTC_4, LTD_4 and 11-trans LTC_4/LTD_4) as well as PAF. Ionomycin (optimal conditions) induced similar PAF formation compared to A23187, while the LT-formation varied in amount and pattern. With increasing pH from 6.8–8.0 the PAF- and LT-formation increased in A23187 stimulated cells. In ionomycin-stimulated cells PAF-synthesis decreased with increasing pH. LT-formation showed a plateau at pH 7.3–8.0. Other stimuli like LPS, PMA, FMLP or SAZ neither induced LT- nor PAF-formation. We could not find a parallelism in respect to the quantity between PAF- and LT-formation until now. However, we have never found the formation of one mediator in the absence of the other(s) using various stimuli.

T cell receptor rearrangements in T cell hybridomas specific for apocytochrome *c* peptide 1–65

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It is still controversial whether the receptor expressed on T lymphocytes recognizes an antigen and the MHC class II (I-A) restriction molecule separately or whether it interacts with a new determinant formed by association between an antigen and I-A. To distinguish between the two hypothesis we investigated if it is possible to correlate antigen or I-A specificity with a specific rearrangement of either the α or β chain gene of the T cell receptor. We analyzed DNA of two groups of T cell hybridomas, all reacting with apo cytochrome *c* peptide 1–65 but restricted to different class II molecules ($I\text{-}A^b$/$I\text{-}A^d$). Southern hybridization with a $C\beta_1$/$C\beta_2$ and $J\beta_2$ probe revealed multiple rearrangements of the T cell receptor β chain gene in each of the two sets of

hybridomas. No correlation was found between antigen specificity or the I-A restriction molecule and a specific rearrangement pattern of the β chain gene. Regarding the α chain gene of the T cell receptor we were not able to see rearrangements in any of the hybridomas with the constant region cDNA probe used.

Levels of expression of the various HLA class II β chain loci and correlation with immunological cell type

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The HLA class II molecules are involved in presentation of antigen to T-cells. The levels of expression of the class II molecules on the surface are important in regulating this aspect of the immune response. Quantitating the levels of the class II molecules is complicated by the presence of multiple loci whose sequences are very homologous. The DP subregion contains two pairs of DP α and β chain genes, whereas the DR subregion contains multiple β and only one α chain gene. The presence of multiple genes poses the question of the levels of their corresponding transcripts and the correlation between these and the immunological function of the class II positive cell. This has been studied using RNase mapping and oligonucleotide Northern blot analysis of the β chain genes in the DP and DR subregions. The levels of the two DP β chain mRNAs are very different with the βI transcript representing a large majority of the DPβ transcripts. In certain established B cell lines the levels of expression of the two active DR β chain loci is equivalent. This comparison is being extended to differences between B cells, macrophages and T cells.

In situ hybridization of immunoglobulin specific RNA in single B cells

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A method for in situ hybridization has been developed which detects immunoglobulin-specific mRNA transcripts in single murine B lymphocytes with radiolabeled, immunoglobulin gene-specific single-stranded DNA probes. The method has been applied to myeloma and hybridoma cells, and to B lymphocytes at various stages of their maturation. A critical step in the procedure is the treatment of the cells with pronase. Single stranded DNA probes of different length have been employed in the hybridization. The number of silver grains over a cell increases proportionally with the length of the probe, and with its concentration in the hybridization reaction. The kinetics of the increase of μ-heavy chain specific RNA molecules in single cells and the appearance of 'switched', γ-heavy chain-expressing cells after stimulation of murine B cells with lipopolysaccharide have been observed.

Assignment of known serological specificities to the product of individual HLA-DR genes expressed in transfected mouse cells

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HLA-DR antigens are the most important class II proteins and their remarkable polymorphism is directly responsible for the phenomenon of Ia restriction in the immune response. There are multiple class II genes, including two active loci for DRβ chains (β_I and β_{III}). Mouse cells transfected with a single HLA-DR gene express on their surface the Ia antigen encoded by that unique gene. Immunochemical studies with transfected cells together with DNA sequence analysis, indicate that locus β_I codes for a highly polymorphic DR chain containing a conserved MT2-specific epitope and that locus β_{III} codes for a much less polymorphic DR chain, also containing a conserved MT2-specific epitope.

The glioblastoma cell derived T cell suppressor factor (G-TsF)

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Previous studies suggested that not yet defined soluble factors may induce a cellular immunodeficiency state in patients with glioblastoma. Results of detailed analyses of glioblastoma cell derived factors provide strong evidence that these cells release factors (G-TsF) which inhibit T cell activation. The G-TsF leads to inhibition of lectin stimulation of mouse thymocytes or human peripheral blood mononuclear cells. Furthermore, it inhibits the growth of mouse neuroblastoma cells in vitro.
We describe a purification procedure for G-TsF which results in an apparently homogeneous preparation. The availability of pure G-TsF will allow detailed studies of both the interaction of the factor with its target cells and the demonstration of analogous factors in glioblastoma patients.

Structure analysis of human MHC-encoded Ia antigens expressed on the cell surface

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The heterogeneity of the human Ia molecular pool, expressed on the cell surface of B cells was analyzed by two dimensional peptide mapping of molecules after lactoperoxidase-glucoseoxidase catalyzed iodination followed by specific immuno-precipitation with distinct anti-Ia monoclonal antibodies.
The results obtained indicate the existence of a fourth Ia heterodimer named DN distinct from DR, DQ and DP molecules, which is present in all HLA phenotype (genotypically homozygous or heterozygous) and thus suggest that a fourth class II locus previously not described is responsible for encoding the DN Ia subset.

β1,4-N-Acetylgalactosaminyltransferase as a marker for cytolytic T cell lines

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We developed a sensitive assay for a β1,4-N-Acetylgalactosaminyltransferase which adds N-acetylgalactosamine to β-linked galactose residues of O- and N-linked oligosaccharides of glycoproteins. The enzyme can be detected in most murine cytolytic T-lymphocyte lines but is only weekly expressed in helper T-lymphocyte lines. Most tissues do not express significant levels of this glycosyltransferase except for intestine. The correlation between the cytolytic phenotype and the expression of the β1,4-N-acetylgalactosaminyltransferase is strengthened by the finding that two monoclonal antibodies (Lefrançois, L., and Bevan, M.J., Nature *314* (1985) 449) reacting specifically with cytolytic T lymphocytes react with a cytolytic T-lymphocyte line, B6.1 but not with B6.1.VV6, a mutant of B6.1 which is deficient in β1,4-N-acetylgalactosaminyltransferase.

Functional expression of transfected T-cell receptor α- and β-chain genes

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The T-cell receptor for antigen and MHC appears to be a membrane spanning glycoprotein composed of at least two disulphate linked polypeptide chains (α and β). To find out whether the α and β chains are sufficient for antigen and MHC recognition, we isolated functional T-cell receptor genes from one cytotoxic T-cell clone and transferred them into T-cells with different specificity. The α and β alleles were isolated from cytolytic T-cell clone BDFL1.1, which lyses targets carrying fluorescein as an antigen and a D^d MHC molecule as a restriction element. The functional α and β genes were joined with a selectable marker (neomycin resistance gene) and transferred by protoplast fusion into T-cell hybridoma SPH1.3 (a killer cell which lyses targets carrying hapten 3-(p-sulfophenyldiazo)-4-hydroxyphenyl acetic acid (SP) and K^k MHC molecule). Out of 40 transfectants, only one (BD7-S17) was found to express the introduced α- and β-chain genes. When tested in CML assay, the BD7-S17 transfectant showed the host cell specificity (SP + K^k) and, in addition, the donor cell specificity (FL + D^d).

Cytochrome c presentation by macrophages to T helper cells: metabolic requirements and chemical characterization of antigenic fragments generated by macrophages

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Antigen specific T helper cell (Th) proliferation and lymphokines production is a process triggered by the H-2 class II restricted interaction between Th and antigen presenting cell (APC). Our goal is to determine the nature of the antigenic determinant recognized by the Th. A monoclonal antibody (Mab SJL 2-4) specific for apo-peptide 1-65 which inhibits lymphokine production and proliferation of apo-peptide 1-65 specific Th clones has been characterized. By using ^{125}I-Mab SJL 2-4 and radio-labeled apo-peptide 1-65 we showed in preliminary experiment that: a) Macrophages (Mø) or glutaraldehyde fixed Mø bound a similar amount of apopeptide 1–65; b) Trypsin treatment abrogated ^{125}I Mab SJL 2-4 binding on apopeptide 1-65 pulsed Mø; c) Trypsinization of apo-peptide 1-65 pulsed Mø did not inhibit Ag presentation. It was concluded that Mø process apo-peptide 1-65 and generate antigenic determinants several orders of magnitude more efficient than metabolically inert Mø. The latter may represent a weak 'cross-reacting' antigenic determinants.

Effects of cyclosporin A and G on renal function of rats

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The immunosuppressant cyclosporin A (CsA) has been shown to impair renal function in man and in spontaneously hypertensive rats after short or long term exposure. The present study was carried out to study the effects of various CsA congeners in order to find one, which has immunosuppressive activities similar to those of CsA but which causes less or no nephrotoxicity. Cyclosporin G (CsG) was shown to have these qualities as shown in a rat model. *Experimental design:* Rats were administered olive oil as a vehicle in a 10-day control phase. In a following 11-day test phase the rats were treated with either 20 or 50 mg/kg day p.o. CsA or CsG, dissolved in olive oil. Treatment was stopped in order to examine the reversiblity of possibly impaired renal function during a 10-day recovery phase. Examinations of renal parameters such as BUN, Creatinine, GFR were carried out throughout the experiment every 2–3 days. *Results:* CsA caused a dose dependent impairment of kidney function which at the 50 mg/kg dose level occurred as of day 4–5 of the test phase. In contrast, CsG did not cause any relevant effects on kidney function at the low dose, and only caused minor impairment at the 50 mg/kg dose level. The adverse reactions observed for both compounds were shown to be reversible but those of CsG reversed more rapidly than those of CsA. Thus, CsG is clearly less nephrotoxic than CsA at dose levels of equal immunosuppressive activity.

Transplantation reactions and MHC expression by *Xenopus* tadpoles

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The amphibian *Xenopus* allows a detailed examination of the ontogeny of the immune system since the young (embryo and tadpole) are numerous, free-living and immunocompetent from very early stages. Like adults, *Xenopus* tadpoles have a fully developed immune system; however, certain transplantation reactions differ (e.g. tadpoles are more prone to be made tolerant depending on the genetic disparity and size of skin grafts). We have found that the ability of tadpoles, but not adults, to reject grafts is dependent upon whether the grafts contain allogeneic (MHC-mismatched) passenger hematopoietic cells. These results may be due to the absence of certain classes of MHC molecules or tadpole cells that are expressed in the adult. The adult *Xenopus* like all other rigorously examined vertebrates, expresses class I and class II MHC-encoded molecules. The tadpole expressed the same class II molecules as the adult, but fails to express class I antigens until the onset of metamorphosis when they then appear on all tissues.

Astrocytes of the brain synthesize interleukin 3-like factors

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Interleukin 3 (IL-3) is produced by activated T-lymphocytes and T cell lines, as well as by a myelomonocytic cell line (WEHI-3B). This lymphokine stimulates the growth of hemopoietic stem and progenitor cells, activates lymphocytes, mast cells and macrophages. Recently we have demonstrated that astrocytes act as immune accessory cells through the secretion of interleukin 1 and the presentation of antigens to T lymphocytes. Here we show that cultured astrocytes from newborn mice release an IL-3 like factor. The 35 kD factor induces the expression of 20-α-hydroxysteroid dehydrogenase (20α SDH) in nu/nu spleen cells and the proliferation of the IL-3-dependent cell line 32DCL. The astrocyte-derived factor also stimulates the incorporation of (^{3}H)thymidine by thioglycollate-elicited adherent peritoneal macrophages.
These results indicate a possible role for intracerebral IL-3 production to the maturation and proliferation of microglia cells, the tissue macrophages of the brain which proliferate during viral encephalitis or multiple sclerosis.

The amino-terminal portion of rabbit secretory components is responsible for binding to secretory IgA

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Rabbit secretory IgA (sIgA) is constituted by two IgA monomers bounded to J chain and high- and low M_r secretory components (SC). SC's are non-covalently associated with the

IgA dimer in the IgAg subclass. Upon time-controlled digestion of milk sIgA with trypsin or chymotrypsin, two SC-derived fragments of ~40 kD and 30 kD were released. N-terminal sequence determinations indicated that the 40 kD (from the high M_r SC) encompassed the three domains from the C-terminal end, whereas the 30 kD (from the low M_r SC) contained only the last two domains of SC molecule. Upon dissociation of the trypsin-treated sIgA complex in the presence of 3 M KCNS, a mixture of two SC-derived fragments (~20 kD and 18 kD) was obtained and peptides were further isolated by repeated gel filtration steps. The 20 kD fragment (from the high M_r SC) represents the N-terminal two domains and the 18 kD fragment (from the low M_r SC), the N-terminal one and a half domain, respectively. As expected, these fragments were shown to bind to SC-depleted intact IgA dimers almost as tightly as the native SC molecules, whereas the C-terminal fragments did not.

Analysis of the tissue-specific Ig heavy chain enhancer

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The murine immunoglobulin heavy chain gene (IgH) enhancer has been localized within a 1 kb XbaI restriction fragment between the J_H and the C_u regions (Banerji et al., Cell *33* (1983) 729). It exhibits a strict cell-type specificity, i.e. increased expression of test genes linked to this enhancer is observed only in cells of the lymphoid lineage. We are trying to delineate important sequence motifs of the IgH enhancer by using small restriction fragments or oligonucleotides cloned as single or multiple copies. In particular, we address the question whether there exist elements responsible for tissue-specificity that can be dissected from 'constitutive' enhancer elements.

The data obtained so far indicate that even small fragments of this enhancer retain tissue-specificity and are inactive in cells of epithelial or fibroblast origin. Oligomerization of these fragments increases their activity in lymphoid cells.

Immunochemical analysis of a melanoma associated differentiation antigen regulated by interferon-gamma

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Monoclonal antibody (Mab) Me 14/D12 identified a new melanoma associated differentiation antigen which is modulated by recombinant interferon-gamma (IFN-γ). The expression of this antigen was significantly enhanced on melanoma cell lines and induced on two Me 14/D12 negative lines. No enhancement or induction was observed with IFN-α. In addition to melanomas, Mab Me 14/D12 reacted with gliomas, neuroblastomas and B cell lines. This Mab immunoprecipitated from S-35-methionine labelled melanoma cells two non covalently linked chains with apparent mol. wt of 33 and 38 kD on SDS-PAGE. Cells treatment with an inhibitor of N-linked glycolisation (Tunicamycin) resulted in reduction of apparent mol.wt of the two chains to 26 and 28 kD. Nonequilibrium pH gradient electrophoresis showed that both chains had an acidic pH of 4.5–5. The two chains structure as well as the modulation of its expression by IFN-γ and not by IFN-α, suggested the Me 14/D12 antigen might belong to a class II like MHC antigen.

Nephrotoxicity of the major human ciclosporin metabolite in rats

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The immunosuppressant ciclosporin (CSA) causes nephrotoxicity in man and rats. The possibility that a metabolite is responsible for toxicity was tested in rats. *Methods:* Rats were given either the major human metabolite (OL17) or CSA at 20 and 50 mg/kg/day for 4 weeks orally. Serum and urine chemistry were performed weekly as well as histologic examination of the kidneys after 4 weeks. *Results:* Whereas CSA caused distinct functional and morphological renal changes, OL17 did not induce any adverse renal effects. In addition, the metabolite OL17 was devoid of any immunosuppressive effect both in vitro and in vivo. *Conclusion:* The major human metabolite OL17 proved to be non toxic in rats. If this holds true for the other metabolites, it seems that the parent molecule exerts the nephrotoxic effect.

Identification on human thymocytes of the idiotypic receptor structures (Ti) from two T cell leukemia lines Jurkat and HPB-ALL

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Monoclonal antibodies (Mabs) were derived from mice immunized with either Jurkat or HPB-ALL T cell leukemia lines. Several Mabs reacting exclusively with the T cell receptor of the immunizing cell line and not with six control T cell lines were selected. When these antibodies defined as anti-T cell idiotype (Ti) were tested by flow microfluorimetry on normal pediatric thymocytes, a surprisingly high percentage of these cells (2–4%) were stained with either anti-Ti HPB-ALL or anti-Ti Jurkat Mabs. Immunohistochemical studies on frozen sections from fetal or pediatric thymus specimens confirmed these findings and showed a scattered distribution of Ti positive cells in both the cortex and the medulla. Anti-Ti HPB-ALL and anti-Ti Jurkat Mabs precipitated disulfide linked heterodimers from lysates of I-125 labeled thymocytes, which were similar to the heterodimers precipitated from I-125 labeled HPB-ALL or Jurkat cells as shown by SDS-PAGE analysis.

Molecules of the axolotl major histocompatibility complex

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The axolotl *(Ambystoma mexicanum)* is a neotenous salamander which requires exogenous thyroxin to achieve external metamorphosis, though slow changes in hemoglobin and serum proteins without thyroxin have been reported. Rabbit antisera made against purified human MHC class I and class II molecules were used to immunoprecipitate homologous molecules from radiolabeled axolotl blood and spleen cells. These molecules are polymorphic and have biochemical properties similar to mammalian MHC molecules. While class II molecules are found even in young axolotls, class I molecules appear only in older animals or after maturation with thyroxin.

Antibody-independent activation of the first component of complement (Cl) does not require the subcomponent Clr

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The activation of Cl is controlled by the regulatory protein $\overline{Cl}$-inhibitor ($\overline{Cl}$-INH). The latter modulates activation by antibody-independent 'strong' and 'weak' Cl activators. Lipid vesicles containing cardiolipin (CL) and phosphatidyl-glycerol (PG) were tested for Cl *activation* in the presence of $\overline{Cl}$-INH. Whereas the latter consistently suppressed activation by PG, a dose-response activation of Cl was observed in the presence of vesicles

containing 40–100 moles % Cl (J. Immun. *135* (1985) 2695). Cls *binding* to Cl ('strong' activator), but not to PG vesicles ('weak' activator) followed the same pattern. This binding required the presence of Clq, but *not* of Clr and was Ca^{2+}-dependent. We also observed a specific cleavage of Clq-bound Cls into its 58 kD and 28 kD chains in the absence of Clr. – Similar results were obtained with a natural activator of Cl, the *E. coli* strain J5. These results suggest that strong nonimmune activators of Cl have the property to bind and cleave Cls in the absence of Clr.

Antitumor activity of polysaccharides from *Solidago* sp.

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The water soluble polysaccharides from the upper parts of *Solidago* sp. were fractionated on an ion exchange resin into a neutral fraction F1 and a weakly acidic fraction F2 which are composed of: F1: D-glucose, D-mannose, D-galactose and L-arabinose; F2: D-galactose, L-arabinose and uronic acid. The antitumor activity of both fractions was tested with the solid Sarkoma 180 in female CD1 mice. Mainly F1-polysaccharide showed a strong inhibition of tumor growth with a comparatively high regression rate.

It is speculated, as it has been shown for other antitumor polysaccharides (i.e. Schizophyllan) that this particular activity of F1 may be caused by an immune modulating effect.

A B-cell lymphoma expressing two isotypes concomitantly

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The B cell lymphoma K46R arose as a spontaneous tumour, B cell lymphoma, of Balb/c mouse. It was reported to produce both IgM and IgG, a phenomenon that was investigated further. A subclone of this cell line, K46R/B7, was established that express both IgM and IgG on the cell surface on more than 90% of the cells as determined in the FACS. This phenotype is stable in culture and the cell line has a diploid karyotype for chromosome 12. Northern blot analysis revealed that the cell line produces normal size transcripts for IgM and IgG_2 at equimolar amounts. Southern blot analysis showed that the cell line has only one IgM allele. The other allele has switched to the IgG_{2a} constant region. Both alleles are rearranged to the J_4 segment but are linked to different V genes. From these data it is concluded that the K46R lymphoma expresses two different isotypes from two different chromosomes and thus represents an exception to allelic exclusion.

T90/44: Biochemistry and function in T cell activation

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T90/44 is a novel surface glycoprotein of human T lymphocytes; 90 kD S–S-bonded dimers and free subunits are detected at the cell surface. T90/44 structurally is homologous to the antigen receptor T_i (MW, pI, S–S-bond), but T90/44 and T_i are not identical, because (a) tryptic fingerprints differ and (b) T90/44 and T3 antigens do not comodulate. Its role in T cell activation was tested with the anti-T90/44 monoclonal 9.3 antibody. 9.3 strongly enhances growth of activated PBL at ≥ 0.1 nM and is mitogenic for T cells in the presence of accessory cells at > 10 nM. The specific activation by *M. leprae* of cloned T_H cells derived from leprosy patients is inhibited by ≥ 1 nM. In order to study a possible relation of T90/44 to the Ig-gene family, the N-terminal amino acid sequence was determined. Preliminary results were obtained in the cloning of T90/44 with synthetic oligonucleotides and cDNA banks.

Expression of high and low affinity receptors for interleukin 2

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Interleukin 2 (IL2) is a T cell derived polypeptide hormone of 133 amino acids which exerts its growth promoting activity via specific cell surface receptors. The gene for the IL2 receptor has recently been cloned and codes for a single chain glycoprotein of 50–65 kD mol.wt. IL2 receptor expression is transiently induced on resting T cells by antigenic stimulation. Subsequent interaction of IL2 with its receptor induces a finite number of cell divisions before the cells return to a resting state. Two classes of IL2 receptor have been identified on the basis of Scatchard plot analysis of equilibrium binding data and by dissociation experiments; a high affinity form and a 10-fold more abundant low affinity receptor. Interaction of IL2 with high affinity receptors results in cell proliferation which correlates with the capacity to rapidly internalize IL2, whereas IL2 bound to low affinity receptors is not internalized. As yet no function has been attributed to low affinity IL2 receptors.

Proteins and antigens of bovine herpesvirus 4

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Bovine herpesvirus 4 (BHV-4) includes isolates recovered from animals with diverse diseases, as well as from healthy cattle. These viruses in general fail to elicit clinical disease in experimentally inoculated cattle or laboratory animals. Present knowledge implies BHV-4 to establish latency in lymphoid cells. Analyses of five isolates with regard to infected cell proteins (ICP) has shown, that the viruses did not shut-off host cell protein synthesis efficiently. Immunoblot and radioimmunoprecipitation assays indicated, these viruses to induce 37 ICP of comparable size, ranging from 14,000 to more than 200,000 daltons. Two proteins (81kD and 41kD) displayed minor but consistent strain specific SDS-PAGE migration characteristics, allowing to differentiate among strains being recovered from 'diseased' animals and from 'normal' cell cultures, respectively. Together the available data indicated BHV-4 to possibly represent the first established non-primate lymphotropic herpes virus.

Antigenic sites in bovine β-lactoglobulin

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β-Lactoglobulin (BLG) which constitutes about 50% of the total whey protein is considered a major allergen in infant's hypersensitivity to cow's milk. Tryptic hydrolysis of the total whey protein drastically decreased allergenicity of this protein in vivo (Pahud, Monti and Jost, J. Pediatr. Gastroent. Nutrition *4* (1985) 408). In a tryptic digest of BLG 4 peptide fragments were found by radioimmunoassay to react with rabbit anti-BLG antibodies. These fragments and their calculated mol. wts were: $Ser_{21} - Arg_{40}$ (2030), $Val_{41} - Lys_{60}$ (2314), Trp_{61} [illegible] Lys_{69} Leu_{149} Ile_{162} (2764) and $Val_{92} - Lys_{101}$ (1065). The capacity of these peptides to elicit anaphylactic reactions in passively sensitized guinea pigs was discussed.

The kinetics of internalization of interleukin-2 and its receptors by an IL-2 dependent cell line

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We have analyzed IL-2 receptor (IL-2R) traffic in an IL-2 dependent murine T-lymphocyte line. The cells express $\sim 10^5$ IL-2R; 10% of these are high affinity receptors (HAR, $K_d \sim 20$ pM), the rest has a K_d of ~ 1000 pM. Our experiments are based on the use of ^{3}H-IL-2 and a monoclonal anti-IL-2R antibody. The half-life of the cell surface receptors is ~ 10 h. IL-2 is internalized only via HAR at a maximal rate of ~ 500 molecules IL-2/cell. min. By monitoring the conversion of trypsin sensitive surface IL-2R into trypsin-resistant molecules we found that receptors are internalized with similar kinetics during the first 40 min at 37°C. After this time the fraction of resistant molecules ($\sim 15\%$ of all molecules initially at the cell surface) remains almost constant. Internalization is not IL-2 dependent. One interpretation which takes into account all our data is that HAR are formed by the association of IL-2R molecules with an accessory molecule which is co-internalized with the HAR but recycles back to the plasma membrane while the IL-2R molecules themselves are not recycled.

A monoclonal antibody against altered LFA-1 induces proliferation and lymphokine release of cloned T cells

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A murine mAb (I-17, IgM) has the following functional effects on murine longterm T cell clones: inhibition of cell mediated lysis, induction of proliferation, release of lymphokines and change of the cell morphology. The determinant detected by I-17 is expressed on longterm T cell lines but not on thymocytes, lymph node cells and spleen cells. I-17 precipitated proteins with a apparent mol.wt of 220 kD, 170 kD, 150 kD and 100 kD. Biochemical studies indicated that the determinant recognized by I-17 is tunicamycin sensitive and that I-17 binds to the α chain of the lymphocyte function associated antigen (LFA-1).

Cyclosporine as a model antigen: what does an antibody see?

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Monoclonal antibodies (McAbs) have been raised against the rigid cyclic undecapeptide cyclosporine (Cs). The fine antigenic recognition patterns of these McAbs were determined using a series of Cs-derivatives showing a skeleton conformation similar to native Cs. McAbs specific for small, well defined clusters of aminoacid residues on the upper or lower face or on the front or reverse side of the Cs-molecule have been characterized. They can detect slight conformational changes of their specific epitope induced by modification of residues located outside this epitope, even if the aminoacid sequence of the epitope itself is unchanged. Defined chemical modification or metabolization of this small, well characterized molecule affects predictably the antigen-antibody interaction as evaluated in a competitive ELISA.

Virtually identical nucleotide sequences of mRNAs encoding autoantibodies against bromelain-treated erythrocytes

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Several IgM,k monoclonal autoantibodies against isologous red blood cells, obtained by fusion of pooled peritoneal cells from NZB or CBA/J non immune mice with Balb/c myeloma cells, were isolated. Idiotypic and partial N-terminal amino acid sequence studies suggested that they constitute a family of structurally highly restricted molecules. Nucleotide sequence determinations of the variable (V) regions specified by the mRNAs purified from hybridomas were carried out. Autoantibodies probably use the same joining (J), diversity (D), and V gene segments, as only a few linked base substitutions in the J, D and V elements were detected when NZB and CBA autoantibodies are compared. The autoantibodies of both strains are encoded by J genes expressed by induced antibodies, and by V genes, which may be expressed as germ line genes rather than somatic variants. The expressed D segments originate from a known germ line D gene family.

The autoreactive B-cell repertoire in normal and autoimmune (B6 × DBA/2)F_1 mice

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Systemic autoimmunity in mice may be better understood with a detailed analysis of the Ig repertoire of autoreactive B cells. Therefore the autoreactive B cell repertoire of normal (B6 × DBA/2)F_1 ($NBDF_1$) mice and that of BDF_1 mice undergoing a graft-vs-host reaction (GVH $BDFG_1$), and as consequence of that suffering from a SLE like disease has been compared. We have screened some 1100 hybridomas derived from $NBDF_1$ mice and 600 from GVH BDF_1 mice for their capacity to bind to self antigens. About 15% of the hybrids of both groups produced antibodies directed against the tested self antigens, and no difference in antigen specificity was observed. An important difference between the two groups of autoreactive hybridomas is the Ig class of the antibodies. Whereas the GVH BDF_1 derived ones we found to be mainly of the IgG class, the ones from $NBDF_1$ are all IgM.

The expression of V_H in these hybridomas was studied using DNA probes for seven mouse V_H-gene families. Nearly 80% of the autoreactive hybridomas derived from GVH BDF_1 mie use V_H-genes belonging to the J558 family, whereas only 50% of the autreactive hybridomas from $NBDF_1$ use V_H-gene from this family, thus indicating a different V_H repertoire for autoreactive antibodies of GVH BDF_1 mice and of $NBDF_1$ mice.

Isolation of the T-cell antigen receptor genes from an alloreactive cytotoxic T-cell clone

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We have isolated the α,β and γ chain genes for the T-cell antigen receptor of an alloreactive cytotoxic T-cell clone (3F9). This cytotoxic T-cell clone is of BALB/c origin and specific for the D^b allele of the major histocompatibility complex (MHC). Surprisingly, the V_α and the V_β genes expressed in 3F9 are identical to the ones used in a chicken red blood cell specific, I-A restricted helper T-cell clone (LB2). These two clones differ, however, in the $D\text{-}J_{(\alpha,\beta)}$ portions of the T-cell receptor. Further, analysis of the γ chain genes in 3F9 reveals that this alloreactive cytotoxic T-cell does not express any functional γ chain genes. This indi-

cates that the D-J regions of the T-cell receptor play a central role in Ag and/or MHC recognition and that functional γ chain genes are not required in alloreactive cytotoxic T-cells.

Cloning of cDNA encoding B cell specific proteins

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To characterize B cell specific proteins which are involved in the differentiation of premature B cells along the B cell maturation pathway, we have constructed a B-T cDNA library in the λgt 11 phage vector from polyA$^+$ mRNA of the membrane-bound fraction extracted from the murine pre-B lymphoma cell line 70Z/3. The single strand cDNA was enriched for B cell specific sequences by hydroxylapatite column chromatography after repeated subtraction hybridization with excess amounts of polyA$^+$ mRNA from the T cell hybridoma K62.
Screening by differential hybridization with ^{32}P-labeled cDNA obtained from either B cell mRNA or T cell mRNA showed that this selected library is at least twenty times enriched for B cell specific sequences compared to the non-selected total cDNA library from the same preparation of polyA$^+$ mRNA. From the primary screening, we selected 200 clones, extracted the insert DNA as labeled radioactive probes, and screened the RNA from B cells of different stages of differentiation and of T cell lines, macrophage lines and fibroblasts for the presence of hybridization sequences.
We have identified four different groups of clones which appear to be expressed preferentially on B lineage cells.

Correlation between fine specificity of alloreactive T cell clones and rearrangements of antigen receptor genes

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We have produced 18 different H-2D^b allospecific cytotoxic T cell clones of BALB/c origin. These clones were analysed with a clone-specific monoclonal antibody for 3F9 (a D^b-specific cytotoxic T cell clone of BALB/c origin). Seven of our D^b-specific CTL's reacted with this mAb indicating that these cells carry the same idiotype (id$^+$). Biological activity of the clones was assayed by measuring cytotoxicity on target cells of different haplotypes. For further characterization, rearrangements of β-chain genes of the T cell receptor were studied by Southern blot analysis. These analyses enabled us to divide the clones into five distinct groups. Interestingly, the id$^+$ clones have deleted the $C_{\beta 1}$ gene contrary to the id$^+$ 3F9 cell where the $C_{\beta 1}$ gene is expressed. Among the id$^-$CTL s we have found four different patterns of rearrangements.

Biochemical characterization of murine A_1 lymphocyte surface antigen

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The monoclonal antibody A_1 (provided by Dr O. Kanagawa) was shown to be specific for the mouse T lymphoma EL-4. This reagent precipitated a heavily labeled membrane protein from lysates of surface iodinated EL-4 cells. This protein of an apparent mol.wt of M_r = 80,000 was converted into a broad band of M_r = 45–50,000 upon reduction, indicating that it exists at the cell surface as a disulfide-linked dimer. Two dimensional IEF-SDS-PAGE analysis of the reduced molecule gave a single band with charge heterogeneity reflecting probably a microheterogeneity at the carbohydrate level. Deglycosylation of the molecule by treatment with endo-F yielded two polypeptides of M_r = 35,000 and 30,000 respectively, with peptide maps identical to those of the intact glycosylated protein. This suggests that the molecule is composed of two identical subunits differing in their glycan units. In addition, no structural relationship with the T-cell antigen receptor or with the human T-44 protein was found.

Role of immunoglobulin binding factors in the regulation of human IgE synthesis

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Immunoglobulin-binding factors are known to regulate the synthesis of B-cell-derived immunoglobulin heavy chain isotypes. We have generated a human T hybridoma line producing IgE binding factors (EBF). On SDS-PAGE, binding activity was demonstrated at apparent mol.wts of 15, 30, 60 and > 120 kD. These IgE binding factors bound strongly to concanavalin A and, therefore, seem to be heavily glycosilated. This T hybridoma derived EBF inhibited the spontaneous in vitro IgE synthesis of B cells from atopic blood donors as well as the IgE synthesis of the U266 B cell line. EBF was absorbed on the surface of IgE receptor positive and negative cell line cells. Such cell associated EBF was able to additionally bind IgE onto cell surface. It is hoped that the study of such effector molecules of human B cell differentiation might provide further understanding and new tools for the treatment of allergic patients.

