Symposia

Complete list of titles of oral presentations in program booklet

S1 1

SUBSTRATES INHIBITING NEURITE GROWTH IN THE CNS

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Two types of macroglial cells exist in the CNS of higher vertebrates; astrocytes appear early in development and express a variety of extracellular molecules which are favorable substrates for neurite growth in vivo and in vitro. Oligodendrocytes start their differentiation in fiber tracts after termination of neurite growth and express two membrane proteins which strongly inhibit neurite growth cone movement as well as migratory movements of many other cell types. Antibodies against these inhibitory proteins injected into optic nerve explants lead to massive nerve fiber ingrowth from cocultured neurons into this CNS tissue, a finding assigning a predominant role to these inhibitory molecules for the normal absence of neurite regeneration in adult CNS. The mosaic-like appearance of differentiated oligodendrocytes and myelin during CNS development suggest a role in territory segregation and in guidance of late growing CNS tracts. In fact, prevention of myelin formation in the thoracic spinal cord by neonatal X-irradation lead to a highly aberrant position of many pyramidal tract axons. Inhibitory myelin constituents appearing in a channel-like fashion around the growing tract may function as "guard rails" and thus exert a guiding function, in addition to stabilization of preexisting tracts.

S1 2

FAMILIES OF NEURAL ADHESION MOLECULES

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Cell surface glycoproteins operationally termed adhesion molecules play decisive roles in the development, stabilization, maintenance and regeneration of nervous system functions. Some neural adhesion molecules belong to the immunoglobulin superfamily and share functionally important carbohydrate structures. The Ca^{++}-independent adhesion molecules L1 and N-CAM cooperate with each other in mediating cell contacts and are stabilized in the surface membrane, with N-CAM connecting to the membrane-cytoskeleton linker protein brain spectrin. Adhesion molecules also influence the cellular and extracellular ionic milieu. Members of the J1 family of extracellular matrix glycoproteins are highly localized at the node of Ranvier, in the vibrissae-related barrel field boundaries of the somatosensory cortex and at the denervated neuromuscular junction, suggesting a crucial role in the formation and maintenance of neuron-glia and neuron-muscle interactions.

S2 3

PRODUCTION OF INTERLEUKIN-6 (IL-6/BSF-2) IN THE CENTRAL NERVOUS SYSTEM IN VIRAL INFECTIONS

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A feature common to viral infections of the brain is the intrathecal production of immunoglobulins by B lymphocytes having infiltrated the brain tissue. The mechanisms leading to B cell maturation in the central nervous system (CNS) are not known. In general, the terminal differentiation of activated B cells into immunoglobulin-producing plasma cells depends on T cell-derived signals such as interleukin 6 (IL-6/B cell stimulatory factor 2, BSF-2).
We have investigated on production of IL-6 in the CNS. When testing cerebrospinal fluids (CSF) of mice ICR (+/+) infected with lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus (VSV) production of IL-6 can be detected within the CNS. VSV-infected athymic ICR nu/nu mice develop meningitis and also IL-6 is produced intrathecally. However, upon LCMV infection ICR nu/nu mice did neither respond with disease nor IL-6 production. As a potential source for IL-6 in the CNS cultured astrocytes and microglial cells established from newborn ICR +/+ mice were found to secrete IL-6 when infected with LCMV. Production of IL-6 in the brain tissue and/or in CSF may be essential for an effective humoral anti-viral immune response in the CNS.

S2 4

ON THE PRODUCTION OF TRANSFORMING GROWTH FACTOR-BETA IN THE BRAIN.

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Transforming growth factors (TGF) were initially discovered in conditioned medium of virus-transformed cells and believed to be specific markers for the transformed phenotype. However, as TGF's are also expressed by a variety of nonneoplastic cells they seem to play a fundamental role as regulators of cell growth and differentiation. Two structurally not related proteins (TGFα and TGFβ) have been described. Several forms of TGFβ have been found and were named TGFβ1, TGFβ2, TGFβ1,2 and TGFβ3.
We have purified and cloned a factor from a human brain tumor (glioblastoma) cell line which was originally termed glioblastoma derived T cell suppressor factor(G-TsF),because it inhibits T cell growth, and subsequently found to be identical to TGFβ2. We have studied the expression of both TGFβ1 and G-TsF/TGFβ2 in normal human brain and brain tumors. Glioblastoma cell lines and fresh glioblastoma tissues both express TGFβ1- and TGFβ2 mRNA, but no such transcripts were found in normal human brain. Differences in the levels of transcripts and the amounts of secreted protein by glioblastoma cell lines suggest a post-transcriptional level of regulation for TGFβ1 expression. (NF grant 3.930.087)

S3 5

FUNCTIONAL REPRESENTATION OF LUMINANCE AND CHROMATIC CHANNELS IN THE PRIMATE VISUAL SYSTEM

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Much psychophysical evidence suggests the presence of luminance and chromatic channels in the human visual system. Although the visual system of the old-world primate is sufficiently similar to that of man to permit exploration of the physiological basis of psychophysical channels, the substrate of these channels has remained unclear. Anatomically, there exist in the primate visual pathway two main cell systems. The one is made up of tonic, cone-opponent ganglion cells which project to the parvocellular layers of the geniculate nucleus and the other of phasic ganglion cells which project to the magnocellular layers of the nucleus. I shall review recent evidence from retinal ganglion cell recordings in the macaque which shows that the phasic, magnocellular system corresponds to the luminance channel of psychophysics while different types of cell in the parvocellular system correspond to chromatic channels. These functionally derived channel concepts thus have a structural and physiological basis.

S3 6

VISUAL ACTIVITY IN EXTRASTRIATE CORTEX DURING REVERSIBLE INACTIVATION OF AREA 17 IN THE MACAQUE MONKEY

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It is generally admitted that, in the monkey, visual information reaches extrastriate visual areas only via a relay in area 17. However, recent anatomical studies demonstrate the presence of several routes by-passing area 17 and linking the superior colliculus or the lateral geniculate nucleus with extrastriate areas. We have tested the role of area 17 as a relay station by cold inactivating it while recording in its primary extrastriate targets, areas V2 and MT. We first measured the temperature gradients in cortex and the blocking temperatures for neurons in area 17. This enabled us to determine a proper recording depth in V2 to avoid direct cryo-blocking of neurons in this area. Recordings in areas V2 and MT were restricted to the region coding the same part of the visual field as represented in the cooled region in area 17. During cryo-blocking of area 17, 98% of the recorded sites in area V2 ceased to respond to visual stimulation. In contrast, numerous neurons remained active in MT in the same conditions. These results suggest that area MT receives convergent information from area 17 and other visual structures, whereas neurons in area V2 depend strongly on area 17 input for their visual responses.

S3 7

ANALYSIS OF CONTOUR AND MOTION IN MONKEY PRESTRIATE CORTEX.

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Studies in primates suggest that color, form and motion are processed in separate, anatomically distinct regions of the visual cortex. We have studied the neural mechanisms of contour perception and perceptual grouping. We recorded from single cells in the visual cortex of awake macaque monkeys trained on a visual fixation task. We defined the function of neurons by correlating their responses with perception. We found that many cells in the prestriate cortex responded to stimuli in which human observers perceive contours or a moving object (real or illusory), and gave weaker responses or none to control stimuli in which no object or contour could be seen. We have also correlated the physiology with the anatomical cytochrome-oxidase pattern. Contour neurons were found in the pale and the thick dark stripes of V2 but not in the thin stripes. Neurons capable of grouping from motion were most often recorded in the thick dark stripes of V2, and in V3. Our results show that, in the awake animal, neural signals in V2 and V3 can often be related to specific and complex visual functions, and that these functions are concentrated in anatomically distinct regions. (NF grant 3.939.84).

S3 8

FUNCTIONAL ROLE OF THE CALLOSAL CONNECTIONS OF THE VISUAL CORTEX

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All areas of the visual cortex in placental mammals are endowed with callosal connections. In most visual areas, however, callosal connections are limited to areal portions that contain the representation of the vertical midline of the visual field. Anatomical findings suggest that in other visual cortical areas callosal interconnections may be more diffuse in character, but upon physiological analysis neurons in these areas that emit or receive callosal fibers appear to have receptive fields abutting or crossing the vertical meridian. The functional significance of the link of the callosal connections with the representation of the vertical meridian is suggested by the analysis of the interactions between the intrahemispheric (thalamic) visual input and the interhemispheric (callosal) visual input to a same cortical neuron. This interaction complies with the Sperry's principle of supplemental complementarity, in that it results in the formation of continuous and homogeneous receptive fields extending into both halves of the visual field. This may have important consequences for the unity of visual perception.

S4 9

NEURAL CIRCUITS FOR MANIPULATION

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The evolution of primates was marked by an increase in manipulative behaviour with its many degrees of freedom. This behavioural evolution finds its counterpart in an increase of neural circuits devoted to the control of manipulation. The following evolving principles in neural organization have been revealed: 1) increase of central representation of the hand (magnification factor); 2) division of labour in multiple somatotopic representations; 3) increase of somatosensory feedback channels; 4) formation of direct corticomotoneuronal connections. The behavioural capacity of the primate's hand to perform fractionated (independent) finger movements and the precision grip depends critically on the integrity of the pyramidal tract. Focal hand movements are complex synergies requiring precise recruitment and timing of many muscles of the hand and, in addition, stabilizing actions of proximal muscles. In view of this complexity, it becomes imperative to learn more about the rules that govern manipulative skills in order to test hypotheses as how the brain masters the degree of freedom problem.

S4 10

GLOBAL VS LOCAL FACTORS IN THE PLANNING OF COMPLEX MOVEMENTS

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One of the most generally accepted hypothesis concerning the organization of voluntary movements holds that motor planning is organized as a multi-stage hierarchical process. More specifically, the suggestion has been put forward that the transition between the general topological description of an intended movement and the actual motor commands is mediated by a parameter-setting stage in motor planning, whereby the temporal and metrical properties of the actual gesture are specified on the basis of both contextual and volitional factors. One problem concerning this hypothesis is the nature of the free variables that are actually set to a specific value in order to obtain the desired result. A second aspect of this general scheme is the interplay between extrinsic properties of the motor control system. I will review recent experimental work on two-dimensional complex hand movements. In particular I will address the processes responsible for the specification of the kinematic variables (angular and tangential velocities) and I will show that a relatively simple factorization of both intrinsic and extrinsic influences accounts very satisfactorily for the observed behavior.

S4 11

SENSORIMOTOR MECHANISMS OF MANIPULATION (PERIPHERAL ASPECTS)

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For successful manipulation using the precision grip, the motor commands have to be precisely tuned to the relevant physical properties of the manipulated objects. This appears to primarily take place on the basis of previous manipulative experiences with the current object or similar objects, i.e. the relevant information is internally represented in sensorimotor memories. If an erroneous internal representation results in inappropriate motor programming, somatosensory signals automatically trigger compensatory actions and update relevant parameters of the sensorimotor memories. Tactile afferent innervation, which conveys information about mechanical events particularly at the interface between the hand and the manipulated object, plays a crucial role in this context. The present paper specifically addresses (1) how tactile afferent units innervating the skin of the fingers actually respond during manipulative tasks, and (2) the significance of those responses for the control of the manipulation.

S4 12

SENSORIMOTOR MECHANISMS OF MANIPULATION (CENTRAL ASPECTS)

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The control of manipulation requires from the central nervous structures several processes:

- the choice of the finger muscles activated either in synergy for movements such as the precision grip or independently in fractionated finger movements.
- the precise dosage of force or contraction strength in the individual muscles.
- the control of other movement parameters such as speed, amplitude and duration.

This complex task is performed by the coordinated action of many neuronal populations located in the premotor, motor and somatosensory cortex, as well as in subcortical regions like cerebellum, basal ganglia and thalamus. We will review the progress made in understanding at the single cell level the control of manipulation, in particular the coding of force, the relation of the neurons to their target muscles, the possible specialisation of identified neuronal populations and the central integration of peripheral afferent inputs.

S5 13

THE PONTINE INPUT TO DOPAMINERGIC CELLS OF THE SUBSTANTIA NIGRA AND NATURE OF ITS NEUROTRANSMITTER IN THE RAT.

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The in vivo firing rate of dopaminergic neurons of the substantia nigra pars compacta (SNPC) results from intrinsec membrane properties and inputs to the substantia nigra. A major input to the SNPC arises from neurons located in the pontine tegmentum. Electrical stimulation of this region evoked a brief short-latency orthodromic activation in 25% of SNPC neurons. This response could be the result of activation of pedunculopontine fibers giving a monosynaptic input to SNPC neurons. The putative neurotransmitter mediating the activatory response might be glutamic acid (GLU) as suggested by the antagonism exerted by GLU antagonists on this response as well as by the presence of GLU-positive neurons in the pedunculopontine region. Evidence is presented that the substantia nigra may influence motor mechanisms at lower brainstem and spinal cord through the pedunculopontine nucleus.

S5 14

BEHAVIOURAL EFFECTS OF DOPAMINE D-1 AND D-2 RECEPTOR ANTAGONISTS IN RATS.

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On the basis of biochemical data, brain dopamine (DA) receptors have been divided into two distinct classes; D-1 and D-2 receptors. Classical neuroleptics, for example haloperidol, are thought to exert their behavioural effects through an antagonism of the D-2 receptor. SCH 23390 is the first developed selective D-1 antagonist. Surprisingly, SCH 23390 was found to exert potent effects in the behavioural models presumed to reflect classical neuroleptic activity. Sulpiride is a selective D-2 antagonist. However, sulpiride does not exert potent effects in behavioural models reflecting classical neuroleptic activity. For example, sulpiride does not potently attenuate operant behaviours, which both haloperidol and SCH 23390 do. Furthermore, sulpiride selectively antagonises effects of low doses of the D-1/D-2 agonist apomorphine, an effect not mimicked by haloperidol or SCH 23390. The results are difficult to reconcile with the idea that D-1 and D-2 receptors are in a simple manner directly related to different behavioural functions. The results can more easily be explained by the existence of a sub-class of D-2 receptors with a specific function.

S6

GENOME INSTABILITY

S7 15

CHROMATIN STRUCTURE BY X-RAY CRYSTALLOGRAPHY

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The histone octamer of chromatin has been combined with defined sequence DNA fragments and the resulting nucleosome core particles are being examined by nuclease digestion and X-ray crystallography. The sequences under investigation are from the 5S RNA gene of *L.variegatus* and the long terminal repeat of the mouse mammary tumor virus. These sequences specifically position the histone octamer to a single base pair.

A 146 bp palindromic sequence has been made in which the center aligns with the dyad axis of the nucleosome core particle. Crystals from these particles may diffract beyond the current 3-4.5Å limit.

In addition, the histones of the H2A/H2B dimer have been expressed in *E.coli*, isolated, and combined with chicken erythrocyte H3/H4 tetramers and 146 bp DNA. The nucleosome core particles produced allow structural studies using histone dimers having sequence alterations.

S7 16

PACKAGING OF DNA IN CHROMATIN: MECHANISMS THAT DETERMINE NUCLEOSOME POSITIONS AND NUCLEASE SENSITIVE REGIONS

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Due to the protection of DNA by histone proteins, the position of nucleosomes with respect to the underlaying DNA sequence might play a decisive role in regulation of transcription or replication. The chromatin structure of many yeast genes is characterized by precisely positioned nucleosomes on the coding region flanked by non-nucleosomal, nuclease sensitive regions (NSR) at the 5'- and 3'-ends. We use specifically designed minichromosomes and reconstitution experiments to study the determinants of particular chromatin structures *in vivo* and *in vitro*, respectively. It could be demonstrated that histone-DNA interactions, flanking nuclease sensitive regions and chromatin folding determine nucleosome positions (Thoma and Simpson, 1985, Nature 315,250; Thoma, 1986, J.Mol.Bio. 190, 177, Thoma and Zatchej, 1988, Cell 55). We currently investigate whether particular sequences like bent DNA or poly dA-poly dT (an element of a constitutive promoter) might exclude formation of nucleosomes.

S7 17

THE NUCLEAR LAMINA

E.A. Nigg, I.S.R.E.C., 1066 Epalinges

The nuclear lamina is a karyoskeletal structure lining the nucleoplasmic surface of the inner nuclear membrane. It is believed to be important for nuclear envelope integrity and the organization of interphase chromatin. Its major constituents, the nuclear lamins, share structural homologies with the intermediate filament class of cytoskeletal proteins. By sequence analysis, two major subfamilies, i.e. A-type lamins and B-type lamins, can be distinguished. A- and B-type lamins are expressed according to cell- and tissue-specific patterns during embryogenesis, and they exhibit strikingly different behaviours during cell division. These observations suggest that different lamin isoforms may carry out different functions. (For further information see also abstracts by Nakagawa and Nigg, and by Vorburger, Lehner, Peter, Kitten, Eppenberger, Bailer, Maridor and Nigg).

S8

GENE EXPRESSION

S9

GENETIC EPIDEMIOLOGY AND STATISTICS

S10 18

FACTORS LIMITING ANAEROBIC PERFORMANCE IN HYPOXIA

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The maximal anaerobic performance of humans is impaired by both acute and chronic hypoxia. In acute hypoxia : 1) the size of the maximum lactic O_2 debt is not influenced by hypoxic breathing down to FIO_2 = 0.09 (P_B = 760 mmHg) as shown by indirect estimates as well as by blood lactate (La_b) measurements; 2) the "true" anaerobic threshold calculated as the maximum work load below which the rate of La_b accumulation ($\dot{La}_b$) = zero, is lower the lower FIO_2, its decrese paralleling the reduction of $\dot{V}O_2max$; 3) the O_2 equivalent of La_b accumulation is the same as in normoxia. In chronic hypoxia : 1) the observed maximum [La_b] decreases with lowering P_B and attains levels of ~ 3mM at 6300 m; 2) the extent to which anaerobic glycolysis operates appears to be limited by a negative feedback mechanism probably triggered by intracellular [H^+] when blood pH falls below 7.1; 3) the O_2 equivalent of La_b accumulation at 5350 m does not differ significantly from the values reported for normoxia and acute hypoxia; natives (Sherpas and Perivian Indians) and acclimated lowlanders show similar adaptations.

S10 19

REMODELLING OF THE RESPIRATORY SYSTEM AS A CONSEQUENCE OF PROLONGED EXPOSURE TO HYPOXIA.

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Maximal O_2 uptake ($\dot{V}O_2max$), and maximal cardiac output ($\dot{Q}max$) are reduced in chronic hypoxia, in spite of the increased blood Hb concentration ([Hb]). Also, a sudden return to normoxia (acute normoxia) is not accompanied by an increase in $\dot{V}O_2max$ proportional to the increase in [Hb]. Some authors therefore concluded that $\dot{V}O_2max$ in chronic hypoxia and in acute normoxia is not limited by the O_2 transport system. Recently, the activity of the key enzymes of the Krebs' cycle appeared to be reduced, and the muscle capillary density to be increased after prolonged exposure to hypoxia. These data, together with data of $\dot{Q}max$ and of [Hb] obtained in acute normoxia, can be introduced in the O_2 conductance equation, to calculate the factors limiting $\dot{V}O_2max$. This appears to be limited mostly (~80%) by the O_2 transport system ($\dot{Q}max$ * [Hb]), thus contraddicting the above reported conclusions. Similar results were obtained in normoxia on trained subjects. It is suggested that, in acute normoxia, the effects of increase [Hb] are offset by a drop in $\dot{Q}max$ and that decreased enzyme activity is counterbalanced by increased capillary density.

S10 20

MUSCLE ADAPTATIONS TO HYPOXIA

Hoppeler, H., Kleinert, E., Schlegel, C., Howald, H. and Cerretelli, P. Department of Anatomy, University, CH-3000 Berne and Department of Physiology, University, CH-1200 Geneva, Switzerland.

A morphometric analysis was performed on biopsy samples from vastus lateralis muscle obtained from 14 male subjects before and after participation at one of two expeditions to the Himalayas in order to analzye the influence of high altitude exposure on skeletal muscle structure. All participants were minimally exposed to an altitude of 5200 m at the base camp for six weeks. The muscle cross-sectional area of the thigh estimated by computed tomography was reduced by 10% (significant, $p<0.05$). Muscle fiber cross-section area was reduced by 20% (s) mostly due to loss of myofibrillar proteins. The oxitative capacity of muscle fibers estimated by the volume density of mitochondria was also reduced by 20% (s). The global oxidative potential of the thigh muscles (muscle volume times the mitochondrial density) was thus reduced by nearly 30% (s). In contrast, muscle capillarity was spared from catabolism so that an essentially unchanged capillary bed served a smaller muscle oxidative capacity after high altitude exposure.

S10 21

Defining the resistance to oxygen transfer in muscle tissue hypoxia. D.P. Jones and T.Y. Aw, Department of Biochemistry, Emory University, Atlanta, GA 30322 USA.

Resistance to O_2 transfer occurs both within cells and in the respiratory and circulatory systems that supply O_2 to cells. We have focussed our studies on cellular oxygenation with the hope that a secure understanding of oxygen supply at the cellular level will also allow a more complete interpretation of O_2 transfer in more complex in vivo systems. Results from studies of freshly isolated cardiac myocytes, hepatocytes and proximal tubule cells have provided evidence that spatial distribution of mitochondria in cells is of great importance in determining the availability of O_2 to support mitochondrial functions. Other factors, including cell size and shape, the mitochondrial O_2 consumption rate, and the effective intracellular diffusion coefficient for O_2, also contribute to the characteristics of cellular oxygenation. At least some of these factors, including mitochondrial distribution, are under dynamic regulation and change during development and in response to physiological adaptations.

S11 22

MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION IN TISSUE OXYGEN SENSING.

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Mitochondrial oxidative phosphorylation consumes most of the oxygen utilized by the body. In cells, on the average the rate of ATP synthesis must equal the rate of ATP utilization. If the steady state oxygen pressure is lowered to a limiting value, ATP synthesis (respiration) is decreased to below the rate of ATP utilization and the cellular [ATP]/[ADP][Pi] begins decreasing. As the latter falls, however, it stimulates mitochondrial respiration until the rate of ATP synthesis again equals the rate of ATP utilization and a new steady state is achieved. Thus the cytoplasmic [ATP]/[ADP][Pi] can, under some conditions, be a measure of the intracellular oxygen pressure and could be an intermediate in oxygen sensing by mitochondria even when the respiratory rate is independent of oxygen pressure. Only when the oxygen pressure is too low to maintain the rate of ATP synthesis does the respiratory rate decrease.

A new optical method for measuring oxygen pressure has been developed which can quantitatively measure the oxygen dependence of mitochondrial respiration throughout the physiological range of oxygen pressures (Vanderkooi et al, J. Biol. Chem. 262 (1987) 5476). The oxygen dependence of mitochondria *in situ* begins above 30 torr in most cells whereas the oxygen pressure for half-maximal respiration is only 0.5 to 3 torr (depending on cell type). In small cells (10-15 um dia.) the diffusion induced difference in oxygen pressure from the extracellular medium to the mitochondria is only a few tenths torr. Supported by grants GM-21524 and GM-36393.

S11 23

OXYGEN PRESSURE AND FORMATION OF ADENOSINE BY ISOLATED CARDIAC MYOCYTES.

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Formation of adenosine (AR) by the intact heart is known to be critically dependant on the supply to demand ratio for oxygen. Which cell type within the heart is responsible for the formation of AR is still a matter of debate. Isolated metabolically stable cardiomyocytes of the rat were incubated at constant PO_2 (0.1 - 100 mmHg) in an oxystat system (Biochem.J.236:765, 1986). The free cytosolic concentration of AR was measured by intracellular conversion of AR to S-adenosylhomocysteine (SAH) in the presence of L-homocysteine (Circ.Res.63:240,1988). SAH levels progressively increased when PO_2 decreased from 3 to 0,1 mmHg. Changes in SAH occured prior to changes in cellular adenine nucleotide levels. Treatment of normoxic cells was oligomycin (10 uM) and deoxyglucose (55 mM) also caused a time dependant accumulation of SAH. Conclusion: Adenosine is formed intracellularly by quiescent cardiomyocytes when ambient PO_2 is below 3 mmHg.

S11 24

ERYTHROPOIETIN PRODUCTION IN THE ISOLATED PERFUSED KIDNEY

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Production of Erythropoietin (Epo) in the kidney is stimulated by diminished renal oxygen supply. It was our aim to develop an in vitro model to study the regulation of Epo production. Isolated rat kidneys were perfused with a substrate enriched Krebs-Henseleit solution containing 2g/dl or 5g/dl bovine serum albumin (BSA). Human erythrocytes were added to adjust hematocrit (hct) to values between 5% and 40%. When kidneys were at an arterial pO_2 of 500mmHg (10% hct, 2g/dl BSA) practically no Epo was detectable in the perfusion medium (RIA) after 3.5 hrs of perfusion. At a pO_2 of 30mmHg kidneys produced 3200±1200 mU/g ($\bar{x}$±SD). The rise in Epo response at low pO_2 was not observed with 40% hct. Furthermore, the kidneys produced significantly less Epo (520±250 mU/g) when BSA was elevated to 5g/dl (10% hct, pO_2 30mmHg). Our results indicate that (1) Epo is produced by the isolated perfused rat kidney (IPRK), (2) in this model Epo production is under the control of hypoxic stimuli, (3)the IFRK can be used for further studies regarding the mechanisms of hypoxia-induced Epo production.

This study is supported by the National Science Foundation (Grant No. 3.165.88)

S11 25

THE PO_2-DEPENDENT RELEASE OF PGI_2 AND EDRF SUGGESTS A ROLE OF THE ENDOTHELIUM AS VASCULAR OXYGEN SENSOR.

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Experiments suggest an O_2-sensing mechanism in the vascular wall being involved in the hypoxic blood flow response. It was tested whether endothelial cells which decisively control vascular tone showed pO_2-dependent changes in the release of the vasoactive autacoids PGI_2 and EDRF. Experiments on native and cultured endothelial cells revealed an enhanced release of PGI_2 and EDRF at low pO_2 (20-40 mm Hg) followed by vasodilation which is consistent with an endothelial O_2 sensor function. The mechanism of this enhanced release is still not fully elucidated. However, at low pO_2 there was an increase of intracellular free Ca^{2+} depending on the presence of extracellular Ca^{2+} (similar results were obtained with low concentrations of cyanide). This indicates that a transmembraneous influx of Ca^{2+} is involved. It remains to be established whether this Ca^{2+}-influx is driven by a membrane hyperpolarization by opening of K^+-channels (as observed during longlasting receptor stimulation). Since a decrease in intracellular ATP is associated with an opening of K^+-channels in several cell types, this mechanism may establish a link between reduced oxidative phosphorylation and enhanced release of autacoids.