Role and expression of the HLA-associated invariant chain

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The HLA-associated invariant chain (INV) is a glycoprotein which is intracellularly and transiently associated with HLA-DR α and β chains. It is thought to play a role in the assembly and/or transport of class II antigens to the cell surface or with antigen processing and recycling of the class II molecules. Transfection experiments of INV negative monkey cells (COS) with DR α and β chain cDNAs alone have led to the expression of these chains on the surface of the cells indicating that the INV chain is not essential for HLA-DR expression. At the biochemical level, the INV chain displays a very interesting heterogeneity. Three major forms of this protein can be resolved on 2D gels after immunoprecipitation with a mAB. The three forms (p33, p35 and p41) are almost indistinguishable by peptide maps and are not metabolic intermediates. The p33 and p35 forms of the INV chain are derived from a unique mRNA by use of two, in-frame, translational starts. The p41 form is the result of an alternative splicing event. The p41 mRNA contains an additional exon in the middle of the coding region. This is, to our knowledge, the first example in higher eucaryotes, of the use of two initiation codons on the same mRNA.

A novel approach for preparing anti-T cell receptor constant region antibodies

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Recent breakthroughs in the molecular cloning of the T cell receptor (TCR) genes coding for α, β and the related γ poly-pep-

tide chains have led to extensive accumulation of information about the gene organization and transcriptional behavior of these genes. Information about the actual receptor proteins is dragging behind, however, because of the lack of optimal anti-TCR antibodies. The constant region genes of the TCR α and β chains, like that of the related γ chain, have very similar genetic organization. The 5′ exons represent the main body of the constant (C) regions and the information coding for transmembrane, cytoplasmic as well as untranslated regions is located in separate exons. The 5′ exons clearly code for immunoglobulin (Ig) like domains. This genetic organization suggested to us that we could solubilize the TCR C region domain by inserting the main TCR C region exon, along with the immediate intronic flanking regions, which include the consensus RNA splicing signals, between the suitable Ig exons at the DNA level. Chimeric proteins could then be produced by myeloma cells which had received the engineered genes through conventional gene transfer techniques and thus the 'antigen' could be purified by using monoclonal antibodies against Ig domain.

Antigenic homology of lytic proteins involved in complement- and cytotoxic T-cell-mediated cytolysis

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The lytic activity of granules from cytotoxic T-cells resides on a 66 kD protein called perforin (Masson and Tschopp, J. biol. Chem. *260* (1985) 9096). Perforin shares many functional properties with the complement component C9. Both proteins occur as monomeric precursor which can polymerize and insert into lipid bilayer forming transmembrane lesions. In order to search for structural homology, two peptides corresponding to amino acids 74–86 and 101–111 of C9 were synthesized. This segment is rich in cysteine and is highly conserved, since it is present, besides in C9, as a repeat unit in the LDL receptor. Antibodies raised against these two peptides reacted specifically with C9, since immunostaining was inhibited by the corresponding peptides. Moreover, the antibodies bound on Western blots to complement components C6, C7, C8α–γ and to purified perforin indicating that these proteins form a new protein superfamily. The peptide corresponding to residues 101–111 of C9 inhibited at a concentration of 100 µM granule-, perforin- and CTL-mediated cytolysis. These results suggest that perforin plays a crucial role in CTL-cytotoxicity and that the cysteine-rich domain shared by perforin and C9 is functionally important.

Augmentation of plaque forming cell response by low dose cyclosporin A treatment

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Cyclosporin A (Cs A) is well known for its immunosuppressive effect. However, we found that Cs A at low doses strongly enhanced the in vitro antibody response as measured by a plaque forming cell (PFC) assay. Cell proliferation, as well as IL-2 secretion were inhibited at Cs A doses which enhanced PFC response. This suggests a differential effect of Cs A on cell proliferation and IL-2 secretion versus the induction of the thymus dependent PFC response. Cs A nontreated cultures reached a peak PFC response around day 5 which then declined after this time. In the presence of Cs A, however, the PFC response was still raising at a time when the response of the untreated culture was already declining. An unknown feedback mechanism controlling the generation of PFC might be affected by Cs A.

Detection of RNA in B cell colonies grown in semisolid agar

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Hematopoietic lineage studies have been greatly facilitated by the development of semisolid culture techniques that allow the differentiation of single precursor cells into colonies containing mature cells. The information that these assays can provide is only limited by the methods available to detect and distinguish the differentiated progeny. Clearly, the ability to monitor differentiation in colonies using molecular tools will greatly extend these assays. Thus, we have developed a rapid direct method for detecting RNA present in heamatopoietic colonies grown in semi solid agar.

The intact layer of agar containing the colonies is transferred to a suitable RNA blotting material and washed under continuous vacuum with 2 × SSC, 0.2% SDS. After blotting, the filters can be hybridized using standard procedures. Using this technique, we have been able to detect μ, κ and λ specific RNA in colonies derived from clonable B cells containing as few as 50 cells. We hae used this technique to explore variable region gene usage. Using specific DNA segments as probes, we have determined the frequency of expression of RNA from eight heavy chain variable region gene families in pre-B and B cell colonies.

Fine specificity of anti-PC IgE and IgG antibodies

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The immune response to phosphorylcholine (PC) elicted in BALB/c mice by PC-keyhole limpet hemocyanin (LKH) is composed of two groups of antibodies with specificity to PC and p-nitrophenyl-phosphorylcholine (NPPC) respectively. They were designated as group I and group II anti-PC antibodies. We demonstrated that anti-PC IgE anti-bodies elicted by PC-KLH and PC-ovalbumin belong to group II and are T15 idiotype negative. Anti-PC IgG1, IgG2a and IgG2b antibodies express group I characteristics in the early response. Later, after four injections of PC-KLH, a clonal change to group II is observed. In contrast, anti-PC IgE are group II antibodies throughout the time of immunization. The regulation of group I and group II antibody expression in serum is independent of the genetic background of the animals.

Symposium 12: Oncogenes

Mammary gland specific expression of a chimeric human Ha-ras gene in transgenic mice

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We subjected the activated human Ha-ras gene to the control of the promotor region of the whey acidic protein (WAP) gene. This gene is regulated by lactogenic hormones and expressed in mammary epithelial tissue. Transgenic mice were derived which have stably acquired the WAP-ras gene in their genome. Three lines do not express the hybrid gene, whereas in two lines we find expression during lactation predominantly in the mammary gland, but also in the brain. After lactation the gene is turned off, suggesting that the hormonal dependence of WAP expression was conferred to the Ha-ras gene. Even after several lactations

we haven't detected mammary tumors. Therefore the observed level of expression of the activated Ha-ras gene during lactation was not sufficient to cause transformation of mammary epithelial cells.

An association of the transforming gene product of polyoma with the cytoskeleton of virus-infected cells

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Middle T antigen (mT), one of the early gene products of polyoma virus (Py), has been shown to be involved in the process of Py induced cell transformation. mT is associated with the plasma membrane of transformed cells and becomes phosphorylated on tyrosine residues through an interaction with a cellular phosphokinase ($pp60^{c\text{-}src}$). Tyrosine phosphorylation of mT is a rare event in intact cells and is more easily studied using immunoprecipitated material or isolated plasma membranes. Further fractionation of cells revealed that kinase-active mT is enriched in focal contacts (f.c.'s), areas of closest cell-substratum contact. F.c.'s are important structures regulating the anchoring of stress fibers to the plasma membrane. Vinculin, a 130 kD protein associated with f.c.'s. interacts with mT as shown in immunoprecipitates formed with a vinculin-specific antiserum. The complex formation between mT, $pp60^{c\text{-}src}$ and vinculin may cause the disruption of the stress fiber-plasma-membrane linkage, one of the earliest events observed in Py induced cell transformation.

Herpes simplex virus immediate early protein ICP4 binds to DNA and promotes transcription

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In Herpes simplex virus infected cells there is a sequential expression of viral genes. In vivo experiments have implicated the 175,000 mol.wt immediate early protein ICP4 in the regulation of viral RNA synthesis, but the mechanism whereby ICP4 regulates transcription of viral genes is at present unknown. In this report we describe experiments with an in vitro transcription system and purified ICP4. Using DNA from the Herpes virus glycoprotein D gene (gD) as the template, we have observed that (i) specific binding occurs between ICP4 and DNA sequences adjacent to the gD gene promoter and (ii) ICP4 stimulates initiation of transcription from the gD gene. The degree of stimulation depends on the amount of ICP4 present in the incubation. The kinetics of RNA synthesis demonstrate that the protein acts at the initiation step of transcription. These results identify ICP4 as a viral transcription factor whose presence on DNA facilitates the formation of transcription complexes.

Vinculin a substrate of $pp60^{src}$ is acylated in a transformation-sensitive manner

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The cytoskeletal protein vinculin (130 kD) has been postulated to be involved in the linkage of actinmicrofilaments to plasma membranes. $pp60^{v\text{-}src}$ phosphorylates vinculin at tyrosine-residues (tyr) in vitro. Furthermore in virally transformed cells tyr-phosphorylation of vinculin is increased 10 times. Thus the phosphorylation of vinculin on tyr has been suggested to be one of the key steps involved in the regulation of cytoskeleton-membrane interactions. However, it is now generally accepted that vinculin phosphorylation on tyr is not necessarily required for the rearrangement of cytoskeletal elements observed during transformation.

Evidence will be presented that vinculin isolated from ^{3}H-palmitic acid labeled chicken embryo fibroblasts contains covalently bound fatty acid. This acylation turned out to be transformation-sensitive.

These results suggest that the covalent modification of vinculin by fatty acids is directly or indirectly involved in the association of this protein with membranes, thereby regulating the anchoring of microfilaments to the plasma membrane.

Effect of transfected oncogenes *(ras, myc)* on non-tumorigenic, immortalized rat embryo fibroblasts

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To approach the question how oncogenes interact in immortalized cells, non-tumorigenic rat embryo fibroblast (clone-4) cells were co-transfected with activated *ras, myc,* both, or no oncogene and a selectable marker (gpt). Six colonies from each group were picked, expanded, characterized in vitro and tested in nude mice for tumorgenicity. Control (0/6) and *myc* transfectants (0/6) were not tumorigenic, *ras* transfectants (5/6) tumorigenic after 22 days latency, *ras* plus *myc* (6/6) were tumorigenic after 12 days latency. Tumor cells were reestablished in culture for comparison with the original cells and examined for production of plasminogen activator, karyotypic abnormalities and tested for possible amplification of the transfected oncogenes.

Tumor-promoter effect on protein kinase C and its relation to growth in human mammary carcinoma cells

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TPA and its analogs inhibited growth of mammary tumor cells (MCF-7 > BT-20 > MDA-MB-231 > = ZR-75-1 > HBL-100) with the exception of T-47-D arguing for specific phorbol ester receptor (i.e. PKC). Undetectable low PKC activity levels were found in T-47-D cells. – TPA (100 nM) treatment (30') caused a reversible time- and dose-dependent shift of cytosolic PKC to the respective membrane fractions without inhibition of cellular growth. TPA treatment (> 10 h) resulted in growth inhibition accompanied by the proteolytic degradation of membrane-bound PKC (MW = 75 kD) into 60 kD and 50 kD fragments and subsequent loss of PKC activity. TPA removal from long-term-treated cells resumed normal growth and correlated closely with the reappearence of the PKC holoenzyme.

Effects of the cloned mos oncogene of myeloproliferative sarcoma virus (MPSV) on murine hematopoiesis

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MPSV severely disrupts hematopoiesis in mice but the primary lesion has been difficult to identify in vivo. Our approach was to infect bone marrow cells in vitro with helper-free virus constructs from ψ_2 cells. These constructs contain the selectable neo gene either alone or with the cloned mos oncogene from MPSV. Cells were grown in methyl cellulose cultures containing various growth factors ±2 mg/ml G418 to select for the expression of the neo gene. Approximately 15% of all late erythroid precursors (CFU-E) formed colonies in G418 and less of the early precursors (BFU-E, CFU-C, CFU-mix) carried the neo gene. Terminal erythroid differentiation always required erythropoietin but cells carrying the neo mos construct formed BFU-E colonies without the addition of IL-3. The data indicate that the mos

oncogene may confer IL-3 independence on early precurors of the erythroid lineage.

Establishment of two rabbit mammary epithelial cell lines with distinct oncogenic potential and differentiated phenotype by microinjection of transforming genes

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To study the mechanism of epithelial cell transformation we have chosen the mammary gland as a model system. Mammary epithelial cells from midpregnant rabbits were cultured in vitro, and by microinjection of SV40 DNA alone or in combination with the activated human cHa-ras gene we were able to establish two distinct cell lines. An immortalized cell line, obtained by injecting SV40 DNA alone, was characterized by a moderate rate of proliferation, contact-inhibited growth and no production tumors in nude mice. A large number of lactogenic receptors were found on the cell surface, however, no induction of genes coding for milk proteins was observed. A transformed epithelial cell line was obtained by coinjecting SV40 DNA and the cHa-ras. Both microinjected genes were stably present and expressed in transformed cells. Their growth was anchorage-independent and the cells were tumorigenic in nude mice. This assay system should be useful in assessing the potential of oncogenes to transform mammary epithelial cells.

Effects of oncogenes in conditional transformation of cultured cells

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We have placed the v-mos oncogene, the activated human H-ras oncogene and the normal cellular H-ras proto-oncogene under the control of the hormonally inducible Mouse Mammary Tumor Virus promoter (MMTV-LTR) and stably transfected NIH 3T3 cell clones were isolated. They were charaterized using several parameters of transformation. While most of the cell clones containing an activated LTR-oncogene construct showed a transformed phenotype and growth in soft agar exclusively in the presence of hormone, cell clones containing the LTR-H-ras proto-oncogene formed only small colonies when treated with hormone. The level of p21, LTR-mRNA and the transcription rate were quantitated upon induction by glucocorticoid hormone. In cells containing a LTR-mos or LTR-H-ras oncogene, the level of transcription and the amount of mRNA as well as protein were down-regulated after 8–25 hs (mRNA) or 24–42 h (protein). No or only a moderate down-regulation was observed in cells containing the LTR-H-ras proto-oncogene.

Suppression of oncogenic lethality by reintegration of the lethal(2)giant larvae gene into the *Drosophila* genome

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Homozygous mutations of the recessive oncogene l(2)gl of *Drosophila melanogaster* produce lethal neoplasms of the imaginal discs and the brain hemispheres in the third instar larvae. Molecular analysis of 50 different chromosomal l(2)gl rearrangements has allowed to assign the l(2)gl gene to a transcription unit covering 12 kb of cloned DNA (Mechler et al., EMBO J. *4* (1985) 1551). Using P-mediated transformation, we have been able to introduce this cloned DNA segment into the germ line of heterozygous l(2)gl/+ flies and to show by backcrossing that the transposed l(2)gl DNA segment was functional and able to completely rescue homozygous l(2)gl deficient animals, which otherwise would have died of brain and imaginal disc neoplasms. Further transformations using shortened l(2)gl DNA segments allowed to map more precisely the functional limits of the gene.

ras oncogene and IL-3 dependence

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PB-3 is an immortalized non-tumorigenic mouse mastocyte line which requires IL-3 for growth in vitro. To study the possible effect of oncogenes on IL-3 dependence, we have introduced the viral H-*ras* gene into these cells using the retroviral Zip vector. We have obtained three cell lines (A2, A3, A4) which display distinct differences from PB-3. A2 requires 1/120 of IL-3 compared to PB-3 for similar growth. In the absence of IL-3, PB-3 cells die over two days, A2 cells over five days. In contrast to A2, A3 and A4 grow in an IL-3 independent fashion. Experiments testing for autocrine growth stimulation will be presented.

Phenotypic effects of mutated *ras* genes in *Dictyostelium* transformants

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Dictyostelium contains a single *ras* gene (Dd-*ras*) which encodes a protein highly homologous to mammalian and yeast proteins (Reymond et al., Cell *39* (1984) 141). At positions associated with a transforming potential in 3T3 cells, Dd-*ras* encodes the amino acids found in cellular *ras* proteins. Dd-*ras* was mutagenized in vitro to encode a threonine instead of the glycine at position 12 and was reintroduced in *Dictyostelium* cells. Transformants containing a high copy number of the mutated gene form multiple tips and seem impared in their response to cAMP during early development. Cells containing high copy number of non-mutated Dd-*ras* develop like wild type. Since a role for cAMP has also been implied during tip formation in *Dictyostelium*, these results suggest that *ras* may be involved in cAMP signaling process.

Detection of mutated (activated) N-ras oncogenes using an oligonucleotide assay

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Ras oncogenes acquire their transforming potential by single base pair mutations located at codon 12, 13 or 61. For recognizing activated N-ras oncogenes in human tumors we established an assay using synthetic 20mer oligonucleotide probes. The hybridization and melting procedure, which uses tetramethylammonium chloride for base composition-independent hybridization, allows to screen the critical two regions with the corresponding probes simultaneously and to detect single base pair mismatch between the DNA and the oligo probe. Applying the method we demonstrate the proper identification of distinct mutations in N-ras containing plasmids and the detection of G→A transitions in codon 12 of the N-ras gene originating from human tumors transfected into N1H/3T3.

Anatomy, Histology and Embryology

Organelle movement in macrophages studied by cytomagnetometry and immunocytochemistry

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Monolayers of macrophages that had previously phagocytized magnetic (Fe_3O_4) particles were studied by cytomagnetometry, immunofluorescence technique and immunoelectron microscopy. The cells were exposed to a magnetizing field and the decay of the remanent magnetic field (relaxation) induced in these cells was measured. Addition of cytochalasin B, a desintegrator of actin filaments, to the cultures caused a significant slowing of relaxation, as did the addition of FCCP, an uncoupler of the resporatory chain, whereas relaxation was not significantly influenced by colchicine, a desintegrator of microtubules. Labelling with antiactin antibodies (gift of G. Gabbiani, Geneva) revealed discrete fibrous structures in association with lysosomes containing magnetic particles. Treatment with antivimentin antibodies also showed a distinct lysosome associated labelling. Anti-tubulin antibody labelling, however, did not show any microtubule-lysosome association. These data provide evidence that organelle movement is an actin-based mechanism; intermediate filaments might also participate but their contribution to motion remains obscure.

Organization of the barrelfield (BF) projections to the thalamus in mice studied with the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L).

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To elucidate the topological organization of the thalamic projections originating in BF, iontophoretic injections of PHA-L were made at different sites in SMI cortex in 12 mice. Following survival times of 8–12 days, labeled axonal terminations could be demonstrated in three thalamic nuclei: reticularis (RT), ventrobasalis (VB) and posterior thalami (PO). Preliminary results indicate that each cortical column, functionally linked with one vibrissa, projects to separate areas in both RT and VB. The projection to PO consisted of only a few scattered terminals, that are larger in size than those in RT and VB. The termination areas in VB resemble the barreloids (VdL, N.Sc.Lett. *2*). Current investigations aim at elucidating the development of these projections using the same technique.

Infrapyramidal mossy fibers and Y-maze discrimination learning of mice: a positive correlation

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The capacity of rats and mice to learn a two-way avoidance task is *negatively* correlated with the extensiveness of the infrapyramidal mossy fiber projection (IIP-MF) in the hippocampus. This study tested whether that circuitry variation is related to a genuine learning ability.

Twenty random-bred mice were tested for the acquisition of discriminated avoidance learning in an automatized Y-maze (visuo-tactile stimulus, 144 trials, 36 trials/day). Morphometry of their hippocampal synaptic fields was done on 10 sections taken from a mid-septotemporal level. The number of correct choices was *positively* correlated with the volume of the IIP-MF projection ($r = 0.75$, $p = 0.001$). Hence, variations of the IIP-MF are not related to learning capacity per se, but rather to the ability of mice to switch coping and choice strategies.

Deoxyglucose (DG) autoradiography reveals mediolaterally reversed representations of mouse whiskers in trigeminal subnuclei interpolaris (Si) and caudalis (Sc)

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In Si and Sc 5 rows of segments of high cytochrome oxidase (CO) activity represent the five rows of large whiskers on the muzzle. After injection of C-14-DG, whiskers C1-3 and E1 (left) of restrained, 12-day-old albino mice were stimulated for 45 min (Melzer et al., Brain Res. *348*). Autoradiograms of transverse brainstem sections showed two areas of high DG uptake in ipsilat. Si and Sc. The smaller area was in the dorsalmost E-row of CO segments; the larger one, in the central C-row. In Si both areas covered *lateral* segments; in Sc, *medial* ones. This transition was abrupt. Our observation confirms the ventral-to-dorsal (= upside down) representation of whisker rows A-E in Si and Sc of rat (Belford and Killackey, JCN *183*). This first functional evidence supports the medial-to-lateral reversal of whisker representations between rodent Si and Sc (Arvidsson, JCN *211*). What are the functional meaning and the developmental basis of this reversal?

Discordant behavior of the sarcoplasmic acetylcholinesterase

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The sarcoplasmic (i.e. non-endplate) acetylcholinesterase (AChE) being high in fast and low in slow muscles, is correlated with the fiber type: highest in the type IIB fibers, absent or very low in the type I, and intermediate in the type IIA fibers. Additionally there exists a size dependence most obvious in the type IIA fibers: the larger the fiber the higher the sarcoplasmic AChE activity. The extensor digitorum longus (EDL) muscle was cross-reinnervated with the soleus (SOL) nerve and vice versa (X-EDL, X-SOL). In the *X-EDL* the sarcoplasmic AChE showed a drastic reduction corresponding to the well known transformation from a fast to a slow muscle. In the *X-SOL* the AChE activity – after an initial increase – returned to the low values of the normal SOL muscle in contrast to physiologic and histochemical parameters indicating the conversion from slow to fast. This suggests that the AChE of the SOL depends more on the function of that muscle than on the type of the imposed impulse pattern.

Immunohistology on semithin cryo-sections

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We have utilized immunohistochemistry on semithin (0.5–1 μm) sections cut at −60 °C with a cryo-ultramicrotome, to detect various antigens by high resolution light microscopy. Since no detergents or organic solvents are used in the pretreatment of the tissue, the antigens are presumably retained in a much more 'natural' configuration. In fact, we have been able to detect a large palette of brain, muscle and kidney antigens with both poly- and monoclonal antibodies. This technique is particularly useful for studies on the coexistence of molecules because serial sections can be cut through the same cell. In the nervous system this method can be easily combined with retrograde tracing techniques. It is conceivable that semithin cryo-sections might be

useful in studies combining immunohistology and in situ hybridization as well as immunohistology and receptor autoradiography.

Development of GABA-like immunoreactivity in the chicken optic tectum

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Neurons having acquired a certain transmitter specificity in the course of their differentiation can be located in tissue by means of transmitter-immunohistochemistry. A monoclonal antibody able to reveal GABA-like immunoreactivity (Matute and Streit, 1985) was applied to the optic tectum of chick embryos and young chickens. The final staining pattern consists of numerous immunoreactive cell bodies in most layers, with accumulations in layers IIb (3), IIc (4) and IIi (10), and of horizontally organized processes in layer IId (5). Several aspects are consistent with findings in [^{3}H]-GABA uptake studies (Hunt and Kuenzle, 1976; Streit et al., 1978). A basic form of the final pattern was observed already about three days before hatching. In earlier phases of development, the distributions of immunoreactive elements are complex. Cell bodies as well as strong fiber bundles including their growth cones are stained even before the sixth incubation day. GABA-like immunoreactivity can possibly be used as marker for certain neurons during their development.

Mapping of the LICR-LON-E36 monoclonal antibody's immunoreactive sites in the rat brain

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Using the indirect immunofluorescence method, the regional distribution of the monoclonal antibody LICR-LON-E36, produced to the acidic fraction of soluble protein extracts of human brain (Sappino et al., J. Histochem. Cytochem. *32* (1984) 1041), has been studied in untreated rat brains. Cell bodies were located principally in: cortex (III–V), septum, hypothalamus and hypophysis (pars distalis). Fibers and terminal dots were specially detectable in: nucleus septi lateralis, amygdala, hippocampus, claustrum, nuclei thalami reticularis and anteroventralis, stria medullaris, habenula, nuclei pontis and cerebellum. These preliminary results show a well-defined distribution of E36 positive cells, fibers and terminal dots, with a similar location and males and females, but with a higher density in females, specially in cortex and hypophysis. We are currently investigating the structure and function of the epitope recognized by E36-antibody.

An interface for three dimensional reconstruction of brain structures from serial sections with a high-level graphic system

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Outlines of the auditory thalamus were digitized from histological sections using a light microscope connected to a computer. Adjacent individual sections were then drawn on a plotter and aligned using blood vessels as landmarks. The core of our software interface is a module with an algorithm sorting region oriented files, with the coordinates of contours of a selected region at all section levels, which are full compatible with the geometrical structure of the MOVIE.BYU system of general purpose computer graphics. This system provides the capability to convert contour line definitions into polygonal element mosaics, use clipping planes and display 3D models as line drawings or as continous tone shaded images. The major interest of our interface is to obtain files compatible with a well known system available on several kind of machines and for several graphic devices.

Early postnatal cholinergic innervation of rat brain

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Choline acetyltransferase (ChAT), a specific marker for cholinergic neurons, was localized immunocytochemically using monoclonal antibodies. Perfused brains from Long Evans and Sprague Dawley rats were incubated using floating methods. At postnatal day 6, pericarya labeled with ChAT are found in basal regions of the forebrain similar to those described in adult rats. Small cells and fibres are located in corpus callosum and hippocampal region. In contrast to adult brain, clusters of ChAT-positive cells are situated in several deep layers in postnatal mediodorsal neocortex, sending out processes to the cortical surface.

Biochemistry

A new highly specific affinity chromatography method to measure NAD^+

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We have developed a simple and highly selective physicochemical method to determine the NAD^+ levels in intact cells. Dihydroxyboronyl-Bio Rex, a boronate resin which specifically binds compounds containing at least two sets of cis-diol groups (Alvarez-Gonzales, R. et al., Analyt. Biochem. *135* (1983) 69), was used to purify NAD^+ from the total acid soluble pools. Boronate purified material was analyzed for NAD^+ content by strong anion exchange-HPLC under isocratic conditions using a low salt buffer system. The overall recovery of this procedure is at least 80%. This new affinity chromatography method was successfully applied to measure the extent of NAD^+ depletion in intact hepatocytes following treatment with different DNA damaging agents. An additional advantage of this method is that it allows for the simultaneous determination of $(ADP\text{-ribose})_n$ from the acid insoluble fraction in the same sample.

Effects of hyperinsulinemia and hyperglycemia on hepatic glycolysis and glycogenolysis in rats in vivo

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The relative effects of hyperinsulinemia and of hyperglycemia on hepatic glucose production, glycogen synthase and glycolytic intermediates levels have been studied in normal fed rats. After 60 min of saline infusion, euglycemic or hyperglycemic (170 and 200 mg/dl) hyperinsulinemic clamp, the liver was rapidly removed, frozen, and the metabolic parameters measured. The data indicate that insulin suppresses hepatic glucose production by increasing glycolysis without modifying glycogen phosphorylase activity thereby decreasing glucose-6-phosphate level. In addition, hyperglycemia inhibits glycogen phosphorylase, leading to a further decrease in glucose-6-phosphate. These data

indicate that suppression of hepatic glucose production by insulin and glucose is mediated mainly by a decrease in glucose-6-phosphate; this decrease being explained by the activation of glycolysis by insulin and the inhibition of glycogenolysis by glucose.

Does nafenopin, a peroxisome proliferator, induce DNA damage in rat livers?

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We have further investigated the genotoxic potential of nafenopin, a peroxisome proliferator which induces liver tumors in rodents. Nafenopin was shown to induce microsomal drug metabolizing enzymes. Thus it was considered possible that it may alter its own metabolic activation. However, nafenopin was not mutagenic to *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538 or TA 102 either directly or in the presence of S-9 fractions isolated from control, aroclor- or nafenopin-treated rats. Treatment of rats with nafenopin (200 mg/kg for 7 days) or the structurally related clofibrate (300 mg/kg for 7 days) was accompanied by slight increases in the rate of elution of hepatic nuclear DNA from polycarbonate filters under alkaline conditions. This effect required repeated administration of the compounds. However, further investigations suggested that it was not a consequence of indirect DNA damage caused by H_2O_2 generated within the peroxisomes. The significance of these findings is under investigation.

The effect of anion-binding on the redox potential of cytochrome *c*

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It is known that cytochrome *c*, a soluble mitochondrial electron carrier, binds anions as a consequence of its highly positive surface. We have shown the binding of certain anions to be specific, and have located the binding sites using analogues of cytochrome *c* prepared by semisynthesis or specific chemical modifications. One of these sites is oxidation-state dependent. We have proposed a physiological role for such binding, and a number of possible mechanisms whereby binding may affect electron transfer: inhibition of association with the mitochrondrial substrates; reorientation of the dipolar moment of the protein; perturbation of the structure and consequent alteration of redox potential, or effects on potential of changes in electrostatic field at the redox center.
In this presentation, we examine the effect of specific anions and of the ionic strength on the redox potential of cytochrome *c*, over a range of concentrations close to the physiological values.

A new method for the determination of epitopes on protein antigens

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Available methods to determine epitopes of protein antigens provide only indirect evidence of the size and structure of antibody binding sites. We propose a more direct approach whereby the antibody binding site is delineated directly in the antigen-antibody complex by differential chemical modification of amino acid side chains (Bosshard, Meth. biochem. Anal. *25* (1979) 273).

The epitope recognized by the monoclonal antibody L2.61 was localized on the 'backside' of horse cytochrome *c*. It is formed by two separate parts of the polypeptide chain brought together in the 3D-structure. This is the first direct evidence for a conformational epitope on cytochrome *c*.

Mitochondrial creatine kinase binds to cardiolipin

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Mitochondrial creatine kinase, a water soluble enzyme, can be released from mitochondria by phosphate treatment. The rebinding of the enzyme can be inhibited by adriamycin, a drug used in cancer chemotherapy. Since adriamycin is known to bind specifically to cardiolipin in the inner mitochondrial membrane, we have performed experiments aimed at determining whether cardiolipin is the membrane receptor for creatine kinase in heart mitochondria. Binding studies of creatine kinase were carried out in depleted heart mitochondria, on liver mitochondria, and on liposomes of different phospholipid composition. Liposomes showed significant binding only if they contained cardiolipin. The binding could be inhibited by adriamycin. Creatine kinase-depleted heart mitochondria bound creatine kinase, and again the binding was inhibited by adriamycin. Liver mitochondria, which contain about the same amount of cardiolipin as heart mitochondria, but normally do not contain creatine kinase, bound creatine kinase to the same amount as creatine kinase-depleted heart mitochondria. The ATP/ADP translocator has been previously discussed as a possible creatine kinase receptor. However, creatine kinase failed to bind to liposomes reconstituted with the ATP/ADP translocator. These results offer compelling support for the concept that cardiolipin is the receptor for mitochondrial creatine kinase.

Preparation of glucagon derivatives of altered sequence at position 1

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Glucagon derivatives have been prepared by replacing histidine-1 with the amino acids glycine, glutamic acid, lysine and phenylalanine. The modifications were carried out by semisynthesis using differential protection of the amino groups of glucagon and subsequent cleavage of histidine-1 by the Edman reaction. Boc-amino acid hydroxysuccinimide esters were coupled on the truncated peptide and deprotected. The derivatives were purified by high performance liquid chromatography. Chemical characterization shows the expected alteration in amino-acid sequence.

Sites of cleavage of insulin by insulin protease

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At last year's meeting we described an intermediate in the degradation of [$Phe^{B1-3}H$] insulin by the enzyme insulin protease E.C.3.4.22.11. We concluded that one point of attack by the enzyme was in the region B7–B9, and another in the region around residues A13–A14. We present further work confirming and refining the above conclusion. The cleavage in the B chain is, with very high probability at the carboxyl group of residues B9, whilst that in the A-chain is almost certainly at the carboxyl group of residue A13. The two truncated chains are held together by a disulphilde bridge, presumably A7–B7. We discuss the probable biological significance of these findings.