S11 26

DIFFERENTIAL EFFECTS OF VARIOUS TYPES OF OXIDATIVE STRESS ON DNA DAMAGE AND PROTEIN SYNTHESIS IN CULTURED ENDOTHELIAL CELLS (EC)

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The production of endothelium derived agents which modulate smooth muscle tone is known to be affected by the presence of active O_2 species. In order to understand the possible relationship between the action of O_2 metabolites and the production of vasoactive substances by EC, we assessed protein synthesis and DNA damage and repair in primary cultures of porcine aortic EC under the effect of hyperoxic exposure or treatment with the enzyme system hypoxanthine-xanthine oxidase (HX-XO) or H_2O_2. These two types of oxidative stress block protein synthesis at the level of the translation, but hyperoxia acts on the elongation, whereas the HX-XO system inhibits the initiation. H_2O_2 treatment, but not a 5-day exposure to 95% O_2, induces DNA damage and the subsequent repair process, characterized by the activation of the enzyme poly-(ADP)ribose polymerase and the NAD and ATP cellular depletion. These experiments indicate that various types of oxidative stress have differential effects on major cellular biological functions.

S12 27

DIFFUSION LIMITATION OF MUSCLE MAXIMAL O_2 CONSUMPTION

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Diffusion limitation of oxygen in the peripheral tissues was studied by left shifting the O_2 dissociation curve (ODC) of the blood perfusing maximally working muscle. Hypoxic hypoxia (HH) was compared to carbon monoxide hypoxia (CMH) in conditions where arterial O_2 content and partial pressure as well as muscle blood perfusion were kept equal. Under CMH conditions the O_2 partial pressure heads for diffusion from the capillary to the mitochondria are reduced and any diffusion dependant O_2 flux should be reduced. We observed that in isolated gastrocnemius working maximally in situ, perfusion with blood containing 30% CO reduces $\dot{V}O_2max$ by 26% (P=0.01). Since in our conditions tissue CO intoxication could be kept at a negligible level, our finding demonstrates that O_2 diffusion limitation is one of the factors that can curtail muscle $\dot{V}O_2max$, especially in hypoxia.

S12 28

CONTROL OF RESPIRATION IN SKELETAL MUSCLE AT REST

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Metabolic rate of resting muscle seems to be larger, under good oxygenation conditions, in nonperfused or saline-perfused preparations of skeletal muscle than in blood-perfused muscles *in situ* (Stainsby & Lambert, Exercise Sport Sci. Rev. 7: 125-151, 1979; Chinet & Mejsnar, J. Appl. Physiol. 66: in press, 1989). It has been said that the high-work to low-work transition in muscle cells is closely analogous to the state 3 to state 4 transition of a suspension of mitochondria (Hansford, Curr. Topics in Bioenerg. 10:217-278, 1980). For the muscle *in situ*, however, it is possible that muscle-cell energy potentials be maintained below the static-head values one could observe in mitochondrial or isolated-cell suspensions. This might be achieved through the heterogeneous distribution, within the organ, of an otherwise nonlimiting oxygen delivery to that organ (Dubois-Ferrière & Chinet, Eur. J. Physiol. 390: 224-229, 1981). Recent comparisons of oxygen consumption and heat production rates at different albumin-saline perfusion flow rates in isolated *gracilis anticus* muscles from rats suggest that the energy content of noncontracting muscle cells may, under artificially "improved" oxygenation conditions, become larger than normal.

S12 29

ENERGY TURNOVER OF VENTRICULAR MYOCARDIUM AT REST AND DURING ACTIVTY - ROLE OF LIMITATION OF OXYGEN SUPPLY by G. Elzinga

The energy (~P) required by the normal heart for steady state function is supplied by substrate oxidation and does not limit ~P turnover. Thus in the well oxygenated heart ~P supply meets ~P demand and equals ~P utilisation. In hypoxia ~P supply occurs through glycolysis, which is insufficient to maintain the heart in a steady state. Thus in hypoxia ~P supply, and ~P utilisation do not meet ~P demand. Since concomittant changes of the ~P stores are found, ~P supply is less than ~P utilisation.
When after a period of hypoxia the myocardium is reoxygenated loss of contractile performance and even cell death is found. How these phenomena relate to the decrease in ~P turnover during the hypoxic period, is not precisely understood. From estimates of ~P demand, ~P supply, and ~P utilisation it appears that the difference between ~P demand and ~P utilisation during hypoxia, i.e. the energy deficit, is an important determinant of the loss of contractile performance thereafter.

S12 30

ELECTRICAL AND IONIC CONSEQUENCES OF MYOCARDIAL HYPOXIA AND ANOXIA

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Reduction of the O_2 content in the coronary perfusate (hypoxia, anoxia) and arrest of myocardial perfusion (ischemia) are associated with rapid electrical and ionic changes. Severe homogeneous hypoxia (P_{O_2}~ 25mmHg), leads to a rapid decrease of the electrical resistance of the extracellular space which is due to edema formation. Almost complete oxygen withdrawal (anoxia) is necessary to produce marked electrical changes (shortening of the action potential, depolarization) and ionic changes (cellular potassium loss). In ischemia, acidosis and a rapid, marked extracellular accumulation of K^+ lead to electrical inexcitability and conduction block within 4-7min. Intracellular Na^+ and Ca^{++} overload, which mark the onset of irreversible cellular damage, occur only with a delay of 10-20 min, concomitantly with cellular uncoupling. Conduction disturbances (ventricular fibrillation) occur in most cases during the reversible stage and, therefore, are not initiated by cellular Ca^+ overload.

S13 31

OXIDANT STRESS AND LIPID PEROXIDATION IN CELL DEATH INDUCED BY DRUGS OR BY ISCHEMIA AND REFLOW. Charles V. Smith. Baylor College of Medicine, Houston, Texas, USA.

Reactive oxygen species have been implicated as mediators of many examples of tissue injury, primarily through studies of the effects of pharmacological agents capable of altering cellular antioxidant defense mechanisms. However, identification and quantitation of chemical changes characteristic of reactive oxygen-mediated alterations of cellular molecules are needed for elucidation of the molecular basis of damage. The measurement of glutathione disulfide is a useful quantitative index of increased production of reactive oxygen species, although a measurable shift in any redox equilibrium or steady state can be defined as an oxidant stress. Studies of reactive oxygen-mediated hepatic necrosis induced by diquat in rats have shown that dramatic oxidant stresses can be produced without killing hepatocytes in vivo. Similar experimental approaches and quantitative criteria applied to studies of ischemia/reflow injury have shown that production of reactive oxygen is much less than is observed with nontoxic doses of diquat. The peroxidation of tissue lipids does not parallel thiol oxidation in all cases and the complex array of primary and secondary products and the potent biological activities of many of these products makes the contribution of lipid peroxidation to pathogenetic processes difficult to evaluate. A greater appreciation of the limitations of chemically nonspecific methods and the developoment and application of specific methods for analysis of products of the peroxidation of biological lipids are necessary for the study of peroxidation in cell damage.

S13 32

STRATEGIES TO LIMIT THE SIZE OF MYOCARDIAL INFARCTION IN MAN: PRESENT STATUS AND FUTURE PERSPECTIVES

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In the past few years convincing evidence has accumulated indicating that myocardial reperfusion by coronary thrombolysis, if implemented within 3 to 6 hours after the onset of symptoms, does result in reduced infarct size, improved left ventricular function, and lower mortality.

The question arises, whether myocardial salvage can be enhanced by adjunctive measures. Interventions examined for this purpose in animal studies can be subdivided into (1) those that delay progression of damage during ischemia, and (2) those that attenuate reperfusion injury.

The clinical relevance of pharmalogical delay of the progression of injury is limited because optimal efficacy requires treatment before or early after the onset of ischemia.

Agents that limit reperfusion injury are conceptually attractive because they could be administered immediately before thrombolysis. Pharmacological control of accumulation of free radicals and intracellular Ca^{2+} may prove efficacious. However, the occurence of reperfusion damage in patients, and its pharmacological modification remain to be established.

S13 33

THE USE OF IN-VIVO NMR IN EXPERIMENTAL STROKE RESEARCH: EFFECTS AND MECHANISMS OF CALCIUM ANTAGONISTS

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The calcium antagonist PN 200-110 (isradipine) reduces the extent of cerebral infarction in a rat model of stroke by 60% as determined in-vivo by magnetic resonance imaging (MRI) (Sauter & Rudin, Stroke 17: 1228 (1986)). This cytoprotective activity might be due in part to cerebrovascular effects, since PN 200-110 improves blood supply to the ischemic region (Sauter et al, Am J Med (1989) in press). Additional sites of action have to be considered as well, since PN 200-110 readily crosses the blood brain barrier. We have therefore studied the drug's effect on the brain energy metabolism. Brain steady-state levels of high energy phosphates (HEPs, i.e. PCr and ATP), measured with 31P magnetic resonance spectroscopy (MRS), were unaffected by PN 200-110. However, results obtained by 31P and 1H MRS in the rat following cardiac arrest, as well as saturation transfer experiments in the normal rat brain, strongly suggest that PN 200-110 reduces the cerebral HEP consumption. This energy spareing effect of PN 200-110 might be an important protective mechanism, since maximum reduction of ATP consumption is observed at similar doses as the optimum cytoprotection.

S14 34

IMMUNREGULATORY MECHANISMS OF THE IMMUNE RESPONSE

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The immunotoxicological properties of chemical compounds affect the immune responsiveness of an organism to various degrees. The tracing of the primary interaction sites usually is a difficult task and the biological activity of an immunotoxic substance can be measured at different levels of the immune response. The aim of this introduction to the session is to describe the basic ontogenetic mechanisms leading to a functional immune system and to present the major pathways of a cellular and humoral immune response.

S14 35

TEST PROCEDURES FOR THE IDENTIFICATION AND CHARACTERIZATION OF IMMUNOSUPPRESSANTS

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For the detection and interpretation of immunotoxicity, we use a structured but flexible approach. Screening for potential immunotoxicity is performed within the framework of conventional toxicological studies in which high dose levels of toxic compounds are used and that are aimed at establishing the target organs of toxicity. Since these routine studies are predominantly performed with rats, we choose this species as the main experimental animal. For a first tier of screening, a set of general parameters of the specific and non-specific defense potential is used, including weight and histology of thymus, spleen and lymph nodes, and analysis of serum immunoglobulin levels. Chemicals that are identified as potentially immunotoxic in this phase will then be subject to further specialized studies. This second tier comprises function tests of non-specific defense mechanisms, cellular immunity, and humoral immunity, and includes host resistance models. Using this approach, we investigated the potential immunotoxicity of 18 pesticides and identified e.g. hexachlorobenzene and tributyltin oxide as potent immunotoxic chemicals.

S14 36

A TOXICOLOGICAL APPROACH TO CHEMICAL-INDUCED AUTOIMMUNITY

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Low molecular weight (LMW) drugs and chemicals are among the best documented environmental agents that induce or exacerbate systemic autoimmune disease (AD) in man. However, knowledge of the causal mechanisms of chemical-induced AD is incomplete and hypothetical because effects appear difficult to reproduce in experimental animals. Analysis of the relative contribution of environmental chemical and individual factors in the etiology and pathogenesis of AD is needed for proper risk assessment. Structure-activity relationship studies may identify the crucial (combinations of) LMW structures required for the induction of AD. Studies with inbred strains may point to genetic requirements for the induction of AD. The popliteal lymph node (PLN) enlargement assay is being evaluated as a short-term test system to screen for the potential of chemicals to induce lymphoproliferative reactions. As the PLN assays allows screening experiments, structure-activity relationship studies (Int.J.Immunopharmac. 1988; 10: 997), immunogenetic and histologic investigations, the application of this assay as a simple method for detecting immune disregulating potential of compounds seems promising.

S15 37

MOLECULAR CLONING AND EXPRESSION OF THE HUMAN AND MURINE INTERFERON-γ RECEPTOR

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Interferon-γ (IFN-γ) is produced by activated T-cells and plays an important role in modulating antigen presentation. IFN-γ shows no structural similarities with other IFNs and initiates its biological effects through its own specific receptor system. The function and structure of this receptor is poorly elucidated. A full length cDNA encoding the human IFN-γ receptor was isolated from a λgt11 expression library using a polyclonal anti-receptor antiserum (Cell 55, 273-280,1988). The gene for this receptor was identified in a cosmid library and transfected into mouse cells. Although the human IFN-γ receptor expressed in mouse cells displayed the same binding properties as in human cells these transfectants were not sensitive to the biological effects of human IFN-γ, suggesting the need for species-specific cofactors in receptor function. As inferred from the cDNA sequence the human IFN-γ receptor is a novel probably single chain receptor with a potential transmembrane domain in the middle of the molecule. It is most likely the product of a single mRNA and a gene located on chromosome 6q. A murine IFN-γ receptor cDNA was identified using human probes. Structure function relationships are currently investigated using hybrids between the human and murine IFN-γ receptor.

S15 38

INHIBITION OF T CELL ACTIVATION BY GLUCOCORTICOIDS AND LIPOXYGENASE INHIBITORS.

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The cytolytic activity of the murine T cell line PC60 is induced by IL-1 plus IL-2 and can be monitored in the cell lysates as haemolytic activity on sheep erythrocytes. IL-1 alone or enhancers of intracellular cAMP also induce the production of the serine esterase BLTE (granzyme A). Glucocorticoids such as dexamethasone ($ID_{50}=5x10^{-9}M$) and hydrocortisone ($ID_{50}=5x10^{-8}M$) have been shown to inhibit BLTE induction in PC60, whereas dexamethasone apparently does not affect BLTE activity in cells expressing the enzyme constitutively; dexamethasone also inhibits the induction of haemolytic activity. It has been investigated whether these two effects are actually mediated by the glucocorticoid receptor. Furthermore, treatment of the cells with lipoxygenase inhibitors such as NDGA or ETYA significantly reduces the cellular response to IL-1 or dibutyryl-cAMP for BLTE induction, in contrast to treatment with specific cyclooxygenase inhibitors. The possible existence of a relationship between the effects of the glucocorticoids on PC60 activation and the inhibition of BLTE induction by lipoxygenase inhibitors will be discussed.

S16 39

PLASMA MEMBRANE CALCIUM PUMP: CURRENT VIEW OF ITS STRUCTURE, FUNCTION AND REGULATION

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Calcium pumping ATPases of plasma membranes play a key role in regulating intracellular free Ca^{2+} levels. The complete primary structure of a human plasma membrane Ca^{2+} pump has been deduced from cloned cDNA, using as screening probes oligodeoxynucleotides made on the basis of peptide sequences of tryptic fragments of the red cell pump. The pump contains 1220 amino acids, corresponding to a Mr of 134,683. Asp-475 forms the acyl phosphate during the reaction cycle, and Lys-601 binds the ATP antagonist FITC. The calmodulin (CaM) binding domain has been identified next to the C-terminus (residues 1100-1127). A sequence stretch rich in Asp and Glu is present on the N-side of the CaM binding domain and may play a role in the binding of Ca^{2+} and in regulating the interaction of CaM with the pump. The pump also contains, near the N-terminus, two 11-amino acid stretches which resemble EF-hands and might thus form Ca^{2+} binding sites. Ten hydrophobic putative membrane-spanning domains have been identified: 4 in the N- and 6 in the C-terminal portion of the pump. Ser-1178 is phosphorylated by the cAMP-dependent kinase, increasing the Ca^{2+} affinity of the pump. cDNA sequencing studies demonstrate that multiple isoforms exist for plasma membrane Ca^{2+} pumps. These isoforms are produced from different genes as well as from multiple mRNAs generated by alternative splicing of the primary transcript of a single gene. Sequence differences in the C-terminal regulatory portion suggest a functional significance for the Ca^{2+} pump isoform diversity.

S16 40

BACTERIAL ION-MOTIVE ATPases: STRUCTURE, FUNCTION, AND EVOLUTION

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In recent years, a number of novel ion-motive ATPase have been characterized in bacteria. Examples are the K-ATPases of Escherichia coli and Enterococcus faecalis and the Cd-ATPase of Staphylococcus aureus. These enzymes appear to be involved in the transport of potassium, protons and other cations. Protein sequences derived from the cloned ATPase genes share extensive sequence homology. Some highly conserved regions are also found in eukaryotic Na-K-ATPases and Ca-ATPases. Thus, all these ATPases, in spite of their widely different transport functions, appear to have a common evolutionary origin and probably function by the same underlying reaction mechanism.

S17 41

CHEMICAL SIGNALS IN THE UNDERWATER COMMUNICATION OF SEAWEEDS

Jaenicke, L., Institut für Biochemie, Universität D-5000 Köln

The brown algae have communication systems during the sexual reproduction cycle that are based on chemotaxis. The pheromone is produced by the female gamete at sexual maturation, excreted into the water, and lures the male into copulation. The pheromones are species-typic alicyclic olefines, in the lower genera C_{11}, in the Fucales mostly C_8. They are biogenetically derived from poly-unsaturated fatty acids by highly stereospecific electron withdrawing decarboxylation and cyclization reactions. The lipophilic substances might be excreted by vesicular transport. Receptors have to be assumed on the male's outer surface or on intracellular sites to which the messenger substance is bound with great affinity and specificity - possibly aided by metal complexing. The stimulus leads to changes in the swimming behaviour due to changes in the beating frequency of the flagella of which the proximal, long one pulls the body of the androgamete; the distal, short one steers it into spiral motions of narrowing radius. Only indirect evidence is yet at hand on the shape of the binding site; systematic studies in different species with synthetic pheromones reveal very specific geometrical and bonding requirements. In the optimal fitting, threshold values down to 10^{-12} M are measured. The answer is so quick that single-hit events have to be postulated. Degradation of the pheromone ends the signal chain.
Lit.: Jaenicke, L., Botanica Acta 101 (1988) 149 - 159.

S17 42

PHOSPHOLIPID-STIMULATED PROTEIN KINASE INVOLVED IN SIGNAL TRANSDUCTION IN PLANTS

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In animal cells transmembrane signalling can be described at the molecular level for several signalling pathways integrated into the plasma membrane. Far less is known about the role of plant protein kinases in signal transduction in plants. We discovered that a lysolipid-like phospholipid, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, activates three reactions: (1) membrane-associated protein kinase which phosphorylates proteins on the tonoplast and the plasma membrane; (2) H^+-ATPases in vitro on the tonoplast and the plasma membrane; (3) proton extrusion from cultured plant cells. This protein kinase is also activated by Ca^{2+} ions. We also found that several plant phospholipids one of which is lyso-PC can activate the protein kinase. This suggests a hypothetical signal transduction chain consisting possibly of a phospholipase A_2, lysophospholipids, protein kinase, and substrate proteins, some being closely associated to the H^+-ATPases. This signal chain with the Ca^{2+}/lysophospholipid-activated protein kinase as a center piece is reminiscent of the protein-kinase C-containing signal chain from animals although it is different in that it is not activated by diglyceride. It may be linked to the PI-cycle by Ca^{2+} only.

S17 43

REGULATION OF CALCIUM TRANSPORT IN PLANT CELLS : IMPLICATIONS FOR CALCIUM AS A POTENTIAL SECOND MESSENGER

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Calcium is known to control a number of cell activities in plant cells and is considered as a second messenger in the coupling of stimuli to biological responses. As it is not metabolized, the amounts of calcium in the cytosol depend only on the balance of uptake/release processes from extra/intracellular stores. Carrot protoplasts are able to import calcium from the incubation medium. The uptake is specifically inhibited by calcium channel blockers of the phenylalkylamine type. Membrane derived from protoplasts bind labelled calcium channel blockers in a saturable and reversible manner. Therefore a direct relationship between site occupancy and biological effects exists. The functioning of the calcium-channel-like components is disturbed by the phytotoxin zinniol that stimulates calcium entry into protoplasts to bring about the death of the cells by competing partially with the channel blockers. Calcium accumulates within the cell into the large central vacuole from where it may be specifically released into the cytosol by another transient second messenger IP_3. The respective role of the calcium channel activity and the IP_3 sensitive pool will be speculated.

S17 44

A TASTE FOR PATHOGENS IN PLANTS: PERCEPTION OF ELICITORS AND INDUCTION OF DEFENSE REACTIONS

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Plants respond to an attack by pathogens with an array of biochemical defense reactions. Cell-free extracts of pathogens, so-called elicitors, can induce many of these defense reactions to a similar extent as a living pathogen. Among the best-known elicitors are cell-wall components of fungi. Many such preparations are active at sub-ppm concentrations, suggesting that plant cells recognize elicitors with similar acuity as specialized taste cells. The defense response induced by elicitors has been studied in great detail. Comparatively little is known about the perception and transduction of the elicitor signal. A high-affinity elicitor-binding protein has been discovered in soybean cell membranes; however, its involvement in chemoperception of the elicitor remains to be demonstrated. Calcium ions, phosphoinositides and cAMP have been implicated in signal transduction but the experimental basis for this is weak. Biosynthesis of the plant hormone, ethylene, is rapidly induced by elicitors, but it remains unclear whether ethylene has a role in the defense response.

S18 45

MOLECULAR BASIS OF THE ANTI-SELF IMMUNE RESPONSE

Kofler, R., Institute for General and Experimental Pathology, University of Innsbruck, Medical School, A-6020 Innsbruck, and Scripps Clinic and Research Foundation, La Jolla, CA 92037

The genes and somatic mechanisms involved in the generation of autoantibodies, particularly those associated with autoimmune disorders, have attracted considerable interest over the last years. Immunoglobulin heavy and light chain gene loci in autoimmune individuals and strains of mice and genes encoding disease-associated and so-called "natural" autoantibodies have been analyzed by modern molecular technics in our and other laboratories. As will be discussed, these studies, although in some respects still incomplete and controversial, have provided substantial insight in the genetic origin and primary structure of self-reactive immunoglobulins and have potential implications for etiologic concepts.

S18 46

GENETIC ANALYSIS OF ANTI-BROMELINIZED MOUSE RED BLOOD CELL (BrMRBC) AUTOANTIBODIES.

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The variable region sequences from three NZB, three CBA/J and one BALB/c monoclonal anti-BrMRBC autoantibodies have been determined. The virtually identical nucleotide sequences indicated that the anti-BrMRBC autoantibodies are encoded by restricted VH(BrMRBC)-D SP2-JH1 and V_K(BrMRBC)-J_K2/J_K4 gene combinations, likely in a germ-line configuration. Moreover, the VH(BrMRBC) nucleotide sequence was less than 80% homologous to members of the ten known VH families, and the genomic restriction fragments detected by the VH(BrMRBC) cDNA probe did not correspond to those defined with probes of the most homologous VH families (X24 and 7183). Therefore, the anti-BrMRBC VH related genes belong to an 11th gene family.

S18 47

NATURALLY OCCURRING ANTIBODIES AND COMPLEMENT IN HOMEOSTASIS OF HUMAN RED BLOOD CELLS

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Senescent but not young red blood cells are continuously cleared from the circulation. Evidence suggests the involvement of naturally occurring antibodies to red cell proteins which have been isolated from normal human IgG. Anti-band 3, one of these antibodies, was also found associated with senescent red cells most likely because it bound to oligomerized band 3 protein, but its numbers were too small to consider phagocytosis exclusively FcR dependent. We studied the opsonic properties of increasing concentrations of anti-band 3 in serum on oxidatively stressed red cells. It stimulated alternative complement pathway C3b deposition, increased formation of covalently linked C3b-IgG complexes (C3b-IgG) and phagocytosis of these cells. In analogy to this, senescent red cells also contained more membrane-associated C3b-IgG than young ones. The specificity of the IgG of cell-bound C3b-IgG was analyzed after cleavage of the complex. It had anti-band 3 specificity and in addition bound weakly to C3. It is the concomitant binding to C3 which increases the chances for anti-band 3 to form covalently linked C3b-IgG upon spontaneous or induced activation of complement. As C3b-IgG complexes effectively nucleate a C3 convertase, antibodies with an affinity for C3 stimulate alternative pathway C3b deposition. Thus, binding to C3 convays to anti-band 3 the ability to stimulate alternative complement pathway C3b deposition. It potentiates the effect of this low titer and low affinity antibody.

S18 48

T CELL RECEPTORS IN MURINE AUTOIMMUNE ENCEPHALOMYELITIS

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Experimental allergic encephalomyelitis (EAE) is an autoimmune disease which results in demyelination and paralysis. In EAE CD4+ T lymphocytes are the mediators of the disease and the autoantigen has been defined. In PL mice the N-terminal nonapeptide of myelin basic protein (MBP) represents the dominant encephalitogenic peptide. In order to determine the heterogeneity of T cell receptors involved in this autoimmune disease, cDNA sequences of TCR molecules of eight independent T cell clones were derived. A very limited heterogeneity of TCR molecules was found, especially usage of V regions was highly restricted. Based on these observations prevention and reversal of ongoing autoimmune disease was achieved with monoclonal antibodies specific for the TCR V region $V_\beta 8$. T cell clones with differences in the D and J regions of the TCR molecules did not exhibit any significant differences in fine specificity as determined by proliferation studies with peptides with single amino acid substitutions and analysis of crossreactions with allogeneic spleen cells. Implications for TCR structure-function and for treatment of autoimmune diseases will be discussed.

S19

NUTRITION AND PHYSICAL ACTIVITY

Poster Session, Thursday

Neurobiology (P1)

P1 1

NERVE GROWTH FACTOR - A MEDIATOR BETWEEN THE NERVOUS AND THE IMMUNE SYSTEM

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Using a monoclonal antibody against human nerve growth factor (NGF) receptor a sensitive immunoprecipitation assay for the quantitation of the human NGF receptor has been established in our laboratory. As expected, NGF receptors were detected on NGF-responsive neurons of the peripheral nervous system (e.g. the superior cervical ganglion contains 15 fmole receptor/mg protein) and central nervous system (cortex and hippocampus contain 2 respectively 3 fmole receptor/mg protein).

Non-neuronal tissues also expressed significant amounts of NGF receptors: e.g. human spleen and lymph nodes exhibit 8 respectively 6 fmole receptor/mg protein. These findings suggest that NGF specifically interacts with cells of the immune system. The assumption is supported by our recent findings that human blood T- and B-cells express specific NGF receptors. Moreover, we have found that NGF stimulates lymphocyte proliferation and immunoglobulin production.

P1 2

NGF ALTERS THE PROPORTION OF THREE PHARMACOLOGICALLY DISTINCT K^+ CURRENTS IN CULTURED DORSAL ROOT GANGLION CELLS

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Beside its well known effects on survival and growth of embryonic sensory cells, nerve growth factor (NGF) has been reported to affect the action potential of postnatal cells (Chalazonitis et al., P.N.A.S. 84:289, 1987). We therefore compared voltage dependent K^+ currents of cultured dorsal root ganglion (DRG) cells grown in the presence of NGF (5ng/ml, NGF+) to those of cells deprived of NGF (NGF-) for 1 to 7 days. DRGs of 4 day old rats were dissociated by trypsin and grown on polyornithine coated plastic dishes for up to 7 days with or without NGF. Whole cell K^+ currents were recorded in CholCl containing bath solution using the patch clamp method. The currents were activated by voltage steps from holding potentials of -80mV to +60mV. Three components of K^+ current were separated pharmacologically: a non inactivating component which was blocked by Cd (calcium activated current I_{KCa}), a non or slowly inactivating component which was blocked by TEA-Cl (delayed rectifier I_K) and a rapidly inactivating component blocked by 4-aminopyridine (I_A). While the withdrawal of NGF did not significantly affect the total K^+ current, the proportion of the three components was altered in NGF deprived cells: I_{KCa} and I_K were slightly larger than in normal (NGF+) cells (2.3 vs. 1.6nA and 6.2 vs. 5.6nA respectively) whereas I_A was smaller in NGF- than in NGF+ cells (3.3 versus 6.6nA). In consequence the ratio of I_A to I_K was significantly lower in NGF- than in NGF+ cells (0.56 vs. 1.08). These findings suggest that NGF influences the regulation, expression or distribution of voltage dependent K^+ channels in postnatal DRG cells.

(Supported by Schweizerischer Nationalfonds Nr. 3.265-0.85)

P1 3

REPETITIVE ADDITION OF A HIGH DOSE OF EPIDERMAL GROWTH FACTOR PROLONGS GLIAL CELL MULTIPLICATION IN A THREE-DIMENSIONAL CULTURE SYSTEM

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Previously, we have studied the effects of epidermal growth factor (EGF) in early aggregate cultures of fetal rat telencephalon. We have found that at low concentration, EGF (5ng/ml) stimulates the maturation of astrocytes and oligodendrocytes, whereas at high concentration (90ng/ml) it enhances both the proliferation and maturation of glial cells. In untreated cultures, cells cease to proliferate around day 12, as they undergo extensive differentiation. At that time, they become unresponsive to EGF.

Here, cultures were treated repetitively on days 6,9 and 12 with a high concentration of EGF. Incorporation of ^{14}C-Thymidine measured on day 14 was doubled with respect to untreated cultures. Total DNA and glutamine synthetase activity measured on day 15 were also greatly increased.

Our results demonstrate that EGF can prolong the mitotic activity of astrocytes in histotypic cultures. However, around day 16, a substantial part of the newly acquired cells were lost by cell shedding.

P1 4

EXPRESSION OF SUBSTANCE P (SP) BY PRIMARY SENSORY NEURONS IN CULTURE IS REGULATED BY EXTRACTS OF CENTRAL AND PERIPHERAL TARGETS.

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Dissociated cell cultures of dorsal root ganglion chick embryos were performed at the embryonic day E6 or E10 (before or after formation of specific connections with peripheral or central targets). SP expression was detected by both immunocytochemistry with monoclonal antibodies and in situ hybridization with tachikinin ^{35}-S-labelled RNA probes. SP was expressed in 98% of the neurons grown at E6, but only in 60% at E10. To determine whether peripheral or central targets exert a control on SP expression, extracts of muscle, skin or brain were added to cultures at E6: SP-expressing neurons were then reduced to 75, 86 or 88% respectively. It is concluded that factors present in muscle and, to a lesser extent, skin or brain repress the transcription of SP in primary sensory neurons grown in vitro.

(supported by N° 3 097.86 grant of the Swiss National Foundation)

P1 5

EXPRESSION OF SUBSTANCE P (SP) IN DORSAL ROOT GANGLIA (DRG) CELLS: ROLE OF PERIPHERAL AND CENTRAL TARGETS IN CHICK EMBRYOS.

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The influence of peripheral and central connections on SP-expressing DRG cells was studied at two stages: 1) at E6, before the appearance of specialized connections, by resecting one hindlimb or by cauterizing the caudal neural tube; 2) at E12, after the establishement of specialized connections with the periphery, by resecting one hindlimb. Operated and control lumbosacral DRG were examined 6 days later. After suppression of central connections, the percentage and cell size of SP-immunoreactive ganglion cells were the same as in control embryos. The effect of interruption of peripheral projections was 1) at E6, a preferential loss of large A-neurons and only a slight decrease in the subpopulation of SP-positive small-B neurons; 2) at E12, a severe reduction in the number and cell size of SP-positive small B-neurons.

Contrary to calbindin expression, the induction of SP-expression by DRG cells does not seem to be controlled by interaction with specific targets. It is indeed concluded that peripheral projections are mainly required for the survival of the SP-expressing subpopulation.

P1 6

REGULATION OF FETAL AND ADULT TYPE ACETYLCHOLINE RECEPTOR EXPRESSION BY MUSCLE ACTIVITY IN MAMMALIAN MUSCLE.

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We have analysed electrophysiologically the effect of muscle activity on the expression of "fetal" and "adult" acetylcholine receptors (AChR.s) and, by northern analysis, on the levels of their specific subunit mRNA.s, i.e. ε and γ . Muscle denervation and mere pharmacological blockade of impulse conduction in the nerve produced similar increases in extrajunctional AChR.s as estimated by binding of ^{125}I-α-bungarotoxin. Their gating and conduction properties determined by noise analysis were similar and were of the fetal type. However, unlike denervation, pharmacological blockade did not cause an increase in γ-subunit mRNA. Chronic stimulation of denervated muscles suppressed the increase in AChR.s and in γ-mRNA. At the endplate, the number and type of (adult) endplate AChR.s remained unaffected by stimulation as was ε-mRNA of total muscle. However, activity did prevent the 8-10 fold decline in the metabolic stability of junctional AChR.s that is observed after denervation. The results show that 1) fetal AChR expression may not be controlled by activity alone and that 2) the stabilizing action of activity on the constant number of adult AChR.s is caused by a) differential resistance of γ-vs. ε-mRNA and by b) increased AChR stability.

P1 7

AXONAL GUIDANCE IN THE EMBRYONIC AVIAN RETINA

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During retina morphogenesis, optic axons grow out from their parent ganglion cells and navigate toward the optic disc. A detailed analysis of the orientation of newly formed axons shows that the initial growth of axons is directed from the very beginning. If one disturbs the local environment of the axons, aberrant fiber tracts are caused suggesting that the information for directed fiber growth is located in the axonal environment. By a mechanical cleavage of the retina, the basal surface of the retina, which represents the environment of axons in vivo, can be isolated and used as a substrate for axonal growth in vitro. Comparative studies of the in vivo-derived matrix with collagen, fibronectin or laminin show that the basal surface of the retina is a far superior in promoting axonal growth than any of the other substrates, however, the retinal basal lamina had no effect on the orientation of axonal growth. Antisera against the basal lamina dramatically disturb the normal neurite extension on basal lamina, but did not alter the principal direction of fiber outgrowth from the explant. The antisera experiments showed that extracellular matrix components from basal laminae are crucial for neurite extension in the retina, but not for the directed outgrowth of the axons.

P1 8

PLASTICITY OF OPTIC NERVE GLIA IN VITRO: I DEVELOPMENTAL ASPECTS.

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Respecting developmental and histological criteria the optic nerve (ON) is a simple part of the central nervous system (CNS). The ON of the newborn rat is composed of naked axons, differentiated astrocytes and progenitor cells. To study developmental aspects of glia, small pieces of ON, called minisegments were cultured as long-term explants and then investigated by means of microscopy and immunocytochemistry. Under the already described culture conditions, five different cell types develop within the minisegment. During the first 2d in culture phagocytes appear; they increase numerically up to 2 weeks and seem to disappear almost compleatly by d 20-30 in vitro. At 3d in vitro, oligodendrocytes differentiate expressing numerous myelin specific antigens. Both oligodendrocytes and astrocytes are observed during the whole time in culture (up to 6 months). After 4-5 weeks in vitro, a neuron-like type of cell is present showing both a typical neuronal fine structure and neuron specific immunoprecipitates. These data are an additional piece of evidence that CNS glia and precursor cells exhibit a high degree of developmental plasticity. (Supported by NSF 3.652.0.87 and Swiss MS Society).

P1 9

AXONAL TARGET REGULATES DENDRITIC DEVELOPMENT IN THE CENTRAL NERVOUS SYSTEM.

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In the mature isthmo-optic nucleus (ION), the neuronal perikarya are arranged in a convoluted lamina from which the dendrites project perpendicularly into the neuropil. By embryonic day 10 (E10), virtually all the dendritic trees are already highly polarized, but, unlike in the mature ION, most are directed ventralwards or ventromedialwards. Between E10 and E14, the dendrites change their direction of polarization so as to produce the adult pattern. The afferents to the ION play little if any role in these events, because by the time they arrive in the ION (about E12) its dendritic arbors are already highly polarized ventralwards, and the first stage of repolarization is virtually complete. In contrast, the axonal targets seem to play an important role. Early removal of both eye primordia is known to cause the death of virtually all the ION neurons, beginning at E12-E13. But our present data indicate that well before this, by E11, the dendritic trees in the target-deprived IONs are smaller and not so highly polarized as in unoperated embryos. This suggests that the axonal target may send an early retrograde signal to the ION neurons that modulates their dendritic geometry before they become dependent on the target for survival.

P1 10

DEVELOPMENT OF LAMINATION IN THE LATERAL GENICULATE NUCLEUS OF TUPAIA

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At birth no cellular lamination can be seen in the tree shrew (Tupaia belangeri) dorsal lateral geniculate nucleus (LGNd) with Nissl staining. However, when the LGNd is stained with the rapid Golgi method, laminae appear as dark bands which are separated by paler interlaminar spaces. This separation of laminae corresponds to the fact that fibres originating from the two eyes are already segregated at birth (Rager et al., Neurosci. Abstr. 6, 662, 1980). Therefore, it has to be assumed that lamination is preformed already at birth although it does not appear in the Nissl staining. If the Golgi technique is applied to the adult LGNd the staining character is reversed in the sense that interlaminar spaces appear darker. Since the Golgi technique does not reveal any identifiable structures in the background staining of laminae and interlaminar spaces, histochemical procedures have to be used to analyse further the development of lamination.
Supported by grant No. 3.518-0.86 from the Swiss NSF.

P1 11

INTRACELLULAR EVENTS IN DYING NEURONS DURING NORMAL DEVELOPMENT AND FOLLOWING TARGET-DEPRIVATION

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During the natural development of the chick embryos' isthmo-optic nucleus (ION), 60% of the neurons die between embryonic day (E) 12 and E17. Some of these die by the autophagic mode of cell death. If the ION neurons are deprived of retrograde trophic maintenance owing to an intraocular injection of colchicine at the beginning of the cell death period, they all die and solely by the autophagic mode. The autophagic dying neurons are ultrastructurally similar in normal and colchicine-injected embryos, and have the following characteristics. 1. Their perikaryon and dendrites contain numerous vacuoles, which are of three types, two being kinds of secondary lysosome. 2. They endocytose an exogenous label, horseradish peroxidase, which is channelled into all three kinds of vacuole but not into the cytosol or nucleus. 3. They lose nuclear DNA into the largest of the three kinds of vacuole and exibit enhanced unscheduled DNA-synthesis, as shown by thymidine autoradiography. Our data indicate that the cells accomplish their own destruction, channelling all their parts - nucleus, cytoplasm and plasma membrane - into the cell's own lysosomes for hydrolysis.

P1 12

HUMAN NEUROBLASTOMA COLONIES GROWN IN METHYLCELLULOSE CLONING ASSAY CONTAIN DIFFERENT CELL SUBPOPULATIONS

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In monolayer culture of the human neuroblastoma cell line IMR 32 at least 2 cellular phenotypes are distinguished: the neuroblast-like (Neuron specific enolase, NSE +) and fibroblast-like (S100 protein +, vimentin +) cells. The attempt to separate the subpopulations in the semisolid methylcellulose medium, frequently used in the cloning of hematopoietic and tumor stem cells, leads to homogeneous colonies of small-sized cells. However, immunocytochemical staining of colony sections reveals a mixed pattern: all colonies contain NSE +, S100 + and islands of negative cells (to almost equal parts at 3 weeks of culture). Some colonies contain single vimentin + cells. Since a colony is generally considered to develop from one stem cell, the identified subpopulations would reflect the expression of distinct phenotypes originating from one neuroblastoma stem cell.

P1 13

EXPERIMENTAL DIASTEMATOMYELIA IN THE CHICK EMBRYO

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Diastematomyelia is characterized by a local division of the spinal chord in two parts, usually separated by a fibrous or osseous septum associated with alterations of the vertebrae. Such a malformation was never reported in birds. The neural tube of 36 to 40 hour-old experimental and control chick embryos was split by microsurgical techniques. A membranous screen was inserted into the gap of the experimental embryos. The embryos were fixed after 10 days of incubation and examined macroscopically then stained in toto for cartilaginous skeleton study. The altered part of the skeleton was then excised and treated for histological examination. Control embryos healed without any malformation or showed open spina bifida. Experimental embryos showed various degrees of spinal cord division associated with axial skeletal defects. Microscopic examination revealed two hemicords each of them containing its own central canal; in some cases one of the hemicords was hydromyelic. Rumplessness occurred in connection with experimental diastematomyelia. Such experiments may suggest a physical mechanism in the genesis of diastematomyelia, involving the closure of each half of the neural groove to give two parallel hemicords.

P1 14

SHORT REACTION TIMES IN MAN

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Extremely short reaction times of saccades have been demonstrated with goal directed eye movements. The aim of the present experiments was to show if similar reaction time could also be achieved with voluntary foot movements. At the onset of the trials two bi-color LEDs in front of the subjects turned on red. One of the LEDs (in a random sequence, the right or left one) turned on green after a variable interval (0 to 200 ms). The subjects were required to perform a plantar flexion ipsilateral to the green light as fast as possible. Reaction times were shortest with gaps of 150 ms (about 320 ms) and longest with no gap (about 270 ms). With further experiments it could be shown that two factors modulated reaction time. (1) The onset of the gap was a warning signal for the subject. (2) Visual stimulus efficacy of the go signal depended on whether the LED was turned off or was turned on red before the green go signal (contrast).

P1 15

NEURONAL ACTIVITY IN THE MONKEY STRIATUM DURING THE PREPARATION OF EXTERNALLY GUIDED OR SELF-INITIATED ARM MOVEMENTS

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Single neuron activity was recorded in the striatum (caudate nucleus and putamen) during the preparation of voluntary arm movements in 3 Macaca fascicularis monkeys. The preparation of externally triggered movements was studied in a go-nogo task in which instruction lights served to prepare the animal for upcoming movement or no-movement reactions. In a second task, animals performed self-initiated arm reaching movements at irregular, self-chosen intervals and without phasic external cues.
Neurons in both parts of the striatum were activated during the preparatory period in the go-nogo task (go: 23%, nogo: 9% of 950 neurons) and 700-3000 ms in advance of self-initiated movements (15% of 680 neurons). In both tasks, premovement activity began before earliest muscle activity and ended immediately before the movement or continued during its execution.
These data demonstrate that some neurons in the striatum are involved in the preparation of purposive voluntary movements. Their activations may reflect the input from cortical areas with similar premovement activity.

P1 16

TWO-WAY AVOIDANCE LEARNING OF ACALLOSAL MICE

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The inbred mouse strain I/Ln is characterized by congenital absence of the corpus callosum, a condition associated with a profound reorganization of intracortical connections. This mouse strain may provide an animal model for behavioral sequelae of human agenesis of the corpus callosum, and for mechanisms of hemispheric lateralization of functions. In a preliminary study, we compared the acquisition and performance in two-way avoidance learning of I/Ln mice with that of three widely used inbred strains, namely C57BL/6, BALB/c and DBA/2. One-way analysis of variance indicated a massive deficit of the I/Ln mice in learning the task and continuing poor performance during subsequent testing (strain rank order DBA/2 >> BALB/c > C57/BL/6 >> I/Ln). This deficit was neither associated with escape failures nor with pretrial locomotor activity, both similar across strains. Since I/Ln mice perform fairly well in a swimming navigation task, a visual handicap can be excluded as well. However, further investigations are needed in order to verify whether the lack of the corpus callosum is a factor responsible for poor shuttlebox-learning (which itself probably reflects an inability to cope with conflicting behavioral tendencies). Supported by SNF 3.206.

P1 17

SEASONAL VARIATIONS OF SUBJECTIVE SLEEP AND MOOD PARAMETERS ARE MODIFIED BY THE INDIVIDUAL "DIURNAL TYPE"

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Seasonal rhythms in humans are known to be modified by age, sex, ethnic origin, bioclimatic factors and geographical latitude (Reinberg,1974). Present data will add as a further potentially influencing factor the morningness-eveningness dimension, as previously suggested by Klöppel (1980).
According to the Horne-Ostberg Scale (1976) administered to 20 healthy subjects (age range:23-52 years), morning-, evening- and intermediate groups of individuals were formed and compared with respect to seasonal rhythm characteristics of sleep and mood parameters, rated monthly during 14 months by Visual Analogue Scales.
The morning- and evening groups showed large seasonal variations in most of the self-rated parameters, while the intermediate group lacked rhythmicity. The seasonal pattern was unimodal with a peak of sleep complaints, fatigability and sadness in winter in the evening group, bimodal with a peak around january/february and a secondary one around july/august in the morning group.
Further work is needed to confirm these preliminary findings. However, diurnal typology merits consideration in seasonal rhythm studies and may provide new insight into mechanisms relating the circadian and seasonal system in men.

P1 18

MODULATION OF SPINDLE RYTHM IN THE THALAMUS IN VITRO. M.Mühlethaler and M. Serafin, Dept de Physiologie CMU,1211 Genève.

Intracellular recordings from thalamic neurons have been obtained so far in situ in the cat and in rodent slices. In order to study their membrane properties and the way they are controlled by the brainstem reticular formation we developed an alternative preparation of an isolated and perfused brain preparation(IWB). We found that thalamic neurons displayed all the properties already described in slices. In addition however they also displayed spontaneous spindles. These spindles were characterized by a duration of 1-10 sec. and consisted of low threshold rebounds and spikes and the interspindle interval was 10-20 sec. These spindles could be affected by either brainstem stimulations or application of drugs in the perfusion. We found in particular that carbachol was able to strongly but reversibly block the spindling. Our studies confirm that acetylcholine might play a role in arousal and indicate that thalamic circuits are preserved in the IWB. These data together with preliminary recordings obtained from the pediculopontine cholinergic reticular formation suggest that the IWB will thus be an excellent preparation for studying intrinsic thalamic mechanisms as well as brainstem-thalamic interactions.(Supported by a Swiss NSF grant no 3.288-0.85)

P1 19

ELECTROPHYSIOLOGY OF CORTICAL ATTENTION USING DYNAMIC RANDOM DOT STEREOGRAMS

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Electrophysiological Investigations of the human visual information processing typically employ static contrast stimuli. Since these stimuli contain monocular cues precortical responses may confound the stimulus-evoked brain potentials (EP). We used dynamic random dot stereograms (RDS) since these stimuli operationally skip all stages prior to the activation of cortical disparity neurons (Area 18). The aim of the present study was to investigate the topographical distribution of the P300-component of the EP, an electric event about 300 ms after the stimulus which is related to the categorization of relevant stimuli. In order to obtain high spatial resolution, 42-channel recordings were used.
The results in 12 healthy volunteers revealed significant differences in latency, amplitude and topography of the P300 to RDS as compared to the P300 evoked by contrast stimuli. The results suggest that the late, so-called endogenous components of the EPs are confounded by peripheral neuronal activation when the stimuli contain monocularly visible non-cortical cues.

P1 20

ELECTROPHYSIOLOGICAL STUDY OF VESTIBULAR NUCLEI NEURONS IN VITRO. M.Serafin and M.Mühlethaler, Dept. de Physiologie,CMU, Genève.

Vestibular nuclei neurons have not yet been studied in vitro and the ionic basis of their firing properties are thus not described. These properties however might play an important role in particular in some aspects of plastic phenomena that these nuclei undergo following unilateral deafferentation. Using brainstem slices and an isolated and perfused preparation (IWB) we found three main neuronal cell types. Type 1 was characterized mainly by a single range of firing, a rather broad action potential followed by a deep single afterhyperpolarization and the presence of a strong rectification probably due to an A current. Under conditions of K and Na channels blockage it only revealed a small calcium high threshold spike that could be potentiated by replacement of calcium by barium. Type 2 was distinguished by the presence of a thin action potential followed first by a fast and then by a slow afterhyperpolarization (the latter due to a strong gK(Ca)). It displayed a secondary range of firing in its response to current injection which was probably due to the presence of calcium and sodium non inactivating plateau potentials. The last neuronal cell type(3) was similar in some respects to type 1 but completely lacked an A type of rectification. Preliminary data using the IWB seem to indicate that second order vestibular neurons are rather from the type 2. (Supported by a Swiss NSF grant no 3.288-0.85).

P1 21

AUDITORY AND LEG SENSORY SYSTEMS IN LOCUSTS ARE HOMOLOGOUS

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Segmental differentiation processes in insects transform a homonymously segmented embryonic nervous system into a segmentally specialized adult nervous system. We are studying these processes in the locust auditory and mechanosensory systems using neuron specific antibodies. The locust ear consists of numerous auditory receptors attached to a tympanal membrane in the abdominal body wall. These chordotonal receptors differentiate as part of an embryonic abdominal proleg. Axons from these sensory cells make contact with an intersegmental nerve, fasciculate and project into the sensory neuropile of the CNS. Virtually identical proliferation, differentiation and pathfinding processes generate the proprioceptors in the proximal femoral chordotonal organ of the true thoracic legs. These receptors are thus segmentally homologous to those of the auditory system. This shows that similar organizational modules in the embryo can give rise to diverse sensory systems in the adult. (Supported by the Fonds National Suisse.)

P1 22

MODIFICATION OF THE FUNCTIONAL SELECTIVITY IN THE AUDITORY THALAMUS OF THE CAT DUE TO CORTICOFUGAL MODULATION

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Several functional quantitative properties of single units (n=184) recorded in nitrous oxide anesthetized feline auditory thalamus were studied before, during and after the inactivation, by cooling, of the ipsilateral primary auditory cortex. Two populations of units, belonging to the same anatomical subdivision, are defined according to the tendency of one property to increase or to decrease during the cortical cooling. The functional selectivity (FS) of one property is defined as the presence of these two distinct populations of units. Out of 7 properties studied in 5 anatomical subdivisions we observed the following cases: (i), n=15 (40%), one FS was visible before and after the cortical inactivation; (ii), n=11 (30%), the FS appeared only during the cortical cooling; (iii), n=6 (20%), the FS was visible only when the cortex was not inactivated; (iv), n=3 (10%), the FS was not clearly distinguishable. The cortical inactivation induced the appearance of FS mostly in the dorsal nucleus of the Medial Geniculate Body and the Suprageniculate nucleus. We suggest that the cell assemblies coding for a functional property could act as adaptable filters, whose parameters are controlled by the cortex. Such filtering could selectively extract information from the incoming sensory signal, according to the cortical activity.

P1 23

SINGLE UNITS IN AUDITORY CORTICAL FIELDS AI, AAF AND PAF: RESPONSE PROPERTIES AND INTERACTIONS.
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Eight spike trains were recorded simultaneously from an array of 8 tungsten microelectrodes aimed in the auditory cortex of nitrous oxide anaesthetized cats. Neuronal activity was recorded during spontaneous discharge as well as in response to noise and tone bursts. The average firing rate of the recorded units ranged from 0.1 to 27 spikes/s. The majority of units (89%) showed non-monotonic changes in their discharge rate as a function of noise burst intensity. Units in AI and AAF were characterized by complex response patterns starting with a short latency excitatory component. In PAF, the response latencies were longer, and over 40% of the units showed exclusively excitatory components during the stimulus. Interactions were observed between pairs of units within the same cortical field (42/103) as well as in different fields (52/429). They were more frequent between AAF-AI (17/68) than AAF-PAF (3/20) or AI-PAF (3/53) units.

P1 24

RECIPROCAL HETEROTOPIC CALLOSAL PROJECTIONS IN AREA 17 OF TUPAIA BELANGERI

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As reported previously neurons projecting via the corpus callosum to the contralateral area 17 in the tree shrew (Tupaia belangeri) are located homo- and heterotopically depending on their location (Pritzel et al. 1988: Exp. Brain Res. 72, 481-493).
The goal of the present study was to clarify the origin of axonal terminals ending in the region close to the area 17/18 border. Therefore we injected WGA-HRP in this region and examined the contralateral striate cortex. We found labeled cells distributed almost over the whole binocular visual field. In coronal sections labeled somata were found extending from the area 17/18 border into the medial bank region. The majority of callosal neurons was detected in supragranular layers II and III. Most of them resembled typical pyramidal cells, but it also existed a band of neurons in basal sublayer IIIc with elongated processes oriented tangentially to the pial surface. This study demonstrates the existence of reciprocal heterotopic callosal projections in Tupaia striate cortex.
Supported by grant No. 3.518-0.86 from the Swiss NSF.