The active-site of calf intestinal alkaline phosphatase (AP): labeling and isolation of a 12-residue tryptic peptide

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The incorporation of radioactive phosphate at a serine residue in the active-site of AP is well documented (Engström, BBA *92* (1964) 79; Whitaker, Clinica chim. Acta *71* (1976) 285). When we started our work, very little was known about the sequence surrounding this reactive serine in mammalian AP. Our enzyme was isolated from calf intestinal mucosa by a procedure previously described (Portmann, Helv. chim. Acta *65* (1982) 2668). The labeling was done by the procedure of Whitaker. After trypsin proteolysis a radioactive peptide was isolated by high voltage electrophoresis and reverse phase HPLC. Its amino acid composition is: Asx, Thr_2, Ser(P), Glx, Pro, Gly, Ala_3, Val, Tyr with 1 mol of NH_3 liberated by hydrolysis. Recently the sequence of a CNBr active site peptide from bovine intestinal AP (140 U/mg) was published (Butler, BBA *831* (1985) 330). The composition of our peptide corresponds exactly to a part of the published sequence. The sequence of our peptide, prepared and isolated in a different way, is actually under study.

Anomalous apparent mol.wts of unreduced IgG on immunoblots from SDS-polyacrylamide gels

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The mobility of a protein on SDS gels is proportional to the log of its MW, if the protein was reduced and not heavily glycosylated. With the widely used immunoblotting techniques and the need to preserve the proteins' antigenicity, unreduced samples are electrophoresed and blotted. Human IgG has an average MW of 150 kD, but its unreduced form, denatured at 100 °C, migrated with an apparent MW of 250 RD. Upon denaturation for 30 min at 37 °C, it appeared within three bands (250, 220, and 200 kD) on Lämmli- and Neville-type gels. Subclass-specific antibodies bound similarly to the three bands, thus it is unlikely that the heterogeneity was due to structural differences of the four subclasses. This finding eludes to a word of caution: any antigen that appears with an apparent MW of 200–250 kD on immunoblots from unreduced biological samples should be revealed directly with labeled antibody, rather than with labeled second antibody.

Phospholamban phosphorylation in cardiac SR results in several distinguishable products

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Phospholamban (PLB) was phosphorylated under different conditions by either the catalytic subunit of the cAMP-dependent proteinkinase (cAMP-PK) and/or the endogenous Ca^{2+}-calmodulin-dependent kinase (CAL-PK). The resulting phosphoproteins were analyzed on SDS PAGE gels and on isoelectric focusing gels (IF). Phosphorylation by the cAMP-PK (60 U/ml) yielded a phosphoprotein of M_r26 kD and pI 6.2. CAL-PK dependent phosphorylation produced one of M_r28 kD and pI 6.4. Phosphorylation by both kinases resulted in the formation of two distinct products of M_r26 and 28 kD and pI of 6.2 and 6.4, respectively. On the other hand, 600 µ/ml of cAMP-PK yielded only one product of M_r28 kD and pI 6.2. Phosphorylation with both high cAMP-PK *and* CAL-PK shifted the M_r of PLB to 30 kD and the pI to 5.1. On 2-dimensional PAGE-IF, the pI 5.1, 6.2 and 6.4 phosphoproteins all had an M_r of 11 kD. It is concluded that PLB migrates on 1-dimensional SDS PAGE as a functional complex of variable M_r (26–30 kD), but is split into 11 kD subunits on IF gels. Double phosphorylation of the same PLB subunit (M_r11 kD, pI 5.1) is obtained only in the presence of CAL-PK and high levels of cAMP-PK.

Ca^{2+} uptake by cardiac SR was stimulated by both 60 U/ml cAMP-PK and CAL-PK. The effects were additive. Further stimulation was observed using higher amounts of cAMP-PK.

Tetrameric detergent-soluble acetylcholinesterase from human caudate nucleus: subunit composition and number of active sites

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Purified tetrameric detergent-soluble acetylcholinesterase (DS-AChE) from human caudate nucleus was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in absence as well as in presence of a reducing agent. In both conditions staining with silver revealed heavy and light monomers (78 kD and 66 kD) and heavy and light dimers (150 kD and 130 kD). The same four polypeptides were also detected by Western blotting and by autoradiography of ^{3}H-diisopropyl-phosphoryl enzyme. The functional molarity of DS-AChE was determined at pH 10.2 with the active site label ^{3}H-diisopropyl fluorophosphate. Four active sites were obtained for the tetrameric enzyme and the turnover number per site was 2.45×10^7 mol of acetylthiocholine iodide hydrolyzed $\times$ h^{-1}.

The interaction of calmodulin with plasma membrane fractions isolated from rat liver

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Purified rat liver plasma membrane contains about 2 µg calmodulin/mg protein which is tightly associated and can not be removed by extensive washing with EGTA. Differential extraction (Triton X-100 solubilization followed by alkaline extraction of the pellet) of plasma membrane proteins indicates an association between calmodulin and cytoskeletal proteins. A 220,000 MW calmodulin binding protein has been detected in the plasma membrane fraction using ^{125}I-calmodulin overlay on blot. A study of the distribution of calmodulin in subplasmamembrane fractions shows that calmodulin is particularly enriched in a fraction which contains basolateral membranes.

Interaction of modified calmodulin with two different calmodulin dependent enzymes

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The mechanism of the interaction of calmodulin with target proteins is still being debated. Work on tryptic fragments of calmodulin has documented differences in the interaction with different target proteins (Newton, D.L. et al., J. biol. Chem. *259* [1984] 4419; Guerini, D., Krebs, J., and Carafoli, E., J. biol. Chem. *259* [1984] 15172). Chemically modified calmodulin has been used to gain insight on the mechanism by which the protein stimulates different enzymes. In the present study methionines, lysines and arginines have been modified, the different products have been separated by HPLC-techniques and then tested for their ability to stimulate either the Ca^{2+}-ATPase of plasma membranes or cyclic nucleotide phosphodiesterase. Modification of 2–3 lys decreased the activation of the phosphodiesterase but did not affect the stimulation of the Ca^{2+}-ATPase. By contrast, modification of methionines reduced the interaction with both enzymes. Interestingly, selective modification showed that the

various met of calmodulin were differently important in the interaction with the two target enzymes.

Reversal of gene fusion of yeast tryptophan synthase

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Amino acid sequence alignment of the single polypeptide chain of yeast and the two subunits of *E. coli* tryptophan synthases suggests that the bifunctional yeast enzyme has arisen by gene fusion. The major difference is the covalent polypeptide connector. We have attempted to reverse the putative gene fusion event by expressing separately the subcistrons of TRP 5, using site-directed mutagenesis. The N- and C-terminal moieties, which correspond to the α and β subunits of *E. coli*, fold normally, and have been purified to homogeneity. They are 100-fold less active than the bifunctional enzyme. Partial activation occurs upon homologous (yeast-yeast), but even better upon heterologous (yeast-*E. coli*) complex formation. These results indicate that the subunit-subunit interacting surfaces have been conserved strongly during evolution. However, the covalent connector has an additional stabilizing role in the yeast enzyme.

Synthesis of glucagon antagonists for the suppression of hyperglycemia in diabetes mellitus

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The peptide-hormone glucagon is involved in the regulation of the blood sugar level, hyperglycemia and ketosis in diabetes mellitus are generally associated with elevated levels of glucagon in the blood. According to the bihormonal hypothesis (Unger, R.H., Metabolism *27* [1978] 1691), overproduction of glucose and ketones by the liver is primarily mediated by glucagon. A glucagon-receptor antagonist would provide direct evidence for glucagon's role in diabetes mellitus. Using solid-phase methodology, the two glucagon analogs (Asp^3, D-Phe^4, Ser^5, Lys^{17,18}, Glu^{21})-glucagon and (D-Phe^4, Tyr^5, I$_2$-Tyr^{10}, Arg^{12}, Lys^{17,18}, Glu^{21})-glucagon were synthesized. Their binding properties and adenylate cyclase activity with rat liver membranes were determined. They were antagonists with respect to adenylate cyclase activity and they inhibited the glucagon mediated increase of the blood glucose level.

Uric acid in rat spinal cord in chronic relapsing experimental allergic encephalomyelitis (CR-EAE)

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Many groups have shown that the blood brain barrier (BBB) permeability is increased in EAE. Haellgren et al. (Stroke *14* [1983] 382) suggest that urate is a marker of BBB dysfunction. Uric acid (UR) was therefore determined by HPLC in nine regions of Lewis rat spinal cord during the first attack (days 13–15 p.i.) and recovery (days 19–20) in CR-EAE and adjuvant controls. Not all rats developed paralysis after EAE induction. In those with paralysis (group 1) UR was greatly elevated in all regions during the attack. In about half of those without paralysis UR was slightly elevated in all regions (group 2), whereas in the rest and in controls UR was not measurable. In both groups 1 and 2 distribution varied along the spinal cord. Values were greatest between T3 and L3 (maximum twice the lowest value) indicating a regional predilection for BBB disturbance. During recovery of group 1, UR values dropped markedly to a slight residual level in all regions. These results suggest a relationship between the UR level (degree of BBB impairment) and clinical symptoms in EAE (SMSG grant).

Nonenzymatic glycosylation (glycation) of proteins: the principal sites of in vitro glycation of RNase A

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The specificity of glycation observed with hemoglobin and albumin is surprising and not understood. In order to find a rational for this site specificity RNase A was glycated and the principal sites of glycation elucidated.

RNase A was incubated with 1M glucose in phosphate buffer at pH 7.4 for four days at 37°C. The glucose-adducts were labeled by reduction with NaBH4*. The denatured, reduced and alkylated protein was subjected to tryptic digestion and the glycated peptides isolated by boronate affinity chromatography. The peptides were separated by HPLC, hydrolyzed, and the amino acid composition determined. Four glycated sites were identified: lys 7, lys 37, lys 41, and lys 98.

Glycation of RNase A decreases its enzymatic activity, which is explained by modification of lys 41 in the active site. In the absence of phosphate glycation proceeds to a lesser extent and does not affect enzymatic activity. Phosphate therefore seems to favor glycation at certain sites.

The role of fusion in the bifunctional enzyme PRAI:InGPS from *E. coli*

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The bifunctional enzyme phosphoribosyl anthranilate isomerase:indole glycerol phosphate synthase (PRAI:InGPS) is the penultimate enzyme in tryptophan biosynthesis. The denaturation and kinetics of the enzyme have previously been studied. Recently, the structure has been solved. In order to explore the possibility of functional interactions between the two domains, we have constructed three mutants. Two correspond to the individual domains and the third has an insertion between them (extended fusion). The isolated domains show reduced activity, but the extended fusion has intermediate activity. The insertion exposes residues susceptible to trypsinolysis. Upon cleavage, the synthase activity is stable, with a reduction in activity comparable to the isolated domain. (Isomerase is degraded). The results will be discussed with reference to the structure and kinetic properties of the wild type enzyme.

A new bioprocess for the selective hydroxylation of nicotinic acid

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The nicotinic acid can be selectively hydroxylated at position 6 by several microorganisms which have been isolated from the soil (sp. *Achromobacter, Pseudomonas*).

After a fermentation under optimized conditions the biomass can be used directly for the biohydroxylation of nicotinate. Under mild conditions (30°C, pH 7) the yield of 6-hydroxynicotinic acid is above 90% and the purity of the crude product exceeds 99%.

In comparison with chemical processes the bioprocess is much more efficient and economical for the production of 6-hydroxynicotinic acid on an industrial scale.

Localization of fibrin polymerization site by photoaffinity labeling

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The polymerization of fibrin is competitively inhibited by the tetrapeptide GlyProArgPro. This peptide is identical with the new N-terminal sequence of the α-chain exposed after fibrinopeptide A release by thrombin. In order to identify the location of the polymerisation site, an azido derivative of the GlyProArg was synthetized. The photoactive affinity label was obtained by reacting t-BOCGlyProArgProLysmethylester with p-azidoazobenzene-4-isothiocyanate in pyridine. Human fibrinogen was incubated with the peptide in the dark. Photolysis was then carried out at 0 °C. Photoaffinity labeled fibrinogen was reduced and alkylated, the polypeptide chains (α, β, γ) were separated on Mono S, a strong cation exchanger. Photolabel incorporation was detected immunologically using a anti-azobenzene-antibody (ELISA). A positive response was obtained with the fibrinogen γ-chain only. It is concluded that the binding site of GlyProArg is located on the γ-chain.

The Na^+/Ca^{2+} exchanger of calf heart sarcolemma: isolation, reconstitution, and partial purification

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The Na^+/Ca^{2+} exchanger of plasma membranes has been tentatively identified in different laboratories as a protein of M_r 82 kD, 70 kD, or 33 kD. This report describes an isolation procedure for the 33 kD protein of calf heart sarcolemma (HSL) with partial retention of exchange activity. Calf HSL was solubilized in the SDS electrophoresis (S-PAGE) sample buffer in the absence of reducing agents. SDS was then extracted with acetone. The protein-phospholipid aggregate was treated with 6 M guanidinium-HCl, cholate and phospholipids were added and vesicles were reconstituted by cholate dialysis. They had Na^+/Ca^{2+} exchange (2–3 nmoles Ca^{2+}/mg/s). On S-PAGE they contained essentially one protein of M_r 33 kD. However, S-PAGE under non reducing conditions yielded only one protein M_r 140 kD. Rabbit-antibodies (RAb) raised with homogenates of the 33 kD S-PAGE region, and fractionated by interaction with different regions of Western blots of HSL-S-PAGE were tested on HSL. Maximal (50%) inhibition was seen with the RAb isolated from the 140 and/or the 33 kD regions. The data indicate that the exchanger of calf HSL is indeed a protein of M_r 33 kD, and suggests that its active from may be a tetramer of M_r 140 kD.

Blood components modulate lymphocyte activation in hypergravity

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We are investigating whole blood cultures of lymphocytes from crew members of two Spacelab missions (D-1 in 1985 and SLS-1 in 1987). When whole blood cultures of lymphocytes (obtained by diluting fresh blood with medium 1:10) are exposed to concanavalin A in hypergravity at 10 × g, a dramatic increase of activation (up to 500% of the control) is observed. The effect is less evident in cultures of purified lymphocytes (up to 130% of the control). In whole blood cultures a similar increase can be induced by pre-incubating the cultures at 10 × g for 2 days prior to exposure to concanavalin A at 1 × g for three days. The influence of autologous plasma and erythrocytes has been investigated: Plasma and hypergravity have a synergistic positive effect on lymphocyte activation, i.e. cultures of separated lymphocytes show the highest attainable activation when incubated at 10 × g and supplemented with autologous plasma. Conversely erythrocytes have a depressing effect on lymphocyte stimulation.

Isolation of the type I insulin-like growth factor (IGF) receptor from human placenta

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The type I IGF receptor exhibits a strong structural homology with the insulin receptor but is not identical. All studies on type I receptor have been made so far either in situ or in crude solubilized preparations.

We have been able to purify the type I IGF receptor from solubilized human placental membranes (Triton X-100) by gel chromatography (Sephacryl S-400) followed by affinity chromatography of IGF I sepharose. The purity of the receptor preparation was shown by SDS-gel electrophoresis. On reducing gels two main bands are found (silverstaining) of M_r 125 kD and 95 kD, very similar to the insulin receptor subunits. The binding affinities of pure type I receptor for IGF I, IGF II and insulin have been determined.

Proenkephalin processing in chromaffin granules: low-density immature granules are enriched in processing activity

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Differential ultracentrifugation of adrenomedullary cell granules allows to isolate a subpopulation of chromaffin granules (about 20% of the total pellet) at the interface of two sucrose layers, 1.6 M and 1.8 M, respectively. The lyzed fraction when assayed with the synthetic proenkephalin precursor fragments Ala-Lys-Arg-Tyr and Try-Gly-Gly-Phe-Met-Arg-Arg-Val-Gly-Arg-Pro-Glu, is shown to be enriched about ten times in processing activity compared to the total population of granules. The enhanced proteolytic activity is not due to lysosomal contaminations which are low and not significantly increased in the more active fraction. The result confirms the current view that hormone precursor processing mainly occurs in (low-density) immature granules and implies an inactivation mechanism of the processing enzymes during granules maturation. Furthermore, the enriched fraction is a convenient starting material for the isolation of prohormone processing enzymes.

Evolutionary changes of myelin composition

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In all Gnathostomata studied, myelin contained the so-called myelin-associated glycoprotein and myelin basic protein. In Elasmobranchii and Chondrostei, CNS and PNS myelin proteins were similar. In PNS myelin, the only major phylogenetic event was the addition of P_2 protein from the reptiles onward. A protein with a M_r of 36 kD (36K) was identified in Holostei and Teleostei CNS myelin. In tetrapods, the glycoprotein P_0 was replaced in CNS myelin by the proteolipid protein (PLP) and Wolfgram protein. In *Protopterus*, P_0 and 36K were absent, but a glycosylated form of PLP was the major protein of CNS myelin. This attests to a close relationship between Dipnoi and Amphibia. Also interesting was the simultaneous presence in *Polypterus* of P_0 and 36 K with PLP. This suggests that polypterids have an unusual position between fishes and tetrapods. These results indicate that myelin proteins could be valuable evolutionary markers. In contrast, myelin lipids varied consider-

ably even within a given order making their use for phyletic reconstruction doubtful.

Effect of minor cartilage collagens on type II collagen fibril formation

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Fibril formation of neutral salt extracted type II collagen was studied in the presence and absence of type IX collagen in vitro. Type II collagen alone formed D-periodic fibrils with a broad diameter distribution, ranging from 150 to 2000 nm. These fibrils appeared as the needle-like tactoids as earlier described by Lee et al. (Collagen rel. Res. *3/2* (1983) 89). A mixture of type II and type IX collagen formed fibrils with a uniform diameter of about 15 nm. They appeared as very long, thin fibrils and where poorly banded. When the concentration of type IX collagen was lowered a critical concentration was found at which, beside the thin uniform fibrils, large wide diameter, D-periodic fibrils (as seen in the type II-experiment) appeared again.
These results support the hypothesis that type IX collagen participates in regulation of type II collagen fibril diameter.

Endocytosis of fluorescein labeled influenza virus

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Influenza virus (A/PR/8/34) was labeled with fluorescein isothiocyanate at a labeling ratio of 100:1. Most of the FITC associated with the viral envelope membrane but 40% was bound to the surface gylcoprotein hemagglutinin (HA) and to the neuraminidase. The labeled virus retained 100% of the hemagglutination titer, 100% of the infectivity and 86% of the hemolytic activity induced by low pH. The labeling procedure did not change the electron microscopic appearance of the viral spike proteins. Adsorption and penetration of labeled virus on MDCK-cells were monitored by fluorescence microscopy with monoclonal anti-HA antibodies. At 0°C, adsorption of virus particles to cells resulted in a disperse distribution of faint fluorescence spots on the cell surface. Partial or complete internalization of virus was induced by incubation of cells at 20°C or at 37°C. By a reaction of the FITC-labeled virus particles with anti-HA antibodies, it was possible to study the fate of the virus in the infectious endosomal pathway.

Crystal structure of neutral protease from *B. cereus*

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The amino-acid sequence of the title enzyme has been determined (Sidler et al., Experientia *41* (1985) 811) and exhibits a 74% homology to thermolysin. Despite this homology the title enzyme is 20°C less thermostable. A high resolution structure of thermolysin is available (Holmes and Matthews, J. molec. Biol. *160* (1982) 623) and was used to solve the neutral protease structure by molecular replacement methods. The arrangement of the molecules in the unit cell (a = b = 76.5, c = 201 Å; $P6_522$) and the intermolecular contacts of the solution (R = 53%) are very reasonable. Rigid-body refinement, replacement of the thermolysin sidechains by those of neutral protease, and rebuilding the model into 'omit-maps' on a graphics display system lowered the R-factor to 49, 44 and 42%, respectively. Hendrickson-Konnert refinement is currently in progress using 3.0 Å oscillation data. When refinement is complete, the molecule will be compared to thermolysin.

Characterization of the polysialic acid capsule in *Escherichia coli* K1 mutants

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Polysialic acid chains of similar chemical and antigenic structure occur in the neural cell adhesion molecule N-CAM and on the surface of two bacteria causing human meningitis, *Escherichia coli* K1 and *Neisseria meningitidis* group B. In order to study the role of polysialic acid in bacterial meningitis, *E. coli* K1 mutants defective in expressing their capsules were isolated by using a K1 specific bacteriophage. The independent 58 mutants were divided into eight phenotypic groups according to their total and neuraminidase sensitive sialic acid content as well as their agglutination by K1 specific antibody. Immunofluorescent staining patterns were identical within each phenotypic group and revealed intracellular accumulations of the antigen in some strains. The localization of capsular sialic acid in the mutants is further studied by electron microscopy including immunolabeling with protein A-conjugated gold. A novel electrophoresis procedure to determine the length of the polysialic acid chains is being developed.

Polyol pattern and chemosystematics of the Eumycetes

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Acyclic polyols occur in almost all higher taxa of the Eumycetes, are the fungal metabolites studied most widely, and appear to be of physiological significance. All data on the systematic distribution of polyols (comprising nearly 600 entries concerning 400 different species) have been compiled for a taxonomic evaluation, using an IBM PC/Lotus Symphony (1985). When delimiting taxa on account of the characters P_0 (no polyols present), P_1 (polyols, but not mannitol present), and P_2 (mannitol present), the three groups coincide with the Oomycetes, the Zygo- and Hemiascomycetes, and the Chytridio-, Euasco-, Deutero-, and Basidiomycetes, respectively, as defined by classical criteria. This chemotaxonomic classification of the fungi agrees with group formations made earlier with respect to the lysine biosynthetic pathway, the sedimentation pattern of enzymes of tryptophan biogenesis, and the cell wall composition. Thus, the polyol pattern represents a marker of high predictive value for both the identification as well as the unraveling of evolutionary relationships within the fungi.

The crystal structure of the bifunctional enzyme N-5′-(phosphoribosyl) anthranilate isomerase: indole glycerolphosphate synthase (PRAI:IGPS) from *E. coli*

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PRAI:IGPS from *E. coli* is a monomeric bifunctional enzyme of M_r 49,500 catalyzing the two penultimate reactions in the biosynthesis of tryptophan. In other organisms these reactions are catalyzed by two separate enzymes or by a multienzyme complex. Using X-ray crystallographic techniques we have solved the 3-dimensional structure to 3.2 Å resolution. The molecule is clearly divided into two separate structural domains with the division at residue 255. The folding of both domains is very similar, consisting of an 8-stranded parallel β-barrel with α-helices connecting the β-strands. The N-terminal domain (the synthase) also possesses a long N-terminal α-helix and a long stretch of random coil. Least squares refinement of the structure has been started.

Enzymic manipulations of protein fragments in cytochrome *c* semisynthesis

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A number of complementary two-fragment systems of cytochrome *c* are known, some of which have considerable biological activity. With certain proteins it has been found that if such systems are enzyme generated, then reverse proteolysis can be used to recouple the fragments. This phenomenon is potentially of great value in the semisynthesis of protein analogues.
We have not found this to be possible with the trypsin-generated 1-38:39-109 complex, but we have used both the proteolytic and synthetic properties of enzymes to manipulate fragments in useful ways. One approach has been to prepare analogues of functional complexes for structure-function studies; another has been to prepare derivatives of one of the fragments that are activated, and thus have the potential to couple to the other under appropriate conditions.

Calmodulin regulation of human platelet adenylate cyclase

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The mechanism of calmodulin dependent regulation of adenylate cyclase from human platelet membranes has been studied. Calmodulin activated adenylate cyclase was biphasically regulated by both Ca^{2+} and Mg^{2+}. The V_{max} of calmodulin dependent platelet adenylate cyclase depended on the relative concentrations of Mg^{2+} and Ca^{2+}. Calmodulin stimulated adenylate cyclase activity increased 2-fold above respective levels of activity induced by either GTP, Gpp(NH)p and/or PGE_1. The stimulatory influence of calmodulin was not additive but synergistic to the effects of PGE_1, GTP and Gpp(NH)p. GDPβS inhibited GTP- and Gpp(NH)p stimulation of adenylate cyclase but was without effect on calmodulin stimulation. Since the inhibitory effects of GDPβS have been ascribed to apparent reduction of active N-protein-catalytic unit (C) complex formation, these results suggest that the magnitude of calmodulin dependent adenylate cyclase activity is proportional to the number of N-protein-C complexes.

Electron transfer by sulfite oxidase from chicken liver

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Sulfite oxidase catalyzes the oxidation of toxic sulfite to innocuous sulfate. The physiological electron acceptor is cytochrome *c*. Binding experiments indicate two equal and non-interacting binding sites for cytochrome *c* per sulfite oxidase monomer, i.e. four sites per active dimer. Steady state kinetics are monophasic. Carboxyl groups are essential for activity. This is shown by chemical modification with carbodiimides and Woodward's reagent K. The carboxyls' role in binding of cytochrome *c* is not yet clear. Modification with diethylpyrocarbonate inactivates sulfite oxidase; the activity can be restored with hydroxylamine. Ten histidines are modified, but only one of them is responsible for inactivation. This residue seems to be essential for electron transfer.

Effects of neurotropic virus on transmitter synthesis in cell culture

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A rat pheochromocytoma cell line, PC12, has extensively been used as a model system for sympathetic neurons, since in the presence of NGF they sprout neurites and develop catecholaminergic characteristiques. Infection of cultures of dissociated rat superior cervical ganglion cells with Herpes virus suis (HVS) causes a rapid, transient increase in tyrosine hydroxylase (TH) activity between 45 min and 180 min p.i. The concentration of norepinephrine increases accordingly. In contrast to these results no early effect was found in PC12 cells. However, at about 9 h p.i. a net increase in TH activity of 150% the control value is noted. While the cells incubated in DMEM without any additives lower their TH activity after 15 h, the infected cultures maintain their level at about 120% of the original value up to 24 h p.i. The possibility of a trophic action of the infection with HVS will be discussed, due either to the virus itself or the infection medium.

Monoclonal antibodies used for the characterization of human serum acetylcholinesterase

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Normal human serum contains minute amounts of acetylcholinesterase (AChE) EC 3.1.1.7, however, a determination of the kinetic parameters of this enzyme is not possible due to the presence of about 1000-fold excess of butyrylcholinesterase (BChE) EC 3.1.1.8, which has overlapping substrate and inhibitor specificity. The low concentration has not allowed a purification with conventional means. We have produced monoclonal antibodies against AChE, and used them for characterization of the serum AChE. The antibodies do not cross-react with BChE, and could be used in a solid phase immuno-assay, in which the bound AChE could be enzymatically characterized. The kinetic parameters K_m and K_{is} were determined to be 199 µmoles/l and 27 mmoles/l respectively. The enzyme was inhibited by BW284C51 but not so by Lysivane. The kinetic parameters and inhibition patterns are similar to those determined for erythrocyte membrane AChE and brain AChE.

Stereospecific labilization of the C4′-pro-S hydrogen of pyridoxamine-5′-phosphate by aspartate aminotransferase

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In the reverse half reaction of enzymic transamination a proton is removed from the pro-S position at C4′ of the pyridoxamine-5′-P (PMP) moiety of the ketimine intermediate. The present study shows that the pro-S hydrogen at C4′ of PMP is labilized by its active site environment independently of the formation of the ketimine intermediate, i.e., in the absence of substrate. Reconstitution of apoaspartate aminotransferase with 4′-[^{3}H] PMP resulted in a stereospecific exchange of the pro-S ^{3}H with solvent water. The [^{3}H] exchange follows first order kinetics ($t_{½}$ = 23 min at pH 7.5, 25 °C). Unbound PMP showed no measurable exchange. Rigorous control experiments excluded the possibility of a transamination reaction of the enzyme with contaminating oxo acid substrates. The observed exchange of pro-S [^{3}H] of PMP procures and experimental model for the study of the protonation/deprotonation at C4′ of the ketimine intermediate.

X-Ray crystallographic studies on the mechanism of action of mitochondrial aspartate aminotransferase (mAAT)

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mAAT is a pyridoxal phosphate dependent, dimeric enzyme of $M_r \simeq 90{,}000$ which functions in amino acid metabolism. It catalyzed the reaction aspartate + 2-oxoglutarate⇌glutamate + oxaloacetate, shuttling between its pyridoxal and pyridoxamine forms and undergoing conformational changes in the process. Crystal structures at 2.3–2.8 Å resolution have been determined for the enzyme and several derivatives which mimic or correspond to intermediates in the catalytic pathway. Recent structure refinement results of the holoenzyme and an enzyme-maleate complex have enabled substantially improved models of the active site region for all derivatives to be built. These, in turn, have revealed a more detailed picture of the mechanism of action of mAAT than has hitherto been reported.

Physical and biological properties of plant mitochondrial cytochromes *c*

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Cytochrome *c* is the best known of all the redox carriers of the mitochondrial transport system. Comparisons between the cytochromes of many different species, of both amino acid sequences and of functional properties, have been used in studies of mechanism and structurefunction relationships. These comparisons have proved useful in elucidating the contributions of specific amino acid residues or regions of the structure. However, though many cytochromes *c* have been isolated from higher plants and sequenced, until now no detailed examination of their properties has been undertaken. This paper presents the results of a study of the cytochromes of *Acer* and *Bixa*.

Morphological and biochemical effects of phorbol esters on rat optic nerve

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Our interest in the involvement of protein kinase C in the phosphorylation of myelin basic protein led us to examine the effects of phorbol esters on intact rat optic nerve incubated in vitro. We report that the biologically active 4β-phorbol 12-myristate 13-acetate (PMA) induces pronounced changes in the axonal cytoskeleton. This reorganization of the cytoskeleton is associated with a marked reduction in axonal caliber such that the axon becomes totally separated from the surrounding myelin sheath. These effects are observed after only 30 min incubation in the presence of 100 nM PMA and are not seen in the presence of a biologically inactive phorbol diester. In an effort to define biochemical correlates of these changes we have initiated studies of PMA induced protein phosphorylation. Specific changes in the phosphorylation of a number of proteins including myelin basic protein have been identified. For the most part, however, these changes are transient and the time-course does not relate to that of the morphological changes.

Domain organization of ClInh

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The highly glycosylated plasmaprotein ClInH binds to the 1st component of human complement Cl and inhibits its activation. Electronmicroscopy and chemical analysis of ClInh and some proteolytic fragments are consistent with the following model: ClInh consists of two domains. The globular domain (0 = 4.5 nm, 50 kD) contains the active site and comprises the C-terminal part of ClInh. The N-terminal rodlike domain (35 nm length) has a proteoglycan like structure: most of the carbohydrate of ClInh (90%) is attached to an elongated, serine, threonine and proline rich peptide chain. The N-terminal sequence of ClInh is known. We found that this part is homologous to the extracellular domain of glycophorin A and B. This is most pronounced for the serine and threonine residues.

Proteolytic cleavage of ClInh at the active site results in a conformational rearrangement of its globular domain and an increased stability against denaturation with guanidine as a consequence. The transition shifts from 3 M (intact ClInh) to 6 M guanidine (modified ClInh). This reflects most probably a similar rearrangement as upon modification of α_1 proteinase inhibitor.

Two forms of bovine growth hormone

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Growth hormone from bovine pituitaries, even after extensive purification under mild conditions, shows two bands on isoelectric focussing (Bell, J.A. et al., J. biol. Chem. *260* (1985) 8520). The intensities of the bands are in the approximate ratio 3:2. The charge difference suggested by the isoelectric focussing experiment is considerably less than that to be expected from, say, the loss of a side chain amide. We have separated the two forms and find that they have a small difference in apparent mol.wt upon polyacrylamide gel electrophoresis in the presence of SDS. We have determined the N- and C-terminal sequences of the two forms. Both forms have the same C-terminal sequence whilst the form present in greater amount lacks the first four residues of the form present in lesser amount.

Involvement of glycoprotein (GP) Ib in activation of platelets by thrombin

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Polyclonal antibodies against the 45 kD globular region of GPIb and anti-glycocalicin antibodies, inhibit binding of von Willebrand factor to platelets and also partly hinhibit binding of thrombin. Elastase treatment, which removes the 45 kD part of GPIb, also reduces binding of thrombin. Active site inhibited thrombin binds to the same extent as active thrombin. Glycocalicin binds to inhibited thrombin coupled to Sepharose implying that the binding site on thrombin for glycocalicin is different from the active site. Pretreatment of control and elastase-treated platelets with inhibited thrombin before thrombin and collagen activation results in a shorter lag phase. Inhibited thrombin did not affect the composition of detergent-insoluble platelet cytoskeleton. These results indicate that while GPIb undoubtedly plays a role in the kinetics of platelet activation it is not the essential receptor for thrombin. It remains unclear how inactivated thrombin influences the activation kinetics via GPIb.

Chemiluminescence determination of H_2O_2 released during the respiratory burst of human neutrophils

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We present a very sensitive method for the determination of H_2O_2 by luminol-dependent chemiluminescence (CL) and illus-

trate its use for the real-time measurement of the respiratory burst of phagocytic cells. A single photoncounting apparatus with temperature control, strirring, injection-device and a time-resolution of 100 ms was constructed. Measurement of the respiratory burst of human neutrophils stimulated with the chemotactic peptide fMLP showed excellent agreement between the integrated CL curve and the progress curve of H_2O_2-release determined fluorometrically. High sensitivity and fast response allowed accurate determination of the onset of H_2O_2-production by stimulated human neutrophils. H_2O_2 was already detectable within 2 s of fMLP addition and was independent on the fMLP concentration, indicating that the transduction-time of receptor mediated respiratory burst is much shorter than generally believed. A longer and concentration dependent onset time was observed with the protein-C kinase activator PMA.

Cell and Molecular Biology

Proteoglycans in the extracellular matrix of normal and neoplastic human mammary tissues

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The quantitative distribution and the biochemical composition of proteoglycans (PG) in the extracellular matrix of normal and neoplastic human mammary tissues was analyzed. PG were morphometrically evaluated on electron microscope pictures (× 77,000). The stereological analysis revealed that the density of PG per unit of surface area in the dense connective zone was roughly two time higher in normal than in malignant tissues. In both types of tissues, the loose reticular zone displayed a significant lower PG density that the dense connective zone, but in malignant tissues the PG/cm^2 of the former layer was seven time lower than in the normal tissues. However, in the malignant tissues the mean lenght of PG was two to three time higher than in the normal tissues. Biochemical data, obtained from CsCl gradient centrifugation and chromatographic separation, showed that PG from neoplastic tissues are larger than that of normal tissues.

Two transcripts of the ecdysterone regulated gene I-18C of *Chironomus tentans*

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In salivary glands of *Chironomus tentans* the molting hormone ecdysterone induces a prominent puff at locus I-18C. We have cloned a 16 kb genomic DNA sequence of this region which gives rise to two different transcripts (4.6 kb and 1.8 kb). Both sequence and Northern analysis reveal that the small transcript is located in an intron of the large one. The synthesis of both is stimulated by ecdysterone. While in salivary gland cells only the large transcript is found, both transcripts are present in an epithelial cell line. The 1.8 kb transcript is found predominantly in the polysomal fraction and it is RNase-sensitive. On the other hand the 4.6 kb transcript is not associated with the polysomes but exists in a relatively RNase-resistent form in both cell types.

Genetic rearrangements following the insertion of ampicillin transposons into the plasmid DNA of *N. gonorrhoeae*

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Penicillinase producing *N. gonorrhoeae* (PPNG) isolated throughout the world harbor a 7.3 kb resistance plasmid or its 5.1 kb deletion derivative: both elements contain 40% of the transposon Tn*A*. In order to better understand the emergence of such plasmids and to establish whether these can originate from multiple events of Tn*A* insertions into a specific site of a core plasmid, we constructed *bla*$^-$ derivatives of the PPNG resistance elements. Transposition experiments performed in *E. coli* showed indeed that these recombinant plasmids have a hotspot insertion site for the Tn*A* transposons. Furthermore, when introduced into *N. gonorrhoeae* by transformation or conjugation, these plasmids originated undistinguishable derivatives, characterized by specific genetic rearrangements and deletions in the Tn*A* element and the gonococcal DNA. These results can be related to the observation that no complete functional Tn*3* transposons could ever be found in gonococci and support the hypothesis that the spread of the gonococcal resistance plasmids might be due to multiple but specific Tn*A* insertional events in a unique core plasmid.

Visible light induces cell morphology changes – an interference-reflection-contrast microscopy (IRM) study

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In a study on cell locomotion (J. Cell Sci. *52* (1981) 289) we investigated *Xenopus laevis* primary cultured cells by IRM. For determination of cell thickness changes (Microsc. Acta *84* (1981) 153) we used the 436, 546 and 577/79 nm lines of a HBO 50, respectively. The intensity was diminished by a ring-shaped aperture resulting in a mean angle of incidence of 21° and adjusted to 0.1 W/cm^2 by neutral density filters; two heat adsorbing filters were used. In the case of blue light a dose of 10 J/cm^2 results in a retraction and later in a detachment of the thin cell margin; 50 J/cm^2 destroyed the cells irreversibly. Observation by green and orange light showed no significant effects during the first 30 min. Then at green light cell spreading becomes slower and the lamellipodia locked crisped. In comparison to phase contrast observations orange light showed no morphological differences up to a dose larger than 700 J/cm^2. Antioxidants like vitamin E and selenium could reduce the damaging irradiation effects.

Expression of cloned cDNA encoding the precursor of mitochondrial aspartate aminotransferase

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Chicken mitochondrial aspartate aminotransferase is synthesized as a precursor (pre-mAspAT) in the cytosol which is post-translationally imported into mitochondria. Three overlapping cDNA segments encoding the entire precursor were used to construct a full-length cDNA in the vector pSP65. A restriction fragment from the 5′ end containing 19 noncoding bp and the DNA encoding the 22 residues of the prepiece as well as the residues up to Thr 80 or the mature protein was ligated downstream of the Sp6 promoter. Two restriction fragments with compatible ends spanning Arg 81 to Val 198 and Asp 199 to Lys 410 were introduced at the 3′ end of the first fragment. This construction (pSP65-premAspAT) was linearized with SAlI and transcribed in vitro. The transscript is being used for in vitro translation studies. In addition, the cDNA from pSP65-premAspAT was inserted into pASl and promotes production of preAspAT in *E. coli*.

Functional analysis of a Vaccinia virus late promoter

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We have recently mapped a sequenced a Vaccinia virus late gene encoding a major structural polypeptide and shown, that 100 bp of its 5'-flanking sequence are sufficient to temporally regulate late gene expression. A large number of mutations, including 5' and 3' deletions, as well as insertions have now been introduced into this DNA. Their effects were studied by CAT assays and by nuclease S_1 analysis both in a transient expression system and in Vaccinia virus recombinants. The results ca be summarized as follows. 1) 5' deletions up to about 15 bp from the initiation site of transcription do not interfer with late gene expression. 2) all mutations that include the mRNA start site abolish late gene expression. A possible mechanism of Vaccinia virus late gene transcription that accounts for these unexpected findings will be discussed.

Morphofunctional changes in the thyroid and pituitary gland of rats with experimental diabetes

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Streptozotocin-diabetes in rats is associated with hypoactivity of the hypothalamo-pituitary-thyroid axis. Structure and hormonal secretion of thyroid and pituitary gland of adult male rats were studied one month after induction of diabetes. Compared to controls, the thyroid of diabetic rats showed a decreased follicle area and epithelium thickness ($p < 0.01$); by electron microscopy, RER cisternae were empty and degenerate, exocytotic apical and endocytotic colloid vesicles scanty, mitochondria swollen; by immunohistochemistry, intracolloidal and intraepithelial thyroglobulin and intracolloidal T3 were reduced ($p < 0.025$). In the pituitary gland thyrotrophs were more frequent ($p < 0.001$) and filled by TSH granules. In diabetic rats plasma T3, T4 and TSH were reduced ($p < 0.001$). TSH response to TRH was blunted ($p < 0.02$), and pituitary TSH concentration increased ($p < 0.02$). This study demonstrates a relationship between structural and functional disturbances of thyroid and pituitary secretion in our animal model.

Chromatin structure upstream of the *Dictyostelium discoideum* rRNA gene

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We have analyzed the chromatin structure of the rRNA gene in its transcriptional active and inactive state. We found three MNase and DNase I hypersensitive sites (HsS) −200, −1500 and − 2300 bp upstream of the transcriptional start site when the gene is inactive. The − 200 bp HsS contains topoisomerase I consensus sequences (Bonven et al., Cell *41* (1984)), the − 2300 bp HsS is located at a structural transition point.

Accumulated mutations in the measles virus genome correlate with subacute sclerosing panencephalitis (SSPE)

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SSPE is a fatal disease affecting the human central nervous system several years after acute measles virus (MV) infection. We have previously found by Northern blotting and in vitro translation of brain RNA from several cases of SSPE that the replicating MV genomes differed substantially from each other; each showed multiple defects in the formation and/or functionality of various viral mRNA species. Cloning and sequencing of the matrix gene and other smaller MV genome regions revealed that the nucleotide sequences varied by 1–2 orders of magnitude more in one SSPE case than in stocks of nondefective MV. This suggests that error-prone replication might be one of the features associated with, and possibly required for, development of SSPE.

Visualization of tissue-specific proteins binding to regulatory sequences of immunoglobulin genes

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In our search for tissue-specific proteins involved in the regulation of immunoglobulin gene transcription, we have started to analyze complexes formed in vitro by incubating nuclear extracts from mouse hybridoma cells with cloned segments of mouse Ig heavy chain genes. Purified complexes made under transcription initiation conditions were visualized in the electron microscope. We have mapped proteins binding specifically to the promotor region, to the coding region of the Igμ variable region gene, and to the heavy chain enhancer region. Only few specific complexes are observed in parallel experiments carried out with nuclear extracts from 3T3 cells. Protein binding seems to induce bending or kinking in the DNA and results in a shortening of DNA segments. Further structural details of transcription complexes will be discussed.

Functional analysis of the glucocorticoid regulatory region of mouse mammary tumor virus (MMTV) DNA

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Mutant DNA molecules in the glucocorticoid regulatory region of the MMTV LTR were produced and tested by quantitative S1 mapping in stable and transient transfection experiments. The hormone response was lost when both in vitro binding sites were removed; it was reduced to ~ 10% when either the distal or the proximal one were deleted. Replacement of the proximal region with a synthetic distal one restored hormone stimulation in certain configurations. In the distal region, linkerscanning mutants with reduced hormone response (10–20% of wt) mapped in a conserved hexanucleotide (- 177/- 173) or 5' of it (- 185/ − 177). No reduction was found with mutations located between the two binding sites. In the proximal region, a reduction was seen in some mutants, not in others. Our data show a functional contribution of multiple sequence elements contained in regions that bind the receptor in vitro.

Cell-type-specific expression of fusion genes containing mouse albumin promoter sequences

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Fusion genes, containing mouse albumin promoter sequences linked to the bacterial indicator gene coding for chloramphenicol acetyl transferase (CAT), were used for transient and stable transfection studies in two hepatoma cell lines and L-cells. While extracts from transfected rat hepatoma cells (FAZA) contain considerable CAT enzyme activity, those from transfected mouse hepatoma cells (BW 1) or L-cells do not. Since BW 1 cells and FAZA cells have fetal and adult phenotypes, respectively, the above observation suggests that albumin expression is regulated differently in fetal and adult hepatocytes. This interpretation appears more likely than an alternative one, namely that FAZA cells contain more positive factors required for albu-

min expression than BW 1 cells. Indeed, the latter cells transcribe the endogenous albumin gene more efficiently than the former. Recently we succeeded in preparing highly active in vitro transcription extracts from adult rat liver nuclei. These extracts utilize the albumin promoter significantly more efficiently than the one of the Adeno virus major late transcription unit. We are currently studying whether the efficient in vitro transcription from the albumin promoter is cell-type-specific.

Transcripts and transcription signals of measles and SSPE viruses

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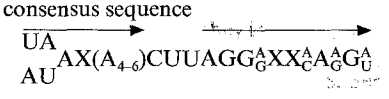
RNA viruses of the families paramyxoviridae and rhabdoviridae synthesize their mRNAs from a linear minus strand genome of about 15 kb. The viral polymerase complex recognizes so-called intercistronic sequences (ICS) at which it processes and polyadenylates the viral mRNAs. ICS sequences of negative stranded RNA viruses are generally strictly conserved, and RNA processing at ICS is usually very efficient. We have cloned and sequenced 4 of the 5 ICS of measles virus (MV), the medically most important paramyxovirus. The MV ICS are not as well conserved as the ICS of other negative stranded viruses and contain the consensus sequence



(plus strand). We have also studied the mRNAs of MV and of the MV-related SSPE viruses. In the lytic infection 5–10% of the MV mRNAs are present as unprocessed bi- or tricistronic transcripts. In one SSPE brain and one SSPE cell line, bicistronic transcripts replace two monocistronic mRNAs.

Myosin heavy chain (MHC) transitions during regeneration of chicken breast muscle (PM): the neonatal and adult isoforms are expressed independant of the nerve

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In order to investigate the role of nerve on regulation of fast myosin isoforms we studied MHC expression in regenerating myotubes which appeared following cold injury in both, normally innervated and three week denervated chicken PM. MHC isoforms were determined by staining cryosections of the regenerating areas with monoclonal antibodies specific for embryonic and adult MHC (EB165), neonatal MHC (2E9) or adult MHC (AB8). At seven days of regeneration in both, normal and denervated muscle a rim of cells just outside of the necrotic area reacted with 2E9, indicating that the expression of neonatal MHC is independant of innervation. The same cells also reacted with AB8, suggesting that the adult MHC appears at a very similar timepoint as neonatal MHC and is also independant of innervation. Since 2E9 reactivity eventually disappeared in both, normal and denervated PM, the suppression of neonatal MHC is independant of the nerve as well.

Human parvovirus B19: identification of a promoter by transcription in vitro of cloned DNA

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The parvoviruses are small, with a 5 kb linear single-stranded DNA genome. Those which infect vertebrates fall into two distinct groups, the defective dependoviruses, and the autonomous parvoviruses. The human parvovirus B19 shares structural features with the two groups. B19 is responsible for aplastic crises in anemics and is the etiologic agent for erythema infectiosum. Till now B19 resisted all attempts to propagate it in tissue culture.

The viral DNA, cloned by Tattersall and al. from a viremic serum, was transferred to the bacterial vector pEMBL. In vitro transcription of several restriction fragments, using a HeLa cell nuclear extract, has allowed us to map a single promoter close to the left end of the genome. This result fits with the presence of a TATA box in the DNA sequence (Astell et al., unpublished). Since all other parvoviruses studied so far have one or two promoters in addition to the one near the left end, it is possible that B19 also has additional promoters which require for expression transactivation by a viral gene product.

Immunological labeling of trichocyst proteins in 2 protozoan cells

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Polyclonal antibodies were produced in rabbits against two groups of *Pseudomicrothorax dubius* trichocyst proteins isolated by SDS-PAGE followed by electroelution: G1 contains two bands at 29 and 30 kD, and G3 shows at least 6 bands at 16–20 kD. On Western blots of isolated trichocysts, anti-G1 reacts with G1, and anti-G3 with G3, as well as with 41, 43, 36, 37 and 26 kD bands. On Western blots of entire cells, the same bands react with anti-G3, but the 43 kD is more strongly labeled, and on cells without trichocysts, anti-G3 recognized the 43 kD only: 43 kD is therefore a trichocyst protein much more abundant in the cytoplasm than in the trichocysts. The results obtained on transfer of unreduced samples suggest that the trichocyst matrix might contain at least one protein < 20 kD and several dimer proteins of 33–36 kD. Both antisera reveal several proteins on Western blots of isolated trichocysts or entire cells of *Paramecium tetraurelia*. On ultrathin sections, anti-G1 and anti-G3 specifically recognize the *P. dubius* trichocyst shafts and anti-G3 labels trichocysts of *P. tetraurelia* as well.

Actin in the ciliated heterotrich protozoan *Climacostomum virens*: first results

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A protein presumed to be actin has been identified in *C. virens* by immuno-labeling with an affinity purified anti-actin. Populations of 0.5–1 million fasted cells were either 1) homogenized in Pollard's fluid (1 mM ATP, 1 mM DTT, 1 mM EGTA, 340 mM sucrose. 50 mM imidazole, pH = 7) and then centrifuged at 120,000 × g for 1 h at 4 °C, or 2) directly solubilized in a sample buffer (SB), then centrifuged at 13,000 × g for 5 min, with or without cold acetone pretreatment. The three kinds of extracts were analyzed by SDS-PAGE allowed by blotting on nitrocellulose. Blots were labelled with an antibody reaised in rabbit against mouse skeletal muscle, and affinity purified on rat skeletal muscle bound to a sepharose column. All three extracts show a major band (MW ca 43,500) which co-migrates with mouse skeletal actin (MW ca 43,000) and two minor bands (MW ca 39,000 and 26,000); the acetone-pretreated SB extract shows a third minor band (MW ca 30,000).

Developmentally regulated transcription termination controls immunoglobulin δ gene expression

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The expression of immunoglobulin μ and δ heavy chain mRNA from the $\mu + \delta$ complex transcription unit changes during B cell

development. We have used transcription assays to examine the role of transcriptional controls in determining the levels of secreted (μ_s) and membrane (μ_m) mRNA and μ and δ expression during development. Transcription of μ_s and μ_m gene segments is equivalent in all developmental stages. This suggests that post-transcriptional RNA processing rather than transcriptional termination controls the levels of μ_s and μ_m mRNA. In IgM-secreting cells, the δ gene is transcribed at a much lower level than in mature B cells. Polymerase II loading measurements showed that termination of transcription could occur 1 kb past the μ_m exons and blocks δ expression. Reintroduction, into plasma cells, of different DNA fragments, using expression vectors should help us to define this control region.

Studies on murine and human antigen F

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The monoclonal antibodies (Ab) C9-D5, D10-B2, and RS-1 were prepared against Type 1 murine liver specific antigen F (Ag F) and used for the purification of murine and human Ag F. Both have a MW of 40 kD as demonstrated by SDS-PAGE and immunoblotting using polyclonal and monoclonal Ab. The isolated molecules retained antibody reactivity as judged by ELISA and RIA. In addition they induced polyclonal antibodies to Ag F. The amino acid analysis of murine Type 2-Ag F gave the following composition: Asx 35, Thr 18, Ser 18, Glx 41, Gly 38, Ala 26, Val 25, Met 6, Ile 19, Leu 35, Tyr 14, Phe 18, His 12, Lys 25, Arg 18. Ag F could be localized immunohistochemically within the cytoplasm of hepatocytes, proximal tubules of the kidney and neurons of the central nervous system. Ag F isolated from kidney and brain have the same MWs as Ag F extracted from liver.

Effect of solutes on aminopeptidase inactivation in extracts from bean cotyledons

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Leucine aminopeptidase extracted from cotyledons of ungerminated bean seeds (low endopeptidase activity) is relatively stable at pH 5.5 and 30 °C. A rapid inactivation can be initiated by the addition of purified papain or extract from cotyledons of seeds geminated for 8 days (high endopeptidase activity). The inactivation was delayed in presence of sucrose, glucose, fructose, glycine, alanine or serine. Only a minor stabilization was observed after addition of lysine or KCl, while the inactivation was accelerated in presence of $MgSO_4$ or $MgCl_2$. The results suggest that the susceptibility of aminopeptidase to proteolytic inactivation is altered by interactions with low mol.wt compounds. Various enzymes may by affected in different manner by the same solutes. This hypothesis was supported by the behaviour of glutamine synthetase in wheat leaf extract.

DNA in situ hybridization to detect and characterize human papilloma viruses in cell scrapes of normal and abnormal cervical mucosa

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Human papilloma viruses (HPV) are known to be the causative agents of warts and other epithelial lesions. Applying molecular biology techniques to the analyses of these viruses, up to now 35 different HPV types were cloned and characterized. A few types are found preferentially in genital and anal lesions: the HPV types 6 and 11 are found mainly in condylomatas and mild dysplasias whereas the HPV types 16 and 18 are found predominantly in severe dysplasias, in in situ and invasive genital carcinomas.

Since in infected genital cells the HPVs are found mostly as viral DNA and not as viral particles, we concentrated on a method to probe for the presence of HPV-DNA: modifying colony screening techniques we developed a simple and rapid in situ hybridization test which enables to screen many samples in parallel and to identify, resp. to characterize the type of HPV even in small samples (10^4–10^6 cells max.).

Transcriptional regulation of the colicinogenic functions in the Col-D plasmid

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The plasmid ColD-CA23, which has a mol.wt of 5.1 kb, encodes for colicin D (an antibiotic protein), for an autolysis function and for immunity against colicin D. We find that the genes for colicin D activity (*cda*), colicin immunity (*cdi*), and mitomycin C inducible lysis (*cdl*) are located contiguously on a 2.4 kb fragment. We established the direction of transcription of the colicin gene by analysis of the truncated proteins in different mutants obtained by insertion of the Omega fragment into the *cda* gene. Such mutants also abolish the lysis function but not the immunity. The colicin D and the lysis genes are transcribed on the same SOS inducible operon, while the immunity gene, which is located between them, has its own transcriptional unit. The enormous expression of the truncated colicin D proteins in the insertional Omega mutants compared to the production of wild type colicin D, after induction, suggests an autoregulative function for colicin D.

snRNP dependent in vitro processing generates genuine histone mRNA 3′ ends by an endonucleolytic cleavage of the precursor

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All known cell cycle dependent histone mRNAs terminate in a conserved hairpin structure followed by an ACC (A) sequence. This 3′ terminus is generated during maturation of a precursor with 3′ extensions.

In order to examine the detailed mechanisms of pre-mRNA maturation we have established an in vitro system capable of correctly processing histone pre-mRNAs. SP 6 generated mouse H4 precursors show conversion into their mature forms upon incubation in a Roeder type nuclear extract prepared from HeLa cells (a kind gift of Angela Krämer and Walter Keller). The reaction also yields a product which corresponds to the expected 3′ cut off piece, as could be shown by reverse transcription. We hope to define essential components of the reaction machinery by complementation studies with various fractions of the extract.

Functional study of a highly repetitive sequence from *Xenopus laevis*

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A highly repetitive DNA sequence has been isolated from *X. laevis* and characterized. It consists of a tandem array of subunits of

77–79 bp. The array is flanked by a direct repeat of 13 bp and is interspersed in the genome with single copy sequences. Transcripts of this repetitive sequence has been found in various embryonic as well as adult tissues. To find out whether the repeat influences the transcription of neighbouring sequences, chimeric genes containing a pol-II promotor located in the vicinity of the repeat have been constructed and assayed by injection into fertilized eggs of *X. laevis*. The data suggest that the repetitive sequence element has a stimulatory effect on transcription.

The stability of bacteriophage T4 gene 32 mRNA; a 5′ leader sequence that can stabilize mRNA transcripts

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The gene 32 monocistronic mRNA has the unusual property of being extremely stable. To study the molecular basis for this stability, we have constructed chimeric plasmids containing the monocistronic promotor and the gene 32 translation initiation sequence fused to either prokaryotic (lac operon) or eukaryotic (TK of Herpes simplex virus) coding segments. The coding sequence is followed by the transcription terminator of gene 32. The hybrid mRNAs from these gene fusions are not stable in uninfected cells. In phage-infected cells, however, the hybrid mRNAs are at least as stable as the gene 32 mRNA itself. An unidentified early phage function is implicated in this stabilization. Analysis of other plasmid constructs indicates that the sequences on the gene 32 mRNA from its 5′ end to slightly beyond the initiation codon suffice to stabilize hybrid mRNA molecules. This was confirmed by studies with a series of deletions of the gene 32 leader sequence which indicate that an RNA sequence in the vicinity of the gene 32 initiation codon is involved. One may envisage stabilization occurring in a variety of different ways; these various mechanisms are discussed.

Stimulation of the respiratory burst in mammalian leukocytes from eight different species

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Reactive oxygen intermediates (ROI) generated by stimulated leukocytes are considered to be important in the host defense against microorganisms. We assessed the respiratory burst activities of leukocytes from eight mammalian species stimulated with eight different agents. The sensitivity of the assay systems for the generation of H_2O_2 (scopoletin oxidation), O_2^- (cytochrome C reduction) and chemiluminescence (OCl^-, H_2O_2) differed as follows: chemiluminescence $\gg O_2^- = H_2O_2$. Differences in the reaction patterns towards FNLPNTL, WGA, ConA, A23187, opsonized zymosan, Sendai virus, C5a, and PMA were noted. The number of samples which can be tested simultaneously in kinetic assays in higher for the H_2O_2 assay (96 samples) than for chemiluminescence (10 samples) or O_2^- (1 sample).

Indirect hormonal regulation of the *Amy*-2^a alpha-amylase gene promoter

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We have identified a regulatory DNA sequence −100 to −150 in the mouse alpha-amylase gene *Amy*-2^a by transient transfection of *Amy*-2^a-CAT fusions in exocrine pancreatic cells of rat (AR42J). The element is a transcription enhancer. It acts at distance and irrespective of its orientation. The activity of the enhancer is dependent on glucocorticoids. Run-on transcription reveals that dexamethasone stimulates *Amy*-2 transcription by a factor of 4. Therefore, the hormone acts directly at the transcriptional level. The effect is pancreas-specific since it is only observed in AR42J cells. Run-on transcription in the presence of cycloheximide shows that the hormonal activation of *Amy*-2 is indirect. We propose a model by which the hormone-receptor complex acts on a pancreas-specific regulatory gene whose product stimulates, either directly or indirectly, *Amy*-2^a transcription. Other pancreas-specific genes, such as those encoding elastase and trypsin, are not dependent on glucocorticoids. This argues against a common regulatory mechanism.

Tubulin gene expression in *Trypanosoma brucei*

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The cytoskeleton of the parasitic hemoflagellate *Trypanosoma brucei* consists primarily of microtubule-based structures. The tubulin genes of the organism are located in a single tightly packed cluster of lo tandemly arranged, alternating alpha- and beta-genes. Pulsed field gradient electrophoresis has demonstrated that no tubulin genes are present outside this clustered array. Therefore, the cluster can be assumed as the locus of tubulin gene expression. Single, homogeneous populations of alpha- and beta tubulin mRNAs are observed both in procyclic and in bloodstream trypanosomes. Both types of mRNA have distinct 5′ termini, which carry a 35 nucleotide mini-exon sequence. This latter sequence is not coded for colinearly with the tubulin genes, and it may be added to the mRNA via a trans-splicing reaction during the processing of the primary transcript. Several aspects of tubulin gene transcription and its control are presently investigated by means of in vitro transcription.

Two transcripts for IgF II in human brain differing in the 5′ non-coding sequence

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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′ non-coding sequence. Partial sequences show that the coding sequence for prepro-IgF II is identical with the published cDNA sequences from human liver, but that the leader sequence is different. Northern blots show two transcripts for IgF II in brain, only one of them hybridizing with the leader sequence. Differential processing of the IgF II mRNA could influence the expression of IgF II in different tissues and/or different stages of development.

Differential regulation of late histone genes in the sea urchin embryo

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In the sea urchin the repetitive early histone genes are expressed from fertilization up to the hatching blastula stage (12 h), whereas different subsets of late histone genes are active during later development. We have isolated a pair of non-allelic late H2A and H2B genes on two cosmid clones. The H2A and H2B genes of cosmid L1 are identical to the previously cloned late H2A-2 and H2B-2 cDNA sequences. The two genes of cosmid L2 have recently diverged from their counterparts on cosmid L1 and code for novel late H2A and H2B variants. S1 mapping experiments revealed that the mRNAs transcribed from both genes of cosmid L1 accumulate to maximal levels in the hatching blastula (12 h) and that their amount remains constant throughout later

embryogenesis. By contrast, the mRNAs of the H2A and H2B genes of cosmid L2 do not reach their maximal levels until the pluteus larva stage (48 h). The four genes analyzed exhibit all the structural features of replication-dependent histone genes and are most likely regulated during the cell cycle in dividing cells. The two different profiles of mRNA accumulation could therefore reflect the expression of the respective histone genes in two distinct cell lineages which differ in their cell division rate during late embryogenesis.

Intermediates of T-DNA transfer from *Agrobacterium tumefaciens* to plant cells

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The T-DNA of a nopaline Ti-plasmid was modified in such a way that we could look for T-DNA intermediates in the transfer between *Agrobacterium tumefaciens* and plant cell using the λ-in-vitro packaging technique. Co-cultivation of *Agrobacterium* with plant cells leads to the induction of packageable T-DNA structures within the bacterial cells. After the packaging reaction, they are recovered in *E. coli* as circular plasmids. The discovery that the junction of these T-DNA circles occurs precisely in the 25-base pair terminal sequence shown to be involved in T-DNA transfer strengthens the suggestion that the induced packageable T-DNA structures are indeed intermediates in the transfer process. Their form in vivo will be discussed.

Watanabe hereditary hyperlipemic (WHHL) rabbits: extensive aortic arteriosclerosis at 200 days of age

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Ibm: WHHL×Ibm: Kobu rabbits exhibit high plasma cholesterol, apo B, and triglyceride levels. In perfusion fixed aortic segments neointima formation was observed between 100 and 200 days of age, with increasing severity from the abdominal to the ascending aorta. At 200 days, the correlation between % luminal narrowing of two thoracic segments taken 1 cm apart ($n = 24$; mean±SEM: 3.93±0.7) and the cholesterol content of their intermediate segment (40.4±5.8 μg/mg) was high ($r_s = 0.914$; $2p < 0.001$). Cholesterol was accompanied by cholestanol (4.6±0.2%; $n = 20$). As in human arteriosclerotic lesions, the neointima contained smooth muscle cells and foam cells, amorphous and crystalline materials. Smooth muscle cells were seen migrating through the internal elastic lamina. Monocytes adhered to and migrated through the endothelium. Media lesions and elastolysis were observed. Conclusions: In this model aortic cholesterol content paralleld neointima formation.

Uptake of exogenously supplied NAD and its conversion into poly (ADP-ribose) in intact hepatocytes

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We have discovered that exogenously supplied NAD can be taken up by rat hepatocytes by a presumably endocytotic mechanism. Uptake was confirmed by enzymatic and chromatographic determination of unlabeled NAD as well as analysis of the intracellular material following (^{3}H-adenine)- and (carbonyl-^{14}C)NAD exposure of cultured cells. Treatment of hepatocytes with the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine stimulated the conversion of loaded NAD into poly(ADP-ribose) as determined by boronate affinity chromatography in combination with HPLC. Taking advantage of this phenomenon, we observed distinct changes of intracellular poly(ADP-ribose) concentrations in response to altered NAD concentrations. This model system offers a new approach to the study of the regulation of poly(ADP-ribose) metabolism and the physiological acceptor proteins in intact cells.

Cell cycle regulation of histone gene expression involves post-transcriptional nuclear event(s)

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A 3′ terminal mouse H4 gene fragment fused to the SV40 early promoter is transcribed in mouse cells into a short RNA which is faithfully regulated during the cell cycle. Deletion mutants completely defective in 3′ end generation cover the RNA 3′ processing site including two conserved, essential sequence elements. Other, partly defective deletion mutants are located 118–232 bp downstream from the processing site, but the low amount of fusion RNA produced by them is still under cell cycle control. Their effect might be to reduce transcription termination, since, by SP6 mapping, we can detect putative nuclear precursor transcripts of the endogenous H4 gene, some of which seem to terminate in this region. The amount of processed nuclear H4 mRNA is greatly reduced in G1-arrested cells, while the putative precursor transcripts are slightly accumulated. This indicates that a nuclear process, most likely the processing step, may be involved in regulating histone mRNA levels during the cell cycle.

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. Genes fusion techniques have enabled us to identify transcriptional events within these regions, upstream of the gene repA. We are currently characterizing these transcripts by in vitro run off transcription and by Northern analysis.

Comparison of the effect of dopaminergic compounds on rat pituitary hormone-mRNA and -release levels in vivo and in vitro

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Rats (♂, OFA, 250 g) were treated s.c. for various length of time with the dopaminergic compounds CB 154 (Parlodel®, Sandoz), CQP 201–403 and CV 205–502 (experimental compounds). In parallel, primary cultures of dispersed pituitary cells were established and treated with the same compounds. Total RNA was extracted from whole pituitaries or from cultured cells and the levels for hormone mRNAs (prolactin, growth hormone) were determined using dot blot hybridization with synthetic oligonucleotide probes. The levels of released hormone were determined by RIA. The results indicate that both in vivo and in vitro the compounds reduced the amount of released hormone as well as the level of corresponding mRNAs in a sequential fashion. Hormone release was affected before significant reductions of mRNA levels could be detected. It remains to be elucidated whether the reduction of RNA levels may be functionally related to the reduction of hormone release.

Qualitative differences between germ line and somatic DNA sequences in *Ascaris lumbricoides*

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From an *Ascaris* germ line DNA library we succeeded in isolating several clones containing low copy number DNA sequences which become completely eliminated from the somatic genome during the process of chromatin diminution in the early embryonic development of the nematode *Ascaris lumbricoides*. One piece of DNA, localized on several overlapping λ-clones, is composed of single copy DNA sequences of over 30 kb length. Complete elimination of low copy and single copy DNA sequences from the presumptive somatic cell lines demonstrates clearly that the diminution process causes the loss of potential genetic information from somatic cells. Elimination therefore leads to qualitative and not only to quantitative differences between the genomes of the germ line and the soma. Detailed analysis of these strictly germ line limited sequences should give us some clues about their possible function.