P1 25

SPECTRAL INTERACTIONS IN THE VISUAL SYSTEM OF TUPAIA

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In the tree shrew (Tupaia belangeri) the spectral sensitivities have been investigated by means of behavioral, physiological, microspectrophotometrical, morphological and immunocytochemical methods. All the data confirm that in Tupaia two different cone photoreceptor types exist. The long-wavelength receptor type with its absorption maximum at about 557nm is much more abundant in the retina (90-96%) than the short-wavelength receptor type (λmax at about 425nm).
The goal of the present study was to examine possible spectral interactions based on the two reported cone types in the visual system of Tupaia. Therefore we performed single-unit studies and found that in the lateral geniculate nucleus (LGN) and in the primary visual cortex (A 17) most neurons so far investigated belong to the broad-band type. They respond to the onset or the offset of a stimulus throughout the spectrum. Color-opponent cell types are much less frequent but they do also exist in both structures, the LGN and A 17. The results of this preliminary study show, that in the dichromatic color vision system of Tupaia spectral interactions occur based on the two different cone receptor types.
Supported by grant No. 3.518-0.86 from the Swiss NSF.

P1 26

EXPRESSION OF AVIAN GENES ENCODING NEURONAL NICOTINIC ACh RECEPTORS.

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Two nicotinic ACh receptors (nAChRs) from chick brain have been purified and one of these consists of only the ligand-binding subunit $\alpha 4$ and the structural subunit non-α ($n\alpha$). We have used voltage clamp and patch clamp techniques to characterize the physiological properties of nAChRs expressed by oocytes 1-5 days after nuclear injection with cDNAs for the two subunits linked to a heat-shock promoter. cDNA injection technique proved to be reproducible and simple since no cRNAs preparation is required. Heat-treated oocytes injected with both $\alpha 4$ and $n\alpha$ construct expressed ACh-induced currents in the µA range that could be reversibly blocked by hexamethonium but were insensitive to α-bungarotoxin. We found that these brain nAChRs incorporate at least two $\alpha 4$ subunits and that their functional properties differ from muscle nAChRs in at least two respects: a) the elementary conductance is considerably smaller (20 pS) and b) channels in outside-out patches stop functionning within a few minutes.

P1 27

PRENATAL NICOTINE EXPOSURE AND ITS EFFECTS TO CENTRAL NICOTINIC BINDING SITES: AN AUTORADIOGRAPHIC STUDY

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The effect of prenatal nicotine exposure was investigated at several postnatal stages (Odermatt et al. Experientia 44 (88)). Time pregnant Long Evans rats were treated with nicotine from gestational day 12 - 19. Alzet minipumps containing either nicotine bitartrate or tartaric acid were implanted subcutaneously in ether anesthesia.
^{3}H-nicotine binding sites were studied in brains of offspring by in vitro autoradiography (Odermatt et al. Experientia 43 (87)). Quantitative densitometric measurements were done with a modified Leitz-ASBA image analysis system. A preliminary analysis of adult offspring indicates an increase in the number of nicotinic binding sites in layers III/IV of neocortex, in the laterodorsal thalamic nuclei and a decrease in substantia nigra pars compacta. Additional measurements were also done in caudate putamen and in the lateral geniculate nucleus. So far no significant differences have been observed in these brain regions.

P1 28

COMPARIZON OF SOME PROPERTIES OF HYDROPHILIC AND AMPHIPHILIC CHOLINE ACETYLTRANSFERASE OF THE *TORPEDO*.

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The Triton X-114 phase partition method was used to separate hydrophilic (88%) from amphiphilic (10%) choline acetyltransferase activities present in purely cholinergic synaptosomes of the *Torpedo* electric organ. Partition coefficient (water-detergent) of amphiphilic ChAT solubilized in the Triton X-114 phase was estimated by extracting this phase with one volume of aqueous buffer: a value of 0.27 was found. In a similar way we extracted the aqueous phase containing hydrophilic ChAT with one volume of Triton X-114 and found a partition coefficient of 4.7. Chromatofocussing analysis (pH gradient 8.3-5) of samples containing hydrophilic or amphiphilic ChAT revealed that the two forms of ChAT had very similar isoelectric points of 6.8. The sedimentation coefficients of the two forms of ChAT on sucrose gradients containing 1% Triton X-100 were 5.8S, suggesting that the two forms did not differ significantly in their molecular weight and shape. In the absence of detergent in the gradient, hydrophilic ChAT sedimented at 5.9S and amphiphilic ChAT between 8.9 and 13.1S indicating that aggregates of amphiphilic ChAT were formed.

P1 29

A MONOCLONAL ANTIBODY INHIBITS CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINE RELEASE IN TORPEDO SYNAPTOSOMES.

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A monoclonal antibody raised against cholinergic synaptosomal plasma membranes (SPM) isolated from the *Torpedo* electric organ was able to completely inhibit hydrophilic and amphiphilic choline acetyltransferase (ChAT). This antibody also blocked the Ca^{2+}-dependent release of acetylcholine (ACh) induced in *Torpedo* synaptosomes either by KCl-depolarization or with the Ca^{2+} ionophore A23187; this inhibition was dependent on the antibody concentration and reached 85% at 220 µg antibody/ml. The antibody was probably not inhibiting ACh release by binding to the depolarization-dependent Ca^{2+} channel. Also, it did not appear to affect the release by blocking ACh presynaptic muscarinic receptors. On immunoblots of SPM the antibody labelled two polypeptides of 135 and 66 kDa. The antibody might inhibit ACh release by acting upon SPM-bound ChAT or/and another protein of the SPM.

P1 30

DIFFERENTIAL EFFECT OF NON-NEURONAL CELLS ON THE CHOLINERGIC PROPERTIES OF RAT EMBRYONIC SPINAL CORD AND BRAIN CELL CULTURES

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In *human* spinal cord cell cultures, we have previously shown that an antimitotic agent (ara C) can decrease CAT activity by eliminating the astrocytes; gamma-IFN can restore CAT activity by protecting astrocytes from the action of ara C. In *rat* spinal cord cultures, IFN-γ also increases CAT activity; this effect can be potentiated by tumor necrosis factor which is known to act synergistically with IFN-γ on antiviral activity. However in *rat* brain cell cultures the elimination of non-neuronal cells by antimitotic agents increases CAT activity. EGF, a mitotic agent for astrocytes, increases CAT activity in spinal cord cultures and decreases this activity in striatal and septal cholinergic cells (14th embryonic day). These results suggest a differential effect of non-neuronal cells on cholinergic CNS cells at this age. Non-neuronal cells seem to provide a permissive environment for cholinergic cells of the spinal cord and a suppressive effect on those cells from the brain.

P1 31

THE EFFECT OF INTRASTRIATAL INJECTION OF NICOTINE ON LOCOMOTOR ACTIVITY IN THE RAT

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Systemic injection of nicotine decreases locomotor activity in nicotine-naive (NN) and induces hyperactivity in nicotine-tolerant (NT) rats. The ventral striatum has been implicated in the control of locomotor activity and contains nicotine receptors. Thus, this structure is a likely canditate for nicotine's effect on locomotor activity. To clarify the role of this structure in nicotine-induced changes in locomotor activity we injected nicotine (40nmol/0.25ul) bilaterally into the ventral striatum (bregma +1.7, 1.5L, 6.8 from flat skull) in NN and NT (14 days of daily injection of 0.4mg/kg nicotine) rats. Control rats were injected with physiological saline. Locomotor activity was automatically registered in the hexagonal tunnel maze, a system of dark alleys which untreated rats explore spontaneously. Saline injected controls showed no significant reduction in locomotor activity. Nicotine injected into the ventral striatum reduced the locomotor activity of NN rats (37% of preinjection activity) but had no effect on the activity of NT rats (98% of preinjection activity). Our results suggest that the ventral striatum might be a cns site responsible for the initial suppressant effect of nicotine on locomotor activity in nicotine-naive rats.

P1 32

OXYTOCIN EXCITES NEURONES LOCATED IN THE GUINEA-PIG VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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The area of the ventromedial nucleus of the hypothalamus (HVM) in the guinea-pig contains high affinity binding sites for oxytocin (OT). In order to ascertain whether HVM neurones are sensitive to OT, single-cell recordings were obtained from 33 HVM neurones in coronal slices of the hypothalamus of adult guinea-pigs. Fifteen neurones were excited by oxytocin applied in the nanomolar range. The response to the peptide was reversible and concentration-dependent; it was exerted directly since it persisted under the condition of synaptic isolation; moreover, it was specific, since it could be mimicked by [Thr^4,Gly^7]OT, a selective oxytocin agonist, and since vasopressin had an effect which was at least 10 fold weaker than OT. These findings suggest that the binding sites for OT detected in guinea-pig HVM represent functional neuronal receptors.

P1 33

AUTORADIOGRAPHIC LOCALIZATION OF VASOPRESSIN (AVP) BINDING SITES IN THE DEVELOPING RAT BRAIN

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The development of AVP binding sites in the rat brain and spinal cord was studied using [^{3}H]AVP in tissue sections from rats aged between embryonic day 12 (E12) and postnatal day 90 (PN90). Specificity of binding was assessed by incubating adjacent sections in presence of unlabelled AVP. Specific binding was first detected at E16 in the brainstem. Many other regions were progressively labelled thereafter until PN3, time at which the distribution of labelling was typical of the "infant pattern". The changeover to the "adult pattern" started at PN13 and took the form of a reduction or even disappearence (in the lateral reticular nucleus) of binding sites. The "adult pattern" was established by day PN21, which corresponds roughly to the period of weaning.

P1 34

LOCALIZATION OF BINDING SITES FOR VASOPRESSIN (AVP) IN THE BRAIN OF THE GOLDEN HAMSTER

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Sites which bind [^{3}H]AVP with high affinity were detected in the brain of adult Golden hamsters by in vitro light microscopic autoradiography. Labelling was optimized by using only 1.5 nM [^{3}H]AVP and 5 nM oxytocin agonist, to prevent binding of radioligand to oxytocin sites. Adjacent sections were incubated with an excess of unlabelled AVP. Specific binding sites were evidenced in the lateral septum, bed nucleus of the stria terminalis, median preoptic nucleus, suprachiasmatic nucleus, anteroventral thalamic nucleus, vestibular nuclei, nucleus of the solitary tract. Other areas showed also binding, though weaker : the cingulate cortex, subfornical organ, medial preoptic area, central amygdala, perihypoglossal nucleus, subiculum, area postrema, central gray. These finding support the view that vasopressin may may play a role in the expression of complex behaviour in hamsters (Ferris et al., Science 224 : 521-22, 1984).

P1 35

NEURONES IN THE NUCLEUS OF THE SOLITARY TRACT ARE EXCITED BY VASOPRESSIN THROUGH V_1-TYPE RECEPTORS

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Single-unit extracellular recordings were obtained from 121 neurones located in the regions of the nucleus of the solitary tract (nts) in rat brainstem slices. Fifty-two responded to vasopressin, at 5 to 2000 nM, by a reversible, concentration-dependent increase in firing rate. The effect of vasopressin was direct, since it persisted under the condition of synaptic uncoupling. The action of vasopressin was suppressed by the antagonist $dEt_2Tyr(Me)DAVP$ and mimicked by [Phe^2,Orn^8]VT, a vasopressor agonist. The antidiuretic agonist, dDAVP, had no effect. Oxytocin was 10-100 times less efficient than vasopressin and HO[Thr^4,Gly^7]OT, a selective oxytocic agonist, was without effect. We conclude that vasopressin, by acting through V_1-type receptors, can modulate the bioelectrical activity of a subclass of nts neurones.

P1 36

LC-EC MEASUREMENT OF PROSTANOIDS IN NERVOUS TISSUE

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We have developed a new method for the determination of prostaglandins (PG's), after derivatization with 4-Nitro-phenacyl bromid according to the procedure of Morozowich and Douglas (*Prostaglandins* 10, 19, 1975). Esterified PG's are then separated on RP-18 column and eluted with a mobile phase (30 % methanol, 20 % isopropanol, 0.1 mM acetate buffer (pH 5) containing 0.1 mM EDTA) maintained at 55°C in order to eliminate oxygen. The detection is performed with an amperometric detector, a negative potential of -0.7 V being applied to the cell. The reduction current is proportional to the amount of injected PG's (1-100 ng). Preliminary results indicate a good separation (reported to be particularly difficult by several authors) of PGE_2 and $PGF_{2\alpha}$, which were previously shown to potentiate the VIP-induced accumulation of cAMP increase in mice cortical slices (Schaad *et al.*, *Nature* **328**, 637, 1987).

P1 37

MECHANISMS OF VIP (VASOACTIVE INTESTINAL PEPTIDE) RELEASE EVOKED BY 4-AMINOPYRIDINE AND K^+ IN MOUSE CEREBRAL CORTEX

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The mechanisms involved in VIP release were investigated in mouse cerebral cortical slices. 4-aminopyridine (4-AP) stimulated in a concentration-dependent manner VIP release (VR) with a significant effect already observed at 50 μM; at 1 mM 4-AP the increase was 562 % over basal VR. 4-AP evoked VR (4-APVR) was completely abolished by Co^{2+} and partially inhibited (73 %) by TTX 2 μM. Mepacrine, an inhibitor of phospholipase A_2 (PLA_2), antagonized 4-APVR with an IC_{50} of 20 μM whereas melittin, a PLA_2 activator, stimulated VR. Nordihydroguaiaretic acid (NDGA), ETYA and caffeic acid, three lipoxygenase inhibitors, antagonized 4-APVR with IC_{50} of 50 μM, 45 μM and 1.5 mM respectively. VR was stimulated by K^+ with a significant effect already observed at 15 mM; at 25 mM the increase was 678 % over basal VR. K^+-evoked VR was unaffected by TTX 2 μM but inhibited by Ni^{2+}, Co^{2+} and Mn^{2+}, three Ca^{2+}-channel blockers, with IC_{50} of 0.5 mM, 0.25 mM and 0.15 mM respectively. Diltiazem 20 μM, nifedipine 10 μM, ω-conotoxin 10 μM and Cd^+ 100 μM did not inhibit K^+-evoked VIP release.

P1 38

HOT SPOTS IN THE BRAIN - ^{18}F-DOPA, ^{18}F-DOPAMINE OR METABOLITES ?

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The metabolism of L-DOPA, precursor of the presynaptic dopaminergic system, whose fluorinated derivatives are already used in Positron-Emission-Tomography (PET), is investigated by means of reaggregating brain cell cultures. Uptake, decarboxylation, methylation and oxidation of L-DOPA in cerebral tissue are measured, circumventing the blood-brain-barrier and without interference of peripheral metabolism. The detected amounts of metabolites were similar to those in tissues. PET requires tracer compounds like 18F-DOPA and its metabolites that accumulate in a defined way in the tissue and reflect biochemical processes in vivo. This work is necessary to develop a model correcting for undesired background radiation from metabolized tracer compounds.

P1 39

THE NEUROTOXIC EFFECTS OF P-CHLOROAMPHETAMINE (PCA) IN THE RAT BRAIN ARE BLOCKED BY SEROTONIN DEPLETION.

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Systemic administration of PCA causes acute release of serotonin (5-HT) and long-lasting degeneration of 5-HT axons in rat brain. However, direct intracerebral injection of PCA does not have toxic effects on 5-HT axons suggesting that a neurotoxic compound is generated in the periphery following systemic administration. To determine whether the toxicity of PCA is influenced by depletion of endogenous 5-HT, PCA was systemically administered to rats pretreated with the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) and/or with the vesicular storage blocker reserpine. Rats treated with PCA alone, with PCPA plus PCA or with reserpine plus PCA exhibited a profound loss of 5-HT axon terminals in cerebral cortex and marked reductions in brain 5-HT levels 2 weeks after drug administration. However, depletion of 5-HT by combined treatment with PCPA and reserpine provided substantial protection against the neurotoxic effects of PCA. This result indicates that the neurotoxicity of PCA depends on the presence of endogenous 5-HT. Treatment with either drug alone produces profound reductions in brain levels of 5-HT, but combined treatment is required to eliminate most sources of peripheral 5-HT. We therefore postulate that a neurotoxic metabolite is formed from 5-HT released in the periphery by the action of PCA on 5-HT storage sites. This mechanism may also account for the similar pattern of neurotoxicity seen with other psychotropic amphetamines such as 3,4-methylenedioxymethamphetamine.

P1 40

EFFECTS OF MEDIUM LEAD EXPOSURE ON THE CENTRAL SEROTONERGIC SYSTEM IN THE RAT

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We have investigated the effects of pre- and postnatal medium lead exposure (Widmer et al.(1987) Experientia 43,653) on the development of serotonergic systems in the striatum and brainstem of the rat. Time-pregnant Long Evans rats were given lead acetate in the drinking water (0,25%) during pregnancy and lactation (maternal blood lead level: 80ug/dl). Serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined using a sensitive modification of HPLC-EC which requires minimal sample pretreatment.

A significant decrease of 5-HT was detected in brainstem at postnatal day 28. At both days 6 and 28, 5-HIAA was found to be decreased in the striatum and brainstem.

Our observations lend further support to the idea of a possible alteration of developing indoleaminergic system by lead exposure.

P1 41

COMPETITION FOR SUCROSE-PELLETS IN TRIADS OF WISTAR RATS: BEHAVIOURAL DISINHIBITION AFTER SUBCHRONIC MDA.

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While daily competing for a limited number of sucrose-pellets within triads of male Wistar rats a high-, a medium- and a poor-performing (P) rat can be discerned (1). The typical, almost complete abstention from competition of P-rats, can temporarily be overcome by attenuating their 5HT-transmission (2). We here report on the effect of (±) methylenedioxyamphetamine (MDA) in P-rats. MDA represents a 'designer drug' which is being increasingly abused. Neurochemically, MDA exerts neurotoxic effects on serotoninergic neurons. After subchronic treatment (6 x 15 mg/kg (±) MDA (s.c.) at 12 hour intervals) a high mortality-rate became apparent. However, in all those P-rats, surviving drug administrations (3 out of 7), a marked behavioural disinhibition was observed: The competition-rate (pre-drug: < 2)increased to maximally 29 (on post-drug day 16). Such observations would indicate once again, that the P-rats' typical abstention from competition (=behavioural inhibition) can temporarily be overcome by attenuating their 5HT-transmission.

(1) Gentsch et al.Behav.Brain Res.27:37 (1988)
(2) Gentsch et al.Prog.Neuro-Psycho.&Biol.Psych.12:639 (1988)

P1 42

CGP 37849 / CGP 39551: NOVEL COMPETITIVE NMDA RECEPTOR ANTAGONISTS WITH ORAL ANTICONVULSANT ACTIVITY

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Much evidence indicates that competitive NMDA receptor antagonists may form a novel therapy for seizure disorders; the aim of this study was to develop antagonists with sufficient potency and oral bioavailability to test this hypothesis. To this end, we examined a series of analogues of the phosphono-amino acid, AP5, for their ability to inhibit L-3H-glutamate binding to postsynaptic densities from rat brain. CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono -3-pentenoic acid) inhibited L-3H-glutamate binding with Ki 0.22 uM, compared with Ki's of 1.5 uM and 0.82 uM for the known antagonists, DL-AP5 and CPP. CGP 37849 showed high potency in a 3H-CPP binding assay (Ki 35 nM), and was weak or inactive at 17 other receptors, including the quisqualate and kainate types of glutamate receptor. Both CGP 37849 and its ethyl ester, CGP 39551, suppressed epileptiform activity in hippocampal neurons *in vitro* and inhibited electroshock-induced seizures when given to rodents by the oral route (see Schmutz et al.); however, CGP 39551 was weak *in vitro*, suggesting that it functions as a precursor of CGP 37849 *in vivo*. CGP 37849 and CGP 39551 are the most potent and bioavailable competitive NMDA receptor antagonists described to date.

P1 43

CGP 37849 / CGP 39551: COMPETITIVE NMDA RECEPTOR ANTAGONISTS WITH POTENT ORAL ANTICONVULSANT ACTIVITY

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NMDA receptor mechanisms have been shown to be involved in burst firing and the generation of seizure activity *in vitro* and *in vivo*. Here, we describe the anticonvulsant properties of two novel competitive NMDA receptor antagonists, CGP 37849 and CGP 39551 (see Fagg et al.), in rats and mice. In both species, CGP 37849 suppressed electroshock-induced seizures with ED-50's of 0.4-1.9 mg/kg after i.v. and i.p injection, and 8-22 mg/kg after oral administration. Relative to CGP 37849, CGP 39551 was more potent after p.o. (ED-50 4-8 mg/kg), and less potent after i.v. or i.p treatment (ED-50 3-6 mg/kg). Following oral treatment, the duration of action of CGP 37849 was about 8 hours, while CGP 39551 still showed good activity after 24 h (ED-50 9-21 mg/kg). Both compounds were anticonvulsant at doses below those at which overt side-effects were apparent. CGP 39551 (but not CGP 37849) delayed the development of kindled seizures in rats at doses of 10 mg/kg p.o. and above, and showed weak anticonvulsant activity against pentylenetetrazol-evoked seizures. Orally-effective doses of both compounds selectively antagonized NMDA-, but not quisqualate- or kainate-evoked neuronal firing in the rat hippocampus *in vivo*.

P1 44

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE SELECTIVE, ORALLY ACTIVE NMDA ANTAGONIST CGP 37 849.

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In male rats anaesthetized with urethane, orally administered CGP 37 849 (30-100 mg/kg) selectively reduced excitations of hippocampal pyramidal neurons (CA1) induced by ionophoretically administered NMDA. The kainate and quisqualate responses were not affected.
In the hippocampal slice preparation CGP 37 849 (1 µM) applied locally to the apical dendrites of CA1 pyramidal cells reversibly blocked the induction of long-term potentiation (LTP) similar to APV (50 µM).
In a hemisected spinal cord preparation of newborn rats NMDA induced a depolarisation of the dorsal- and ventral-root DC-potentials and an increase of the spontaneous activity. CGP 37 849 blocked both effects reversibly. CGP 37 849 itself slightly reduced the spontaneous activity.

In conclusion CGP 37 849 is a potent, orally active and selective new NMDA antagonist.

P1 45

DIFFERENTIAL CONTRIBUTIONS OF GLUTAMATE RECEPTOR SUBTYPES TO LONG-TERM POTENTIATION IN HIPPOCAMPUS.

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High frequency stimulation of a group of afferents in hippocampus results in an increase in synaptic efficacy referred to as long-term potentiation (LTP) and implicated as a possible substrate for learning and memory. The mechanisms responsible for this form of plasticity are still unknown. In an effort to restrict the number of possibilities, we have analysed the contribution of the two classes of glutamate receptors that mediate transmission in area CA1 of hippocampal slices. Application of DNQX, a quisqualate receptor antagonist, blocked 85% of the field potential to single pulse stimulation, leaving a small response that was sensitive to D-AP5, an NMDA receptor blocker. Comparison of this residual D-AP5 sensitive response on control and previously potentiated inputs showed no difference. High frequency stimulation in presence of DNQX did not result in the development of robust LTP, although frequency facilitation was considerably enhanced. Washout of the drug revealed then the potentiation effect. It appears that activation of NMDA but not quisqualate receptor is necessary for LTP induction, whereas, conversely, only quisqualate but not NMDA receptor mediated responses express the potentiation effect. (Work supported by FNRS 83.392.0.86 and 3.173.0.88)

P1 46

MODULATION OF ENDOGENOUS GLUTAMATE RELEASE FROM RAT BRAIN SYNAPTOSOMES BY PUTATIVE PRESYNAPTIC RECEPTORS

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We have used a purified synaptosomal preparation for measuring the release of the putative neurotransmitter L-glutamic acid. A bioluminescence assay was used for determining the concentrations of the amino acid in the medium collected at the end of the incubation period. High concentrations of K^+ stimulated the release of endogenous glutamate in a partially Ca^{2+}-dependent fashion. (L)-2-amino-4-phosphono-butyric acid (L-AP4) inhibited the Ca-dependent release of L-glutamate in a dose - dependent manner, without affecting basal or Ca - independent release. This effect of AP4 was found in hippocampal and striatal, but not in cortical synaptosomes, suggesting a possible action of the compound through presynaptic receptors not uniformly distributed throughout the brain. The pharmacological characteristics of these putative presynaptic receptors suggest that they do not belong to the NMDA or kainate types of excitatory amino acid receptors.

P1 47

$GABA_B$ RECEPTORS IN NORMAL AND EPILEPTIC HIPPOCAMPAL SLICES. (Göril Karlsson and Hans-Rudolf Olpe, Res.Dept, Pharm.Div., CIBA-GEIGY Ltd, BASEL)

In several animal models both pro- and anticonvulsant actions of the $GABA_B$ agonist baclofen have been reported. We have investigated the role of $GABA_B$ receptors in rat hippocampal slices using baclofen and the $GABA_B$ blocker phaclofen. Normal slices: Baclofen attenuated paired-pulse inhibition evoked in the CA1 region. This effect was antagonized by phaclofen. Phaclofen had no effect on the amplitudes of evoked population spikes and had only a weak disinhibitory effect on paired-pulse inhibition. This effect was observed only when the stimuli were delivered at long interpulse intervals (50 - 150 ms). Epileptic slices: Baclofen potently inhibited penicillin-induced epileptic-like activity in the CA3 region. This effect was moderately reduced by phaclofen. The frequency and amplitudes of bicuculline- and penicillin-induced multiple population spikes in the CA1 region were not affected by phaclofen. In conclusion, whereas the findings with baclofen are contradictory, the data suggests that the $GABA_B$ blocker phaclofen has no proconvulsant activity in the hippocampal slice.

P1 48

APOMORPHINE'S EFFECT ON GABACULINE-INDUCED GABA ACCUMULATION REFLECTS SYNTHESIS INHIBITION.

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Study of the effect of apomorphine on GABA turnover showed a marked decrease in the gabaculine-induced accumulation of GABA in the mouse cortex. To check whether this was due to an effect on the rate of GABA synthesis or to a modification of the inhibitory effect of gabaculine on GABA-T, the action of apomorphine on GABA turnover and GABA-T activity was studied concomitantly.
In vitro, apomorphine did not alter the concentration-dependent inhibition of GABA-T by gabaculine. An ex vivo study showed that the simultaneous administration of apomorphine and gabaculine resulted in a 68.5 % inhibition of GABA turnover, and in a slight but significant increase in GABA-T activity compared to the gabaculine group. Gabaculine alone caused a dose-dependent increase in GABA levels which was linearly correlated with the inhibition of GABA-T activity. This linear regression demonstrated that the apomorphine-induced change in GABA-T activity was only marginal. Thus, the effect of apomorphine on the gabaculine-induced increase in GABA levels is primarily due to an action on the rate of GABA synthesis.