Yolk protein evolution: comparison of amphibian, avian, insect and nematode vitellogenins

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Yolk serves as a source of amino acids, lipids, sugar and inorganic phosphate in embryonic development. Vitellogenin, the major precursor of the yolk proteins, is produced under hormonal control in egg-laying organisms. Vitellogenin gene sequences from evolutionary distant organisms such as amphibian, avian, insect and nematode are compared. Chicken and *Xenopus* vitellogenin genes show the same exon-intron organisation and high homology in the coding sequence, with an exception in the serine-rich phosvitin region. Caenhoraebditis vitellogenin gene five exhibits a completely different exon-intron organisation. However it codes for a protein similar in size and amino acids to the two other vitellogenins, but lacking a serine-rich domain. In contrast the *Drosophila* vitellogenin and Yp1 codes for a much smaller protein and show very low, if any, homology to the three other vitellogenins.

The comparison reveals the evolutionary close relation between chicken and *Xenopus* yolk. Caenhoraebditis yolk seems to be more closely related to the vertebrate yolk than to that of the fruit fly.

Chromosomal arrangement and functional aspects of intervening sequences containing rDNA in *Ascaris lumbricoides*

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About 5% of the rDNA repeats in *A. lumbricoides* contain an intervening sequence (IS) in their 26S coding region. These IS^+ units seem to be clustered in the genome rather than being intermingled with other organizational forms of rDNA. We have isolated various clones from a cosmid library which contain several repeats of the IS^+ rDNA. From one of them we have subcloned a Hind III-fragment which starts within the nontranscribed spacer and extends to the first half of the IS. Detailed analysis of this fragment enables us to determine the precise degree of homology between the promoter regions of both, the IS^+ and the IS^- organizational forms of *Ascaris* rDNA. In vivo transcription of the IS^+ repeats is tested by using the technique of Northern blotting as well as by Sl protection experiments. Furthermore, transcription of the IS^+ repeating unit is probed in a homologous in vitro polymerase I system.

Regulation of transferrin receptor expression by iron chelators is abolished by a deletion in the 3'-untranslated region

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Cellular uptake of iron from serum is mediated by a specific cell-surface receptor for transferrin (TR). Chelation of iron by desferrioxamine leads to increased TR expression. The human TR gene codes for a 5.0 kb mRNA of which 2.7 kb are 3'-untranslated sequences. Plasmid constructions carrying the human TR cDNA downstream from the SV-40 or TR promoters were expressed both transiently and stably in transfected mouse L cells. Deletion of 2.3 kb within the 3'-untranslated region resulted in a 5- to 20-fold increase in transient expression after 64 h. This effect was observed with both promoters. In stably transfected L cell clones both TR mRNA and protein levels increased in the presence of desferrioxamine whereas TR cDNA constructions with the 2.3 kb deletion did not respond to the iron chelator. Our results suggest there is mutual feedback regulation of TR expression and intracellular iron concentration. Whether iron controls TR mRNA levels by a cotranscriptional or a post-transcriptional mechanism is under investigation.

The epiplasmic cytoskeleton of ciliates and its immunological relationship to constituents of other protozoan cells

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The cytoplasmic side of the innermost cell membrane of certain ciliates, eg. *Pseudomicrothorax dubius*, is composed of a cytoskeletal layer, the epiplasm, which specifies the position of secretory organelles and basal bodies. To determine the immunological relationships among epiplasms of different organisms, and to ascertain if such relationships exist with organisms lacking a clearly-defined epiplasm, antibodies were raised against *P. dubius* epiplasm and used to label Western blots of whole cells or cell fractions of ciliates, flagellates (including trypanosomes), amoebae and sporozoa (including Plasmodium). Immunolabeling for electron microscopy was done for several of these. Proteins immunologically similar to the epiplasm were found in all organisms examined, indicating that epiplasm-like proteins may have a greater functional significance than previously suspected.

The 'pol' coding region of the cauliflower mosaic virus is expressed as a part of large polyprotein-precursor homology to retroviruses

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The region of the cauliflower mosaic virus (CaMV) genome, encompassing the putative reverse-transcriptase coding region (ORFV), was cloned in an expression vector in *E. coli*. Protein which was synthesized in *E. coli* and corresponded exactly to the coding region of ORFV (78 kD) did not show reverse transcriptase activity. This protein was also used to produce antibody in rabbits. Using this antibody we showed that the ORFV of CaMV is translated from two open reading frames as a large polyprotein precursor in infected plants and is proteolytically processed yielding mature proteins. The similarity in expressing reverse transcriptases of CaMV and retroviruses is discussed.

Gene for interferon-induced guanylate-binding protein maps to mouse chromosome 3

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Gbp-1 is the predominant species of a family of guanine nucleotide-binding proteins synthesized in mouse cells in response to IFNs alpha, beta or gamma. IFN-inducibility of this 65,000-Da protein is controlled by two alleles at a single autosomal locus, *Gbp-1* with allele *a* encoding inducibility and allele *b* noninducibility. Using recombinant inbred strains and classical linkage analysis of offspring of two point and three point backcrosses we demonstrate that *Gbp-1* is linked to *Adh-3* (encoding alcohol dehydrogenase C_2) and *Va* (varitint-waddler-Jackson) located on the distal part of chromosome 3. The relevant RFs (±SE) were 3.5 (±1.1) and 11.7 (±2.8) percent, respectively. We further show that strain B6.C-*H-23*^{c}By(HW 53), congenic for a small segment of chromosome 3, carries the BALB(cBy alleles at both the *Gbp-1* and the *Adh-3* locus and not the alleles of the B6 background strain confirming the chromosomal location and close linkage of the two loci.

Transposition of IS elements in resting *E. coli* cells?

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Earlier studies have shown that a majority of spontaneous P1 prophage mutations were due to the activities of mobile genetic elements, i.e. either to deletions mediated by the resident IS1 element or to insertions (Arber et al., Cold Spring Harbor Symp. quant. Biol. *45* (1980) 38), and suggested a higher frequency in stationary phase than in exponential growth phase of the bacterial host. Here we studied a different target DNA, plasmid pHFG34 in *E. coli* strain W431, which allowed direct selection of galactose-resistant (Gal^R) mutants. After exponential growth the mutation was due to insertion of IS1, IS2 or IS5 into the target plasmid in only about 10% of the mutant clones. After six days stationary phase the fraction of cells harboring a plasmid with an insertion was increased by at least a factor of 10. Indeed, DNA insertions are more abundant in stationary growth phase and are probably in part due to transposition in resting cells.

Selective isolation of specific transcripts from complex brain mRNA populations: application in sleep research

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Due to the complexity of the nervous system, most proteins characteristic of a given function and structure are expressed at a low abundance. To identify mRNA transcripts for such proteins, a 10-fold enriched recombinant library was generated from cDNA transcribed from rat forebrain mRNA after subtraction of cerebellar sequences. Clones specific to forebrain could be revealed by colony hybridization with cerebellum subtracted probes. Clones corresponding to transcripts present at an abundance as low as 0.0001% could still be detected. About 5% of specific probes were recognized with an enriched forebrain probe. Based on the assumption that the regulation of sleep-enhancing peptides is transcriptional, the technique is being applied to detect changes in rat brain induced by sleep deprivation.

Developmental analysis of myelin basic protein synthesis in normal and *mld* mutant mice

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Myelin-deficient (*mld*) is an autosomal recessive mutation in mice exhibiting a drastic reduction of myelin basic protein (MBP) synthesis in both the CNS and PNS. In the characterization of the *mld* mutation at the molecular level, we examined the amount of MBP-specific mRNA in control and *mld* mouse brains. Combining in vitro translation followed by radioimmunoprecipitation and filter hybridization after dot or Northern blotting, we found that in control mice, the amount of MBP-specific mRNA is very high at 18 days of age and then decreases during development to reach a base level at about 72–85 days. In contrast, the amount of MBP-specific mRNA in *mld* mice remained low and constant during development, similar to that observed in controls at 85 days. Our results, together with those of others, suggest that the *mld* mutation is not a deletion in the coding region of the MBP gene.

Contribution of cryotechniques to the ultrastructural immunocytochemistry of nuclear antigens

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The antigenic activity of a number of components of the cell nucleus is rather sensitive to the preparation procedures to which the cells and tissues are submitted prior to immunolabeling. For the direct labeling of ultrathin sections with specific antibodies, the specimens are usually fixed with aldehydes and then embedded either in epoxy resins or by the low temperature procedure using Lowicryl K4M. A helpful alternative for certain nuclear constituents sensitive to dehydration and resin treatment is cryoultramicrotomy. A more recent approach avoiding conventionally used 'chemical' fixatives is a procedure based on cryofixation followed by cryosubstitution with very low temperature embedding into Lowicryl K11M.

The above cryotechniques have been applied in a series of collaborative projects aiming to visualize the nuclear distribution of various enzymes such as RNA polymerases I and II, poly(ADP)ribose polymerase and topoisomerases I and II. In addition, localization of ribonucleoproteins as well as of certain, supposedly structural, nuclear proteins have been analyzed using specific monoclonal or polyclonal antibodies.

Effects of hydroxyurea on ribonucleotide reductase of mammalian cell-cycle mutants

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A heat-sensitive (hs, arrested in G1 at 39.5 °C) and a cold-sensitive (cs, arrested in G1 at 33 °C cell-cycle mutant of the P-815-X2 line were tested for ribonucleotide reductase (RR) activity, using cells made permeable to nucleotides. After transfer of mutant cells to the respective non-permissive temperature, RR activities decreased to 5% (hs mutant) or 15–20% (cs mutant) of initial values, while numbers of DNA-synthesizing cells decreased to near-zero levels. RR in arrested permeabilized cells was not inhibited by 4 mM hydroxyurea (HU). In proliferating cells, HU (1–20 mM) caused a partial inhibition of RR, and residual RR activities reached a plateau of 5% (hs mutant) or 15–20% (cs mutant) of control values at HU concentrations exceeding 2 mM. RR activities in arrested mutant cells were nearly the same as HU-resistant RR activities in proliferating cells, indicating that the HU-sensitive, but not the HU-resistant fraction of RR may be under cell-cycle control.

33 ways to make gene transfer

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In our laboratory we are using a variety of gene transfer techniques such as calcium phosphate coprecipitation, DEAE-Dextran, protoplast fusion, electroporation and microneedle injection. Depending on the booster method (DMSO shock or chloroquine), the assay (transient expression versus stable transformation), and the experimental goal, each technique has advantages and drawbacks which will be listed and discussed.

Oligonucleotide-directed mutagenesis of sea urchin histone 3′ conserved sequence blocks delimits nucleotides important to U7 RNA-dependent H3 processing in *Xenopus* oocytes

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Base-pairing between sea urchin U7 RNA and two conserved sequence elements flanking the site of the mature histone RNA 3′ end has been proposed to be relevent to 3′ processing (EMBO J. *3*,2801). Consistent with this model, the nucleotides GAAAG of the CAAGAAAGA element are shown here to be essential to U7 RNA-dependent 3′ processing; as predicted, the CAA nucleotides are not. Of the suggested 13 bp complementarity over the stem-loop element, a strong sequence dependency is observed only for the loop; substitution of an alternative stem, not capable of base-pairing with U7 RNA, modestly decreases U7 RNA activity. This, in addition to previous data demonstrating the necessity of the stem-loop structure (Cell *35*, 433), suggests that presentation of the nucleotides, TTT, in a secondary structure is sufficient to 'kiss' U7 RNA although an additional 'hug' by the stem is appreciated.

Termination of RNA polymerase III transcripts

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Termination of *Xenopus* 5S RNA transcription occurs efficiently within T_4 tracts surrounded by GC-rich DNA (Bogenhagen and Brown, 1981). Such a tract is found 26 bp downstream of a *X. laevis* $tRNA^{Phe}$ gene, but, surprisingly, termination is inefficient at this site in homologous S-100 extracts. The most obvious feature of the DNA between the mature tRNA-coding sequence and the T_4 tract is its potential to form a stable stem and loop. Oligonucleotide-directed mutagenesis shows that the poor termination is not due to this potential stem and loop but rather to sequences just upstream of the T_4 tract. In particular, a substitution that makes this region more AT-rich leads to more efficient termination. The 5S RNA termination rules are thus not applicable to every RNA polymerase III gene. Indeed, additional experiments show that the 5S rules are not applicable to 5S RNA genes in micro-injected oocyte nuclei.

Purification of murine monoclonal antibody NMS-1 by euglobulin precipitation and anion exchange HPLC

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A simple, fast, and highly reproducible method for the purification to homogeneity of a murine IgM monoclonal antibody (moAB) is described. MoAB NMS-1, directed to a periodate-sensitive carbohydrate epitope on the surface of human polymorphonuclear leukocytes (PMNLs) was enriched ~ 10-fold from mouse ascites fluid by euglobulin precipitation (20 mM Tris-HCl, pH 7.5). MoAB NMS-1 was further purified on a computer-monitored Perkin-Elmer Series 4 HPLC system by anion exchange chromatography using a BIO RAD TSK-DEAE-5PW column (linear gradient of 0.015–0.3 M sodium phosphate buffer, pH 7.3). Purified moAB NMS-1 (SDS-PAGE analysis) eluted at 120 mM sodium phosphate. Binding of purified moAB NMS-1 to PMNLs was quantified by indirect immunofluorescence (FACS) and ELISA (adherent glutaraldehyde-fixed PMNLs). Detection limits: FACS: 5 ng/ml; ELISA: 10 ng/ml.

The tubulins of *Trypanosoma brucei*

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The cytoskeleton of the parasitic hemoflagellate *Trypanosoma brucei* primarily consists of microtubule-based structures. Three microtubular systems can be distinguished: a) the soluble pool of unstable spindle microtubules, b) the table, membrane-associated pellicular microtubules, and c) the microtubules of the flagellar axoneme. Trypanosomes have been demonstrated to contain about ten tubulin genes in their genome. Nevertheless, only a single primary translation product is synthesized in vivo for alpha and for beta tubulin, respectively. In contrast, the mature tubulins exhibit considerable microheterogeneity when analyzed by isoelectric focusing. This heterogeneity, which is different in different cellular compartments, is most probably caused by posttranslational modifications. One type of modification, the reversible tyrosinolation of the carboxy-terminus of alpha tubulin, has been analyzed in more detail.

Genomic organization of the vitellogenin genes in *Xenopus laevis*

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Vitellogenin, the precursor of the major yolk proteins, is encoded in four related genes (A1, A2, B1 and B2) in *Xenopus laevis*. Previously, we have shown that the A1 and B1 genes are linked. The organization of the vitellogenin gene family was further analyzed by a genetic approach taking advantage of restriction fragment length polymorphisms in the *Xenopus* population. The results demonstrate that the A2 gene is linked to A1–B1 complex, whereas the B2 gene seems to be located elsewhere. These observations challenge the hypothesis according to which the A1–A2 and B1–B2 gene pairs arose simultaneously as the result of a whole genome duplication about 30 million years ago. However they help interpreting earlier findings demonstrating that the gene flanking regions and introns in the A1–A2 pair are less conserved than in the B1–B2 pair. Altogether these results suggest that the gene pairs arose by independent duplication events.

Intrinsic radiosensitization of malignant neuroblastoma cells in *Drosophila* by DNA repair deficient loci

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The circumvention of tumor cells' intrinsic radioresistance has been the objective of many approaches in attempting to optimize tumor radiotherapy. We have succeeded in establishing in vivo malignant neuroblastoma cell lines in *Drosophila* carrying DNA repair deficient mutations. Comparisons of survival ratios of proband host flies inoculated either with DNA repair proficient malignant cells (C-43,1(2)gl) or DNA repair deficient malignant

cells (C-43-9,mei-9^a;1(2)gl) revealed, for the first time experimentally, that dose modification factors of at least 2, in favor of the curative effect, can be achieved by genetic impairment of the malignant cells' DNA repair capacity.

The activation of Amy-1^a and PSP during parotid gland differentiation

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Parotid gland acinar cells proliferate and differentiate mainly between birth and weaning (3 weeks). During this time period, the two most abundant mRNAs of these cells, encoding α-amylase (Amy 1) and parotid-specific protein (PSP), accumulate in concert from undetectable to adult levels. In situ hybridization experiments show that the increase in accumulation of the two mRNAs is due to a progressive commitment of acinar cells to the expression of the two mRNAs. For Amy-1, the transcriptional activity of the gene and the accumulation of its mRNA rise in parallel. Unexpectedly, however, PSP is transcribed at close to adult rates, already 10 days after birth, a week before its mRNA reaches significant levels in the cytoplasm. Three independent experiments indicate that the post-transcriptional control of PSP mRNA accumulation occurs in the cytoplasm by changes in mRNA stability during parotid differentiation: 1) All PSP pre-mRNAs are equally abundant in parotid nuclei from 10 days old and adult animals. 2) After a 3-h labeling period similar levels of newly synthesized PSP mRNAs appear in the cytoplasm of young and adult animals. 3) The poly A tract of cytoplasmic PSP mRNA is about 200 nucleotides longer in young than in adult mice. Poly A shortening has previously been associated with mRNA aging.

Expression studies on the two promoters of the α-amylase gene Amy-1 from mouse and rat

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The α-amylase gene Amy-1 is transcribed from two promoters in both mouse and rat. The upstream strong promoter is exclusively utilized by acinar parotid cells in both rodent species. In mouse, the weaker downstream promoter is active in liver, parotid and pancreas. Surprisingly, in rat, this latter promoter shows significant activity only in liver. Structural comparison of Amy-1 from mouse and rat reveals that the distance between strong and weak Amy-1 promoters is more than double (6 kb) in rat that previously reported for mouse (2.8 kb). Moreover, the multiple start sites of the rat liver Amy-1^a transcripts are located between 160 and 200 nucleotides usptream of the ones observed in mouse, in spite of the more than 90% sequence homology within the corresponding regions of mouse and rat Amy-1. Transfection studies with various fusion genes containing strong and weak mouse Amy-1 promoter sequences reveal that 1) the weak but not the strong promoter is utilized in non α-amylase producing cell lines, 2) that only the former promoter responds to an SV40 enhancer and 3) that deletion of sequences upstream of the strong promoter leads to its constitutive expression.

Induction of chemiluminescence in cultured murine glia cells

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Induction of luminol-enhanced chemiluminescence (CL) is studied in glia cell cultures from newborn mice, containing 80–90% astrocytes. A burst of CL can be induced by phorbol myristate acetate (TPA), zymosan A, and antibody-coated bovine red blood cells, whereas Sendai virus and several other agents known to induce CL in myeloid cells are ineffective. An optimal CL response is reached at 4×10^5 glia cells/0.75 ml and 70 nM TPA; CL depends on the presence of Ca^{+2} and is not inhibited by sodium azide, suggesting a myeloperoxidase-independent pathway. In parallel experiments, subpopulations of the glia cells are shown to reduce nitroblue tetrazolium and to be capable of phagocytosis. The results suggest that microglia cells and astrocytes are the main contributors to CL. Glia cells of fetal calves are also CL-inducible. The capacity of some glia cells to generate an oxidative burst may reflect their role in immune defense.

p60, a potential microtubule/membrane linker in *Trypanosoma brucei*

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The dominant structure of the cytoskeleton of the parasitic hemoflagellate *Trypanosoma brucei* is a tight layer of singlet microtubules which underlays the entire cellular membrane and which forms close contacts with it. This unusually tight microtubule/membrane complex is both an attractive model for studying microtubule/membrane interactions and a potential target site for chemotherapeutic attack. Earlier observations in this laboratory have demonstrated that neuroleptic phenothiazines disrupt these connections in vivo in micromolar concentrations. Phenothiazine affinity chromatography of proteins extracted from the microtubule/membrane complex has produced at 60 kD protein (p60). p60 behaves like a cytoskeletal protein by a number of criteria, and it behaves like a membrane protein by a different set of criteria. The observations led to the tentative conclusion that p60 might represent a linker between microtubules and the cellular membrane. Biochemical and molecular biological analyses of p60 are in progress.

Immunochemical characterization of antibodies to myelin glycolipids

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Antisera against myelin glycolipids have been widely used in neurobiology to study the development of oligodendrocytes and Schwann cells. The specificity of two monoclonal antibodies and one polyclonal antiserum reported to be directed against galactocerebroside (GC), has been tested against purified sphingo- and glycerolipids by ELISA, and against lipid extracts from dissociated mouse CNS cultures, by immunochemistry after thin layer chromatography. All three reacted with galactose-containing sphingo- and glycerolipids in addition to GC, but not with sulfatide or glucocerebroside; had a very high titer to psychosine (although this lipid was not present in the cultures); and reacted differentially with mono- and digalactosyldiglycerides, lipids which are concentrated in myelin. These results are discussed in relation to the plasma membrane immunofluorescence of oligodendrocyte populations in mouse CNS cultures. The usefulness of antibodies against myelin glycolipids in the identification of oligodendrocytes is discussed.

Xenopus laevis and *X. tropicalis* sarcomeric actin genes

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A *X. laevis* cDNA library representing mRNAs extracted from embryos and a genomic library have been screened for α actin

sequences. We identified two cDNAs representing a cardiac and a skeletal muscle actin and three different types of α actin genes. Two of them correspond to the isolated cDNAs and the third, which is a processed gene, appears to represent a second type of α skeletal actin gene. The α cardiac and α skeletal actin genes are coexpressed in embryos. Their promoters show homologies in the vicinity of the CAAT and TATA box and proximal to the transcriptional initiation site. These areas are also characterized by the presence of inverted repeats and GC rich motifs. Genomic blot analysis suggests that at least two copies of each gene exist in *X. laevis* but only one in *X. tropicalis*. We have recently isolated three different α actin genomic clones from *X. tropicalis*. Work is in progress to define their promoter areas and to compare them to the corresponding areas of the *X. laevis* genes.

Cloning of cDNA and gene for rat heme oxygenase

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Two cDNA clones for rat heme oxygenase have been isolated from a rat spleen cDNA library in λgt11 by immunological screening using a specific polyclonal antibody. To confirm that the isolated cDNA encodes heme oxygenase, we transfected monkey kidney cells (COS-7) with the cDNA carried in a simian virus 40 vector. The heme oxygenase was highly expressed in endoplasmic reticulum of transfected cells. The cloned cDNA was used to analyse the induction of heme oxygenase in rat liver by treatment with $CoCl_2$ or with hemin. RNA blot analysis showed that both $CoCl_2$ and hemin increased the amount of hybridizable mRNA. To study the molecular mechanims how these substances increase the amount of heme oxygenase, we have isolated one genomic clone for heme oxygenase from a rat genomic library, and we are characterizing this gene.

Expression of hepatitis B surface antigen from an AAV (adeno-associated virus) vector in mammalian cell

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We have previously used the human parvovirus adeno-associated virus (AAV) as a vector for transient expression of the gene coding for chloramphenicol acetyl transferase (CAT) as well as for stable expression of the dominant selectible marker Neo in mammalian cells. Upon infection of susceptible cells in the absence of helper adenovirus AAV naturally integrates its genomic DNA into host cell DNA. We have now designed an AAV vector which allows cloning and expression of a nonselectible gene. This vector contains the Neo gene expressed from the AAV P40 promoter and an SV40 early gene promoter for expression of a second gene. To test this construct we cloned a 1.35 kb DNA fragment coding for hepatitis B surface antigen (HBs) into such an AAV vector. The recombinant vector could be packaged into AAV virus particles and was subsequently delivered into mammalian cells by viral infection. Cell cultures which had acquired the HBs gene secreted HBs antigen into the culture medium. Production of HBs antigen either transiently or from stably transformed cell cultures will be discussed.

Functional analysis of the 5′ end region of the vitellogenin gene B1

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The estrogen-regulated vitellogenin genes of *Xenopus laevis* and chicken possess sequence similarities in their 5′ flanking region. There are four blocks of sequence which are conserved in all the analyzed genes and therefore they could play a role in their hormonally regulated expression. Using the X1 vitellogenin gene B1 as a model, we are currently analyzing the functional significance of these blocks. We have constructed chimeric genes with various portions of the 5′ flanking region of gene B1 driving CAT gene expression. Transfection of these constructs in various cells containing or lacking estrogen receptors indicate the presence of enhancer-like sequences in the 5′ region of the analyzed gene.

Does the MMTV-LTR confer tissue specificity to the virus?

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Mouse mammary tumor virus (MMTV) is thought to be highly specific for mammary gland. In a cell line derived from a kidney adenocarcinoma, however, multiple newly integrated copies of exogenous MMTV were detected and the expression of this virus was regulated by glucocorticoid hormones. We have cloned the MMTV variant by constructing a λ-gene library of this kidney cell line. Restriction enzyme analysis confirmed the exogenous origin of this variant and also revealed some differences when the physical map was compared to the presumed parental virus involved in mammary tumor formation. By heteroduplex analysis we found evidence for a considerable divergence between the two viruses in the LTR which contains the regulatory elements for the expression of the virus. These sequences will be determined and compared to the sequences of the parental virus.

The hemagglutinating activity of Berne virus

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Berne virus is a member of the proposed Toroviridae family. It was originally isolated from a horse with diarrhoea and grows to high titers in an equine dermis cell line.
We here report that Berne virus possesses hemagglutinating activity. In decreasing order, human, rabbit and guinea pig erythrocytes were agglutinated whereas no agglutination was observed with rat, goose, chicken, horse and bovine red blood cells. This pattern is clearly different from that seen with the closely related Breda virus (Woode et al., Vet. Microbiol. 7 (1982) 221). Agglutination was prevented in the presence of Fetuin and gangliosides and was abrogated by pretreatment of the erythrocytes with periodate suggesting that the virus binds to glycoproteins and/or glycolipids on the erythrocyte surface. As seen from the co-sedimentation with viral infectivity in sucrose gradients, the hemagglutinin is part of the virus particle. Treatment of the virus with Tween-ether destroyed infectivity but not the hemagglutinating activity, suggesting that the hemagglutinin is associated with the viral envelope.

Cloning of cDNA coding for chicken cytosolic aspartate aminotransferase (cAspAT)

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Recently, a cDNA encoding the precursor of chicken mitochondrial aspartate aminotransferase (pre-mAspAT) has been cloned in our laboratory (Jaussi et al., J. biol. Chem., in press). We have now cloned the cDNA corresponding to the homologous cytosolic isoenzyme.
A cDNA expression library (chicken muscle cDNA inserted into lac Z of λgt11) was kindly supplied by Dr. U. Rosenberg. Screening of 40,000 plaques with affinity-purified rabbit anti-cAspAT antibodies gave ten positive clones. Three of them were re-screened using an oligonucleotide probe designed on the basis of the known amino acid sequence and all gave strong signals. One clone was purified to homogeneity; its DNA was isolated and cleaved with EcoRI. The cDNA was 500 bp long. It was subcloned into M13mp18 and sequenced. It encodes part of the cAspAT protein.

Genetics

Fidelity of *Drosophila* mutagenicity analysis in the prediction of chemical carcinogenicity: the case of pyrrolizidine alkaloids

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Pyrrolizidine alkaloids (PAs) represent the most widespread group of natural chemicals suspected to cause cancer. Typically in mammals, PAs are primarily hepatotoxic. This indicates that biotransformation by liver enzymes is necessary to produce the reactive metabolites responsible for the adverse effects of PAs. Those metabolites that bind to DNA are likely to induce cancer. The mutagenic potency of 16 PAs was determined quantitatively in *Drosophila* by means of the wing spot test which assays for induced somatic mutation and recombination (Graf et al., Environ. Mutagen. *6* (1984) 153). The mutagenic activity of PAs was found to depend on specificities in the chemical structure. The same predictive rules apply to mutagenicity in *Drosophila* and to hepatotoxicity in rodents. Apparently bioactivation of PAs follows the same or similar pathways in *Drosophila* and in mammals.

Genomic alterations in human breast cancer

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Cultures of mammary epithelial cells from primary tumors and normal breast tissue were set up in MM medium. Metaphase harvests were obtained from primary, secondary and tertiary passages of subconfluent cultures of cuboidal cells. The G-banding technique was used for karyotype analysis.
Chromosome counts of eight tumor samples (35–80 metaphases analysed per sample) showed a distribution of 6–39% of metaphases with 46 chromosomes and 11–28% with 45 chromosomes. In a culture of normal epithelial cells, metaphases with 46 chromosomes occurred in over 60% of cases whereas 45 chromosomes were present in 2% of the metaphases. The level of aneuploidy was generally higher in tumor samples than in normal tissue. Numerically normal metaphases from a tumor were further investigated for chromosomal abnormalities. In the majority of such metaphases translocations, deletions and marker chromosomes could be identified.

The tRNA-Tyr genes of the dpp-complex

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Two *Drosophila* tRNA-Tyr genes localized in the dpp-region contain introns, but of different size (21/48 bp) and sequence. In yeast the ψ modification localized in the middle of the tRNA-Tyr anticodon is dependent upon the presence of the intron in the tRNA-Tyr gene (Johnson and Abelson, Nature *302* (1983) 681). The two *Drosophila* introns share a consensus sequence CGAA with the yeast intron. Both *Drosophila* genes containing the introns, after injection into *Xenopus* oocyte nuclei, support the ψ formation in the synthesized tRNA-Tyr. However, removal of the intron leads to lack of this modification. These results suggest, that the consensus sequence CGAA is the recognition site of the ψ modification enzyme in yeast and in *Xenopus*. *Xenopus* contains two types of tRNA-Tyr genes: one containing a 12 bp, the other a 13 bp intron (Gouilloud and Clarkson, J. biol. Chem., in press). Only one of them contains the sequence CGAR. We shall inject the two *Xenopus* genes into oocytes do determine whether the CGAR sequence is a prerequisite for ψ formation or not.

RSF1010 derived cloning vectors: a small RSF1010 fragment is involved in the copy number control

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RSF1010 is a small antibiotic resistance plasmid which is can replicate in a broad range of gram-negative bacteria. Several gene cloning vectors had been derived from this plasmid; they all contained the origin of replication, the three replication genes, *rep*A, *rep*B and *rep*C, and the functions for conjugative mobilization. We have isolated a mutant RSF1010 plasmid (pJFF13) which has a ten times higher copy number than the native RSF1010 plasmid. This mutant RSF1010 has another particularity: It cannot longer replicate in bacterial species distant from *E. coli* such as *Pseudomonas putida*. We have constructed from this mutant RSF1010 plasmid (pJFF13) a serie of gene cloning vectors for *E. coli* which have the advantage of a very high copy number (80–100 copies per cell), to be compatible with pBR322 and to be independent on the host's *pol A* gene product. The plasmid pJFF13 is also used to study the broad host specificity of RSF1010.

Pharmacology and Toxicology

Isolation and identification of paraxanthine (Px) glucuronide as the major caffeine metabolite in mice

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Comparative metabolic disposition of [1-Me^{14}C] caffeine in animal species showed that the mouse was characterized by the presence of an unknown polar metabolite amounting to 22±3% of the urine radioactivity (Arnaud, M.J., Drug Met. Disp. *13* (1985) 471). This metabolite isolated from urine of mice exhibited an ultraviolet absorption spectrum similar to xanthine. DCI-MS data showed that it corresponded to an hexuronide of a dimethylxanthine. The ^{1}H-RMN spectrum displayed the typical pattern of singlets of Px, with additional signals of the hexuronide residue consistent with its attachment in the β configuration at the 3 position of the xanthine nucleus. Further proof of structure for the aglycone part of the molecule was brought by the release of Px through the hydrolytic treatment of the compound with TFA. Work is still in progress to definitely ascertain the hexuronic part of the molecule, but the data already suggest that the main caffeine metabolite in mice is the 3-β-D-glucuronide of Px.

Hepatoprotector properties of a dibenzo (a, c) cyclooctene derivative from *Fructus schizandrae chinensis* evaluated in rat with the ^{14}C-aminopyrine/demethylation model

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In a previous study (Benakis et al., Int. Cong. Pharmac. Tokyo, 1981) we have used the model of ^{14}C-aminopyrine (^{14}C-AMPY) demethylation giving $^{14}CO_2$, in order to assess the effectiveness of hepatoprotector agents after CCl_4 and D-galactosamine (d-GAL) intoxication. The same model has been used in the present study to evaluate the hepatoprotector properties of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2,2'-dicarboxylate (DDB), a synthetic derivative of a compound isolated from *Fructus schizandrae chinensis*. Pharmacological properties of schizandrae derivatives and DDB, particularly with regard to hepatic function, have been extensively studied (Pao Tien-tung et al., Chin. med. J. *3* (1977) 173, and K.T. Liu et al., Chem. biol. Inter. *39* (1982) 301; *39* (1982) 315; *41* (1982) 39).
Results of the present study in rats (6 male Sprague-Dawley, 220 g) showed that DDB pretreatment (3 × 200 mg/kg oral) reduced the demethylation value of ^{14}C-AMPY (25 mg/kg) after CCl_4 intoxication (0.2 ml/kg) from 33% to 23%. When d-GAL (500 mg/kg) was used as the intoxication agent in DDB pretreated rats the reduction in demethylation value was from 32% to 19%. Although present results show DDB to be an effective hepatoprotector agent, further studies are needed to determine the dose required for optimal effect.