P1 49

EFFECT OF MIDAZOLAM(M) ON HUMAN VISUAL RECEPTIVE FIELDS(VRF)

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Psychophysical experiments allow correlations between single cell recordings in cats and human VRF. In the cat, bicuculline decreases the activity of the inhibitory surround of Y-on-centre cells. Subthreshold summation was used to determine center and inhibitory surround of human VRF. Stimuli were three vertical parallel lines subtending a visual angle of 1.5°displayed for 60 ms on a CRT. The contrast of the flanking lines was 3/8 of the contrast of the central line. Criterion was the perception of the central line. Contrast threshold was measured dependent on the distance between the central and the flanking lines. The sensitivity function obtained is analogous to one obtained with single cell recordings from retinal ganglion cells. In two independent experiments with 6 subjects (S) each, two h after M (7.5.mg p.o.) the strongest increase of contrast threshold was found in the region of lowest sensitivity (0.24° of visual angle, ANOVA, $p<0.02$). After six h baseline values were obtained. In dark-adapted S no inhibitory surround was found neither at baseline nor after M. This suggests, that GABA may be involved in the regulation of human VRF. In dark-adapted S no GABA may be present and bdz therefore have no effect.

P1 50

NEURONS IMMUNOREACTIVE FOR GLUTAMATE DECARBOXYLASE AND PARVALBUMIN ARE RESISTANT TO THE ISCHEMIA-INDUCED DELAYED NEURONAL DEATH IN GERBIL HIPPOCAMPUS

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A bilateral transient 5 min occlusion of common carotid arteries in gerbils results after a delay of 2 to 3 days in a loss of pyramidal cells in the hippocampal field CA1. This delayed degeneration is thought to be brought about by glutamate-triggered Ca^{2+} intoxication. Using an antibody directed against the GABA-synthetizing enzyme glutamate decarboxylase, GAD, and the Ca^{2+}-binding protein parvalbumin, the fate of the parvalbumin containing GABAergic innervation was studied immunocytochemically during the development of delayed neuronal death and for further 30 days. In CA1 local GABAergic neurons and their innervation showed no change at the light and electron microscopic level over the whole period investigated. Also the pattern of parvalbumin immunoreactivity persisted. Thus, it seems possible that the Ca^{2+}-binding properties of the hippocampal GABAergic neurons protect them from the effects of ischemia.

P1 51

ADENOSINE AND CYCLOHEXYLADENOSINE INHIBIT THE CAT'S OPTIC NERVE ACTION POTENTIAL

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Effects of intraarterially applied adenosine and cyclohexyladenosine (a selective A_1 agonist) were studied in isolated, perfused cat eyes. The electroretinogram (ERG) and the light-evoked action potential of the optic nerve (ONR) were recorded after intraarterial injection of adenosine (0.1-20 µmolar) or CHA (0.6-8 µmolar) under selective rod- or cone stimulation.
Both drugs caused depression of all components of the ONR in a dose-dependent and reversible manner. We also observed enhancement of the rod b-wave of the ERG. A concomittant increase in perfusion flow rate reflected vasodilation, that would cause increase rather than decrease of the ONR. The increase in rod b-wave amplitude exceeded what could be attributed to the increase in flow and might reflect a glial response to adenosine and CHA.
We conclude, that adenosine modulates retinal function mainly as inhibition of the optic nerve action potential under both, rod- and cone-stimulation conditions.

P1 52

CENTRAL NORADRENERGIC PATHWAYS IN THE RAT ARE AFFECTED BY INTERLEUKIN-1

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The functional and metabolic activity of monoaminergic pathways in the CNS changes during the immune response. A possible mediator of these changes is interleukin-1 (IL-1), a cytokine which plays an important role in immune processes. We have shown that the i.p.injection of IL-1 is followed by a significant increase in 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG), the main metabolite of norepinephrine (NE). In order to confirm the influence of IL-1 on the NE metabolism we determined the NE turnover rate (blockade of the tyrosine hydroxylase with α-methyl-*para*-tyrosine) in different microdissected hypothalamic and brain stem regions in rats injected with IL-1 or control medium. The levels of NE, MHPG, the amino acids tyrosine and tryptophan, and several other compounds were determined by HPLC. In the IL-1 group which showed marked increase in plasma corticosterone levels there was, apart from changes in the amino acids, a significant enhancement of the MHPG level, and of the NE turnover rate.
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P1 53

MODULATION OF CATECHOLAMINE AND SEROTONIN METABOLISM IN SYNAPTOSOMES BY A NEUROREGULATORY FACTOR FROM BRAIN.

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Neuroregulatory factor purified from mammalian brain (tentatively called neurocatin) modulated the metabolism of catecholamines and serotonin in synaptosomes isolated from rat brain. Neurocatin caused inhibition of oxidative deamination of dopamine, decreased formation of 3,4-dihydroxyphenylacetic acid, increased formation of norepinephrine and its N-methyl derivatives and increased release of catecholamines. It also inhibited oxidative deamination of serotonin, decreased formation of 5-hydroxyindole acetic acid and increased formation of 5-hydroxytryptophol. Neurocatin appears to act, at least in part, by increasing the calcium permeability of the plasma membranes of the synaptosomes. Omission of calcium from the suspending medium completely blocked the neurocatin induced release of catecholamines and decreased by about 80% the effects on catecholamine and serotonin metabolism. These results are consistent with it activating receptor modulated calcium selective ion channels. Neurocatin has been purified to near chromatographic homogeneity. It is sensitive to trypsin and chymotrypsin and has an apparent molecular weight between 1,500 and 2,500 daltons. Analysis of its chemical structure is in progress. Supported by GM-21524.

P1 54

CHRONIC I.C.V. CRF ADMINISTRATION STOPS THE DEVELOPMENT OF OBESITY IN fa/fa RATS.

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Adrenalectomy prevents the development of obesity in fa/fa rats. This has been attributed to CRF effects on the autonomic nervous system. A chronic (7 days) i.c.v. oCRF administration was performed in genetically obese fa/fa rats. While vehicle-treated obese rats gained 30 g during the 7 days, the CRF-treated ones stopped their body weight gain, despite the pair-feeding of the two groups. Although no significant changes were observed in plasma ACTH and corticosterone levels, basal insulinemia was halved in the CRF-treated rats compared to the controls. CRF treatment decreased liver glycogen content and epididymal fat pad weight, but increased interscapular brown adipose tissue weight.
Thus, i.c.v. CRF in fa/fa rats prevents body weight gain, an effect that might be related to alterations in the activity of the autonomic nervous system.

P1 55

FUNCTIONAL CHARACTERIZATION OF ASTROCYTES IN RAT OPTIC NERVE MINISEGMENTS.

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To study functional and developmental aspects of CNS glia in vitro, we cultured small explants of newborn rat optic nerve called minisegments.
Intracellular recordings were performed with glass microelectrodes filled with 2 M potassium acetate in minisegments of 40-60 days mounted in a conventional recording chamber superfused with prewarmed Krebs solution. The mean membrane potential recorded from 71 astrocytes was -74.8 mV, and the mean input resistance and time constant were 10.9 Mohms and 0.36 ms (N=22), respectively. When $[K+]_o$ was increased up to 100 mM and the observed changes in membrane potentials were plotted against the logarithm of $[K+]_o$, a linear Nernstian relationship was obtained. Experimental data diverged from Nernstian values at $[K+]_o$ lower than 4.7 mM indicating a slight permeability to ion(s) other than K+.
These data together with morphological and immunocytochemical properties correspond to those of in situ astrocytes.

P1 56

PLASTICITY OF OPTIC NERVE GLIA IN VITRO: II FUNCTIONAL ASPECTS.

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In situ glia of optic nerves is composed of microglia, oligodendrocytes and astrocytes; these cells are in close contact with axons, which in a first step are fasciculated by astrocytic processes and then myelinated by cell processes of oligodendrocytes. To study functional aspects of glia in vitro we cultured small pieces of optic nerves of newborn rats, called minisegments, as explants in which the axons of the ganglion cells degenerate. After various time-points, minisegments were removed for morphological, immunocytochemical and electrophysiological investigations. At 3d in vitro oligodendrocytes, form loose and compact myelin around cell processes, cell bodies or empty cavities. The myelin shows the typical fine structure, and immunocytochemical experiment confirmed the presence of myelin specific constituents. During the main period of myelin formation in vitro, the resting membrane potential of individual astrocytes was determined. The mean value of -75mV (N 72) corresponds to that of astrocytes in situ. We conclude that the in vitro glia cells show functional properties, ascribed to oligodendrocytes and astrocytes in vivo. (Supported by NSF 3.652-0.87 and MS society).

P1 57

HIGH AMOUNTS OF CREATINE KINASE IN BERGMANN ASTROCYTES AND BLOOD-BRAIN BARRIER CELLS OF THE VASCULAR SYSTEM OF THE CEREBELLUM:Immunolocalization of B-CK and mitochondrial CK.

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Brain-type B-CK and mitochondrial Mi-CK were identified by native electrophoresis and immunoblotting of extracts from several parts of chicken brain. Both CK isoenzymes were present at relatively high levels in the hemispheres,optical lobes, brain stem and the cerebellum. In the latter indirect immunoflourescence localization with anti-B-CK AB revealed pronounced staining of Bergmann astrocyte cell-bodies, and their processes in the molecular layer of the cerebellum and particularly at the membrana limitans gliae. Strong staining of blood-brain barrier cells (glia cells and possibly also endothelial cells) of the vascular system in the cerebellum was found by both anti-B- and anti-Mi-CK AB's. The staining pattern of capillaries obtained by anti-Mi-CK AB was strikingly similar to that observed with anti-GFA AB. The surprisingly high energy status of glia cells, and cells involved in the blood-brain barrier will be discussed with respect to their function in the context of the postulated phospho-creatine circuit model.

P1 58

INDUCTION OF GLIA-DERIVED NEXIN AFTER THE LESION OF THE SCIATIC NERVE

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Glia-derived Nexin (GDN) is a serine protease inhibitor produced by glial cells and by fibroblasts. In vitro studies showed a neurite promoting activity of GDN demonstrating sensitivity of growth cones towards proteases. GDN is constitutively expressed in the olfactory system where neurogenesis and axonal regeneration permanently occurs. But in a peripheral nerve GDN-RNA is down regulated and is dramatically induced after a lesion. Parallel to the increase of GDN-RNA the amount of extractable gene product increases as well. Immunohistochemical studies show the presence of infiltrating macrophages during the period of maximal GDN transcription. Nerve explant cultures are used for the identification of the inducer molecule(s) for the GDN gene. As the regenerating axons reach the distal nerve stump during biosynthesis of GDN this protein might act as a permissive factor for neurite promotion in vivo.

P1 59

SINGLE-CHANNEL RECORDING OF A POTASSIUM CURRENT ACTIVATED BY INTRACELLULAR SODIUM IN SENSORY NEURONS

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Whole-cell recording suggested that a K current activated by intracellular Na (K_{Na}) exists in neurons. Here we describe the single-channel activity induced by Na applied to the intracellular face of inside-out patches excised from quail trigeminal neurons. Ion substitution experiments indicated that K was the major charge carrier of this current. In the presence of Na, channels of high conductance were seen (170 pS, with K_{in}=50 mM and K_{out}=150 mM), which showed little voltage dependence between -75 and +50 mV. Channel activity could be evoked even when the Na concentration was as low as 12 mM. Openings occurred in bursts and the burst frequency increased with the Na concentration. Ca, Li and choline did not induce K_{Na} single-channel activity. Whole-cell recording in neurons suggested that K_{Na} could be activated by a single action potential. K_{Na} could also contribute to the resting potential of neurons since single-channel recording shows that this current can be activated already at low sodium concentrations.

P1 60

A NEW ANTIBODY ELUTION TECHNIQUE FOR THE SUCCESSIVE LOCALIZATION OF 2 ANTIGENS BY IMMUNOCYTOCHEMISTRY (ICC).

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Selected areas of formalin fixed mammalian and human brains were cut on cryostat(8-12µm).Sections mounted on chrom-alun gelatinized slides were first pretreated with KMnO4 0.25% in PBS 0.1M pH7.6,max.6min. After washing 1x5min with PBS (W), they were treated with the Pal's modified solution (oxalic acid 1g% and K-disulfite 1g% in PBS) 2min.Rat antiserum to LHRH or to DSIP were employed as the first antiserum (1:1000, 1hr)W 5x,2nd incubation with IgG-FITC(1:800,1hr).W 5x. After photography, antibodies were eluted by immersion in KMnO4 0.25% in PBS,10-12min;W 1x;followed by immersion in Pal's solution, 2-3min.W 5x. At this step no fluorescence was detectable.Then the ICC technique for the other antigen was repeated and photographied again.Controls included replacement of each of the first antisera with nonimmune rat sera, omission of the first antisera,test with other antisera,2nd incubation with IgG-FITC alone in the second stage procedure. This method is simple, efficient and preserves the antigenicities. The immunoreactivities can be observed in cell bodies as well as in nerve fibers.

P1 61

G-PROTEIN REGULATION OF PGE_2 BINDING IN CHICK SPINAL CORD

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Spinal cord (SC) synthesizes PGE_2. Regulation of PGE_2 binding was studied in membrane preparations of chick SC. After 2 differential centrifugations, 75% of the specific binding sites for ^3H-PGE_2 were recovered in a 17,000 g pellet. The binding sites were highly specific for PGE_2. Scatchard analyses demonstrated 2 classes of binding sites,the first one with a high affinity (Kd_1 1.3 nM) but a low capacity and the second one with a low affinity (Kd_2 2.2 µM) but a higher capacity. The evidence that the high affinity binding sites are regulated by a G-protein is based on the following arguments: 1) the affinity for PGE_2 was much more reduced by GTP or its analog Gpp(NH)p than by ATP; 2) the dissociation kinetics was accelerated by Gpp(NH)p; 3) a desensitization observed after preincubation with PGE_2 was reversed by Gpp(NH)p. In conclusion high affinity binding sites for PGE_2 are regulated by a G-protein in the chick SC. S.N.F. 3.397.86.

P1 62

SELECTIVE LOCALIZATION OF PROSTAGLANDIN D_2 ISOMERASE IN CHICK DORSAL ROOT GANGLIA (DRG). A LIGHT AND ELECTRON MICROSCOPIC STUDY.

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DRG synthesizes actively prostaglandin D_2 (PGD_2). The origin of PGD_2 biosynthesis was investigated by immunocytochemistry with polyclonal antibodies raised to rat brain PGD_2 isomerase. In the light microscope, specific immunostaining was detected in most of the small B ganglion cells (60%) and their emerging axons, and in about 2% of the large A neurons. In the perikaryon, the immunoprecipitates were either homogenously distributed or aggregated in clumps. Small ganglion cells which displayed clumps of immunoprecipitates were examined in the electron microscope; they possessed the ultrastructural characteristics of the B1 subclass of sensory neurons (SNF N° 3397.86).

Molecular genetics + cell and molecular biology (P2)

P2 63

TWIN HOMEOBOX GENES AT THE *DACHSOUS* LOCUS OF *DROSOPHILA*

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Two closely linked homeobox genes, PPH13 and PPH25, have been isolated in *Drosophila* with a homeobox probe derived from the segmentation gene *paired*. Both genes map cytologically to the 21C8-D1 region correlating genetically with the *dachsous* (*ds*) locus. The *ds* gene belongs to a group of "leg-genes" (*d, fj, cg*) showing similar mutant phenotypes as, for example, shortened legs, close crossveins, or tarsal segmentation distortions. Combinations of such "leg" mutations show impressive qualitative changes in histogenesis resulting, for example, in twinning of legs and antennae. Breakpoint analysis of *ds* chromosomal rearrangements demonstrates that the two genes, PPH13 and PPH25, represent *ds*. In addition, we present genetic results providing further insight into the function of *ds*. Of particular interest in this context is the mutant, $net^{87h1(48)}$, isolated by one of us (M.M.G.). This mutation affects the expression of a number of genes located between 21B and 22E,F. Our simplest current hypothesis, consistent with our results, is that this mutation is a cytologically invisible deficiency or insertion inactivating both *ds* genes, PPH13 and PPH25.

P2 64

DETECTION AND HETEROLOGOUS EXPRESSION OF A PROTEASE OF HEPATITIS A VIRUS

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From molecular data and analogy of hepatitis A virus (HAV) to other picornaviruses it was hypothesized that the HAV genome codes for a protease 3Cpro. The protease was predicted to be contained in the P3 region of the typical picornavirus polyprotein. We have subcloned the cDNA of this region into a vaccinia virus/T7-hybrid expression system. Expression of the protease was detected by antibodies to a synthetic peptide that corresponds to the carboxyterminus of 3Cpro. The analysis revealed that 3Cpro was not only expressed but also cleaved its own precursor P3 to the final products predicted from the structure of the HAV genome in this region. Furthermore, a peptide corresponding to 3Cpro was detected in cells infected with HAV. We conclude, that the HAV genome codes for a protease 3Cpro and that the heterologously expressed enzyme faithfully processes the peptide precursor.

P2 65

DNA SEQUENCE OF THE PSEUDORABIES VIRUS IMMEDIATE EARLY GENE, ENCODING A STRONG TRANS-ACTIVATOR PROTEIN

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We report the complete DNA sequence of the pseudorabies virus (PRV) immediate early (IE) gene and its flanking nucleotide sequences, together comprising 5091 base pairs. An open reading frame starts with an ATG codon in position 263 from the transcription initiation site and ends with a TGA codon in position 4601, thus encoding a predicted protein of 1446 amino acids (150 kDa). The PRV IE protein exhibits significant homology with the functionally related trans-activator proteins, ICP4 of herpes simplex virus 1 (HSV1), and p140 of varicella zoster virus (VZV). The extent of homology varies widely along the three sequences: two regions of the PRV IE protein extending from amino acids 482 to 659 and 959 to 1350 exhibit 50-60 % identity with the cognate sequences, whereas the remaining sequence reveals little homology apart from a common polyserine stretch. The base composition of the PRV IE coding region is 80% G+C, compared with 81.5% for HSV1 and 64.1% for VZV. Yet the PRV IE protein appears to be as closely related to VZV p140 as to HSV1 ICP4. The regions of strong homology are also apparent in plots predicting secondary structure.

P2 66

STRUCTURAL ANALYSIS OF THE TERMINAL PALINDROMS OF THE HUMAN PARVOVIRUS B19 GENOME

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The human parvovirus B19 has a 5.4kb single-stranded DNA genome with terminal palindromic sequences which fold over to give typical hairpin structures. We have established a full length clone in E.coli JC8111 (recBCsbcBrecF). To determine whether the hairpins are identical or, alternatively, represent unique sequences we have analyzed the primary structure of both ends by restriction enzyme analysis and sequencing. This also allows a prediction of the secondary structure of the hairpins which are important for self-priming during parvoviral DNA replication and for packaging of the genomic DNA into virus capsids. Our results suggest that B19, in contrast to all autonomous parvoviruses analyzed so far have identical termini as has been found for another parvovirus isolated from humans, the adeno-associated virus (AAV). However, the secondary structure and the size of the hairpins of these two viruses seem to differ considerably.

P2 67

A RETROTRANSPOSON-LIKE ELEMENT INTERRUPTS THE TUBULIN GENE CLUSTER IN TRYPANOSOMA BRUCEI

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The tubulin genes of T.brucei are located in a single cluster in which genes coding for alpha- and beta-tubulin alternate in a regular manner. Each of the two genes is present in 10 nearly identical copies. Both upstream and downstream of the 40kb cluster 1.7kb of DNA have been sequenced. The cluster starts with an intact and therefore probably functinal beta gene: some promotor-like elements could be seen. Downstream the cluster ends in the middle of a beta gene. The sequence outside of this pseudogene shows a high degree of similarity to the retrotransposon-like element TRS/ingi found previously elsewhere in the genome. TRS/ingi has LTRs and may code for reverse transcriptase. Here we have shown for the first time that such an element interrupts a protein-coding gene in this parasite. The invasion of the cluster cannot have been very recent as judged by comparing the tubulin cluster of different species.

P2 68

THE ARGININOSUCCINATE LYASE GENE OF C. REINHARDTII: CHARACTERISATION AND USE IN THE RESCUE OF ARGININE-REQUIRING MUTANTS BY BIOLISTIC TRANSFORMATION.

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Argininosuccinate lyase (ASL) is the last enzyme in the arginine biosynthesis pathway. The ASL gene of Chlamydomonas reinhardtii has been isolated from a λEMBL 3 genomic library using four oligonucleotide probes, the sequences of which were derived from regions of conserved primary sequence between ASL from various organisms. The gene is present on a 12.6kb BamHI fragment. Nucleotide sequence analysis has identified most of the coding DNA including the four conserved regions. The gene is interrupted by a number of introns, some of which contain highly repetitive DNA. Several cDNA clones for ASL have been isolated and are being used to characterise the gene further. Introduction of the 12.6kb BamHI fragment into the cells of arginine-requiring mutants of C. reinhardtii using a high velocity particle gun (biolistic transformation) has resulted in a number of transformants with a wildtype phenotype. Measurement of ASL activity in these transformants reveals levels comparable with that of wildtype cells. We are currently analysing the transformants at the DNA and RNA levels using probes derived from the ASL gene.

P2 69

CHARACTERIZATION OF THE GENES FOR THE 17.9 AND 8.1 kDa SUBUNITS OF PHOTOSYSTEM I FROM CHLAMYDOMONAS REINHARDTII

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cDNA clones encoding two Photosystem I subunits of Chlamydomonas reinhardtii with apparent molecular masses 18 and 11 kDa (thylakoid polypeptides 21 and 30) have been isolated and sequenced. Protein structure predictions indicate that the proteins encoded by the cDNAs are extrinsic membrane proteins. The molecular masses calculated from the sequences are 17.9 and 8.1 kDa. The sequences of the transit peptides indicate that the proteins are routed towards the lumenal and the stromal sides of the thylakoid membrane, respectively. The size of the mRNA is 1400 nucleotides for the larger protein and 740 nucleotides for the smaller one. Southern analysis suggests that both proteins are encoded by single copy genes.

P2 70

ISOLATION OF A GENOMIC ONCOMODULIN CLONE AND FULL LENGTH PLACENTAL CDNA FROM RAT

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Oncomodulin is a Ca2+-binding protein, closely related to parvalbumin. It's expression is restricted to rodent and human tumors and the placental cytotrophoblasts. When we searched the GeneBank(R) for sequences homologous to the rat hepatoma oncomodulin (Gillen et al. J.B.C.262, 5308 (1987), a striking similarity of the 5' leader sequence and a long terminal repeat (LTR), a regulatory sequence element typical for retroviruses, was evident. This indicated that oncomodulin might be under the control of an LTR promoter. To study this possibility, we initially isolated a full length oncomodulin cDNA clone from a lambda gt11 library. Synthetic oligonucleotides corresponding to the rat hepatoma oncomodulin cDNA were used as hybridisation probes. Sequencing of a positive clone revealed 100% identity of placental and hepatoma oncomodulin. Genomic oncomodulin clones were isolated using this 700 bp cDNA probe and synthetic oligonucleotides. Several independent clones are currently analyzed in order to gain new insights into mechanisms leading to tumor-specific gene expression.

P2 71

CLONING OF PIG TNF-α BY IN VITRO AMPLIFICATION OF TOTAL RNA USING THE POLYMERASE CHAIN REACTION

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Monocytes isolated from whole blood were stimulated for 4-6h with LPS, a known inducer of TNF-α mRNA. Total RNA was isolated from the cells and antisense primer of exon 4 of TNF-α was used for the specific reverse transcription of TNF-α mRNA. This primer starts with the translation stop codon and is located at a highly conserved region as deduced from sequence comparisons of the human, rabbit and murine TNF-α genes. A conserved sense primer of the propeptide region was then used to amplify the single stranded cDNA in vitro by the polymerase chain reaction (PCR). After amplification and gel electrophoresis, the amplified fragment was cloned for subsequent sequencing. Comparison with human, rabbit and mouse sequences revealed that pig TNF-α differs mainly in the N-terminal part of the peptide. The procedure shown here screens already during the amplification of the cDNA for full length transcripts and yields microgram amounts of the desired DNA fragment.

P2 72

INDUCTION OF TRANSCRIPTION FROM THE SV40 EARLY PROMOTER BY IL1 AND BY TNF IN RODENT T CELLS

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We have found that in a rodent T lymphocyte line that appears to represent an immature stage of T cell differentiation interleukin 1 (IL1) or tumor necrosis factor (TNF) can induce a strong increase in the transcription of genes under the control of the SV40 early promoter (PvuII-HindIII). The increase can be observed with stably or transiently transfected genes. It is rapid (10 fold in three hours) and cannot be prevented by cycloheximide, suggesting that it involves the activation of preexisting transcription factors. The response to IL1 and to TNF is, at least in part, due to sequences within the SV40 enhancer, and we are, at present, mapping the responsive elements.

P2 73

CLONING OF RF-X, THE MHC CLASS II PROMOTER BINDING PROTEIN AFFECTED IN A HEREDITARY DEFECT IN CLASS II GENE REGULATION

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MHC class II antigens are cell surface molecules playing a crucial role in the control of the immune response. They, are encoded by a family of coregulated genes. Several nuclear proteins bind to conserved cis-acting sequences in class II promoters. In a form of hereditary immunodeficiency (CID) characterized by a defect in a transacting regulatory factor controlling HLA class II gene transcription, we have observed a defect in the binding of one of these nuclear proteins (RF-X) to its target sequence (the class II X box). A cDNA encoding RF-X was isolated by screening a phage expression library with an X box binding site probe. The recombinant protein has the binding specificity of RF-X, including a characteristic gradient of affinity for the X boxes of HLA-DR, -DP and -DQ promoters. RF-X mRNA is present in the regulatory mutants, indicating a defect in the synthesis of a functional form of the RF-X protein.

P2 74

DIFFERENT PROMOTERS OF THE MHC CLASS II MULTIGENE FAMILY EXHIBIT DISTINCT AFFINITIES FOR TWO DNA BINDING FACTORS, RF-X AND NF-S

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MHC class II genes are organized in three distinct subregions, HLA-DR,-DQ and -DP and the regulation of all these genes is generally coordinated. We have now identified 5 distinct proteins that bind to specific DNA sequences within the first 145 base pairs of HLA class II promoters. Among these, RF-X is of special interest since mutants affected in the regulation of MHC class II gene expression have a specific defect in RF-X binding. Unexpectedly, RF-X displays a characteristic gradient of binding affinities for the promoters of DRA> DPA> DQA genes. The same observation was made with recombinant RF-X protein. We also describe a novel factor, NF-S, which binds to the spacer region between the X and the Y boxes of class II promoters and which exhibits a reverse gradient of affinity, binding to the promoters of DQA> DPA> DRA. At high protein concentration, both RF-X and NF-S bind simultaneously. As expected, RF-X binds well to the mouse IE promoter, while NF-S binds well to IA. The genes of each of the three MHC class II subregions differ therefore drastically in their respective binding to these two protein factors.

P2 75

INTERACTION OF GLUCOCORTICOID RECEPTOR WITH GLUCOCORTICOID RESPONSE ELEMENTS (GREs)

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We have described a transcriptional inhibition of glucocorticoid-dependent promoters by the products of the H-ras, v-mos and v-src oncogenes which is mediated by the glucocorticoid response element GRE. The immunologically detectable GR was decreased to about 40% within 24 hours of hormone treatment in normal NIH 3T3 cells and to about 15% in transformed cells. Based on a gel retardation assay we analyzed the binding of the glucocorticoid receptor to specific DNA recognition sequences representing dimers or trimers of the GRE. Activation of cytoplasmic GR and addition of hormone to the binding reaction both resulted in an increased binding in vitro. Binding of the GR could be competed by the addition of a non-labeled HRE (=hormone response element wich contains four GREs) but not by monomeric GRE. Binding of the GR to the GRE was only partially inhibited in cells expressing transforming p37v-mos.

P2 76

EXPRESSION OF THE TANDEM DUPLICATED MBP GENE IN *mld* MICE

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Myelin deficient mutant mice (*mld*) are characterized by a severe deficit of myelin basic protein (MBP) expression, resulting in poor myelination of the central nervous system. In *mld* mice, the MBP gene is tandem duplicated and the upstream gene copy contains a large inversion of its 3' end. Although the overall transcription rate of the MBP gene is normal, only 2 to 5% of MBP and its corresponding RNA are present in the cytoplasm. Hybridization of the 5' flanking region of the gene with nuclear run-off RNA, showed that both MBP gene copies are transcribed from their own promoter. In order to explain the low levels of cytoplasmic MBP and its corresponding RNA, we hybridized run-off RNA to sense and anti-sense RNA probes. Although the transcription rate of each copy of the gene is somewhat reduced in *mld* mice, it could not explain the drastic reduction of RNA found in the cytoplasm. Nuclear RNA levels measured by dot blots corresponded to the levels of transcription of the downstream gene instead of the levels found in the cytoplasm. These results suggest that the low levels of MBP mRNA found in *mld* mice are the result of reduced transcription rates and post-transcriptional events.

P2 77

INDUCIBLE BINDING OF SEQUENCE-SPECIFIC NUCLEAR FACTORS ON THE TNF-α PROMOTER REGION UPON INDUCTION OF TNF-α TRANSCRIPTION.

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Tumor necrosis factor (TNF)-α is a cytokine with many functions and is in particular an essential mediator of the inflammatory response. Its synthesis by macrophages is strictly regulated both at the transcriptional and posttranscriptional levels. Endotoxin (LPS) is the strongest stimulator of TNF transcription, which appears to be under the control of short-lived protein repressors, since arrest of protein synthesis by cycloheximide (CHX) leads to a strong and transient increase in transcription. (Collart et al. J. Exp. Med. 1986, 164: 2113). The binding of DNA by protein from nuclear extracts from non induced or induced (by LPS or CHX) macrophages was analyzed with a variety of DNA fragments covering the 1 kB region upstream of the TNF gene and downstream of the lymphotoxin gene. Two fragments were bound differently by nuclear extracts, from non induced or induced cells: a) fragment - 161 to - 432, containing a Y-box consensus sequence, which is a site of DNA binding as shown by G-methylation interference; b) fragment - 695 to - 432, containing an AP-1 consensus sequence as well as a sequence CYT-1 conserved in several cytokines, which is essential for DNA binding during increased transcription, as shown by lack of binding after cleavage within this sequence. Functional assays are currently undertaken in order to determine the importance of these induced protein-DNA complexes in the activation of TNF gene transcription.

P2 78

CLONING AND ISOLATION OF DBF 1. DBF 1: ANOTHER DECAMER BINDING PROTEIN?

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The mouse immunoglobulin promoter region contains several controlling elements. One of these is the decamer motif ATGCAAATTA, which is also found in controlling regions of other genes. We isolated a protein from a mouse hybridoma nuclear extract by its property to bind specifically to the decamer motif of a murine immunoglobulin μ promoter. We developed a novel approach to determine the NH2-terminal sequence of this protein factor. Specific protein-DNA complexes were gel-purified and microsequenced, using the fluorescence detection method. Oligonucleotide screening allowed to isolate several clones from a genomic as well as from a cDNA library. We are currently investigating the structure of the DBF1 gene and its homology to other factors binding in the controlling region of the immunoglobulin genes.

P2 79

OCTAMER TRANSCRIPTION FACTORS BIND TO TWO CONSERVED BUT DIFFERENT SEQUENCE MOTIFS (HEPTAMER AND OCTAMER) OF THE IgH PROMOTER

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The octamer motif ATGCAAAT is found in all immunoglobulin (Ig) heavy and light chain promoters and in the Ig heavy chain enhancer. B-cell specific transcription of Ig genes has been shown to be dependent on the presence of an intact octamer motif. Ig heavy chain promoters contain a second conserved motif, the heptamer element (CTCATGA), located 2 to 22 nucleotides upstream of the octamer element. We demonstrate that the B-cell specific octamer factors OTF-2A and OTF-2B, as well as the ubiquitous octamer factor OTF-1 from HeLa cells bind to both the octamer and the heptamer element, despite their different sequence. Protein binding to these two sequences occurs in a cooperative manner. Nevertheless, binding to the heptamer element does not depend on an intact octamer motif, and the heptamer element can by itself stimulate transcription in lymphoid cells.

P2 80

A CLONED OCTAMER TRANSCRIPTION FACTOR (OTF-2) STIMULATES SPECIFIC TRANSCRIPTION IN NON-B CELLS.

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The "octamer" motif TNATTTGCAT is found in all immunoglobulin (Ig) promoters and also in the Ig heavy chain (IgH) enhancer. B-cell-specific transcription of Ig genes has been shown to be critically dependent on the presence of an intact octamer motif. The cDNA coding for a lymphocyte-specific transcription factor binding to the "octamer" sequence has been cloned. This cDNA hybridizes to RNA only expressed in lymphoid cells. The protein sequence reveals a homology to the "POU" domain and the homeobox domain which together define a new class of DNA-binding region. Expression of this cDNA in HeLa cells is sufficient for a strong transcriptional activation of B-cell-specific promoters. This finding suggests that a) the OTF-2 represents one of the key factors for B-cell-specific expression of immunoglobulin genes and b) that no further B-cell-specific modification mechanism is needed for its activity. Surprisingly, the activity and target specificity of OTF-2 is similar to *Ultrabithorax* (*Ubx*) and *Abdominal-B* (*Abd-B*), two homeotic (and homeobox containing) transactivators of Drosophila.

P2 81

IDENTIFICATION OF A NOVEL, LYMPHOID SPECIFIC OCTAMER BINDING PROTEIN (OTF-2B)

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The octamer sequence ATGCAAAT, present in the promoter and enhancer of immunoglobulin heavy chain (IgH) genes, is a major determinant of the cell type specific expression of IgH genes in B-cells. Paradoxically, this sequence serves also as a promoter or enhancer element in some housekeeping genes. The differential usage of this regulatory sequence is thought to be mediated by different species of octamer binding proteins. One species of 100 kDa (OTF-1), is present in all cell types and may exert its activating function only when it can interact with additional adjacent transcription factors. The lymphoid cell specific protein of 60 kDa (OTF-2A) specifically stimulates immunoglobulin promoters which consist essentially of a TATA-box and an octamer sequence upstream of it. Here we present evidence for yet another, B-cell specific octamer binding protein of 75 kDa (OTF-2B) and propose a role for this factor in the long range activation by the IgH enhancer. A novel technique, proteolytic clipping bandshift assay (PCBA), was used to distinguish the three different forms found in B-cells. This analysis indicates that the 75 kDa species OTF-2B is closely related to the 60 kDa species OTF-2A.

P2 82

THE SV40 ENHANCER STIMULATES TRANSCRIPTION FROM THE RABBIT ß-GLOBIN PROMOTER EVEN WHEN ATTACHED VIA A PROTEIN

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Enhancers stimulate transcription of RNA polymerase II genes and can be located thousands of base pairs upstream or downstream of the initiation site. Two models have been proposed to explain the activation of a gene by a remote enhancer: (i) enhancer and promoter directly interact via proteins bound to them (looping model); (ii) RNA polymerase II (or another factor) binds to the enhancer and then slides along the DNA until it reaches a promoter (scanning or entry site model).
So far, it has been reported that enhancers transmit their effect on a promoter only via covalently closed DNA, i.e. in a cis-configuration. The looping model would predict, however, that an enhancer is able to transmit its effect also in certain trans-configurations. Indeed, we find that the SV40 enhancer, non-covalently attached to the rabbit ß-globin promoter via the protein streptavidin, can stimulate transcription in vitro. These findings are consistent with the looping model, rather than the scanning model.

P2 83

CpG methylation of the cyclic AMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation

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In mammals and other vertebrates, cytosine methylation in CpG sites is often negatively correlated with gene activity. Since methylation of the promoter region is most crucial for this effect, the simplest hypothesis is that CpG methylation interferes with the binding of specific transcription factors. We have examined this hypothesis with two different transcription factor binding sites which contain a CpG dinucleotide, namely the cyclic AMP (cAMP) responsive element (CRE) TGACGTCA and the Sp1 binding site CCGCCC. We have previously reported that CpG methylation of the Sp1 binding site affected neither factor binding nor transcription in HeLa cells, which may be related to the fact that Sp1 is typically associated with promoters of housekeeping genes (Höller et al., 1988, Genes & Dev.2, 1127-1135). By contrast, cAMP-responsive elements are often associated with promoters of cell type-specific genes such as the one encoding the human glycoprotein hormone alpha subunit. CpG methylation of these CRE consensus sequences resulted in loss of specific factor binding as well as loss of transcriptional activity in vitro and in vivo, in both HeLa cells and PC12 cells. This suggests that the inactivity of methylated promoters can, at least in some cases, be explained by their inability to bind specific transcription factors.

P2 84

CIS-TRANS MUTATION ANALYSIS REVEALS UNUSUAL FEATURES OF THE RAT GLUCOCORTICOID RECEPTOR

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The transactivation properties of wild type glucocorticoid receptor (GR), deletion mutants or chimaeras were assessed by co-transfection with various reporter genes into mammalian cells. In the reporter genes the number, geometrical arrangement (= clustering) and position (with respect to transcription initiation) of target glucocorticoid response elements (GREs) was changed. Four major conclusions can be drawn from our experiments: (a) The GR amino-half (residues 1-405) can be functionally replaced by a shorter, synthetic region encoding the yeast GAL4 activator region II(aa. 658-881), suggesting that the GR amino-half contains a general activating domain of the "acid blob" type. (b) The constitutive GR-fragment (aa. 3-556) can act as a promoter as well as an enhancer activating factor. This "dual" function seems to be unique to the GR so far, for no other single transcriptional factor seems to be capable of activating a combination of an upstream (="promoter") and downstream (="enhancer") target combination in a more-than-additive fashion. (c) A reporter gene in which *all* promoter elements (including the TATA-box!) are deleted, can still be transcriptionally activated via and adjacent GRE cluster, suggesting that under some circumstances, one single factor may be sufficient for regulation of correctly initiated transcription. (d) In studying deletion mutants, we observed that the presence of the carboxy-domain of the GR (which harbours functions for hormone binding and, perhaps, protein:protein interactions) seems to lead to a preferential interaction with a particular type of clustering of the DNA-targets in chromatin, thus revealing an unexpected *DNA-target discriminatory* function of this GR domain.

P2 85

REGULATION OF THE VITELLOGENIN B1 PROMOTER IN PRIMARY CULTURES AND IN IN VITRO TRANSCRIPTION

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In the liver of adult *X. laevis*, vitellogenin genes are expressed in a sex-specific manner under the strict control of estrogen. However, males that normally do not express these genes can be artificially stimulated by a hormone injection. In order to study the regulation in vivo of these genes, primary cultures of adult male hepatocytes were established. A battery of 5'- and 3'-deletions of the promoter region of the vitellogenin B1 gene cloned in front of the CAT reporter gene were transfected by the calcium phosphate technique into the cultured hepatocytes exposed to estrogen or left unstimulated. The same set of mutants was analyzed in an homologous in vitro transcription system derived from liver nuclear extracts. We present evidence that constructs assayed in both systems generate a similar, but not identical response at the level of inducibility and basal transcriptional activity.

P2 86

FACTORS INVOLVED IN THE CONTROL OF TISSUE-SPECIFIC EXPRESSION OF THE VITELOGENIN B1 GENE

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Elements controlling tissue-specific expression of the Xenopus vitellogenin gene, *in vitro*, reside within the 140 bp upstream of the transcriptional start site. The DNAase I footprinting assay reveals that at least four distinct factors present in nuclear extracts from Xenopus livers interact with these sequences. No difference was observed between extracts from male animals not producing vitellogenin and female animals in which the gene is active. Extracts from non-hepatic cells that do not regulate expression of the vitellogenin gene *in vitro* display a different pattern of DNA-protein interactions. Competition experiments reveal a complex interplay of factors along the promoter. One of these factors is similar to mammalian nuclear factor I (NF-I) as suggested by both DNAase I footprinting and gel-retardation assays with a preparation of purified NF-I obtained by DNA-affinity chromatography.

P2 87

P-ELEMENT MEDIATED ENHANCER DETECTION: APPLICATIONS IN THE STUDY OF *DROSOPHILA* OOGENESIS

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We have generated roughly 600 fly stocks that carry single copies of a novel P-element enhancer detector, P[lArB]. In this P-element the *lacZ* gene from *E.coli* is fused in frame to the P-transposase gene, so that it is regulated by the weak but constitutive P-transposase promoter. In insertion strains, this promoter comes under the control of nearby transcriptional regulatory elements. The expression of the *lacZ* gene can easily be detected in whole mount ovaries, embryos and in larvae. The resulting staining patterns provide a visual "record" of the temporal and spatial specificity of the detected regulatory elements.
Ovaries of all 600 stocks have been dissected and stained with X-gal. The spatial and temporal distribution of ß-galactosidase activity in some relevant stocks will be described. We have identified many strains that will be useful as markers for all ovarian cell types and some subsets of these cell types.
Several of the regulatory elements that have been detected appear to control bona fide neighbouring Drosophila genes. P[lArB] is designed to facilitate rapid cloning of adjacent genomic sequences and genetic analysis of the neighbouring genes. Therefore, enhancer detection is a powerful method to screen for and analyse genes expressed during oogenesis.

P2 88

STRUCTURE AND EXPRESSION OF TWO NEW HOMEOBOX-CONTAINING GENES OF DROSOPHILA MELANOGASTER

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Using probes from *cad (caudal)* and *msh (muscle segment homeobox)* two new homeobox-containing genes, W13 and W26, were isolated. W13 maps at 88A and detects a 2.3 kb RNA during embryogenesis. The gene is expressed in a head segment, tracheal pits and posterior spiracles as monitored by *in situ* hybridization to sections and staining of W13-*lacZ* gene fusions. This localized expression correlates well with the regions of defects in the mutant *ems (empty spiracles)* mapping at 88A, suggesting that W13 corresponds to the *ems* gene. This hypothesis will be tested by mutant rescue experiments.
Clone W26 maps at 57B, and encodes two transcripts of 2.3 kb and 2.7 kb that are detectable during embryogenesis. The gene contains two separate promoters and eight exons spanning 20 kb. Using *in situ* hybridization to sections and antibody staining the expression pattern was studied. Expression is first seen in the proctodeum, and later in the hindgut, anal pads, and in the central nervous system. The 2.7 kb transcript appears to be expressed preferentially in the nervous system, whereas the 2.3 kb transcript is found in the other tissues. This differential expression is being investigated further by ß-gal fusions of both promotor regions.

P2 89

A NOVEL APPROACH FOR IDENTIFYING *ANTP*-REGULATED GENES

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Homeotic genes specify the unique identities of the different body segments of *Drosophila melanogaster*. These homeotic genes act as **selector genes** that are thought to initiate specific developmental pathways by controlling the expression of genes (the **realizator genes)** that are involved in structural and cellular differentiation.
The homeotic gene *Antennapedia (Antp)* is required for the correct development of the thorax. During larval development it is normally expressed in the leg imaginal discs which are the primordia of the adult legs. In order to detect possible realizator genes, we have screened stocks in which a detector transposon (P[lArB]) has inserted into chromosomal loci that are expressed in the leg imaginal discs. By crossing such stocks which express ß-galactosidase in the leg discs with *Antp* mutants, a possible regulatory interaction between *Antp* and those loci can be detected. In several crosses the ß-galactosidase expression pattern in antennal discs is altered to a pattern resembling that in the leg discs. We are now analyzing these strains to ascertain whether the enhancer detector has inserted near an *Antp*-regulated gene.

P2 90

ANALYSIS OF THE *FTZ* UPSTREAM ELEMENT: A DEVELOPMENTALLY REGULATED ENHANCER

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The *fushi tarazu (ftz)* gene is a homeobox-containing gene required for the establishment of the segmental pattern in the *Drosophila* embryo. It is expressed in seven stripes at the cellular blastoderm stage. Analysis of fusion genes containing *ftz* 5'flanking sequences and the protein coding region of the *E.coli lacZ* gene revealed different cis-acting control elements necessary for normal *ftz* expression. A 2.6 kb region-"the upstream element"- that is required for normal expression in the stripes appears to act as a typical eucaryotic enhancer element. Furthermore, the "upstream element" generates a *ftz*-like striped pattern when linked to a heterologous TATA box. In order to identify the functional enhancer region(s), series of sequential deletions from either end of the "upstream element" were generated. These deletions and several internal fragments were then fused, in either orientation, upstream of a TATA box and assayed for expression in transformant flies. The "upstream element" appears to contain several functional domains including a tissue specific enhancer required for the expression of the *ftz* stripes in the ectoderm. In order to identify trans-acting factors interacting with defined cis-acting elements within the *ftz* enhancer, we are analyzing expression of the constructed fusion genes in different embryonic mutants and in *in vitro* transcription assays.

P2 91

THE HOMEOBOX ENCODES A DNA BINDING DOMAIN

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Several genes controlling *Drosophila* development share a characteristic 180 bp DNA segment, the homeobox, which encodes a conserved polypeptide region called the homeodomain (HD). The HD shares a small but significant homology with the yeast mating type proteins Mat a1 and Mat α2 and with prokaryotic helix-turn-helix type DNA binding proteins. These observations, as well as the putative gene regulatory function of the HD-containing proteins, suggest that the HD represents a DNA binding domain containing a helix-turn-helix motif.
In order to test this hypothesis, the HDs encoded by the *Antennapedia (Antp)* and the *fushi tarazu (ftz)* gene of *Drosophila* were overproduced in *E.coli* using a T7 expression vector. These HDs (and several mutant versions thereof) were assayed for *in vitro* DNA binding by DNaseI protection experiments and mobility shift assays. Both HDs recognize the same DNA sequence elements. The stoichiometry of binding of the HDs was determined. In addition, the DNA binding properties of the *Drosophila*-expressed *Antp* gene product were analysed. Our studies show that the HD indeed represents a DNA binding domain of HD-containing proteins. NMR analysis of the *Antp* HD revealed a striking structural similarity with the helix-turn-helix motif found in many prokaryotic DNA-binding proteins.

P2 92

TRANSCRIPTIONAL REGULATION OF *CAUDAL* IN *DROSOPHILA MELANOGASTER*

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The *caudal (cad)* gene is one example of a maternally active gene thought to be involved in establishing the spatial pattern of the embryo. The protein product synthesised as a result of expression from a maternal promoter becomes organised in an anterior-posterior gradient peaking at the posterior of the embryo. The *cad* gene also has a zygotic promoter that gives rise to a slightly different transcript encoding an identical protein; the protein synthesised from this second promoter is expressed in a transverse stripe at the posterior of the embryo. The presence of two promoters, controlling transcripts that encode the same protein, offers an interesting system for studying the regulatory specificity of *trans*-acting transcription factors.
Analysis of ß-galactosidase staining patterns from transformant embryos, where the *lacZ* gene is under the putative control of DNA fragments taken from the 5´upstream and 3´downstream regions of *cad* will identify enhancer elements of the *cad* gene. Using these elements it will be possible to identify putative transcription factors acting at these regions.

P2 93

POSITIVE FEED-BACK REGULATION OF THE P04 PROMOTER OF MINUTE VIRUS OF MICE BY THE NON-STRUCTURAL PROTEIN NS-1

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We studied the regulation of transcription from the P04 promoter of the minute virus of mice (MVMi) by MVMi-coded proteins. The P04 promoter was cloned upstream of the gene coding for chloramphenicol acetyl transferase (CAT) and the expression of CAT monitored in transient transfection experiments with EL-4 cells. Co-transfection with cloned DNAs able to express separately the non-structural and capsid proteins of MVMi showed, unexpectedly, that the non-structural protein NS-1 has a positive feed-back effect on the activity of the P04 promoter. Thus NS-1 activates its own promoter. A DNA sequence at position -100 in the P04 promoter is highly homologous to the tar element, which is believed to mediate transactivation by NS-1 of the MVM capsid protein gene promoter P39. We have cloned the NS-1 gene in an SP6 vector, which allowed us to use SP6 RNA polymerase for the synthesis of NS-1 mRNA; this RNA was then used as a template for in vitro translation, yielding labeled NS-1 protein. We also raised anti-NS-1 antibodies by injecting a bacterial fusion protein containing a part of the NS-1 protein into a rabbit. We plan to use these reagents in order to elucidate the mechanism of transactivation by NS-1.

P2 94

MAPPING OF HPV6 PROMOTERS BY TRANSCRIPTION IN VITRO

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We are interested in the DNA sequences and proteins controlling human papilloma virus mRNA synthesis. We subcloned and purified the two fragments of HPV6b DNA generated by cutting with EcoRI (position 2188) and BamHI (position 4722). DNA fragments were transcribed in vitro using a nuclear extract of HeLa cells and RNA initiation sites estimated from specific run-off transcript lengths. Three regions functioning as promoters in this assay were identified. One corresponds to the TATA box indentified at position 64 near the junction of the noncoding region and the "early" open reading frames. A second is 0.4 kb downstream from there. And the third, around position 4100, lies just upstream from the start of the L2 "late" open reading frame.

P2 95

GENE EXPRESSION OF BOVINE HERPESVIRUS 1 DURING LYTIC INFECTION

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Protein synthesis of bovine herpesvirus 1 (BHV-1) is regulated in a temporal cascade producing immediate-early (IE), early, and late proteins. The spatial and temporal distribution of transcripts was studied by Northern blot technique. Permissive cells were infected with BHV-1 strain Jura or K22 at an m.o.i. of 5-10 PFU. The protein synthesis inhibitor cycloheximide was used for enrichment of IE RNA, whereas early and IE RNA was isolated from cells treated with the DNA synthesis inhibitor cytosine arabinoside. These RNA preparations were compared to RNA isolated between 1 and 8 hours post infection in the absence of metabolic inhibitors. A total of 49 BHV-1 transcripts were found ranging in size from 0.58 to 8.5 kb. Five major and one minor IE transcripts mapped mainly within inverted repeat sequences, whereas the early and late transcripts showed dispersed locations over the genome. Four of nine identified late transcripts were encoded by the HindIII fragment K (m.u. 0.677-0.733). All of the 34 defined early transcripts were encoded by unique viral sequences. Most abundant transcripts were derived from sequences within m.u. 0.017-0.188 and 0.852-0.904. One of the BHV-1 IE transcripts exhibited homology with the IE gene of pseudorabies virus. The genome region shown by Rock et al. (87) to be transcriptionally active during latency (m.u. 0.734-0.748) encoded two IE transcripts. The number of IE and late transcripts is in good agreement with [^{35}S]methionine in vivo labeling and immunoblotting experiments showing the presence of five (possibly seven) IE and six late proteins.

P2 96

ANALYSIS OF THE VACCINIA VIRUS 11KD GENE REGULATORY REGION USING AN IN VITRO TRANSCRIPTION SYSTEM

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We used extracts from infected HeLa cells to transcribe a chimeric gene consisting of the 11Kd late gene regulatory sequence fused to the CAT gene coding region. Nuclease S1 analysis with a probe containing a poly(A) stretch upstream of the translation initiation codon, shows that a 5'poly(A) leader of about 30 nt is present on the mRNAs synthesised in vitro, similar to what has been reported for mRNAs made in vivo. In vitro transcripts were compared with those obtained from transfected plasmids. This showed that specific transcription initiation, as compared to random initiation, is about 5 times less efficient in vitro than in vivo. Furthermore, specific initiation in vivo is about 15 times more frequent than random initiation, indicating that the 11Kd gene 5' flanking sequences act as a promoter element. Analysis of a 30 bp promoter fragment allowed us to identify 4 functional domains: the sequences -19 to -11 and -4 to +1 are essential for transcription initiation. The two other regions modulate the level of transcription .

P2 97

REGULATION OF THE IFI-78K GENE, THE HUMAN EQUIVALENT OF MURINE Mx GENE, BY INTERFERONS, CYTOKINES, AND VIRUSES.