Correlation between metabolism, clinical efficacy and side-effects of maprotiline

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Two genetically controlled hydroxylation defects have been described: debrisoquine (D) and mephenytoin (M) polymorphism. In 1974, Angst und Rothweiler published the results of a double-blind trial comparing 150 and 225 mg per day maprotiline (MP). 10 years later, 16 out of the 20 patients could be reexamined using a combined test procedure for the examination of the D- and M-polymorphism. All patients were extensive metabolizers for M (EM-M). Four patients (25%) could be classified as poor metabolizers for D (PM-D). Two out of these four patients had developed very high blood levels of MP and were nonresponders. The two other PM-D had normal blood levels of MP and were responders. A prospective study was done with 80 newly hospitalized depressed patients. The D-M test was performed before treatment. Psychopathological and somatic symptoms were documented with the AMDP-system. One patient could be classified as PM-M and four as PM-D. PM did not differ from EM concerning blood levels of MP, improvement of depressed symptoms and of side-effects.

Noradrenaline (NA) turnover in locus coeruleus (LC): Pharmacological characterization and effect of vincamine (V)

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V and other nootropics increase the firing of LC NA neurons (Olpe and Steinmann, J. neural Transm. *55* (1982) 101). This should result in an increase in NA turnover in this area. A method for reproducible dissection of LC was developed. The dopamine (DA) metabolite, DOPAC, which reflects NA turnover in NAergic areas, was determined in LC by HPLC with coulometric detection. The DA-β-hydroxylase inhibitor, FLA 63 (40 mg/kg i.p.), increased DOPAC to 346±42% of controls, the α_2-antagonist idazoxan (I) at 10 and 20 mg/kg i.p. to 193±23% and 294±24%, resp. The effects of I and FLA 63 were additive. Prazosine (1–10 mg/kg i.p.) and haloperidol (1 mg/kg i.p.) were inactive. Clonidine (1mg/kg) reduced DOPAC to 57±9% of controls and pargyline to 0%. These results suggest that alterations in LC DOPAC indeed reflect changes in LC NA turnover. V (10 mg/kg i.p.) had no effect and dit not enhance that of 10 mg/kg I.

Uptake and storage of ^{3}H-MPP$^+$ in blood platelets

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The oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to 1-methyl-4-phenyl-pyridinium (MPP$^+$) by monoamine oxidase-B and the selective accumulation of MPP$^+$ by dopamine (DA) neurons in the substantia nigra seem to be important steps in the neurotoxic action of MPTP. The uptake of ^{3}H-MPP$^+$ was studied in blood platelets. ^{3}H-MPP$^+$ is actively taken up by human platelets with a K_m value (20 µM) similar to that for DA. The uptake is inhibited by serotonin (5-HT) uptake blockers, KCN and ouabain. Drugs that interfere with the storage of amines in the 5-HT organelles reduce the uptake of ^{3}H-MPP$^+$. By reducing the proton gradient at 5-HT storage sites, ionophores induce the release of radioactivity from platelets loaded with ^{3}H-MPP$^+$. Subcellular fractionation of rabbit platelets preincubated with ^{3}H-MPP$^+$ shows the drug to be localized mainly in the dense granules fractions. These data indicate that MPP$^+$ enters the platelets via the 5-HT pump and is accumulated in the amine storage organelles.

Rapid measurement of the MAO-B activity in human platelets by a newly developed assay with ^{3}H-MPTP as substrate

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Accumulation of ^{3}H-MPP$^+$ into platelet rich plasma (PRP) after incubation in ^{3}H-MPTP is proposed for measurement of platelet MAO-B activity (Neurosci. Lett. *57* (1985) 257). Human PRP (200 µl) was incubated for 30 min at 37°C with various MAO-B inhibitors and then exposed for 60 min to ^{3}H-MPTP (10 nM). The formation of ^{3}H-MPP$^+$ was blocked in icecold Tyrode (1 ml). The samples were passed through GF/C glass-fiber filters which were rinsed with cold Tyrode (2 × 2 ml) and then transferred to counting vials. Radioactivity was expressed in dpm/h/mg platelet protein (PP). IC50 values (calculated from results with five different concentrations) for L-deprenyl, Ro 16-6491, MDL 72145, AGN 1135 and almoxatone (MD 230928) were: 8, 10, 40, 50, 100 nM PP, resp. These values are very similar to those obtained using conventional MAO-B substrates e.g. ^{3}H-PEA. This new, simple method is well suited for the rapid, precise assay of MAO-B in clinical studies with fully reversible inhibitors, e.g. Ro 16-6491 (J. Neurochem. *44*, suppl. (1985) S94).

DNA binding and mutagenicity in the Ames test with trenbolone

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The anabolic steroid hormone analog trenbolone (Tb) is generally regarded as non-mutagenic. We found, however, that low doses of Tb increase the number of revertants of *Salmonella* strain TA100 reproducibly and in a dose-dependent manner. Relative mutagenicities of 1.2–1.3 were induced after preincubation of the bacteria with doses between 30 and 60 µg per plate. Above this dose the number of revertants decreased to control values, together with a reduction in survival. The addition of S9 eliminated the mutagenicity. Tb was irreversibly bound to DNA isolated from *Salmonella* after incubation of the bacteria with [^{3}H]Tb. Covalent DNA binding is therefore proposed as the mechanism of mutagenicity. It is concluded that bactericidal or sparingly soluble compounds which give negative Ames test results at high doses should be carefully reevaluated at low doses.

Acetylcholine release: involvement of creatine kinase and effects of presynaptic toxins

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In the rat sympathetic ganglion treated with the black widow spider venom (Brain Res. *44* (1972) 289) and in the Torpedo electric organ treated with botulinum toxin, the enzyme creatine kinase was found to be inactivated. We have tried to mimick the action of these toxins on cholinergic synapses by using agents such as fluorodinitrobenzene or diamide (a thiol-oxidizing compound). Both inhibited creatine kinase, blocked synaptic transmission and provoked a large increase in the spontaneous release of transmitter, mainly in the form of miniature and subminiature potentials. It is concluded that the quantal release of acetylcholine requires energy provided by a ATP-creatine phosphate system. Directly or indirectly, the above presynaptic toxins may act by inactivating this system.

Effects of 3 compounds with anxiolytic properties on the EEG of rats in comparison with maprotiline and scopolamine

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The effects of the anxiolytic substances diazepam (DI), meprobamate (ME) and phenobarbital (PH) on the cortical and hippocampal EEG (coEEG, hcEEG) of freely moving rats were studied by spectral analysis and compared with those of the monoamine reuptake inhibitor maprotiline (MA) and the anticholinergic scopolamine (SC). In the coEEG, DI, ME and PH increased power in all frequency bands above 12 Hz in a dose and time dependent manner. MA increased power in the 6–12 and 12–20 Hz ranges, SC in the slow bands. In ther hcEEG, all compounds tested increased power in the 0–5 Hz band, but differences were found in other spectral components. DI and ME, but not PH, decreased power between 5 Hz and 9 Hz, whereas ME and, to a lesser extent, DI and PH, increased power above 16 Hz. MA had no effect on power above 5 Hz, SC increased power in all ranges below 32 Hz. These results confirm the selective property of three chemical classes of anxiolytics to induce fast frequencies in the coEEG.

The benzodiazepine midazolam enhances visual backward masking

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In psychopharmacology few models are available for precise and specific assessment of drug effects. Visual information processing in humans might prove to be a promising example. We therefore studied effects of midazolam 12 mg p.o. on visual backward masking with 6 healthy 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 almost tripled from 5,3% at the baseline up to 13.5% 2 h after drug administration ($p < 0.01$), and it was still slightly raised compared to the baseline after 8 h. At a SOA of 120 msec, however, no drug effects were observed, suggesting little influence of nonspecific factors such as attention.
It is concluded that midazolam (and presumably all benzodiazepines) specifically interferes with some aspects of visual information processing.

Glu-containing dipeptides do not modulate excitatory amino acid receptors

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Dipeptides of the structure X-Glu (X = Phe, Leu, Met) have been proposed as allosteric modulators of excitatory amino acid receptors in rat brain membranes (Ferkany et al., Neurosci. Lett. *44* (1984) 281). Here we report that these dipeptides reduce the binding of L-H-Glu to N-methyl-D-aspartate (NMDA) receptors in isolated postsynaptic densities (Fagg and Matus, PNAS *81* (1984) 6876) with potencies (Phe-Glu, Ki = 8 μM) similar to those reported by Ferkany et al. However, several observations suggest that the effects of these peptides are mediated by Glu liberated via the actions of a membrane-bound amino-peptidase (and not by allosteric receptor modulation). 1) The relative potencies of the dipeptides are similar at all excitatory receptors, suggesting a common underlying mediator (e.g., Glu). 2) N-Acetylated dipeptides are poor substrates for aminopeptidases and are poor inhibitors at NMDA binding sites. 3) Bestatin, an aminopeptidase inhibitor, reduces the inhibition of NMDA receptor binding by the dipeptides.

Modulation of onset of type II collagen induced arthritis

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Sensitization of DBA-1 mice with bovine type II collagen (CII) in complete freunds adjuvant (CFA) can cause polyarthritis (CiA), however, the role of cellular and humoral immune response(s) to CII are still under debate. To study the role of cellular immunity in vitro restimulated lymph node and spleen cells from CII/CFA primed DBA-1 donors were tested for their ability to adoptively transfer arthritis. Transfer of disease with up to 50×10^6 cells/recipient has proven unsuccessful. However, subsequent active immunization for CiA of lymph node cell recipients resulted in the onset of arthritic symptoms over one week sooner than in control animals. Spleen cell recipients on the other hand showed a delay in onset of arthritis of one week. Both cell populations were capable of transferring delayed type hypersensitivity to CII.
From these results it can be concluded that the popliteal lymph node cell population is predominately of T helper phenotype whereas in spleen the CII specific cells are primarily suppressive cells in nature.

Some fiber tracts contain more opioid binding sites than do the nuclei in the guinea pig brain: a quantitative autoradiographic study

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In vitro autoradiography using [^{3}H]-(–)-bremazocine was performed on cryostat sections of guinea pig brain. Quantification of nuclei was accomplished using standard samples of tritium mixed in brain gray matter. Fiber tracts were quantified using standards made from white matter to compensate for the quenching caused by myelin. Several fiber tracts such as cerebellar white matter and corpus callosum contained densities of [^{3}H]-(–)-bremazocine binding sites equal to or higher than the most densely labeled nuclei. The dorsal hippocampal commissure and the splenium of the corpus callosum contained 2200 fmol sites/mg protein, 2.5 times more than one of the most densely labeled gray areas, the external plexiform layer of the olfactory bulb. These sites may be receptors in transport, in which case the terminal axonal arborization is probably very large and the turnover of receptors high in these neurons.

In vivo binding characteristics of benzodiazepine receptor ligands: quantitative autoradiography and image analysis

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An in vivo binding assay with anatomical resolution has been used to determine the ID_{50} values for various benzodiazepine receptor ligands in 12 mouse brain regions. In competition binding experiments, the ligands (1, 3, 10, 30, 100 mg/kg) were administered p.o. 15 min prior to a bolus i.v. injection of the antagonist ^{3}H-Ro 15-1788 (1 mCi[= 3.5 μg]/kg 5 min). Brain sections were evaluated by quantitative autoradiography with image analysis. Nonspecific binding (in the presence of 100 mg/kg p.o. diazepam) was 15–20%. The mean ID_{50} for diazepam (4 mg/kg p.o.) corresponds well with that determined biochemically. This assay will be used to study partial agonists at benzodiazepine receptors whose atypical pharmacological profiles might be the result of local differences in receptor structure,

receptor conformation or in fractional receptor occupancy and reserves in brain regions. Future applications of the assay will include studies of the 1-cycloserine-induced GABA shift, the correlation between duration of binding and of pharmacological action, and finally receptor regulation during acute and chronic drug tolerance.

Isolated perfused adipose tissue of rats, uptake of lipophilic compounds

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Uptake of xenobiotics into isolated perfused rat adipose tissue was studied. Aorta and vena cava were cannulated and ligations were placed so that only an epididymal fat pad was perfused. Perfusion experiments were performed in situ and non-recirculating, for up to 350 min, with buffer containing 4% albumin. The functionality of the preparation was tested. Thiopental (THP), imipramine (IP), chlorpromazine (CPZ), DDE, and 2, 4, 5, 2', 4', 5'-hexachlorobiphenyl (6-CB) were used at influx concentrations of 2–8 μM. All five model compounds were readily taken up. Rate of uptake tended to decrease initially and to reach a constant value. Mean relative uptake in % was: THP 38±8, IP 69±4, CPZ 85, DDE 56, 6-CB 13±1. Thus, IP and CPZ, which do not accumulate in adipose tissue in vivo, are even more rapidly taken up into the isolated perfused adipose tissue, i.e., in the absence of other tissues, than is THP.

Influence of dietary fats on striatal phospholipid fatty acid profile and acetylcholine (ACh) release

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Since dietary fatty acids (FA), according to their degree of saturation, alter the FA composition of cellular membrane, it is assumed that they could have an influence on choline metabolism. In the present study, four nutritionally adequate diets containing fats differing in their degree of saturation (safflower oil, grape-seed oil, beef tallow, coconut oil) were fed to adult rats for three months. The proportion of saturated to polyunsaturated FA in the liver phospholipids was to some extent a picture of the dietary fats. In the striatum, changes were somewhat less significant; however differences were observed in the proportion of 18:2 ω6, 22:5 ω6, and in the ratio ω6/ω3. Both tissue contents and release of ACh and choline from striatal slices were not affected by the different dietary treatments. It is concluded that the observed changes in phospholipid FA profile had no influence on the cholinergic parameters studied.

Pharmacological characterization of tachykinin receptors on the endothelium of isolated pig coronary artery

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Substance P (SP), neurokinin A(SK), neuromedin K(NK), physalaemin (Phy) eledoisin (Eld), kassinin (Kas) were used to identify and locate the tachykinin receptors and their subtype in the isolated pig coronary artery. The open rings were suspended in oxygenated Krebs solution (34 °C) and were studied as changes in isometric tension. All the tachykinins relaxed the rings (with endothelium) precontracted with acetylcholine (10^{-5}), while they had no effect on the rings without endothelium. The order of potency was SP $\geq$ Phy > Eld > Kas > SK > NK with ED_{50} values 0.38±0.1, 0.38±0.5, 1.2±0.2, 2.6±0.9, 8.3±0.6 and 34±4 nM respectively. Three new SP-analogues were studied for their antagonistic properties. It was observed that two of them effectively blocked the vasodilator effects of SP, NK, SK and Eld in a dose dependent manner, but not to Phy. The third could partially block the responses to Phy as well at a conc. 2×10^{-6}M. We may conclude that coronary artery possesses the receptors of SP-P subtype on the endothelium.

Immunostimulation by a PGE_2 analogue in rats

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Prostaglandin E types (PGE) reportedly play a role in the regulation of cellular and humoral immune responses, where they act as feedback inhibitors (J. clin. Immun. 3 (1983) 295). The aim of the present investigation was to compare the modulatory effect of a PGE_2 analogue (dimethyl-PGE_2, $dmPGE_2$) with cyclosporin (CSA) on the in vivo immune response in rats. The methods used were measuring the humoral (plaque forming cells, PFC, against sheep erythrocytes) and the cellular immune response (local GVHR) in Wistar/Furth F_1 rats. $DmPGE_2$ was given at 10 μg/kg s.c. by minipump, CSA orally at 40 mg/kg. Results: $DmPGE_2$ enhanced dose dependently the number of PFC against sheep erythrocytes as well as the local GVHR. CSA inhibited both humoral and cellular immune responses. $DmPGE_2$ reversed the immunosuppressive effect of CSA.

The antiarrhythmic agent cibenzoline inhibits the cardiac Ca^{2+} inward current

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Cibenzoline, a novel antiarrhythmic drug with predominant class 1 (local anesthetic type) properties, was investigated for possible effects upon the myocardial Ca^{2+} inward current. In voltage-clamp experiments on isolated guinea pig cardiac myocytes, cibenzoline caused a concentration-dependent inhibition of the Ca^{2+} current (IC_{50} value 14 μM). The magnitude of Ca^{2+} current inhibition depended upon stimulation frequency (greater block at higher frequencies) and on the membrane potential (greater block at −35 mV than at −80 mV membrane potential). Cibenzoline also caused negative inotropic effects in isolated guinea pig papillary muscles (IC_{50} = 35 μM) and in isolated rat aortic strips (IC_{50} = 55 μM). It inhibited [^{3}H]nitrendipine binding to guinea pig cardiac membranes at concentrations above 10 μM. Thus, cibenzoline appears to possess Ca^{2+} channel blocking properties (class 4) in addition to blocking Na^+ channels.

Nicotine stimulation of intramural inhibitory nerves in the guinea pig Taenia caeci

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Relaxations in response to nicotine (Nic) of strips of the taenia contracted by carbachol or by histamine (atropine added) were compared with those following field stimulation (FS). The responses were unaffected by α- and β-adrenoceptor antagonists, but were suppressed by apamin. Indomethacin decreased the responses to Nic, but not to FS, and this effect was reversed by prostaglandin E_2. Drugs with membrane stabilizing action (lidocaine, dl-propranolol) antagonized the Nic responses at concentrations ($\leq 10^{-5}$M) inactive on FS. The enzyme nucleotide pyrophosphatase (NP, 0.5 U/ml), which breaks down ATP to AMP, reversibly inhibited the responses to Nic, but not to FS. Assuming that ATP is the transmitter, the failure of NP in inhibiting the responses to FS may be due to an insufficient concentration of the enzyme. It could be, however, in view of the modulation by

prostaglandins and of the sensitivity to low concentrations of membrane stabilizing agents, that Nic stimulation is inhibited by a fraction of the enzyme unrelated to NP activity.

Improvement of radial maze patrolling in senescent rats by 3,4-diaminopyridine

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Female Sprague Dawley rats (25 mo) were tested for 15 days in a radial tunnel maze arrangement. According to the patrolling efficiency shown on experimental days 12–15, two experimental groups were formed. Group 1 consisted of 10 poor maze learners, whereas the 10 animals in group 2 constantly showed an efficient patrolling of the maze. On experimental days 16–18 both groups were injected with 0.5 mg/kg b.wt 3,4-diaminopyridine (3,4-DAP) prior to behavioral testing. 3,4-DAP significantly enhanced the patrolling efficiency of the poor maze learners. On the other hand spatial performance of group 2 was significantly decreased. On days 19–21 all animals received saline injections and performance returned to the original level.

Evidence for the release of 1-methyl-4-phenylpyridinium (MPP^+) from rat striatal neurones in vitro

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^{3}H-MPP^+ (1-methyl-4-phenylpyridinium) is taken up into rat striatal small slices, a temperature-, time- and Na^+-dependent process which can be blocked by inhibitors of the uptake of dopamine (DA) but not of noradrenaline and 5-hydroxytryptamine. In superfusion experiments, authentic ^{3}H-MPP^+, previously taken up by these slices, is released by 15 mM KCl. This release is inhibited by (–)N-propyl-norapomorphine and enhanced by (–)sulpiride, just as observed under the same conditions for the ^{3}H-DA release from ^{3}H-DA-preincubated slices. Together with the finding that unlabeled MPP^+ decreases endogenous DA in striatal slices and enhances the spontaneous release of ^{3}H-DA from preloaded striatal slices, we conclude that striatal DA-ergic nerve endings to not discriminate between DA and MPP^+ for their active transport, storage and depolarization-induced and autoreceptor-regulated release.

Marked MPTP-induced dopaminergic neurotoxicity in black mice with high cerebral MAO-B activity

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The neurotoxic activity of MPTP for brain dopaminergic neurons displays a large variability among different species and strains of animals. The mechanism by which MPTP produces toxic neuronal effects is based on its conversion to MPP^+ by the monoamine oxidase type-B (MAO-B) (Markey et al., Nature *311* (1984) 464). We found that the black mice (BM) C57 Bl/6J and C57 Bl/H-2K have a brain MAO-B activity which is twice (5 nmoles PEA deaminated/h/mg fresh tissue) that of Füllinsdorf albino mice (FAM). After s.c. injection of ^{3}H-MPTP, the amout of ^{3}H-MPP^+ in the striatum of the BM remained at high levels for more than 16 h whereas only trace amounts of radioactivity were measured in FAM 8 h after ^{3}H-MPTP injections. In BM, the brain dopoamine (DA) level was dramatically reduced 12 days after 4 daily s.c. injections of 30 mg/kg each of MPTP (> 80% DA decrease). In contrast, FAM were markedly resistant to MPTP (only 40% decrease of brain DA). MPTP appears to induce more severe neurotoxic effects in BM, perhaps because of their high cerbebral MAO-B activity.

Hemostatic effects of vasopressin analogues: sheep as an animal model

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Deamino-8-D-arginine vasopressin (DDAVP) enhances the plasma levels of the blood clotting factor VIII (F VIII) in humans. The mechanism of this response is unknown. Despite of its side effects, DDAVP is currently used in the treatment of hemophilia A. Searching for an animal model, we tested DDAVP in sheep. Dose-response behavior was investigated in groups (3 animals) treated by doses of 0.01–10 µg/kg. Blood samples were withdrawn shortly before, 5 and 60 min after the injection. F VIII concentration, relative to time zero, was assayed by a clotting test (Merz and Dade) in dilution series of 1:5 to 1:200. The bell-shaped dose-response curve showed a maximum at approx. 1–2 µg/kg DDAVP. Time course of F VIII after DDAVP injection (0.1–3 µg/kg) displayed an initial increase (max. 20–30 min) followed by a rebound and a second increase phase (max. in 2 h). The mechanism of this non-monotonic time course is not clear, it may be accounted for by concomitant release of plasminogen activator due to DDAVP administration.

Debrisoquine/sparteine-type polymorphism (DSP) of drug oxidation: kinetic characterization of the defective human hepatic enzyme system using bufuralol, debrisoquine and dextromethorphan as prototype substrates

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The enzymatic deficiency causing the genetic polymorphism of drug oxidation known as DSP was studied in microsomes from human livers with tree prototype substrates in four extensive (EM) and one poor metabolizer (PM) phenotype: Kinetic parameters of debrisoquine (D) 4-hydroxylation, bufuralol (B) 1'-hydroxylation and dextromethorphan (Dex) O-demethylation were determined: The apparent Km's for D and B were 60 and 25 µM in EM-, 600 µM und 150 µM in PM microsomes. This increase in Km in the PM was not observed with Dex, the O-demethylation of which was biphasic with a high and a low affinity component, only the high affinity V_{max} being decreased in PM microsomes (4–15 nmoles/mg/h to 0.1 nmole). These data suggest functionally distinguishable P450 isozymes which are affected in PM subjects in different ways.

Properties of hepatocytes cultured in the presence of DMSO

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Rat liver hepatocytes were maintained in primary culture in HCD medium supplemented with 2% DMSO (HCDD medium) for at least 40 days as described by Isom et al. (PNAS USA *82* (1985) 3252). In HCD medium the content of cytochrome P450, a monooxygenase which activates several procarcinogens, declined within 24 h of plating to 36% of the level in fresh hepatocytes. In HCDD medium this level was partially maintained for at least 14 days, and the addition of phenobarbital increased the content of this enzyme. The cultures were also susceptible to induction of peroxisomal β-oxidation by nafenopin, a non mutagenic carcinogen and peroxisomal proliferator, and to induction of DNA synthesis by nafenopin and the mitogen EGF. The above findings indicate that such a culture system may prove useful for long-term in vitro studies on the mechanisms involved in hepatocyte transformation by genotoxic and non-genotoxic hepatocarcinogens.

Distribution of biodegradable drug-carrier after i.v. application in mice

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Whole body autoradiographs of mice, injected with ^{14}C-polycyanoacrylate nanoparticles, reveal a dotted instead of a smooth appearance of radioactivity (RA) in organs with reticulo-endothelial system (RES). This phenomenon increases with time while in peripheral organs RA drops gradually. Investigations on light (LM) and electron microscopic (EM) levels using autoradiography were carried out elucidating precise localization and state of the drug-carrier in lungs and liver as well as morphological integrity of its target tissues.
In lungs embulus-like clumps of RA were found after 5 min in LM along endothelial walls of alveoli. This cluster formation increases with time. In liver RA is homogeneously distributed in sinusoids as well as in hepatocytes and clusters were found only after 6 h increasing their size with time. On the EM level cluster formation could be verified in both organs and RA is localized within all cell types. No impairments of the tissue were found ultrahistologically.

Dopamine synthesis and release in rabbit retina in vitro: effects of amfonelic acid and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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As previously described (Ofori et al., Experientia *40* (1984) 648), rabbit retinas were used to study dopamine (DA) synthesis and/or release in vitro. 100 µM amfonelic acid (AMF) induced a release of endogenous DA. Thus, tissue DA dropped by 39% and 31% when incubation was done in AMF, in the presence or absence of extracellular Ca^{++} respectively. AMF did not significantly affect DA synthesis, suggesting that its action is not the result of an interaction with DA autoreceptors. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) also released DA, resulting in a 53% decrease in DA stores. In the presence of 100 µM pargyline, MPTP was still able to release DA (35%). Thus it appears that in rabbit retina in vitro, MAO is only partially involved in the pharmacological effect of MPTP.
Rabbit retina in vitro may therefore be used to investigate the modulation of DA release and specifically, the mechanism(s) of action of MPTP, in relation to the study of iatrogenic Parkinsonism.

Combined action of Cd^{2+}, Hg^{2+} and X-rays on V79 cells

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Depending on the concentration and time of treatment, heavy metal ions can modulate radiation effects. By saturating free SH-groups of detoxifying molecules (for example of glutathion) heavy metals can reduce the radical scavenging potential of cells. On the other hand pretreatment of cells with heavy metal ions can increase the endogenous levels of molecules carrying SH-groups. Furthermore heavy metals can induce cascades of enzymatic reactions that generate toxic levels of active oxygen species.
We have studied effects of $CdCl_2$ or $HgCl_2$ alone or in combination with X-irradiation on V79 cells. The induction of primary damage and its repair or enhancement during posttreatment incubation were investigated. The following biological and biochemical endpoints were compared: DNA strand breaks (bulk alkaline unwinding method, single cell assay), clonogenicity (plating efficiency), interphase death (release of radiolabelled adenine from damaged cells).

The effects of a social stress on brain monoamines in C57BL/6 and DBA/2 mice

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The effects of a social stress (defeat) on central monoamines were investigated in two mice strains. In C57 mice, defeat enhanced dopamine (DA) turnover in olfactory bulbs (OB) and the hypothalamus (HYP). DA content was significantly enhanced ($p < 0.02$ left hemisphere (L); $p < 0.1$ right hemisphere (R)) in the OB and in the HYP ($p < 0.1$). Similarly, levels of DA metabolites DOPAC ($p < 0.001$ L; $p < 0.01$ R; $p < 0.05$ HYP) and HVA ($p < 0.001$ L; $p < 0.05$ R; $p < 0.05$ HYP) were significantly enhanced. In DBA mice defeat increased serotonin (5-HT) levels in the OB ($p < 0.02$ L; $p < 0.05$ R) and the HYP ($p < 0.02$). Moreover, DA levels in the HYP were also increased ($p < 0.02$). Thus, defeat enhanced DA turnover in the OB and in the HYP of the C57 mice. In DBA mice defeat enhanced the 5-HT content of both the OB and the HYP, and also increased hypothalamic DA content. Defeat activated the dopaminergic system in C57 mice, but preferentially the 5-HT system in DBA mice.

Role of cellular electrical events in drug- or mediator-induced vasodilatation

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In strips of rabbit main pulmonary artery (RMPA) mechanical and electrical events in response to nicorandil (2-nicotin-amidoethyl nitrate), atrial natriuretic factor (ANF) and acetylcholine (ACh) were studied. All three agents inhibited noradrenaline-induced contractions of the vascular strips in a concentration-dependent manner. This inhibition was dependent on an intact endothelium with ACh, and hence due to the release of endothelium derived relaxing factor, but not with ANF. Nicorandil hyperpolarized the membrane of the vascular cells, reduced depolarization by α-adrenoceptor agonists and decreased the space constant λ. Both ANF and ACh had no effect on resting membrane potential. However, ACh repolarized the membrane previously depolarized by noradrenaline. All three agents have been reported to increase intracellular cGMP. It is concluded that a causal relationship between change in membrane potential and relaxation is unlikely for ANF and ACh, but possible for nicorandil.

TNF in combination with cytostatic agents

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The antitumor activity of partially purified tumor necrosis factor (TNF) was analyzed in combination with cytostatic drugs against intradermally transplanted Meth A sarcoma. Tumors were allowed to grow until they reached an average diameter of ~ 8 mm. TNF was given once i.v. (6000 units as determined in L-M cells) and the cytostatic agents adriamycin (1–10 mg/kg), 5-FU (3–100 mg/kg), cytoxan (50–200 mg/kg) were applied once i.p. 4 h after TNF injection. The strongest antitumor effects and necrosis in normal Balb/c mice were observed when TNF was combined with the following doses of cytostatic agent: cytoxan 100 mg/kg, adriamycin 5 mg/kg, 5-FU 30–100 mg/kg. The most effective combinations induced complete regressions. Experiments were repeated in Balb/c nu/nu mice. Two major differences were noted in comparison to normal mice: 1) tumor necrosis was less pronounced, 2) the antitumor effects were moderate, partial regressions were only obtained with the highest doses of cytoxan.

TNF might have a potential as an anticancer agent in combination with cytostatic drugs.

Assay of rat brain normetanephrine (NMN) and 3-methoxy-tyramine (3-MT) by a new HPLC-AD method: effect of MAO- and amine reuptake-inhibitors

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NMN and 3-MT, the 3-0-methylated metabolites of noradrenaline (NA) and dopamine (DA), were measured by reverse phase high pressure liquid chromatography with amperometric detection (HPLC-AD) in the hypothalamus, striatum, frontal cortex (FC) and pons/medulla of rats administered neurotropic drugs. To block MAO and COMT activities, rat brains were submitted to microwave irradiation 210 s after decapitation. The MAO-A inhibitors moclobemide, brofaremine and isocarboxazid enhanced preferentially the 3-MT levels in the FC after a single dose. Monoamine uptake inhibitors e.g. diclofensine and (–)oxaprotiline induced acutely a transient increase which was followed by a decrease and, subchronically, a prolonged elevation of NMN and 3-MT levels. This method is useful for measuring NMN and 3-MT formed extraneuronally at high turnover rates and for studying the mechanism of action of neurotropic drugs on the catecholaminergic system.

Application of in vivo NMR to CNS pharmacology

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Brain levels of ATP, PCr, P_i and pH, measured in the living, intact rat by ^{31}P-NMR-spectroscopy, using a surface coil, were found to be identical to values obtained by biochemical methods. Following cardiac arrest the levels of ATP and PCr decreased rapidly with a corresponding P_i increase and pH drop. Dihydropyridine Ca-antagonists, such as PN 200–110 and nimodipine (3–10 mg/kg i.p.), slowed down the fall of ATP and build-up of P_i significantly, PN being 3 × more potent. These possibly beneficial effects for treatment of stroke were further evaluated by NMR imaging in rats with left middle cerebral artery occlusion. Well defined areas of enhanced NMR signal were visible in coronal brain sections 24 h after occlusion, slightly increasing over the next 24–48 h. Infarct size and location were highly reproducible. Pre- as well as posttreatment with PN or nimodipine (3 × 0.3–0.6 mg/kg/day s.c.) resulted in significant reductions of infarct size. Again, PN was more potent than nimodipine. In vivo NMR is a promising tool for investigations of drug effects since it is non-invasive and hence also applicable to man.

In vitro binding characteristics of benzodiazepine receptor ligands in the CNS: Quantitative autoradiography and image analysis

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Regional differences in the structure or conformation (heterogeneity) of benzodiazepine receptors or in receptor reserves in the CNS are alternative explanations for the atypical pharmacological profiles of some recently described partial agonists at benzodiazepine receptors. In order to investigate the former possibility with anatomical resolution, the in vitro binding characteristics of several benzodiazepine receptor ligands on rat brain sections were studied in competition binding experiments with ^{3}H-meclonazepam. The binding data (B_{max} and K_D values for the radioligand as well as IC_{50}/K_I values for each ligand) were determined by quantitative autoradiography and image analysis of twelve brain regions. The regional binding profiles in vitro of the various ligands tested to date revealed no major receptor heterogeneity. Whether partial agonists possess regional differences in their ability to undergo a GABA-shift in vitro (thereby reflecting their local agonistic or antagonistic activity) is currently being investigated.