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In response to interferon alpha human diploid cells synthesize and accumulate a cytoplasmic protein of Mr 78000 (p78) which has been shown to be the human homolog of the murine Mx protein (Horisberger and Hochkeppel, 1987. J.Interferon Res.7:331-43). The corresponding gene, the IFI-78K gene, is located on chromosome 21 (Horisberger et al.,1988. Somatic Cell Mol.Genet.14:123-31). We have studied the regulation of the IFI-78K gene within the cytokine network. Induction of p78 has been measured by protoblot-ELISA using specific monoclonal antibodies. Gene transcription has been studied by RNA blot analysis of in vivo mRNA levels and by in vitro run-on assays with isolated nuclei. We report that the IFI-78K gene can be regulated by various factors including interferons, cytokines, and viruses.

P2 98

A SENSITIVE ASSAY FOR INTERFERON-INDUCED Mx mRNAs IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Two interferon (IFN)-activated human genes on chromosome 21 have a high degree of homology to the murine influenza virus resistance gene *Mx*. Selected fragments of corresponding cDNAs specifically detected the cognate mRNAs, designated MxC and MxE, when used as S1 probes to assay preparations of total cellular RNA. We have optimized this protocol for analysis of as few as 1.5 x 10^6 lymphocytes isolated by centrifugation of venous blood through a Ficoll-Paque gradient. Cells are either stimulated in vitro with IFN for 3 hours or are left untreated before RNA is isolated by a single-step method. In non-stimulated human lymphocytes from healthy donors only faint signals of Mx-related mRNAs were detected. In contrast, about 50 fold higher pools of MxC and MxE mRNAs were found in IFN-stimulated cells. We plan to use this assay to monitor IFN therapies. We will also measure MxC and MxE mRNA pools in IFN-treated and untreated lymphocytes of patients suffering from acute viral infections and Down syndrome.

P2 99

ISOLATION AND SEQUENCE ANALYSIS OF A cDNA CLONE CODING FOR AN ISOFORM OF XENOPUS LAEVIS CREATINE KINASE.

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The CK isozyme system of X. laevis appears to involve at least 4 different genomic loci which show developmental and tissue specific expression (Bürki, Biochem. Genet. 23, 1985, 73; Wolff et al., J. Exp. Zool. 234, 1985, 471; Robert et al., Biochem. Genet., in press). To investigate this isozyme system, we have screened a cDNA library from X. laevis embryos using chicken B-CK and M-CK probes. 30 positive cDNA clones were isolated; one of them was sequenced. Its deduced amino acid sequence shows more than 80% homology to published CK sequences. Moreover, the Cys residues are conserved at 4 positions when compared to mammalian or avian species, and the sequences immediately surrounding the active site appear to be highly conserved. Northern blot analysis indicates that this cDNA recognizes a 1.5 kb RNA present in gonads and embryo tissues, but absent in adult skeletal muscle. This distribution is in agreement with the tissue specificity of the X. laevis CK-IV isozyme.

P2 100

OOCYTE AND SOMATIC $tRNA^{Tyr}$ GENES IN XENOPUS

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Oligonucleotide probes have been used to define, by Northern blot analyses and primer extension sequencing, the kinds of $tRNA^{Tyr}$ genes that are expressed during early X.laevis development. Two kinds of oocyte-type genes were detected: they are very active during oogenesis, transiently expressed in blastula and gastrula embryos, and repressed in somatic cells. Two kinds of somatic-type $tRNA^{Tyr}$ genes were also found, one major and one minor. Unspliced transcripts of the major somatic-type gene are first detectable in blastula embryos but are then very abundant from the neurula stage onwards. These changes are contrasted with the oocyte-somatic switch in Xenopus 5S RNA gene expression.

P2 101

T-KININOGEN GENE EXPRESSION IS INDUCED BY AGING

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We have constructed a cDNA library from aged (24 month old) rat liver mRNA, and by differential screening, we have selected clones whose expression is increased by aging. The majority of the clones (9/11) coded for kininogen (also called Major Acute Phase protein, MAP). Sequencing of the inserts showed that they all belong to the T class of genes, suggesting that it is only T, and not K kininogen expression which is induced by aging. Nuclear elongations indicate that the effect is controlled at the transcriptional level, while RNase mapping analysis showed that the induction due to age operates preferentially at one of the three start sites of the gene. Acute phase (inflammation) also induces this gene at the level of transcription; however, two of the three start sites are induced by inflammation. We are currently trying to determine the mechanism of induction by aging at the molecular level, via transfection into primary hepatocytes, footprinting, and in vitro transcritpion analysis.

P2 102

THE EXPRESSION OF TWO BEAN CHALCONE SYNTHASE GENES IN TRANSGENIC TOBACCO PLANTS

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The expression of two bean (*Phaseolus vulgaris,* L.) chalcone synthase (CHS) genes was studied in transgenic tobacco plants (*Nicotiana tabacum,* L.). 5'-flanking sequences up to the translational start side of these genes were used to drive the expression of the β-glucuronidase (GUS) reporter gene. Whole tobacco plants were regenerated from cells transformed with these constructs. The tissue specific expression of these CHS-GUS chimeric genes closely resembled that of CHS genes in bean. Both genes are strongly expressed in roots , the pigmented parts of petals and in pollen grains. One chimeric gene was also inducible by wounding, fungal elicitor and $HgCl_2$.

P2 103

DEVELOPMENTAL AND CELL-SPECIFIC EXPRESSION OF A BEAN CELL WALL PROTEIN IN TRANSGENIC PLANTS

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In the bean plant, a glycine-rich cell wall protein is specifically synthesized in protoxylem cells of the vascular system. We have isolated the gene encoding this particular cell wall protein. A fusion of an upstream promoter fragment of this gene with a reporter gene was analyzed in transgenic tobacco plants. We found the gene correctly expressed in vascular tissue of roots, stems, leaves and flowers. It was developmentally expressed in both primary and secondary xylem. Moreover, it was rapidly induced (in less than 15 min) after wounding. In stems, the wounding response was located in pith parenchyma cells, where vessel regeneration is known to occur. The wound induction of this gene was not only found at the surface of the wounded tissue but also 1 to 2 mm into the tissue. Thus, a promoter fragment of 420 bp contains all the information for correct regulation. The induction of this gene is one of the earliest events in vascular differentiation and regeneration of wounded tissue.

P2 104

IDENTIFICATION AND ISOLATION OF TELOMERES IN ASCARIS LUMBRICOIDES AND C. ELEGANS

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We have isolated a repetitive , 18 kb long DNA element (Pas) which is present in about 50 copies per haploid germ line genome of A. lumbricoides. The copies of this element are not clustered; all of them are completely expelled from the somatic cell lineage during the process of chromatin diminution. The different chromosomal copies of Pas are very conserved in structure. This points towards a possible biological function which would have to be germ line specific. The following observations suggest that Pas elements may be associated with telomeres: A specific fragment of this element crosshybridizes to the genome of C. elegans. It contains a block of a tandemly arranged, 6 bp long repeating unit, similar to those found at the telomeres of some lower eukaryotes. In Southern blot experiments, the repeated Pas sequence hybridizes indeed to nuclease Bal 31 sensitive sequences. Hence, this sequence has to be located at the telomeres of A. lumbricoides and most likely also at those of C. elegans.

P2 105

HOMEO BOX CONTAINING GENES IN C. ELEGANS

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Using the homeo box AHB-1 from A. lumbricoides as a hybridization probe, two homeo box containing clones have been isolated from a genomic λ library of C. elegans. The first one codes for a polypeptide which is 57% identical to the Drosophila Antennapedia homeo domain. This homeo box is not interrupted by an intron. The second clone contains an intron upstream of the codon for the aminoacid at position 45 of the putative homeo domain. An intron at the same location has been found in three other homeo boxes, namely in C. elegans (JM # L1001 and CHEB-2) and one in D. melanogaster (lab).

P2 106

A TRANSCRIBED SINGLE COPY SEQUENCE OF ASCARIS LUMBRICOIDES IS ELIMINATED

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A differential screening of an A. lumbricoides germ line DNA library was carried out in order to isolate DNA sequences specifically transcribed in early embryonic stages. First strand poly A^+ cDNA from 4-cell embryos was tested against L1 larval stages. Several positive clones were obtained crosshybridizing only with RNA from 4-cell embryos. One of them, λ 94121, is of special interest, because it contains a single copy sequence shown to be eliminated from somatic cells during the process of chromatin diminution. In Northern blot experiments with total RNA from 4-cell embryos, a subclone of λ 94121 hybridizes to two bands, corresponding to RNA molecules of 400 and 800 b length. Moreover, clone λ 94121 crosshybridizes to three single copy DNA bands within the germ line genome of Parascaris equorum, which become also eliminated from the somatic cells during chromatin diminution.

P2 107

THE EXPRESSION OF ACTIN ISOFORMS AND DESMIN DURING IN VITRO MYOGENESIS

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The use of cytoskeletal proteins, e.g actin isoforms and intermediate filaments proteins as differentiation markers is well established. During *in vitro* differentiation of striated muscle cells, myoblasts (mononucleated cells) proliferate and synthesize β- and γ-cytoplasmic actins; then they fuse to form multinucleated myotubes, elaborate functional myofibrillar sarcomeres and express α-skeletal and α-cardiac actins. Desmin is expressed in myoblasts and myotubes where it is organized around Z bands. *In vivo* myogenesis is characterized by a switch from an α-cardiac actin predominance to an α-skeletal actin predominance. Recent studies have shown that α-smooth muscle (α-sm) actin, an isoform characteristic of smooth muscle cells, is present in sarcomeres of rat foetal skeletal muscles. To investigate the time course of appearance of actin isoforms and desmin in *in vitro* myogenesis, we have plated myoblasts isolated from new born rat skeletal muscle and studied the expression of cytoskeletal proteins by immunocytochemistry with three antibodies specific for: 1) α-sm actin, 2) α-striated (α-sr) actin i.e recognizing the α-skeletal and α-cardiac actins, 3) desmin. Before fusion myoblasts are positive either for α-sm actin or for desmin or for both; just after fusion, stress fibers of myotubes are positive for α-sm actin, α-sr actin and desmin; then α-sm actin gradually disappears when α-sr actin and desmin are organizing into sarcomeres. Therefore, α-sm actin is expressed transiently during *in vitro* myogenesis. These results suggest that α-sm actin plays a role in sarcomere organization.

P2 108

MODULATION OF GELSOLIN IN QUIESCENT AND PROLIFERATIVE RAT AORTIC SMOOTH MUSCLE CELLS

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Gelsolin is a Ca^{2+} and polyphosphoinositide regulated modulator of actin polymerization. Cytoplasmic gelsolin has been first described in highly motile cells such as leukocytes, where actin polymerization is very dynamic; however aortic smooth muscle cells (SMC), despite their highly stabilized actin bundles, express high levels of gelsolin. Actin isoform expression in SMC has been extensively studied in our laboratory in three models: development, intimal thickening after experimental endothelial injury and growth in culture. Gelsolin modulation was investigated in these models by immunohistochemistry, Western blotting and Northern hybridization. When related to the protein and mRNA contents of adult quiescent SMC, gelsolin is expressed about 50% in SMC of 5-day-old rats, 20-30% in migrating and replicating SMC in intimal thickening 15 days after endothelial injury and in SMC growing in culture, and reacquires the control level in intimal thickening 60 days after injury (at which time SMC are back to quiescence). The high content of gelsolin in SM tissues and the down regulation with proliferation and migration raises the question as whether gelsolin in these cells has other functions than the control of actin filament length. The similar modulation patterns of gelsolin and α-SM actin suggest a preferential interaction between these two proteins.

P2 109

MYOSIN LIGHT CHAIN EXPRESSION AND SKELETAL MUSCLE FIBER TYPES

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Muscle fiber types are usually distinguished by the myofibrillar ATPase stain, reflecting the myosin heavy chain isoform content. We are looking into the fiber specific isoform distribution of additional myofibrillar proteins. In a first study, the expression of "fast" myosin alkali light chains LC_1f and LC_3f, which are encoded on the same gene, was investigated at the RNA level by *in situ* hybridization on cryostat sections of chicken and human muscles. In the six chicken leg muscles studied and in human *m. vastus lateralis*, LC 1f and LC 3f mRNA occurred at about equal levels in the fast (type IIA and IIB) fibers. In the slow (type I or type III) fibers, LC_1s and LC_2s are normally the major light chains, LC 1f and LC 3f mRNA , however, were detected at variable levels. In chicken *m. gastrocnemius* and *m. adductor medialis*, they were very low, close to background; in chicken *m. semitendinosus*, the LC 3f mRNA level was very low, the LC 1f mRNA level was intermediate; whereas in the innermost part of chicken *m. sartorius* and in human *m. vastus lateralis*, quite surprisingly, no differences in LC_1f and LC_3f mRNA-levels in fast and slow fibers exist. It is concluded that the expression of myosin LC 1f and LC 3f does not strictly parallel the fiber type reflected by the myosin heavy chain isoform.

P2 110

EXPRESSION OF LAMIN AND rRNA GENES IN ALL CELLS

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Assuming that in human acute lymphoblastic leukemia (ALL) clonal proliferation inducing regulators might have pleiotropic effects interfering with the differentiation of B and T lymphocyte precursors, we are interested in assessing cellular processes essential for differentiation. We therefore analysed the expression of the nuclear lamin genes and the pre-rRNA cleavage pathways using Northern and Southern blotting techniques. The nuclear lamina is thought to provide nuclear envelope stability and interphase chromatin organization. In higher vertebrates it is composed of three nuclear lamins forming a polymeric meshwork closely apposed to the nucleoplasmic surface of the inner nuclear membrane. First results indicate that lamin A and C mRNAs are only expressed in common ALL cells, i.e. a very early differentiation stage of the B-cell lineage. Concerning the pre-rRNA cleavage pathways preliminary results indicate that ALL cells do not follow the so called "HeLa" rRNA processing pathway.

P2 111

THE MOLECULAR BASIS FOR PANCREAS-SPECIFIC GENE EXPRESSION

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Genes encoding the abundant products of the acinar pancreas of rodents contain, within their enhancer regions, a conserved sequence motif which interacts *in vitro* with the same cell-specific DNA-binding activity. The cognate sequences for this factor enhance transcription *in vivo* in a cell-specific manner. This identifies the nuclear protein as a transcription factor (PTF 1) and suggests that the expression of genes expressed in the pancreas is subject to regulation by a common mechanism. During mouse development, PTF 1 DNA-binding activity is detected for the first time at the stage when pancreas-specific promoters are activated. This observation suggests a key role for PTF 1 during pancreas differentiation. PTF 1 has been purified by affinity column chromatography and its interaction with DNA has been studied by site-directed UV-crosslinking. PTF 1 belongs to a novel class of eukaryotic transcription factors. It is a heteromeric oligomer consisting of 48 kd and 65 kd subunits both of which interact with DNA.

P2 112

MITOCHONDRIAL PROLIFERATION IS AN EARLY PROCESS IN MORPHOLOGICAL DIFFERENTIATION OF MASTOCYTOMA CELLS

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Proliferation of a cold-sensitive cell-cycle mutant isolated from an undifferentiated murine mastocytoma line is reversibly arrested at the nonpermissive temperature of 33°C, and the arrested cells undergo morphological differentiation as expressed by the formation of metachromatic granules. Following transfer of these mutant cells from the permissive temperature of 39.5°C to 33°C, a transient increase in both cytochrome c oxidase and DNA polymerase γ was observed, and the ratio of total mitochondrial volume to cell volume nearly doubled within 6 days, while numbers of mitochondrial cross-sections per cellular cross-section and inner mitochondrial surface area per cell as determined in electron micrographs increased 3- to 4-fold. Addition of chloramphenicol (100 µg/ml) to the mutant cell cultures 6 days prior to transfer from 39.5°C to 33°C prevented the increase in the ratio of total mitochondrial to cell volume. Furthermore, chloramphenicol markedly inhibited the increase in granule number per cell that normally is observed after transfer of cultures to 33°C, suggesting that mitochondrial proliferation may be an obligatory step in the process of morphological differentiation of these mastocytoma cells.

P2 113

EFFECTS OF NGF-INDUCED DIFFERENTIATION ON TWO CLASSES OF Ca CHANNELS IN LIVING PC 12 CELLS

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We have used PC 12 rat pheochromocytoma cells as a model system to study the role of Ca channels during neuronal differentiation. Undifferentiated PC 12 cells have a low density (7 fM//10^6 cells) of voltage-dependent L-type Ca channels that could be labelled by ^{3}H-(+)-isradipine. In the same cells the density of specific binding sites for ^{125}I-ω-Conotoxin G VI A (ω-CgTX) was 10 - 15 fM/10^6 cells. ω-CgTX is known to label both, L- and N-type neuronal Ca channels (McCleskey et al. PNAS 84, 4327, 1987). Treatment with 100 ng/ml NGF for 4 days in culture caused morphological and biochemical differentiation of PC 12 cells into neuron-like cells. This process was associated with a significant 2-fold increase of specific ω-CgTX binding, whereas isradipine binding remained essentially unchanged. NGF-independent differentiation of PC 12 cells was not accompanied by a change in Ca channel density. We conclude that NGF induces specifically the formation of new N-type Ca channels.

P2 114

LOCALIZATION OF MICROTUBULE-ASSOCIATED PROTEINS AND THEIR mRNA IN THE DEVELOPING BRAIN

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Neuronal microtubule-associated proteins (MAPs) are developmentally regulated, and can be categorized as either "early" or "late". We have used MAP-specific monoclonal antibodies and cDNA probes to localize the "early" MAPs in the developing brain via immunohistochemistry and in situ hybridization. Low-molecular weight MAP2 is found in developing axons and in glia, unlike high-molecular weight MAP2 (HMW-MAP2), which is found only in dendrites. MAP5 is consistently found in extending processes, suggesting a role for this protein in neurite outgrowth. The developmental regulation and distribution of the major MAPs have been confirmed by in situ hybridization. Like the protein, the mRNA for HMW-MAP2 is concentrated in developing dendrites. This is the first description of a specific RNA transcript within a developing neuronal process, and it suggests that the local, immediate regulation of MAP2 synthesis is fundamental to dendrite development.

P2 115

DETECTION OF GLIA-DERIVED NEXIN IN THE OLFACTORY SYSTEM OF THE RAT

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Specific polyclonal and monoclonal antibodies have been raised against glia-derived nexin (GDN), a 43kd cell-secreted protease inhibitor with neurite promoting activity. Immunohistochemistry shows that GDN is abundant in the primary olfactory projection. The strongest staining is seen in the olfactory nerve layer of the olfactory bulb and in the submucosa, whereas a weaker staining is detected in the olfactory epithelium. In situ hybridization with a GDN cDNA probe shows that not only GDN but also its mRNA is most abundant in the olfactory nerve layer of the olfactory bulb. This suggests that GDN is synthesized by non neuronal cells which are in close contact with the olfactory nerve. In primary cultures of olfactory epithelium and submucosa GDN positive cells are also stained with antibodies to the Schwann cell marker S-100. The secretion of GDN by non neuronal cells in the olfactory system may be related to the unique degeneration and regeneration phenomena taking place in this system.

P2 116

NUCLEAR DNA CONTENT OF CULTURED ENDOTHELIAL CELLS

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Venous endothelial cells (VECs) can produce in vitro an athrombogenic inner surface of small-diameter vascular prostheses. Transformed human cell lines usually have chromosome numbers in the subtetraploid range. Therefore, the aim was to detect, in serially subcultured VECs, increasing nuclear populations with stable subtetraploid (XC) DNA content using DNA cytophotometry. Such a population was not observed in confluent VEC cultures of human umbilical cord, however populations with tetra- (4C) and octoploid (8C) DNA content. Similarly, no stable XC populations occurred in confluent cultures of canine VECs. The percentage of individual XC or 4C DNA containing nuclei was 15 to 30 % in human VEC cultures, in contrast to 3 to 5 % with canine VECs. Transformation as indicated by stable subtetraploid populations, could be excluded up to 27 or 8 passages for human or canine VECs, respectively.

P2 117

CELL LINEAGE ANALYSIS IN THE AVIAN NEURAL CREST

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The neural crest (NC) is the origin of many differentiated cell types including neurons and glial cells of the peripheral nervous system, endocrine cells, melanocytes and mesectoderm. This raises the question to know whether the NC includes cells possessing all these potentialities or precursors for each particular lineage. We have investigated the developmental potentials (proliferation and differentiation) of individual quail mesencephalic NC cells by cultivating them under permissive conditions. We show that NC cells express highly heterogeneous proliferation and differentiation abilities when cultivated in identical conditions. In particular we report the existence of 1) a progenitor giving rise to virtually all the cell types we are able to identify, i.e. different kinds of neuronal and non-neuronal cells, melanocytes and cartilage, 2) a common precursor for neurons and Schwann cells, 3) a precursor for non-neuronal cells only. These results allow us to propose a tentative scheme of the filiations that could exist between the different types of progenitors observed.

P2 118

CULTURE OF MOUSE NEURAL CREST CELLS IN A CHEMICALLY DEFINED MEDIUM.

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To study the neural crest (NC) cells of mouse embryos, we made use of *in vitro* techniques. The very low yield of NC cells obtained from a single neural tube in culture, makes it difficult to work with. Therefore we tried to modify the culture conditions in order to stimulate the multiplication of the undifferentiated NC cells. Since NC cells don't migrate out of the neural tube when directly placed in serum-free, chemically defined medium (SFM), E8.5 truncal neural tube explants are cultured in MEM supplemented with Ultroser G. The NC cells are allowed to migrate in successive waves onto the culture dish. After 48-60 h, the neural tube is removed and the NC cells are further cultured in SFM. The culture conditions we developed promote not only cell division, but also differentiation of the crest cells into the neuronal pathway. Neuronal phenotype was identified by indirect immunocytochemistry with anti-neurofilament monoclonal antibodies. Cells with the malic enzyme null mutation were used in combination with wild-type cells to distinguish two populations of outgrowing NC cells. The proliferating and finally differentiating cells seem to originate from the second wave of migrating NC cells.

P2 119

CYTOPLASMIC MALIC ENZYME AS CELL MARKER IN THE STUDY OF MOUSE NEURAL CREST CELL DIFFERENTIATION *IN VITRO*

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When E8.5 mouse truncal neural tube explants are cultured in MEM supplemented with fetal calf serum, the neural crest cells migrate in successive waves to form several differentiated structures after 1-2 weeks. We distinguish four concentric cell layers around the mass of the neural tube. In addition, ganglion-like structures, neural-like processes and more rarely premelanocytes develop, mainly on the epithelial-like sheet of the second concentric area. In an attempt to sort out which structure derives from which initial cell type, we combined wild-type malic enzyme positive (ME^+) cells with ME^- ones, recessive for the null mutation *Mod-1*^{n}. At the end of the culture period, a ME specific staining is performed to determine the origin of each cell. Some differentiation pathways are also characterized by the detection of specific end-products (neurofilaments, catecholamines, tyrosinase, etc).

P2 120

SYNTHESIS OF BOTH uPA AND tPA BY MOUSE NEURAL CREST CELLS : AGREEMENT BETWEEN *IN VITRO* AND *IN SITU* RESULTS ?

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Neural tubes of E8.5 mouse embryos were dissected and cultured for 48 h in Ultroser G supplemented medium, allowing neural crest cell (NCC) emigration. The neural tubes were then removed and NCC cultured for 12 h in serum free medium. The supernatant of the culture was run on SDS-PAGE and a zymography was performed to detect the presence of both urokinase- and tissue-type plasminogen activators (uPA and tPA). Ten um thick cryostat sections of stage 13-14 (8-20 somites) mouse embryos were hybridized with antisense S^{35}-RNA probes to the mouse uPA or tPA mRNA. *In vitro*, NCC produce more uPA than tPA with an important increase of uPA secretion by the cells which emigrated out of the neural plate area, near the Hensen's node. *In situ*, no signal for the presence of uPA mRNA was detected in the E8.5 embryos, whereas the tPA mRNA was found throughout the embryo, with areas showing higher signal depending on the level of the section. In the later stages, uPA appears in cells where NCC are expected to be localized and tPA is detected in specific regions of organogenesis.

P2 121

IGF-2-MEDIATED MOTONEURON SPROUTING: THE DIFFUSIBLE SPROUTING ACTIVITY IN INJURED MUSCLE ?

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Partial denervation or paralysis of mammalian skeletal muscle results in massive sprouting from intact neighboring motoneurons, a reaction which might initiate reinnervation. Such intramuscular sprouting has been shown to be due to a diffusible activity released by the inactive muscle fibers. Recently, insulin-like growth factor II (IGF-2) has been shown to promote sprouting of sensory neurons in vitro. In addition, IGF-2 mRNA in muscle correlates with neurite growth phases: IGF-2 mRNA declines rapidly at the onset of synapse elimination and is rapidly and transiently induced by denervation. A possible direct involvement of IGF-2 in motoneuron sprouting was therefore investigated. The following findings are interpreted as being consistent with a direct physiological role of IGF-2 in intramuscular sprouting:1.) rapid and massive stimulation of neurite growth from purified motoneurons by IGF-2; half-maximal effect by 0,2 nM IGF-2; activation probably due to an IGF-1-type of receptor; 2.) direct high-affinity and specific binding of IGF-2 by the fine processes of motoneurons in vitro (identified by retrograde labeling); 3.) induction of intramuscular sprouting in intact adult mouse gluteus muscle by non-lesioning subcutaneous injections of low doses of IGF-2.

P2 122

OLIGODENDROCYTES INHIBIT NEURONAL GROWTH CONE MOVEMENT

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We have used high resolution video time lapse microscopy to analyze the in vitro interaction of growth cones of newborn rat dorsal root ganglia with dissociated young rat CNS glial cells. To provide optimal conditions for neurite outgrowth cocultures were grown on laminin and in NGF supplemented L15 medium. Observations of 24 growth cone - glial cell encounters revealed a drastic difference in the growth cone behavior upon filopodial contact with CNS glial cells:
- growth cones maintained their normal configuration and velocity (10-18um/hr) when growing along or on top of astrocytes - in contrast, repeated filopodial contact with oligodendrocytes resulted in a permanent arrest of the growth cone mobility, often followed by a retraction This contact inhibition phenomenon was exclusively found with oligodendrocytes and could be prevented by antibodies IN-1 against oligodendrocyte-associated, neurite growth inhibiting membrane proteins (Caroni and Schwab, Neuron 1: 85-96).There defined proteins (35 and 250 kD) of oligodendrocytes and myelin may be crucial for the lacking nerve fiber regeneration in the adult CNS.