Pharmacokinetics of obidoxime-chloride – a potential reactivator of alkylphosphate inhibited acetylcholine esterase

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The distribution and kinetics of ^{14}C-labeled obidoxime-chloride (bis-[-4-hydroxy-iminomethyl-pyridinium-(1)-methyl]-aether, Toxogonin®) were studied by whole-body-autoradiography in male and pregnant mice. Time intervals between intravenous administration of ^{14}C-obidoxime-chloride (0.1 mg/g) and the death of the animal ranged from 90 s to 1 h. In further experiments animals were exposed to sarin (1.5 µg/g i.v.) and the effect of ^{14}C-obidoxime-chloride was tested when given either prophylactically or therapeutically. The CNS (brain and spinal cord) was free of radioactivity whereas kidneys and liver showed high amounts of radioactivity diminishing primarily by renal excretion and secondarily by biliary excretion. Increased amounts of radioactivity in pregnant mice and a similar distribution pattern indicate a delayed elimination. The application before or after Sarin dit not change the distribution pattern, elimination however was delayed. These results indicate a retardation of elimination of obidoxime and no change of the blood-brain-barrier by obidoxime or sarin.

Effects of prenatal exposure to diazepam on the electroretinogram (ERG) of adult rats

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The ERG of adult rats was investigated in chloral hydrate anesthesia after administration of diazepam (1.25 mg/kg·day, s.c.) during the third week of gestation. In the absence of any drug, the ERG of adult offspring (D-rats) of diazepam-treated dams did not differ from that of offspring (K-rats) from vehicle-treated dams. Acute administration of diazepam caused a decrease of the b-wave in a dose-dependent manner. This decrease was delayed in D-rats. Under the influence of haloperidol the amplitude of the b-wave increased significantly in both D- and K-rats. This increase was significantly higher in K- (+58%) than in D-rats (+37%). On the contrary, apomorphine caused a decrease of the b-wave . With the lowest dose, a significant effect was only seen in D-rats. Neither of the two dopaminergic drugs did alter the a-wave. Prenatal exposure to diazepam seems to cause a persistent subsensitivity to benzodiazepines and a supersensitivity in the DA system of the retina.

Phenobarbital protects hepatocytes against the cytotoxic activity of a hepatocarcinogen

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The cytotoxic and genotoxic activity of the liver carcinogen N-nitrosomorpholine (NNM) and of the liver tumor promoter phenobarbital (PB) were investigated in freshly isolated cultured rat hepatocytes. 2N, 4N and 8N cells were analyzed by flow

cytometry. After treatment with NNM (0.1–100 mM) a dose-dependent nuclear fragmentation, a decrease in the 4N and an increase in the 8N cell population was found at the 5th day. PB alone (10^{-3} mM and 1 mM) had no effect on the ploidy of the hepatocytes. In cells treated with PB one day after NNM, the cytotoxic activity of NNM (0.1 mU and 1 mU) was reduced. Data suggest that the protection of cells by the tumor promoter facilitates the expression of the genotoxic alteration leading to preneoplastic cells.

The 14-C-dextromethorphan test for the radiometric assessment of polymorphic drug oxidation in vitro

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Rapid and sensitive methods for the determination of polymorphic drug hydroxylation in liver biopsies and extrahepatic tissues are still lacking. In man, the oxidative O-demethylation of dextromethorphan co-segregates with polymorphic debrisoquine hydroxylation and we therefore synthesized in 14-C-labeled dextromethorphan (14-C-O-methyl) in order to use the 14-C-formaldehyde formation rate as an expression of the debrisoquine type cyt P-450 activity in vitro. To validate the optimal experimental conditions, rat liver microsomal preparations were incubated at varying concentrations of microsomal protein, semicarbazide and 14-C-labeled substrate for different time periods. The Michaelis-Menten kinetics in the formation rate of 14-C-formaldehyde was studied in female rats of the Sprague-Dawley (SD) and the dark Agouti (DA) strain which represents an animal model for genetic drug hydroxylation deficiency. K_m-values of 0.03 mM versus 0.14 mM and V_{max}-values of 180 versus 80 pmoles/min/mg protein were calculated in SD and DA rats, respectively. These findings indicate that the radiometric formaldehyde determination after dextromethorphan incubation is a valuable tool in the assessment of the debrisoquine-type cyt P-450 activity e.g. in liver biopsies and extrahepatic tissues.

Physiology

Effects of bradykinin (BK) and Des-Arg^{9}BK on the mechanical tension and transmembrane potential of smooth muscles of isolated pig coronary arteries: role of the endothelium

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Transversal strips of pig coronary arteries (left descending branch) are incubated in vitro. Transmembrane potential is recorded intracellularly and isometric tension of the whole strip is measured simultaneously. Bradykinin (BK) relaxes muscle contracted with acetylcholine 10^{-6}M ($ED_{50} = 2.6 \pm 0.6$ nM; $n = 5$). This relaxation is accompanied by a transient hyperpolarization (17.4mV±1.8; $n = 10$) for a submaximal dose of (200 nM BK). BK however has no effect on a precontracted strip which has been freed of its endothelium. Des-Arg^9 BK has no effect on a precontracted strip, but it contracts a relaxed strip independently of its endothelium. This contraction is not accompanied by a significant change in the muscle's transmembrane potential ($n = 28$ cells using 12 tissues). We conclude that pig coronary arteries have BK B_2 receptors on their endothelium that mediate an hyperpolarization and relaxation of smooth muscles. BK B_1 receptors develope on smooth muscles during in vitro incubation. They mediate contraction by a pharmacomechanical coupling.

Synthesis of placental proteins by the in-vitro perfused placenta

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Many pregnancy-specific and pregnancy-associated proteins of mol.wt of up to 1,000,000 daltons increase in concentration in the maternal circulation during pregnancy. For most of them the function is yet unknown. In addition, we do not know which of these substances are synthesized exclusively by the placenta and which can be produced elsewhere (decidua, liver, plasma cells etc.) upon stimulation by pregnancy. An in vitro perfusion, system with separate perfusion of fetal and maternal compartment in an isolated lobe of human placenta provides information on the placental synthesis of these proteins. At regular intervals, an aliquot of the perfusion buffer is removed from both circulations and the placental proteins assayed. The system will be presented and the course of synthesis of placental proteins under various conditions shown. hCG, hPL, and SPl are constantly released into the medium on the maternal and the fetal side during the 4-h examination interval. PAPP-A and α_2PAG show the same pattern on the maternal side but they could not be detected in the fetal circulation. There is considerable variation from one placenta to another, which reflects the wide range of in vivo concentrations of these markers in normal pregnancy,

Reabsorption of ^{14}C-pyrazinoate (PZA) by the proximal tubule (PT) of rabbit kidney

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PZA, the active metabolite of the tuberculostatic pyrazinamide (which induces hyperuricemia) is both actively secreted and reabsorbed by the renal PT. We studied PZA reabsorptive mechanisms in PT S_2 segments by the in vitro tubular microperfusion technique. At 37°C, PZA reabsorption was saturable to a plateau of about 5000 fmoles/min/mm tubular length with 3 mM luminal PZA, value which was depressed to 16% of controls at 25°C. Peritubular ouabain (10^{-4}M) decreased reabsorption of 0.1–0.15 M luminal PZA by 75±12% ($n = 5$). 10 mM luminal lactate inhibited PZA reabsorption by 66%. Peritubular ethoxyzolamide (10^{-4}M) inhibited PZA reabsorption by 26±6% ($n = 5$). It is concluded, in agreement with brush border membrane vesicle data, that PZA reabsorption was active and might share the sodium-dependent mechanism of lactate, and that part of this reabsorption might be linked to H^+ secretion.

Acute effect of hypothalamic lesions on insulin secretion promoting activity present in the plasma of normal rats

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Previous work from this laboratory permitted to purify a peptide having insulin secretion promoting activity from rat hypothalamus. To investigate whether this peptide would behave as an humoral factor, the plasma from rats was submitted to the same purification procedures; that permitted to demonstrate the presence of a substance having a mol.wt and a biological activity similar to the one shown by the hypothalamic factor. To study the possible hypothalamic origin of the active principle present in the plasma, a group of rats was submitted to bilateral hypothalamic lesions. Their plasma was collected and tested in vitro for its insulin secretion promoting activity. Bilateral hypothalamic lesions significantly reduced the insulin secretion promot-

ing activity present in the plasma. These results suggest that the factor present in the hypothalamus is released in the blood and that it could participate to the hypothalamic control of insulin secretion by reaching the pancreas through the circulating route.

Organotypic tissue culture of rat spinal cord

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The structural complexity of the synaptic junctions formed by muscle spindle afferent fibers upon motoneurons (MNs) handicaps the analysis and interpretation of experimental results obtained from these junctions in the intact animal. Therefore, it would be desirable to have an in vitro spinal cord preparation with good accessibility to microelectrode exploration and good visibility of the neuronal elements. In such a preparation the organotypic organization of the spinal cord, together with some of the afferent and efferent pathways should be preserved.
Slices of fetal rat spinal cord with attached dorsal root ganglia (DRG) are cocultured with pieces of skeletal muscle by means of the roller tube method (Gähwiler, J. Neurosci. Meth. *4* (1981)). Detailed morphological features of DRG-cells and putative MNs could be obtained by intracellular staining with HRP and Lucifer Yellow. Electrical stimulation of one DRG resulted in contraction of the cocultured muscle fibers, demonstrating the functional integrity of the monosynaptic reflex arc.

Insulin: its role in thermogenesis

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Respiratory exchange measurements were performed on 8 young men for 1 h before and 3.5 h of somatostatin infusion. After 30 min somatostatin alone two different protocols were performed. A hyperinsulinemic (1 mU/kg·min) euglycemic clamp (phase 1) was followed by (1 mU/kg·min) insulin at a glycemia of ~ 170 mg/dl (phase 2). On a separate occasion hyperinsulinemic (1 mU/kg·min) hyperglycemia ~ 170 mg/dl (phase 1) was followed by hyperinsulinemia (6.45 mU/kg·min; phase 2) at the same glucose infusion rate as in phase 1.
The thermic effects of glucose/insulin, phase 1 both tests, were 7.1±1.4 and 6.5±0.2% (NS). From phase 2 it was possible to calculate the termic effect of a step increase in either glucose uptake 6.3±0.9% or insulin 1.1±0.7%. The latter only correlated with a change in plasma norepinephrine levels. Much of the thermic response to glucose/insulin infusion is due to glucose metabolism alone. That of insulin is small and appears to be mediated by the sympathetic nervous system.

Involvement of noradrenergic systems in the modulation of cutaneous reflexes in the rat

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Descending noradrenergic fibers have a widespread termination in the spinal cord, including the ventral horn. We present evidence that tizanidine (Sirdalud, Wander), a clonidine derivative, has dose-dependent depressant effects on flexor reflexes of intact rats. This depression did not occur when the animals were pretreated with the α-2 yntagonist yohimbine. When flexor reflexes were tested in spinalized animals, tizanidine failed to depress flexor reflexes and, in the majority of cases, even enhanced the responses. Our current interpretation of results is as follows: Depressant effects of tizanidine are mediated by activation of α-2 receptors at brainstem levels (autoreceptors and presynaptic receptors) which would reduce a tonic noradrenergic facilitation at spinal level. Facilitation of spinal reflexes by locus coeruleus stimulation have been described by Strahlendorf et al. (Neuropharmacology *19* (1980) 225). This may also explain the uncovering of facilitation by tizanidine when its supraspinal action is not transmitted to the cord after its transection. We currently investigate whether the facilitatory effects in spinal rats are mediated by a α-1 receptors.

Release of sulphur containing excitatory amino acids (S-AA): homocysteate as NMDA-receptor agonist

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The S-AA cystein sulphinic acid, cysteic acid, homocystein sulphinic acid and homocysteic acid (HCA) have been determined in fluid superfusing rat brain slices by HPLC. They were differentially released by K^+ depolarization in a Ca^{++} dependent manner. HCA release, observed in all regions, was most prominent in cortex and hippocampus. These S-AA are known to be excitatory and their endogenous release suggests that they play a role in synaptic transmission. Microiontophoretic application of L-HCA to intracellularly recorded cat caudate neurons depolarized their membranes and induced a bursty firing pattern typical for N-methyl-D-aspartate (NMDA) agonist. These excitations could be selectively antagonized by a potent NMDA antagonist D-2-amino-7-phosphonoheptanoic acid. Furthermore, L-HCA and NMDA clearly increased both amplitude and duration of cortically evoked excitatory postsynaptic potentials. These results suggest that L-HCA might be an agonist at caudate NMDA receptors and is capable of modulating EPSPS.

The role of parasympathetic innervation in circadian rhythms of airway resistance and bronchial responsiveness to histamine

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Specific airway conductance (SGAW) and airway response (RH) to dosimetered histamine (PD 35 SGAW) were measured plethysmographically in eight healthy subjects at five identical intervals throughout 24 h during two consecutive days, one day without, the other with blockade of the parasympathetic lung innervation by ipratropium bromide (200 μg) inhaled one hour before each measurement. Circadian rhythms of SGAW, RH and heart rate (HF), quantified by cosinor-analysis, show analogous acrophases during both days, i.e., with and without ipratropium. Parasympathetic blockade, however, results in a significant reduction of SGAW-amplitude, while RH decreased without changes in rhythm amplitude. Conclusion: The results found in SGAW and HF suggest that oscillations of the central vagal tone are the common source of these rhythms. The circadian rhythm of bronchial responsiveness, however, seems to be independent from central vagal tone as well as from airway resistance.

A simple, food-motivation apparatus for testing vibrissal function in mice

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The apparatus is a darkened chamber in which mice, food-deprived for 20 h, are trained to obtain 30–40-mg pieces of food

presented at one of two round openings, containing either a rough or a smooth inner surface. The adjacent openings are double-walled, constructed so as to permit vibrissal contact with the stimuli while prohibiting visual discrimination. The mouse must either remain at the correct (rough) hole for 5 s to obtain the food or, if his initial choice was the other hole, must switch to the correct hole within 5 s, necessitating moving away from the reward in order to obtain it. During sessions of 30 such trials, the stimuli are removed at the start of each 60-s intertrial interval and reversed, or not, according to a pseudo-random order. The training of 3 strains of mice in this test has been undertaken. Also, in preliminary sessions following trimming of all the vibrissae to skin level, it was found that the performance of the trained mice, previously accurate to 70–90%, was reduced to chance levels.

Transport of the organic cation tetraethylammonium (TEA) by renal epithelial cell cultures

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Suspended cells from the LLC-PK1 line, which show many characteristics of the renal proximal cell, accumulate TEA. Steady-state uptake is temperature-dependent, saturable, and inhibited by other organic cations (mepiperphenidol and quinine). We measured transepithelial transport on monolayers of cells grown on collagen-coated filters, glued to lucite rings. [^{14}C]-TEA (0.02 mM) fluxes from the basolateral to apical ($J_{TEA}b \rightarrow a$), and from the apical to basolateral sides ($J_{TEA}a \rightarrow b$) were compared to those of [^{3}H]-mannitol, a compound which is not transported, $J_{TEA}b \rightarrow a$ was 7-fold larger than mannitol flux. In the presence of 1 mM quinine in the medium, $J_{TEA}b \rightarrow a$ was only 1.6 times larger than mannitol flux. $J_{TEA}b \rightarrow a$ was equal to mannitol flux, and was not inhibited by quinine. It is concluded that LLC-PK1 cells are able to secrete TEA, as do cells of the proximal tubule.

The effects of exercise on maximal instantaneous anaerobic power in man

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The maximal instantaneous anaerobic power ($\dot{w}$), as determined during a high jump off both feet on a force platform, was assessed on 8 subjects, starting 1) from rest, 2) from a baseline of steady-state cycloergometric exercise, requiring 30, 50, 70 and 100% of $\dot{V}O_2^{max}$, 3) from a supramaximal exercise (120%, of $\dot{V}O_2^{max}$), and 4) during the transition between rest and each of the above workloads. Blood lactate concentration, [La_b], was measured before and 8 min after the end of each workload. When assessed at steady-state, $\dot{w}$ was found to be constant, independent of exercise duration, but lower the higher the work intensity. Up to 50% $\dot{V}O_2^{max}$, $\dot{w}$ decreased linearly with the exercice intensity; for greater loads, associated with an increase of [La_b], the decrease of $\dot{w}$ was progressively larger. During rest to work transients, $\dot{w}$ decreased exponentially, with a half-time of 22–24 s, independent of workload, and for a given $\dot{V}O_2$, it was equal in transient and steady-state conditions. These data seem to suggest that neuromuscular fatigue is not responsible for the observed drops of $\dot{w}$.

The latter is probably attributable to a decrease and/or to a lesser efficiency of the high energy phosphate stores brought about by exercise directly or via a reduction of intracellular muscle pH due to lactate accumulation.

Sexual maturation (vaginal opening) in Roman high- (RHA/Verh) and low-avoidance (RLA/Verh) rats: is there a pineal influence?

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RHA/Verh and RLA/Verh rats are selectively bred for the rapid- vs non-acquisition of two-way shuttle-box avoidance, respectively. Further differences between these two rat lines have been described in many behavioral and neurochemical parameters.

In the present study sexual maturation in female rats of the two Roman lines has been determined. Vaginal opening (v.o.), an anatomical marker of puberty-attainment, was found to differ by several days (RHA/Verh: day 41, RLA/Verh: day 35). Factors previously shown to influence the time of v.o. are, among others: individual housing (v.o. advanced), constant light (advanced), melatonin administration (delayed). Whether the differences in v.o. between the two Roman lines might be due to the weight differences seen in the pineal glands (males: RHA/Verh 2.4 mg; RLA/Verh 1.0 mg; females: RHA/Verh 1.7 mg; RLA/Verh 1.0 mg) is at present unknown, and neuro-endocrinological tests are presently being conducted to investigate this possibility.

Effects of nafenopin on rat food efficiency and brown adipose tissue peroxisomes

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Nafenopin (Naf) is known to induce peroxisome proliferation in liver. Peroxisomes degrade fatty acids by an energy-dissipating β-oxidation pathway. The effects of Naf on rat food efficiency, body temperature and brown adipose tissue, the major effector of thermogenesis in rodents, were tested. In rats kept at 22°C, Naf decreased both brown adipose tissue weight and mitochondria succinate dehydrogenase specific activity (1.5. and 1.2 times, respectively) whereas it increased peroxisome catalase and acyl CoA oxidase specific activities (1.9 and 2.8 times, respectively). Food efficiency and body temperature were not affected. In rats kept at 32°C, Naf did not change brown adipose tissue weight and succinate dehydrogenase activity but increased peroxisome catalase and acyl CoA oxidase specific activities (2.9 and 3.7 times, respectively). Food efficiency was decreased (1.9 times) and body temperature increased by + 0.57°C. The results will be discussed in terms of stimulation of heat dissipators other than brown adipose tissue.

On the mechanism of hypothermia induced by glucose deprivation in the rat

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Hypoxia or glucose deprivation is followed by hypothermia in mammals. The possible involvement of a central inhibition of nonshivering thermogenesis was studied by using 2-deoxy-glucose (2DG), an inhibitor of glucose utilization. Blood flow distribution and circulating catecholamines were measured 40 min and 120 min after 2DG injection (350 mg/kg, i.v) in conscious rats acclimated either at thermoneutrality (32°C-rats) or at 22°C (22°C-rats). At either temperature, blood glucose and catecholamines were similarly increased after 2DG injection, and most organs and tissues studied responded in a similar way. However, while the blood flow fraction to brown adipose tissue (BAT) was unaffected by 2DG in 32°C-rats, it was largely decreased (60–80%) in 22°C-rats. Noradrenaline locally injected in BAT, after 2DG injection, could increase blood flow fraction to BAT by 2.5 times, at either temperature. These findings are compatible with

a central inhibition of the neural control of BAT thermogenesis following 2DG injection.

Is angiotensin III the centrally active form of angiotensin?

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Paraventricular neurons of the rat were examined using microiontophoretic techniques. In all cases angiotensin III (AIII) was more potent than angiotensin II (AII), as evidenced by lower thresholds, shorter latencies, and higher spike frequencies/nanoampere of applied current. The superior potency of AIII was further exaggerated in spontaneously hypertensive rat (SHR) when compared to normotensive WKY rats. The response to AIII also exhibited much shorter latencies than for AII. Although no difference was observed between AII and AIII, postactivity was greatly elongated in SHR. This appeared specific since no elongation in acetylcholine postactivity was seen in SHR. These data support the notion that AIII is the centrally active form of angiotensin and are consistent with an obligatory conversion of AII and AIII prior to activation. The selective enhancement of postactivity observed in SHR to angiotensins suggests a possible defect in signal termination.

Genetically determined ability of pigs to release ACTH after CRF and vasopressin injection

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Domestic pigs tend to respond excessively to various stressors. To clarify the genetic component of this sensitivity, we have investigated some morphological and functional features of the hypothalamo-hypophyseal-adrenal axis in pigs of two genetically well characterized breeding lines defined as positive ('lean') and negative ('fatty') in respect to production traits. In the positive line proportion of zona fasciculata in adrenals is about 10% greater, the nuclei of the fasciculata cells are significantly smaller; the corticotrophs of the anterior pituitary are enlarged. These differences indicate a hereditary disposition to stress. Release of ACTH following a CRF administration (1 µg/kg i.v.) is doubled in the negative line. ACTH respones to 1) a combined i.v. injection of CRF and lysine-vasopressin (1 µg/kg; 0.1 IU/kg) and 2) CRF (1 µg/kg) after metyrapone (30 mg/kg) and 3) metyrapone alone were similar in the two lines. This discrepancy requires further study.

Vestibulo-cerebellar control of optokinetic nystagmus in the rat as revealed by selective lesions

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Horizontal optokinetic ocular nystagmus (OKN) was studied in brown rats 1–4 days after bilateral flocculectomy (bF), labyrinthectomy (bL), combined labyrinthine-floccular bLF) and labyrinthine-flocculonodular (bLFN) lesions by rotating a dot pattern projected onto a cylindrical screen at constant (2–60°/s) or sinusoidally varying speeds (0.05–2.0 Hz; 15 or 5°/s). Initial slow phase velocity (SPV) of step responses was unaltered in bL-rats, but reduced by about 30–50% in bF- and bLF-rats. Correspondingly, gain of sinusoidal OKN decreased and phase-lag increased strongly with increasing frequency. Some bF-rats exhibited a pendular nystagmus. Steady-state gains were in the normal range in bL-, bF- and bLF-rats for step stimuli up to 10, 20 and 2°/s, respectively. Optokinetic afternystagmus (OKAN) was normal in bF- but virtually abolished in bL- and bLF-rats. However, after additional ablation of the cerebellar nodulus, the rats (bLFN) were again able to build-up SPV and OKAN of normal or even increased duration reappeared.

Fusimotor activity patterns known from reduced preparations appear not to prevail in normal cats

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Direct recordings of fusimotor γ-activity have been achieved in reduced preparations but not in freely moving animals. However, it has become possible to test concepts based on such data for their ability to account for spindle afferent responses, chronically recorded during normal locomotion, by simulating movements and efferent activity profiles in anesthetized cats.
When reproduced movements were accompanied by largely tonic γ_S- and modulated γ_D-action (Murphy et al., J. Neurophysiol. *52* (1984)), simulated responses failed to match the chronically recorded targets, unless γ_D modulation was very small. The inverse pattern (modulated γ_S, tonic γ_D; Appenteng et al., J. Physiol. *305* (1980)) fared even worse. Best matches were obtained with tonic γ_S drive alone, even when α-activity was deeply modulated. Thus it appears that CNS control of α-, γ_S-, and γ_D-activity does not obey a single simple law.

The impact of the oral cavity on glucose utilization in rats

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Freely moving chronically catheterized rats were tested, using a double isotope technique, to measure total glucose appearance and disappearance, gut glucose absorption and hepatic glucose production after the ingestion of 1 ml of a 60% glucose solution or after giving the same glucose load directly into the stomach via a chronic gastric catheter. Glycemia and insulinemia were always higher when the glucose was given intragastrically. The defect explaining the higher glucose values in the intragastric group was a decreased rate of glucose disappearance. It is concluded that when glucose is normally ingested, reflexes are elicited that improve glucose tolerance by increasing the efficiency of glucose utilization despite a smaller insulin output.

Mechanism underlying the activation of the diaphragm during apnoea induced by high-frequency oscillatory ventilation

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HFOV inhibits spontaneous breathing in animals with intact vagus nerves, but leads with a given volume, frequency and pressure-range at the same time also to a tonic diaphragmatic activity. To identify the vagal afferents involved in this diaphragmatic response, the vagus nerves of anesthetized rabbits were gradually cooled (from 30 °C to 0 °C). At each temperature the effects of HFOV were compared with those of static lung inflations. Between 15 °C and 10 °C the lung inflation reflex was weakened or blocked, whereas the HFOV-induced apnoea persisted and the accompanying diaphragmatic activity was strongly enhanced. Under 10 °C this diaphragmatic reaction was also elicited by large static inflation. Under 5 °C the effects of both HFOV and inflations were abolished. The results indicate that during HFOV the stimulation of lung irritant receptors

outweighs the concomitant stimulation of lung strech receptors giving rise to the 'paradoxical' activation of the diaphragm.

Food selection and circadian rhythmicity in chronic methamphetamine and quinine treated rats

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Chronic methamphetamine (MA, 100 mg/l drinking water) and quinine HCl (Q, 250 mg/l) treated rats had reduced daily food and water intake and b.wt. development compared with controls. Chronic MA, but not Q treatment, induced a circadian rhythm disturbance in feeding and drinking not via its aversive character. These groups were given a food preference test (protein- vs carbohydrate-rich chow). Both MA and Q treated animals reduced their daily protein intake and slightly increased carbohydrate intake. All groups showed a positive correlation of daily water intake to %protein/total food intake. Thus the reduced protein preference after chronic MA treatment may be mediated by the reduced water intake. All groups similarly showed a higher preference for carbohydrate at the beginning of the dark phase than at the beginning of the light phase. However at the beginning of the light phase only MA treated rats had increased carbohydrate preference. Both circadian and drug effects on carbohydrate preference can be related to α2-adrenoceptor stimulation in the medial hypothalamus.

Motor unit activation by superimposed voluntary and reflex contractions

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Human subjects were performing steady plantar or dorsal contractions 1) in a tracking paradigm or 2) before a conditioned plantar flexion of a foot in a choice reaction time situation. H reflexes were evoked on both sides during the steady contraction. In both situations, the same results were obtained: 1) During steady plantar flexions, the size of the H reflex was independent of the intensity of the steady contraction in most subjects and 2) the size of the H reflex decreased with increasing dorsal steady contraction. We concluded that plantar steady contractions and H reflexes add linearly. Based on this conclusion, using the known exponential distribution of the contraction forces of motor units and assuming that the size principle is respected, we computed the relative size of EPSPs of motor units as a function of their contraction forces. The computed relation fitted well the experimental data obtained by Harrison and Taylor (J. Physiol. *312* (1981) 455).

Paralytical rabies: peripheral motricity is normal

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In animal and man, the rabies infection shows most commonly either the furious or paralytical forms the pathogenesis of which is incompletely understood. We have tested the nerve excitability and conduction, neuromuscular transmission and muscle contractility in rats inoculated with the CVS strain of rabies virus into the triceps surae muscle. At day 7 after infection, a complete monoplegia and analgesia were observed in the distal extremity ipsilateral to the side of inoculation. As shown by immunofluorescence, the neuromuscular spindles in the injected muscle, the axons and somata of motoneurons and of many interneurons in the corresponding spinal segments were infected. Despite of these facts, all physiological parameters recorded from the infected nerves and muscles were comparable to those obtained in control animals. These results exclude the possibility of peripheral origin of the paralysis and suggest to study the functional properties of the infected motoneuronal somata.

Effects of local cooling on acral rewarming and glucose response in depression

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Although a positive correlation between rate of rewarming and glucose mobilization after cooling (handbath, 15 °C, 5 min) has previously been described by Tramèr for healthy subjects and dermatological patients, our results in depressed patients (34♂, 41♀) show only a weak correlation ($p < 0.05$), that completely disappears after pharmacological intervention. Detailed analysis, however, reveal that the degree of coherence depends upon season and even more on gender. Thus, in female patients the correlation between the thermoregulatory and glucose response is highly significant. Rate of rewarming und glucose mobilization also varies according to gender and season, women showing a greater seasonal amplitude in both parameters. The observation that in winter 90% of the female patients did not mobilize glucose in response to cold compared to 55% in spring and summer and 66% in autumn, is of interest with respect to Seasonal Affective Disorder, a subgroup of depression occurring regularly in winter, with a very high proportion of females, and particularly characterized by increased carbohydrate craving.

Salicylate (SAL) transport in renal brush border membrane vesicles (BBMV)

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Salicylate (SAL) is an organic anion secreted and reabsorbed in the mammalian kidney. We investigated its transport mechanisms by using rabbit BBMV. In voltage- and pH-clamp conditions, uptake of ^{14}C-SAL into vesicles preloaded with either 12 mM unlabeled SAL or 12 mM nicotinate was 1.8±0.2 (n = 5) or 1.5±0.2 (n = 7) times larger than that into unloaded vesicles, respectively. Probenecid (10 mM), p-aminohippurate (10 mM), urate (5 mM), and DIDS (5 mM) inhibited the stimulated uptake of SAL by 80, 50, 50, and 40% respectively. The existence of a salicylate-anion exchange mechanism was thus demonstrated at the renal brush-border membrane. SAL uptake was not stimulated by the presence of a Na^+ gradient (out-to-in), thus SAL is not a substrate for the Na^+- organic anion cotransport mechanism.

Smoking, rapid information processing and event related potentials: effects of inhalation

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The effects of cigarette smoking on rapid information processing and on simultaneously recorded event related potentials (ERP) were compared between two groups of preselected light and deep inhaling smokers. According to the different response contingencies of the stimuli, we obtained ERP's with a late negative shift (LN) reflecting expectation and ERP's with a late P300-positivity (LP) reflecting correct stimulus evaluation.
The light inhalers performed generally better and showed larger LN than the deep inhalers. Pre-to post-smoking improvement were obtained in both groups and these correlated in the deep inhalers with LN increases but not in the light inhalers which

even showed pre-to postsmoking decreases of LN. It appears thus that the two groups differed both with respect to their baseline performance and with respect to the effects of smoke inhale.

Quantal acetylcholine release is correlated by the occurrence of large presynaptic intramembrane particles (IMP) in *Torpedo* electric organ

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ACh release was analyzed electrophysiologically using a loose patch clamp technique and morphologically using rapid freezing and cryofracture. In normal conditions, transmission of a nerve impulse evoked the release of about 1.3 quanta or 8500 ACh molecules per μm^2 of presynaptic membrane. Concomitantly, a large increase in IMP density was observed on both leaflets of the presynaptic membrane (500–600 extra IMP on P-face; 300–400 extra IMP on E-face). This change started 0.5 ms after stimulation and lasted for 2–3 ms. At 5 °C, both the electrophysiological response and the IMP change were significantly slowed down. In presence of 4-aminopyridine, ACh quanta were released during about 600 ms following a single stimulus and the IMP change occurred with the same time course. Decreasing Ca prevented ACh release and the IMP change. We propose that this increase in presynaptic IMP is related to the release of ACh quanta.

Effects of buphenine and β-adrenergic blockers on mammalian retinal function in vitro

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Testing the retina for β-adrenergic mechanisms we applied the agonist buphenine and the antagonists propranolol, exprenolol and ICI 118 intraarterially to perfused cat eyes. Buphenine (5–120 µM) caused dose-dependently and reversibly increase in electroretinogram (ERG) b-wave, decrease in c-wave, and configurational changes in the optic nerve action potential. There were no changes in standing potential, light peak, vascular resistance or diameter of retinal vessels. The β-antagonists per se depressed the b-wave, enhanced the c-wave, and also changed the optic nerve action potential. They inhibited dose-dependently (10–60 µM) the ERG responses to buphenine, with propranolol being the most effective.
These data, together with evidence for β-adrenergic binding sites in cat retina (Bruinink, Dawis, Niemeyer, Lichtensteiger, Exp. Eye Res., in press) imply β-adrenergic contribution to signal processing the the mammalian retina.

Beta-blockade and physiological stress reactivity in type A and B subjects

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Effects of beta-blockade on the coronary prone type A behavior and on vegetative reactivity (plasma catecholamines, blood pressure (SBP, DBP), heart rate (HR), finger pulse amplitude, pulse transit time (PTT), EMG, respiration and skin conductance reactivity) were compared between type A (4 type A1, 6 type A2, 4 type XA) and type B (9 type B3, 7 type B4) middle-aged male employed subjects before and after a 6-week period of treatment with oxprenolol or placebo as a randomized double blind trial. The structured type A interview and a self-disclosure procedure served as stressors. Type A's showed stronger stress induced peripheral vasoconstriction and an enhanced HR level after stress onset. Without altering global type A behavior, oxprenolol generally decreased adrenalin levels and reduced HR and PTT stress reactivity but decreased SBP among the typea A's only.

Psychophysiological reactivity during different mental stress tests in type A and type B individuals

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Two speech tests, the type A structured interview (SI) and a self-disclosure task (SD), a rapid information processing trial (VT30), and a time estimation task (DRL) were presented to 100 randomly selected, middle-aged men. ECG, pulse transit time, fingerplethysmographic amplitudes, EMG, bodily movement, respiration and skin conductance were recorded continuously, while blood pressure measurements were taken before and after each stress period.
All stressors, in particular the SI and SD, elicited vegetative stress responses, although quantitatively and qualitatively different. The individual rank positions of the magnitudes of the physiological reactivities remained highly constant across the different stress periods. Comparison of extreme type A and B subjects suggested that the SI type A's differed from SI type B's in a series of noncardiovascular parameters, while Bortner type A's differed from Bortner type B's mainly in cardiovascular measures.

Excitatory action of bombesin on hippocampal non-pyramidal neurones

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The rat hippocampus possesses binding sites for the tetradecapeptide bombesin and has fibers containing bombesin-like immunoreactivity. The effect of bombesin on non-pyramidal neurones was assessed using unitary extracellular recordings from rat hippocampal slices. In 26 out of 27 neurones tested, bombesin at 0.01–1 µM caused a reversible, concentration-dependent increase in firing rate. These neurones were still excited by bombesin under the condition of synaptic blockade. A synthetic structural analogue of substance P, known to antagonize bombesin in the pancreas, did not suppress the effect of bombesin in the hippocampus. Brain receptors for this peptide may thus be distinct from the peripheral receptors. These and previous results from our laboratory indicate that rat hippocampal non-pyramidal neurones bear receptors for peptides having excitatory effects – bombesin, tachykinins and neurohypophysial peptides – as well as for peptides exerting an inhibitory action – opioid peptides.

Transport of organic cations in renal brush border membrane (BBMV) vesicles of the rabbit

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The transport of three organic cations (N^1-methylnicotinamide (NMN) tetraethylammonium (TEA) and morphine) was studied in rabbit BBM vesicles under voltage clamp conditions. A proton gradient (pHi = 6, pHo = 7.4) stimulated the uptake of TEA (0.2 mM) and morphine (0.1 mM), producing a transient overshoot of 260 and 220% respectively above the equilibrium. In contrast, no overshoot of NMN (0.05 mM) was observed suggesting that NMN might be transported by another mechanism.

This hypothesis is favored by the following arguments: 1) while TEA transport (15 s uptake) was inhibited by 79% by mepiperphenidol (1 mM), inhibition by NMN was only 22%. 2) While preloading the vesicles with 1 mM mepiperphenidol resulted in a 223% stimulation (15 s uptake), preloading with 1 mM NMN had no effect on TEA uptake. Our data suggest the presence of a proton: organic cation exchange mechanism in the rabbit BBM for which NMN has only low affinity.

Modulation of testosterone (T) conversion by prolactinemia (PRL) in men

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The role of PRL on T production and conversion is matter of debate. To further evaluate PRL actions, we compared in male the variations in levels of plasma and salivary T (pT and sT), plasma estradiol (E2) and 3α,5α-androstanediol (3α) after hCG or T injection, under normoPRL, hypoPRL induced by lisuride (L) or bromocriptine (B) and mild hyperPRL provoked by sulpiride (S) or metoclopramide (M) administration.
It appeared that the response of pT to hCG was significantly greater during hyperPRL than hypoPRL. On the contrary, sT, E2 and 3α formations were favored under L hypoPRL and attenuated by S induced hyperPRL. After T injection under M hyperPRL, T conversion was also attenuated. Moreover, androgens/estrogens ratio was favoured toward androgenicity during T compared to hCG injection, both during normoPRL and hyperPRL. We concluded that PRL affects the level of pT and the degree of conversion without modifying the androgen/estrogen ratio which depends only from the origin of T elevation.

Dopamine synthesis in TIDA neurons and prolactin concentration in pituitary gland are modulated by thyroid hormones

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There have been discrepant reports on the influence of thyroid hormones on PRL secretion in the rat with no mention of effects on hypothalamic dopamine (DA). In the present study DA synthesis in TIDA neurons (estimated by DOPA accumulation in median eminence after inhibition of DOPA decarboxylase) and PRL secretion were studied in male rats thyroidectomized (TX), sham-TX, TX treated with T4 (2 µg/100 g b.wt daily, 7 days) or vehicle and in intact rats treated similarly with T4 or vehicle. In TX rats a net increase of DA synthesis ($p < 0.001$) was observed with a marked reduction of pituitary PRL concentration ($p < 0.001$). Treatment of TX rats with T4 reversed these effects ($p < 0.001$), DA synthesis and pituitary PRL being identical to those in sham-TX rats. Treatment of intact rats with T4 did not affect DA synthesis nor pituitary PRL. DA concentration in median eminence and plasma PRL did not vary between groups. These results show an influence of thyroid status on the biosynthetic activity of DA neurons regulating PRL secretion.

Functional interactions between neurons at different tangential distances in the cat auditory cortex

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From 6–8 spike trains were recorded simultaneously from different points of the auditory cortex of nitrous oxide anesthetized cats with a square matrix of 4 by 4 microelectrodes. Spontaneous or acoustically driven activity was analyzed by crosscorrelation techniques to assess the presence of interactions between single unit pairs. From 13 recording configurations obtained in 3 cats, 333 unit pairs were selected for their stability and isolation quality. Presence and types of interactions were analyzed along tangential inter-unit distances. Signs of direct excitatory or inhibitory synaptic connections were rare and limited to adjacent units (4/24). The probability of finding common inputs was high for adjacent units (0.79) and decreased progressively with distance, reaching 0.07 at 5 mm. About 40% of these common inputs were modified by the presentation of an acoustic stimulus.

Change in hypothalamic activity during the immune response

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Brain lesions or stress have been shown to alter the immune response, indicating an influence of the brain on the immune system. Conversely, in this study we observed that neuronal activity in specific brain regions of the rat appears to be dependent on the immunological state of the animal. Fifty-six rats were immunized with sheep red blood cells or injected with Ringer's (controls). Five days later, when the maximum number of antibody-producing cells can be found in the spleen, or one day before, we measured the firing rates of 492 hypothalamic neurons using extracellular recording. Five days after immunization we observed a more than threefold increase in the firing rate in the ventromedial hypothalamus, while one day before no change was observed.
The increased activity was site-specific since the preoptic area and the anterior and dorsomedial hypothalamus exhibited no change in firing rate at day 5. Our results suggest that information about the actual immunological state can reach the hypothalamus and cause a specific change in neuronal activity.

Failure of a 1000 kcal supplement of dietary fat to promote fat oxidation in man

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Seven lean young men spent 3 nights and 2 days in a respiration chamber where their rate of energy expenditure and substrate oxidation were continuously measured by indirect calorimetry. During the first 24 h period they ingested a mixed maintenance diet (MD) containing 30% of calories as fat. An additional amount of 106±6 g of fat/24 h was added to this diet (MD+F 52% of calories as fat) during the following 36 h. Energy balance on the MD was close to zero. The supplement of dietary fat (987±55 kcal/d) led only to an unsignificant increase in 24 h energy expenditure (from 2780±230 kcal/d to 2820±280 kcal/d, $\bar{X}$±SD) and failed to promote the use of fat as a metabolic fuel since fat oxidation was unchanged: 1032±205 kcal/d to 1042±205 kcal/d. Indeed overall energy balance was closely correlated with the fat balance ($r = 0.96$, $p < 0.001$) but not with the carbohydrate balance ($r = -0.12$, n.s.). Appreciable imbalances between intake and oxidation appear therefore to be much more likely for fat than for carbohydrate.

A light collection device with high efficiency and small angular divergence for fluorescence measurements

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A collection efficiency close to 100% for luminescence measurements can be obtained by placing a sample at one focal point of a surrounding elliptical mirror with the detector in the second focal plane. (Oetliker et al., Experientia *41* (1985) 832). In fluo-

rescence measurements the detection limit of this arrangement could not be lowered below 0.1 pM of Na-fluorescinate in 20 µl, mainly because traces of scattered exciting light excited the inherent fluorescence of the ordinary cut-off filters used and caused detector saturation. Interference long pass filters could help overcome this problem. Unfortunately these exhibit angle dependent spectral characteristics and function optimally only with parallel light. To meet this requirement combinations of paraboidal reflectors have been tested on a computer model for their efficiency in transmitting light with tolerable deviations from the parallel from source with finite dimensions to a detector.

Single unit properties of the auditory part of the reticular complex of the thalamus (RE)

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The neural activity of 75 single units was recorded in the RE in nitrous oxide anesthetized cats. Each unit was characterized during spontaneous activity by its firing rate and bursting pattern; the proportion of bursting cells was about 80%. From anterior plane A8.0 the bursting tended to decrease more rostrally. For most cells the response patterns changed with frequency, and a CF was hardly definable. The width of the response range was always more than 3 octaves, significantly larger than in the medial geniculate body (MGB) and no tonotopic organization could be described. The lower limit of the response range to pure tones tended to increase from 0.2 kHz at A6.9 to 1.7 kHz at A9.2. The response patterns to white noise showed a high proportion of sustained excitatory responses along all the postero-anterior axis, whereas in the MGB the responses are mainly transient.

Physical fitness and psychophysiological stress reactivity

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Twenty-four highly fit subjects (at least tree aerobic trainings a week) were tested two times for their psychophysiological responses to stress. Three stressors were administered during the test session: 10 min Stroop-test, 4 stressful movies of 90 s each and an auditory discrimination task. Systolic and diastolic blood pressure (SBP, DBP), heart rate (HR), state-trait-anxiety (STAI), agression (FAF) and the maximal oxygen uptake were measured. Before the second test session 12 subjects had to interrupt their training program for two weeks.

No differences in personality traits (FPI) were seen between the two groups. Across the whole sample there was a significant negative correlation between maximal oxygen consumption and HR. All of the three stressors elicited a significant stress response in both test sessions. Although HR, SBP, DBP and STAI reactivity were not affected by the break in training, HR level tended to be higher after this interruption.

Long term evaluation of implanted pulsed ultrasonic doppler (PUD) transducers for hemodynamic studies

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Many hemodynamic studies require long term recordings of cardiac output, its distribution as well as blood flow analysis in large and small vessels of conscious animals. In young pigs and adult mini-pigs, small cuffs (20 MHz piezoelectric crystal) were implanted around the prox. and dist. femoral (D = 4 mm) as well as the lateral femoral circumflex (D = 2.5 mm) arteries. For more than 60 days, the velocity profiles were recorded by an 8-gate PUD device and the center line velocities by a simultaneous 3-channel PUD device. The animals moved normally and showed little tissue reaction to the implanted cuffs. Our results indicate that PUD cuffs have the following advantages: No damage to the vessel wall, little tissue reaction, small and cheap transducers, fine wires, no restrictions to the animal. As PUD may also be applied non-invasively to humans, results from animal experiments can be compared to data of patients and test persons.

Insulin effects on glucose utilization of individual tissues in lean and genetically obese (fa/fa) rats in vivo

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Glucose utilization of different tissues is measured, in lean and genetically obese *fa/fa* rats, after an injection of tritiated 2-deoxyglucose associated with the euglycemic clamp technique. In normal animals, insulin-mediated increase in total glucose utilization is explained mainly by an increase in glucose metabolism in muscles and brown adipose tissue. In obese rats, no significant stimulation of total glucose metabolism by insulin is observed. In muscles, white and brown adipose tissues, basal glucose metabolism is decreased (30–40%) in obese rats when compared to control animals, and the insulin action is clearly decreased. In liver of obese rats, the insulin sensitivity of the inhibition of hepatic glucose production is decreased by 25-fold. These results show a severe insulin resistance of muscles, white and brown adipose tissues as well as liver in genetically obese *fa/fa* rats.

Sleep regulation in the rat: Effect of light, circadian phase, and sleep deprivation

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Sleep states and EEG-parameters were determined in unrestrained rats by telemetry. Two days with a light-dark (LD) cycle and two days under continuous darkness (DD) were followed by 24-h sleep deprivation (SD) ending in the middle of the habitual dark-period, and by two recovery days in DD. In the baseline LD light-period, rapid-eye-movement sleep (REMS) and the EEG-amplitude of non-REMS (NREMS) were lower than in the corresponding baseline DD period. SD caused an immediate enhancement of REMS, NREMS, the slow wave sleep (SWS) fraction of NREMS, and NREMS EEG-amplitude. Although REMS, NREMS and SWS showed a second peak at habitual light-onset, it did not exceed baseline. Subsequently, all parameters exhibited a marked negative rebound. We conclude that light suppresses REMS; EEG-amplitude and frequency are differently affected by light and SD; and a circadian influence on sleep homeostasis may account for the short duration of the SD-induced SWS increase.

Localization of vasopressin, oxytocin, and their binding sites in the guinea pig brain: an immunocytochemical and autoradiographic light microscopic study

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The distribution of specific recognition sites for vasopressin and oxytocin was determined in thin sections of guinea pig brains

using tritiated arginin vasopressin, tritiated oxytocin and tritium sensitive ultrofilm. The most intensely labeled areas were: the septum and the area postrema-nucleus tractus solitarius region with vasopressin, the taenia tecta, the nucleus ventromedialis and the amygdala with oxytocin. The immunocytochemical detection of the vasopressin and oxytocin innervation was performed by using specific antibodies and the PAP technique. Immunoreactivity was present in fibers and/or in 'terminal-like' elements in most of the structures containing receptors but absent in some of them. These results support the hypothesis that oxytocin and vasopressin may play a role in centrally regulated processes.

A comparison of heteronymous and homonymous connectivity in the spinal monosynaptic reflex arc

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To account for the differences in the heteronymous and homonymous projections from muscle spindle afferent fibers to pools of intermingled motoneurons (MNs) a process of species recognition between the afferent fibers and their targets has been proposed. To evaluate this proposition against the hypothesis of topographically ordered connectivity we compared the projections of 28 medial gastrocnemius (MG) spindle afferent fibers to the overlapping region of the MG and lateral gastrocnemius-soleus MN-pools in the spinal cord of the anesthetized cat by means of spike-triggered averaging. In three experiments the projections to 68 MNs were analyzed.

This analysis revealed that differences between the homonymous and heteronymous connectivity to these closely operating synergistic MN-pools can be accounted for solely by the topographical relations between the locations of the motoneurons and the entry point of the afferent fibers into the spinal cord without further specification by cell species.

Effect of enteral versus parenteral nutrition on energy metabolism in healthy women

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The acute thermogenic, substrates and hormonal responses to nutrients administered either parenterally (IV) or intragastrically (IG) were measured in 10 healthy young women (mean weight±SEM: 52±1 kg). After 1 h of baseline measurements using indirect calorimetry, a mixture of D-glucose, balanced amino acids (Nutriflex®) and lipid (Intralipid®) corresponding to 52%, 18% and 30% of the energy infused respectively was administered IV or IG for 3 h at a rate equal to two times resting energy expenditure (REE). During 6 h following nutrient administration, mean REE (±SEM) rose from 0.98±0.02 (IV) and 0.99±0.02 (IG) to 1.13±0.03 (IV) and 1.13±0.02 (IG) resulting in a nutrient induced thermogenesis averaging 10±0.7% (IV) and 9.3±0.9% (IG) of the energy infused. The plasma glucose and insulin elevations were significantly higher using the IV compared to the IG route. Thus, both routes of nutrient administration induced comparable thermogenic response and substrates oxidation; however with the IG route the responses were delayed.

4-Hour infusions of human atrial natriuretic peptide in normal volunteers: hemodynamic, renal and endocrine effects

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Human ANP (hANP), sequence containing 26 amino acids, 0.5 and 1.0 μg/min, or its vehicle (V) were infused for 4 h in six healthy volunteers. Each subject received the two doses of hNAP and the V in a single-blind randomized order at one-week intervals. The experiments were performed after a 5-day period of sodium chloride loading. When compared to V, hNAP 1.0 μg/min changed neither heart rate, blood pressure, inuline clearance, phosphaturia nor calciuria. However, it decreased PAH clearance (by 20% during the 4th hour, $p < 0.05$) and increased $U_{Na}V$ (by 50% during the 3rd hour, $p < 0.05$), and $U_{Mg}V$ (by 40% during the 2nd and 3rd hour, $p < 0.01$). Fractional sodium excretion increased progressively 1.5-fold during the hours 3 and 4 of the infusion. U_KV showed only a slight tendency to fall. The low dose of hNAP produced no significant changes. In 3 additional volunteers, the skin blood flow response to hNAP, 1 μg/min i.v. for 4-h, was studied using a laser-Doppler flowmeter. Skin blood flow increased significantly during the first 2 h of infusion and returned to baseline levels thereafter. These data demonstrate that, in normotensive volunteers, a 4 h infusion of hNAP at a dose which has no blood pressure lowering action can have a natriuretic effect, decrease renal blood flow without altering glomerular filtration rate, and dilate the skin vascular bed.

On the problem of multiple hand representations in area 4 of the alert *Macaca fascicularis*

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The question whether several specialized hand representations exist in the precentral cortex of *Macaca fascicularis* has been investigated in monkeys trained to exert finely graded forces between thumb and forefinger. In one monkey the two-dimensional reconstruction of the recording sites revealed differences between the bank of the central sulcus and the convexity. From the 75 neurons well related to the task and assigned to one of the five major classes (phasic, phasic-tonic, tonic, decreasing and mixed), 90% of the phasic and 68% of the decreasing neurons were located on the convexity. Microstimulation at the recording sites elicited single and simple digit movements in the bank of the sulcus, whereas on the convexity multidigit movements were more frequent. Furthermore 15 neurons on the convexity were also related to ipsilateral hand movements. In conclusion, neurons located in the rostral and caudal parts of area 4 may have different functions in the precision grip, the rostral region having complex motor output and containing predominantly neurons with less metrical relation to force.

A neuron net in the pond snail, *Planorbis corneus*

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In view of the fact that simple nervous systems can be used to demonstrate how neurons coordinate at cellular level, we studied neurons of the pleural ganglia in isolated brain preparations. Using multitrace intracellular recording, current injection and Lucifer Yellow intracellular staining, we observed 1) the projection of pleural neurons into many peripheral nerves which innervate different regions of the body wall; 2) non-rectifying electrotonic coupling both within and between ganglia; 3) excitatory input to pleural neurons in different ganglia, these silent neurons being electrically coupled; 4) synchronous and rhythmic, neuronal discharge in about 50% of the preparations, the rhythm persisting during Ca^{2+} free perfusion while the evoked EPSPs were abolished, indicating that the rhythm generation is independent of chemical transmission. This initial characterization of the neuron net of *Planorbis* should provide a firm basis for further study.

Electron microscopy of an impulse transmitting synapse in the fish brain (*Tinca tinca* L.)

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The impulse transmission from Mauthner axons (MA) to medullary and spinal neurons occurs at both axo-axonic and axo-dendritic synapses. Their common morphological features are: 1) Singularity of the presynaptic elements (= heavily myelinated MA collaterals with a dome shaped tip exposing four specialized districts: nodal membrane, synaptic membrane with presynaptic grid, gap junction and intermediate junction representing the anchoring site of postsynaptic 'dense bands') for a given postsynaptic cell. 2) Multiplicity of synaptic contacts alternating with intermediate junctions induced by the repeated branching of short postsynaptic processes arising either at a node of Ranvier or at a specialized dendrite from which the initial segment of the postsynaptic cell originates. 3) Close proximity of the pre- and postsynaptic nodal membranes to either the chemically or electrically transmitting synaptic complex; and 4) extended extracellular spaces surrounding nodal and synaptic regions.

Plant Physiology

Photosynthesis and chlorophyll content of the lichen *Parmelia sulcata taylor* from different sites of a mid-sized Swiss city

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Lichens have been successfully used as biomonitors of air pollution. Transplant experiments in other cities showed a decrease of chlorophyll and net photosynthesis at sites with increased levels of air pollution. We wanted to know whether lichens that had grown at various parts of a city would give similar results. 13 sections in the city of Biel were chosen. *Parmelia sulcata* was collected from 5–9 different trees at each section. CO_2-fixation was measured as follows: Measurement 1) of $^{14}CO_3^{2-}$-fixation and 2) of net photosynthesis (NPS) by ultra red absorption. Dark respiration (DR) was measured in the same manner as NPS. NPS was lowest in the central parts of town and was 4–5 times higher in suburbs. Neither ^{14}C-fixation nor DR not the protein content were significantly different. The chlorophyll content was highest in the centre and lowest at the peripherie. The results of NPS are in agreement with literature, while the results of the chlorophyll content are inverse to previous reports of transplant experiments. The above results indicate, that a higher percentage of algae is required in the center to let the lichen survive.

Regulation of adenosine 5′-phosphosulfate solfotransferase activity of *Lemna minor* L. by sulfate

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Adenosine 5′-phosphosulfate sulfotransferase (APSSTase) is an enzyme of assimilatory sulfate reduction. The effect of 0–17.6 mM Na_2SO_4 in the culture medium on the extractable activity of this enzyme was studied in *Lemna minor* L. APSSTase activity was increased by 100–200% after transfer from 0.88 mM to 0 or 0.0088 mM Na_2SO_4. 17.6 mM Na_2SO_4 induced a 50% decrease in enzyme activity. Both effects were readily reversible when the plants were transferred back to the original nutrient solution. In these experiments high internal sulfate concentrations were correlated with high thiol concentrations and low APSSTase activity. In control experiments, in which no nitrogen source or NH_4^+ instead of NO_3^- was available to *Lemna*, no corresponding correlations were detected. This indicates that APSSTase activity is regulated by different mechanisms by nitrogen and sulfur sources.

A newly discovered cytochrome P450 is involved in the oxidative metabolism of phenylurea-herbicides in Jerusalem artichokes

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The herbicide chlortoluron (CTU, 3-(3-chloro-p-tolyl)-1,1-dimethylurea) is oxidized by a microsomal preparation from Jerusalem artichoke tubers, predominantly by N-monodemethylation. Cofactor requirement, inhibition by CO and the effects of various substrates of plant monooxygenases provide strong evidence that the reaction is catalyzed by a cytochrome P450 (P450) which is distinct from other known plant P450's. Two compounds structurally related to CTU 3(3-methyl.4-chlorophenyl)-1,1-dimethylurea and 3(3,4-dichlorophenyl)-1,1-dimethylurea were very potent competitive inhibitors of CTU demethylation (Ki 89 and 63 µM) and may therefore be substrates of the enzyme; other phenylurea analogues were weak competitors of not inhibitory at all. These data suggest that chlortoluron demethylase represents a previously not described plant cytochrome P450 isozyme with distinct substrate specificity.

Fermentative activity of pea and soybean seeds: enzymatic and GLC analyses of carbohydrate reserves

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Changes in carbohydrate reserves and adenylate energy charge (AEC) in two germinating legumes, *Glycine max.* (L) *mer.* and *Pisum sativum* (L), were followed under anoxia. AEC in pea, but not soybean, axes attached to their cotyledons, and AEC in excised axes from both seeds incubated in presence of glucose, was higher than AEC in excised axes alone. Soluble carbohydrate content decreased in excised axes from both seeds. Sucrose accumulated in pea, but decreases in soybean, axes attached to their cotyledons, while other oligosaccharides content decreased in both seeds. Starch content dropped from 220 to 130 and from 2 to 0.5 µmol starch-glucose/cotyledon in pea and soybean, respectively. Protein content remained stable. It is concluded that carbohydrate reserves in pea, but not soybean, are sufficient to maintain high AEC values under anoxia and to accumulate sucrose in the axis, thus ensuring the high osmotic pressure required for rootlet extrusion, although fermentative capabilities are potentially sufficient in both seeds.

Characterization of a temperature-sensitive, auxin-auxotrophic mutant of *Hyoscyamus muticus*

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A tissue culture line of *H. muticus* has been isolated after MNNG treatment of haploid protoplasts 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 restrictive temperature. This trait is recessive in protoplast fusions. The line was recloned from protoplasts and ca. 10^6 colonies examined for reversion. No revertants were detected. The addition of indoleacetic acid-ethylester at 0.25 µM is sufficient to give 50% of normal growth at the restrictive temperature. Current experiments involve: a) Analysis of the metabolism of indoleacetic acid

(IAA) in the mutant. Tracer experiments feeding (2-^{14}C)-IAA to the mutant and analysis of its metabolites by HPLC revealed one peak which is different between the wild-type and the mutant. Presently, we do not know whether the temperature-sensitive phenotype of the mutant could be causally ascribed to this difference. b) Analysis of the metabolism of ^{3}H-indole in the mutant. c) Transformation of mutant cells with genes 1 and 2 of the T-DNA of *Agrobacterium tumefaciens*.

Studies of homologous recombination in the plant genome

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There is still a virtually complete lack of studies on homologous DNA recombination in plants except for the specific extra chromosomal case of the DNA virus Cauliflower Mosaic Virus. Direct, vectorless transformation of plantprotoplasts has opened new possibilities of studying the DNA recombination process in plants. Using this method we have constructed two lines of tobacco plants, one containing bacterial plasmid sequences and partial non-functional copies of a plant selectable marker gene [hybrid APH(3')II gene] and the other containing a functional nopaline synthase gene derived from the *Agrobacterium tumefaciens* Ti plasmid. The foreign DNA in these strains is now being used as a target for homologous recombination of DNA into the genome after DNA transformation.

Genetic analysis of a hybrid foreign gene introduced into tobacco by direct gene transfer

I. Potrykus, J. Paszkowski, M. W. Saul and R. D. Shillito, Friedrich-Miescher-Institut, Postfach 2543, Ch-4002 Basel

Transgenic tobacco plants have been regenerated from leaf protoplasts incubated with the bacterial gene for neomycinphosphotransferase under 5'/3' expression signals from CaMV gene VI. Genetic analysis accompanied by molecular analysis revealed that the foreign gene is normally inherited as a single dominant Mendelian factor and that it is maintained through several sexual generations and over a time periode of more than two years without selective pressure. Deviations from this rule have, however, also been found.

Portulaca grandiflora: A model plant for the study of secondary metabolites biosynthesis in in vitro plant cells

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Only a few model systems are available for a systematic analysis of secondary products. We have been looking for a plant which allows 1) genetic analysis, 2) in vitro cultivation and 3) interesting secondary metabolites. *P. grandiflora* can be easily propagated in vitro with a short interval between germination and flowering, and is fairly easy to pollinate in vitro. This plant synthesizes pigments of the betalain family and many genotypes are available; According to Adachi (Jap. J. Breed. *35* (1985) 183) at least three genes are involved in the control of the pigments biosynthesis. After sterilization of seeds, we got steril plantlets expressing different genotypes (mainly in the colour of the corolla). Vegetative subculturing is easy: an apex, either vegetative or floral, is cut 2–3 cm long and put into fresh medium. Two days later root formation is visible. The vegetative apex continues to grow up to the floral stage. Cell cultures can be initiated by cultivating any part of the plant in a ½ strong MS medium containing 2,4-D. Different morphogenetic pathway can be obtained by modulating the hormonal concentrations. Cross breeding can be achieved in vitro, the life cycle between germination and flowering beeing only about four weeks. Fingerprint from HPLC analysis of the different genotypes (or their crosses) will be shown.

High efficiency direct gene transfer to plants

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The efficiency of direct gene transfer to plant protoplasts has been increased 1000-fold over that previously reported. In the order of 2% of all colonies recovered without selection were transformed. The improvement was due to: treatment of the protoplasts with a high voltage electric pulse (electroporation), optimization of the polyethylene glycol concentration, addition of the PEG after the DNA and the application of a heat shock. These factors are of equal importance in achieving high transformation frequencies.

Three forms of nitrate reductase (NR) from soybean

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Soybean leaf extract was loaded onto Blue Sepharose. Sequential elution with NADPH and NADH separated a constitutive NAD(P)H:NR (c_1NR,EC 1.6.6.2) from both const. & inducible NADH:NRs (c_2NR & iNR,EC 1.6.6.1). Subsequent purification with FPLC-ion exchange chromatography enabled separation of the two NADH:NR forms. Subunit M_r of c_1NR was 106 kD, of c_2NR 112 kD, and or iNR 115 kD. Nicking could not be prevented, despite addition of protease inhibitors. All three forms had similar absorption spectra indicating the presence of a Cytochrome b_{556}. The results demonstrate that it is possible to separate three NR forms from wildtype soybean leaves. They confirm the similarity of the NRs regarding their Cytochrome moiety, and their difference in size.

Selection of auxin-auxotrophic *Nicotiana plumbaginifolia* cell lines

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The isolation of auxotrophic plant cell mutants, desirable research tools, has been obstructed by the absence of both a replica-plating technique and a negative selection system suitable for plant cells. The major common problem in published attempts at developing an enrichment system for plant cell mutants has been insufficient reproducibility. The thymidine analogs BUdR (5-bromo-2'-deoxyuridine) and FUdR (5-fluoro-2'-deoxyuridine) were reexamined as selective agents using mesophyll-protoplast derived cells of haploid *Nicotiana plumbaginifolia*. Comparing N-starved and growing cells, reproducibly selective conditions were established. Applying selection at 33 °C in the absence of exogenous auxin, the concentration of auxin-auxotrophic variants after UV mutagenesis was increased 100 × from 1×10^{-4} to 1×10^{-2}. The isolates show a stable, absolute requirement for exogenous auxin. Regenerated shoots are abnormal. No temperature-sensitive auxin-auxotrophic line has yet been recovered.

Aflatoxin contamination in soybeans

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Soybeans contain heat labile (Kunitz type) and heat stable (Bowman-Birk) trypsin inhibitors. Though their functions are

not well understood, some data indicate the involvement of proteinase inhibitors in the defense of plants against pathogens and insects. Since soybean trypsin inhibitors are antinutritional factors, new varieties lacking these inhibitors are being bred. The absence of proteinase inhibitors, however, may enhance the risk of fungal contamination of seeds.

In contrast to raw soybeans, autoclaved seeds are an excellent substrate for aflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus*, thus ruling out inhibition by heat stable trypsin inhibitor. Fungal growth and toxin accumulation were not repressed by heat labile trypsin inhibitor either. Furthermore, the availability of zinc, an essential mineral for aflatoxin synthesis by *Aspergillus*, apparently did not improve upon substrate autoclaving. It could be shown that growth of *A. flavus* on soybeans and subsequent aflatoxin accumulation are controlled by seed coat integrity and moisture content of seeds.

Announcements

Prizes

1986 Doerenkamp/Zbinden Scientific Award

Two prizes of 50,000 DM each will be awarded for outstanding scientific contributions leading to the replacement of whole animals in teaching and research. Specifically, areas of interest for 1986 include:

The replacement of dogs and cats in biomedical research.

Applications should be sent to: Prof. Dr. med. Diether Neubert, Institut für Toxologie und Embryopharmakologie, Freie Universität Berlin, Garystr. 5, D-1000 Berlin 33 (FRG).

The development of alternatives to whole animals in research on antitumor and antiinfective chemotherapy.

Applications should be sent to: Dr. Alan M. Goldberg, Associate Dean for Research, The Johns Hopkins University, School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, Maryland 21205 (USA).

Applications may consist of written description, illustrations, audiovisual presentations, etc. There are no formal application materials and more than one prize may be awarded in the same area. Deadline for submission: 31 December, 1986.

Honma Prize in Biological Rhythm Research

The first Homna Prize in Biological Rhythm Research has been awarded to Joseph S. Takahashi of Northwestern University (Evanston, USA). The prize oy Y 1,000,000 was established in 1983 by the Honma Life Science Foundation for the purpose of promoting original research in the field of biological rhythms. On recommendation of the International Selection Committee, the prize is awarded every two years to a young scientist who has done particularly important work early in his or her career. Members of the Selection Committee are Jurgen Aschoff, Brian K. Follett, Tsutomu Hiroshige, Michael Menaker and Colin S. Pittendrigh. In addition, the Foundation supports a Symposium on the general subject of Biological Clocks and Rhythms which is held in alternate years in Sapporo, Japan. The prize winner is invited to the Sapporo Symposium to give a lecture on his or her research. It is hoped that the prize will be understood to be symbolic of the rewards widely deserved by the many excellent young scientists entering this important and rapidly growing field.

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Reviews (one-man and multi-author reviews)
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Papers reporting on work that is preliminary in nature, or wherein animal experiments have been conducted without the appropriate anesthesia, will not be accepted.

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   Molecular Biology
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6. Ethology, Ecology
   Natural Product Chemistry

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