P2 123

BRAIN MACROPHAGE MIGRATION IN CNS TISSUE IN VITRO, INVOLVEMENT OF A METALLOPROTEASE.

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Differentiated oligodendrocytes and CNS white mater have strong inhibitory substrate properties for neurite growth and cell migration (Schwab and Caroni, 1988, J.Neurosci. 8: 2381-93). Despite this fact malignant rat C6 glioblastoma cells infiltrate optic nerve explants, adhere and spread on cerebellar white matter, on oligodendrocytes and CNS myelin. A metalloproteolytic activity was shown to be involved in this infiltrative behaviour of C6 cells (Paganetti et al., 1988, J.Cell Biol., 107: in press). In the CNS resident brain macrophages are activated by various stimuli. These cells are involved in immune functions and in the phagocytosis of cell debris. We show now that isolated brain macrophages overcome the inhibitory substrate properties of CNS myelin, and that this occurs by means of a metalloproteolytic activity very similar to that of C6 cells. This activity is specifically blocked by o-phenanthroline and the dipeptides cbz-GF-amide or cbz-YY and might be crucially involved in the migratory behaviour of activated brain macrophages in the CNS.

P2 124

Na,K-ATPASE GENE EXPRESSION IN XENOPUS OOCYTES AND EMBRYOS

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Ouabain-sensitive sodium transport in Xenopus laevis is carried out by the plasma membrane Na,K-ATPase made up of an α (98 kDa, catalytic) and a β (49 kDa, glycoprotein) subunit. In adult tissues, α and β subunits are synthesized and assembled in a stoichiometric and coordinated manner. Interestingly, at stage VI oocyte, we observe a much lower level of β subunit synthesis than of α subunit synthesis. By Northern blot and primer extensions analysis, we can show that a vast excess of αI mRNA over β mRNA is detected, ruling out the possibility of a large untranslatable β mRNA pool. Since Na,K-ATPase activity resumes upon fertilization and is expected to increase upon formation of the blastocoele, we have followed the level of α and β mRNA through early development. β mRNA is much less abundant than α mRNA but the α/β ratio decreases at the morula and the neurula stage suggesting that the β subunit synthesis might be the limiting factor in Na,K-ATPase assembly.

P2 125

INTERRELATIONSHIP BETWEEN NERVOUS AND MUSCULAR TISSUE IN TESTICULAR TERATOMA OF STRAIN 129/Sv-ter MICE

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In strain 129/Sv-ter mice teratomas grow spontaneously. We investigated the interrelationship between nervous and muscular tissues in teratomas of 4-week-old 129/Sv-ter mice. The muscle fibers appeared normal. They contained one end-plate per fiber and were probably innervated by a single motoneuron. However, the differentiation into distinct fiber types was only partial. This was probably due to lacking functional stimulus. While both myofibrillar ATPase and sarcoplasmic AChE activity displayed a largely uniform distribution, the amount of cytochrome c oxidase as well as that of the Ca^{2+}-binding protein parvalbumin varied considerably among different muscle fibers. Experimentally induced muscle cells in teratocarcinoma-derived cell lines remained embryonal in the absence of nervous tissue. Maturation of skeletal muscular tissue in mouse teratoma is thought to be dependent on innervation. In human testicular teratomas skeletal muscle tissue is also occasionally found. We assume that this differentiated tissue might be similarly innervated as is the case in mouse testicular teratomas.

P2 126

TERMINAL NEUTROPHILIC DIFFERENTIATION OF HL-60 CELLS LEADS TO INCREASED EXPRESSION OF A PROTEIN OF THE CARCINOEMBRYONIC ANTIGEN FAMILY.

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Normal human granulocytes (GR) express a cell surface glycoprotein (NCA-95) belonging to the carcinoembryonic antigen (CEA) family (Buchegger et al, Int. J. Cancer, 1984). The recent cloning of CEA and NCA proteins has confirmed their close homology and revealed their relationship to other cell surface glycoproteins of the IgG superfamily (cell adhesion molecules, some growth factor receptors, etc.). Using a specific monoclonal antibody (AK-47) we have studied the expression of NCA-95 on the surface of GR and of the human promyelocytic leukemia cells HL-60. Scatchard analysis of equilibrium binding of ^{125}I-labelled AK-47 has shown that NCA-95 sites on GR vary to a large extent depending on the blood donor (20 000 - 100 000 sites/cell) and that HL-60 cells express less NCA-95 (about 6 000 sites/cell). Treatment of HL-60 cells with 1.25 % DMSO for 7 days, which leads to terminal neutrophilic differentiation of these cells, results in a 4 fold increase in NCA-95 on their cell surface. This system will be used to investigate further the regulation of NCA-95 expression on normal and leukemic myeloid precursor cells.

P2 127

REGULATION OF MOUSE HISTONE GENE EXPRESSION BY RNA 3' PROCESSING

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The tight coupling of histone gene expression to DNA replication is known to be controlled by three mechanisms: transcription, RNA 3' processing and mRNA stability. We have previously shown RNA processing to play a predominant role under conditions where cells are growth-arrested in G1 or G0. We have now analysed its importance during the cell cycle proper. In cells synchronized by thymidine block, we did not detect any fluctuations of processing activity. In contrast, in CHO cells synchronized by mitotic shake-off, a SV40/histone H4 fusion transcript, known to be regulated exclusively at the level of histone-specific RNA processing, accumulates between mitosis and S-phase. Since mitotic shake-off is the more efficient and physiological method, we conclude that histone RNA processing plays a role in regulating histone mRNA levels during the cell cycle. However, the complete elucidation of how the three different mechanisms co-operate in the control of histone gene expression during the cell cycle requires further investigation.

P2 128

SPLICING OF MESSENGER RNA PRECURSORS IN VITRO

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The removal of introns from nuclear messenger RNA precursors (pre-mRNA) can be separated into multiple steps in vitro. First, the RNA is assembled into a multicomponent complex, the spliceosome. Within this complex, the pre-mRNA is cleaved at the 5' splice site, a reaction that gives rise to the splicing intermediates. The RNA is then cleaved at the 3' splice site, the exons are ligated, and the final reaction products are released from the spliceosome.

We are studying these reactions in nuclear extracts or chromatographic fractions from Hela cell nuclei. Assembly of the complexes involves the sequential binding of the major U-type snRNPs and at least two protein factors to specific regions within the intron. Additional proteins are necessary for the subsequent cleavage and ligation reactions. The sequence requirements of spliceosome assembly and splicing itself are analyzed with mutant RNA substrates or a chemical modification/interference method that allows the detection of single nucleotides essential in the different reaction steps.

P2 129

Characterisation of mouse U7 snRNPs involved in 3' processing of histone pre-mRNAs.

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The U7 snRNP, an essential cofactor of histone RNA 3' processing , is immunoprecipitable with both anti-Sm antibodies and antibodies directed against the 2,2,7-trimethyl-guanosine (m3G) cap stucture. By affinity chromatography with anti-m3G antibodies, snRNPs can be highly enriched from nuclear extracts of mammalian cells. Further chromatographic fractionation by anion exchange resin (Mono Q), followed by a U7-specific affinity medium should yield essentially pure U7 snRNPs. Such a preparation will be used to study the protein composition of U7 snRNPs and the requirement of other factors for the processing reaction.

A second approch for elucidating the structure and function of U7 snRNPs consists in developing faithful snRNP reconstitution systems. Incubating U7 RNAs with nuclear extracts produces several distinct high molecular weight complexes. One of them comigrates with native U7 snRNPs on a non-denaturing gel. We are characterising these complexes according to several criteria and testing other sources of snRNP proteins for the reconstitution, such as mammalian cytoplasmic and Xenopus egg extracts.

P2 130

TRANS-SPLICING OF A CHLOROPLAST mRNA: A MOLECULAR AND GENETIC APPROACH IN CHLAMYDOMONAS REINHARDTII.

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In *C. reinhardtii*, the three exons of the *psaA* gene are widely scattered on both strands of the chloroplast genome where they encode a reaction center polypeptide of photosystem I. We have isolated numerous choroplast and nuclear mutants that are affected in *psaA* mRNA maturation. They can be grouped in three general classes: class A mutants are able to splice exons 1 and 2, but fail to join exons 2 and 3. Conversely class C mutants fail to join exons 1 and 2. Class B mutants are blocked in both steps and accumulate three separate transcripts. Thus the three exons are transcribed independently as separate precursors which are then normaly assembled *in trans*. The nuclear mutations probably affect factors required for the maturation of *psaA* mRNA. While some of the chloroplast mutations may act *in cis*, there is also at least one chloroplast locus (*coc-1*) which is required *in trans*. With the help of biolistic (particle gun) transformation, we are now localizing the *coc-1* gene in order to define its product.

P2 131

PARAMYXOVIRAL RNA EDITING YIELDS ADDITIONAL PROTEINS AND IS PROBABLY COTRANSCRIPTIONAL

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The measles virus (MV) phosphoprotein (P) gene encodes two known proteins, P (M_r ~70,000), involved in viral transcription and, in a different reading frame, C (M_r ~20,000). By a combination of cDNA cloning, cDNA and RNA sequencing, and *in vitro* translation, we demonstrate here that the MV P gene also expresses a third product (M_r ~46,000) containing the amino-terminal region of P but a different, cysteine-rich carboxyl-terminal motif. This third protein is translated from mRNAs in which one G residue has been inserted after three genomically encoded Gs, a modification found in about 50% of the P mRNAs. A smaller fraction of transcripts contains several additional Gs. We suggest that intragenic nucleotide addition in MV and other paramyxoviruses is cotranscriptional, and that it is due to predisposed stuttering of the polymerase on genomic RNA.

P2 132

POLY(A) LEADERS IN VACCINIA VIRUS LATE mRNAS

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We have analyzed the vaccinia virus late mRNA encoding a 37Kd polypeptide. Primer extension and cDNA cloning demonstrated that this mRNA contains a poly(A) leader sequence which is not encoded in the viral DNA. S1 analysis of the mRNA transcribed in vivo or in vitro showed that the poly(A) leader consists of about 35 residues. We have previously reported a similar structure for another late mRNA encoding an 11Kd polypeptide. In some 11Kd-specific cDNA clones, however, we had also found additional sequences upstream of the poly(A) tract. Electron microscopy of the 37Kd mRNA provided no evidence for the presence of such additional sequences. Re-examination of the 11Kd mRNA showed that the primer extension products vary according to the source of reverse transcriptase used. This strongly suggests that the chimeric structures of the 11Kd mRNAs described previously represent a cDNA artifact. This is consistent with the discrete length 11Kd mRNAs obtained from a recombinant virus carrying a transcription termination sequence downstream from the 11Kd gene and thus producing defined 3'ends.

P2 133

An intron fragment of a Xenopus homeobox-containing gene contains a tissue-specific enhancer-like element which is functional in *Drosophila*

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Homeodomain-containing proteins may bind to regulatory elements in their own genes and in other homeobox-containing genes. We are interested in determining whether the sequences to which they bind have been conserved during evolution. The Xenopus X1Hbox7 and X1Hbox2 genes are two closely linked homeobox-containing genes. They are both expressed in the embryonic spinal cord. A *lacZ*-X1box7 fusion protein specifically binds *in vitro* to a 154 bp Sau3A fragment, which is located in an intron of the X1Hbox7 gene, about 1 kb upstream of the transcriptional start site of the X1Hbox2 gene. Two copies of the Sau3A fragment were cloned in front of a *lacZ* hybrid gene in which the ß-galactosidase expression is under the control of a basal promoter. This construct was introduced into the *Drosophila* genome by P-element mediated transformation. *lacZ* expression in embryos of three independent transformed lines is restricted to cells of the central and peripheral nervous system. A DNaseI protection analysis indicates that there are three binding sites within the Sau3A fragment for a 68 amino acid protein that basically consists of the *Antennapedia* homeodomain. These results suggest that the interaction of homeodomain-containing proteins with sequences within the Sau3A fragment may be responsible for the observed tissue-specific gene expression.

P2 134

HSP18 IS A I-18C GENE PRODUCT OF *CHIRONOMUS TENTAS*

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By differential processing a gene from the early ecdysterone-inducible puff site I-18C of *C. tentans* gives rise to various transcripts (1.8 kb, several approx. 4.6 kb, and 6.5 kb) having the same 5' end. Several heat shock responsive elements occur in the promotor of the gene. Northern blot analysis indeed proved the heat-inducibility of the unspliced 1.8 and 6.5 kb RNAs. This inducibility is developmentally regulated. The spliced 4.6 kb RNAs do not show the same induction pattern, neither during normal development nor after heat shock, suggesting a heat shock effect on splicing. The 1.8 and the 6.5 kb RNAs contain an open reading frame (ORF1), which is spliced out in the 4.6 kb RNAs. Immunoprecipitation experiments with antibodies raised against an ORF1-derived peptide indicate that the cellular protein encoded by ORF1 is 18 kD, very basic, and heat-inducible. By two-dimensional gel electrophoresis and V8 peptide mapping the identity of this protein with hsp18 of *C. tentans* is demonstrated.

P2 135

3' ENDFORMATION OF MESSENGER RNA PRECURSORS IN VITRO

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We have separated and purified three factors from HeLa cell nuclei that together can accurately cleave and polyadenylate pre-mRNAs containing the adenovirus L3 or the SV40 late polyadenylation signal. One of the factors is a poly(A) polymerase (PAP) with a molecular weight of 50 kd. The second is a cleavage factor (CF) with a molecular weight of 70 - 120 kd. The third component is a cleavage and polyadenylation factor (CPF) with a molecular weight of 200 kd.

In the presence of manganese ions PAP is able to polyadenylate RNA unspecifically. However, in the presence of magnesium, PAP depends on CPF to specifically polyadenylate pre-mRNAs that contain an intact AAUAAA-sequence and end at the poly(A) addition site. PAP in combination with CF and CPF is also required for the correct cleavage at the poly(A) site of the pre-mRNA. Since the two activities of the PAP fractions, poly(A) polymerase and cleavage activity, could not be separated by extensive purification, we conclude that the two activities reside in a single component. Extensive purification of CPF demonstrates that CPF activity resides in a ribonucleoprotein and copurifies with U11 snRNP.

P2 136

PRODUCTION OF VIRAL RNAS IN HAV INFECTED CELLS

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Adaptation of hepatitis A virus to cell culture usually leads to the establishment of persistent infection. Such a virus/cell relationship might be caused by the presence of defective interfering particles (DIP). Indeed, DIP with different distinct deletions in their genome could be demonstrated in various HAV isolates, cell culture systems, and after a varying number of in vitro passages. To test at which level defective vRNAs interfere with replication of genomic vRNA, the appearance and presence of various species of vRNAs during the replication cycle of HAV was examined. For this purpose production of total vRNAs, genomic RNA, and of molecules with internal deletions was measured in two cell culture systems infected at different multiplicities. The quantity of genomic vRNA oscillated througout the period of observation. Defective RNAs could always be demonstrated, peak amounts usually being present after genomic RNA reached peak concentrations.

P2 137

A COMPLEX SPLICING PATTERN AND MULTIPLE PUTATIVE PROMOTER SEQUENCES IN THE B-CREATINE KINASE GENE OF CHICKEN

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In higher vertebrates most tissues with a high energy consumption express one or more members of the Creatine Kinase isoenzyme family. The CK isoforms have multiple tasks in the cellular energy buffering system. The ubiquitous embryonic or brain-type isoprotein B-CK is expressed either together with or without the mitochondrial analogue Mi-CK. Two B-CK subspecies, Ba- and Bb-CK have been described, which both show additional heterogeneities due to different posttranscriptional processes (see poster T. Soldati et. al.). According to this, a heterogeneity on the transcript level has been found by cDNA cloning. Three distinct B-CK like cDNAs, H4, 18c and 18b have been sequenced. While the 5'-ends of the full length clone H4 and 18c are different, they are identical from codon 65 to their 3'-ends. As shown by "in vitro" translation of synthetic RNA; H4-transcripts give rise to Bb-CK and a construct of the 18c specific 5'-terminus with the H4 derived 3'-end codes for a CK not distinguishable from Ba-CK. These B-CK transcripts arise by a complex splicing mechanism from a single gene. Several putative promoter sequences have been observed, the most perfect ones between exon 1 and exon 2, others in 5' of exon 1 with noncanonical TATA boxes. In addition there is also a potential polymerase III dependent promoter element.

P2 138

RAPID ASSOCIATION OF MICROINJECTED SEMLIKI FOREST VIRUS (SFV) CAPSID (C-)PROTEIN WITH THE NUCLEUS OF THE TARGET CELL

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As shown elsewhere, purified C-protein of SFV microinjected into various target cells affects protein synthesis in a pleiotropic way (Elgizoli et al., manuscript submitted). Small amounts (10^3 to 10^4 copies per cell) induce the synthesis of specific M_r classes of cellular proteins, whereas high amounts (10^5 to 10^6 copies per cell) act as a general synthesis inhibitor. Here we show that similar to induction, repression of protein synthesis lasted only for about 2 h after delivery of C-protein which was followed thereafter by an unexpected, short lived induction of protein synthesis. Evidence is presented showing that microinjection of C-protein resulted in its rapid association with the nucleus at the expense of C-protein present in the cytoplasm. The implications of these findings are discussed.

P2 139

CHARACTERISTICS OF HEPATITIS A VIRUS PARTICLES RELEASED BY SEVERAL CELL LINES

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Earlier reports describe a variety in number, buoyant density and sedimentation characteristics of HAV particles produced in cell culture. We cultivated HAV in different cell lines (BGM, BSC-1, FRHK, MRC-5 and VERO) and analysed virus particles by CsCl/sucrose gradients followed by sedimentation through sucrose gradients. Comparison of sedimentation profiles showed that all cell lines except BSC-1 cells produced the same particle species with sedimentation coefficients of 156 S (mature virus), 130 S (defective virus), 76 S and 59 S (heavy and light capsids), respectively. We suppose that the 59 S particles are a decomposition product of 76 S particles, because after freezing at -20° C and RNase digestion most of the 76S particles sedimented at 59 S. Slot blot cDNA-RNA hybridization experiments revealed that both forms contain traces of RNA. In light of this fact, we suppose that no RNA-free capsids are released in HAV-infected cells.

P2 140

TRANSLATION INITIATION IN SACCHAROMYCES CEREVISIAE

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Initiation of translation is a complex chain of biochemical reactions and the regulation of these reactions contributes to overall regulation of gene expression. Several lines of evidence indicate that S.cerevisiae is a valid model system to study eukaryotic translation initiation. Among them is our recent demonstration that the mouse translation initiation factor eIF-4E which recognizes the 5' end of eukaryotic cytoplasmic mRNA can substitute for its yeast homolog in vivo. To elucidate the molecular mechanism of translation initiation in S.cerevisiae we have created temperature-sensitive mutations in initiation factor genes to study initiation factor function in vivo and in vitro. Recent experiments with mutant eIF-4E give insight into the function of the cap structure and cap binding proteins in initiation. Most notably, they show that a cap-independent initiation pathway may exist in S.cerevisiae.

P2 141

ANALYSIS OF THE TIF1 AND TIF2 GENES CODING FOR A TRANSLATION INITIATION FACTOR FROM THE YEAST S. CEREVISIAE

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The genes TIF1 and TIF2 from Saccharomyces cerevisiae were sequenced and found to be almost identical, producing two completely identical proteins. The Tif1/Tif2 protein is highly homologous to the translation initiation factor eIF-4A from mouse (65% identical) and strong evidence suggests that the TIF genes code for the yeast analogue of eIF-4A from mouse. The Tif protein also contains motifs conserved in other proteins from yeast and from other organisms such as mammals, Drosophila, amphibian and E. coli. These motifs are most likely involved in interaction with ATP and polynucleotides and define a new family of ATP-binding proteins (D-E-A-D-proteins). As expected for a translation initiation factor, the inactivation of both genes is lethal to the cell, whereas inactivation of either gene individually has no effect on growth.

We have constructed a conditional TIF1 gene under the control of a galactose promoter. A strain having this TIF gene as the only source for the initiation factor can grow on galactose-containing medium, but stops growing after a few generations if it is transferred to glucose-containing medium. We are using this conditional system to isolate temperature sensitive mutations in the TIF1 gene.

P2 142

NUCLEAR GENES FOR PROTEIN FACTORS INVOLVED IN THE ELONGATION STEP OF PROTEIN SYNTHESIS OF EUGLENA GRACILIS

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The biogenesis of the complex chloroplast proteosynthetic apparatus requires the coordinate expression of chloroplast and nuclear genes. This is also true for protein factors involved in the elongation step in case of Euglena, i.e., EFTu is chloroplast encoded and EFTs and EFG are of nucleo-cytoplasmic origin. The chloroplast gene (tuf) was sequenced and shown to be expressed as a 1.95 kb mRNA. The synthesis of EFTu and EF G seems to be light stimulated (Spremulli, 1982, ABB 214:734) but nothing is known about the coordinate and controlled expression of these genes. We started cloning nuclear genes coding for elongation factors using a poly A^+ mRNA derived cDNA library from Euglena gracilis, the aim being to eventually characterize the whole set of genes (chloroplast and cytosol specific) coding for catalytic components of the elongation steps. Results obtained sofar indicate considerable sequence similarity with the corresponding counterparts from other lower eukaryotes. Various DNA probes will be used to follow the dynamics of transcription during light induced chloroplast development.

P2 143

EFFECTS OF MUTATIONS AFFECTING THE TRANSLATIONAL READING-FRAME PATTERN OF THE TRANSPOSABLE ELEMENT IS1 OF E.COLI.

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The bacterial insertion element IS1 contains two essential translational reading frames, insA and insB, arranged in tandem. A translational initiation site has been identified for insA but not for insB (Zerbib & al., EMBO J. 6, 3163 (1987)). We have introduced several site-specific mutations into the region including the putative intergenic sequence and the putative insB initiation codon. A new transposition-cointegration assay was developed to determine the relative transposition frequencies of mutant and wild-type elements in vivo. The results indicate that translation of reading frame insB depends on initiation further upstream, involving either translation of its 5'-extension (insB') or a translational (-1) frameshifting event within the 3'-terminal region of insA.

P2 144

PAIRED GENE EXPRESSION DURING *DROSOPHILA* EMBRYOGENESIS

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The *paired* (*prd*) gene of *Drosophila* is a segmentation gene of the pair-rule class. It encodes a protein of 67 kD whose amino terminal consists of a homeo- and a paired-domain. Using immunofluorescence staining of *Drosophila* embryos, we show that the *prd* protein is localized in the nucleus and that its pattern of expression corresponds to that of its RNA. The protein is expressed during two phases. The first phase is initiated by the appearence of a single anterior stripe during cellularization of the syncytial blastoderm. A periodic pattern is then elaborated through a dynamic process resulting in the appearence of seven stripes, each about six nuclei wide and separated by two unlabeled nuclei. When all seven stripes have acquired about the same intensity, the pattern further evolves, resulting in a pattern of 14 stripes at the onset of gastrulation. This first phase ends at the fully extended germ band stage when the *prd* protein has disappeared. During a second phase of expression, which does not exhibit a spatially repeated pattern, the *prd* protein is detected in patches of cells of the labial and maxillary lobe and in the clypeolabrum and persists in a few cells almost to the end of embryogenesis.

P2 145

INTERNAL INITIATION OF TRANSLATION ADDS TO PHYSIOLOGICAL PROTEIN DIVERSITY IN HIGHER EUKARYOTES.

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In higher eukaryotes three different types of creatine kinases (CK) are expressed: the muscle specific M-CK, the ubiquitous cytoplasmic B-CKs and the mitochondrial Mi-CKs. They fulfill multiple tasks in cells with an intensive energy metabolism. Isolated B-CK can be resolved by twodimensional gel electrophoresis into a major acidic Ba-CK and a major basic Bb-CK protein species, but close inspection of the gels indicated further heterogeneities. The two major B-CK isoproteins are very likely produced from the unique chicken B-CK gene (see poster of Wirz,T. et al.). Further diversity is generated at two distinct post-transcriptional steps. First, post-translational phosphorylation was shown to contribute to heterogeneity of both the Ba- and Bb-CKs, and appears to modulate their enzymatic activity. In addition, internal initiation of Bb-CK synthesis was shown to occur *in vivo* as well as *in vitro*, resulting in proteins differing in the length of their N-termini. Using site directed mutagenesis to "switch off" each of the first four Met codons of a full length Bb-CK cDNA, we were able to correlate each of the protein produced with one distinct translation start site. We show that each of these sites is also used *in vivo*.

P2 146

RNA BINDING DOMAINS OF THE XENOPUS LA PROTEIN

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The 49 kd La protein (427 amino acids) transiently binds to the U-rich 3' ends of all nascent RNA polymerase III transcripts. To delimit the domain(s) involved in this highly selective RNA binding, the Xenopus laevis La protein has been produced in wheat germ extracts and incubated with two kinds of tRNA precursors that terminate with either $UUUU_{OH}$ or $GAUC_{OH}$. Immunoprecipitation with anti-La antibodies shows that the complete protein preferentially associates with the precursor ending with U residues. Truncated proteins that have lost up to 204 amino acids from the C terminus exhibit the same specificity, but proteins missing 110 or 183 amino acids from the N terminus bind both substrates equally well. These results suggest the presence of two RNA binding domains in the La protein, one needed for binding in general and the other conferring specificity to the binding reactions.

P2 147

SELECTION OF FUNCTIONAL REARRANGEMENTS OF THE RAT GLUCOCORTICOID RECEPTOR.

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The rat Glucocorticoid Receptor (rGR) contains the characteristic Cys-rich DNA binding domain which is highly homologous among members of the receptors superfamily (Science **240,** 889). The Cys residues can be grouped into two distinct clusters which are encoded by separate exons and are thought to fold into "zinc finger" like structures. With the aim of studying the effects of internal structural modifications, we have generated a library of receptors bearing random deletions and insertions within the Cys-rich region. These recombinants consist of "carboxy"-resected (Bal 31), **mutant** cDNA, combined with "amino"-resected **wild type** cDNA. We show that it is possible to identify functionally reassembled cDNAs by systematic pool-analysis of the generated library, with the help of a recently developed, assay for trans-activation in mammalian cells (EMBO J. 7, 2503). The data indicate that there is a remarkable degree of "flexibility" with regard to amino acid number, composition, and sequence, in particular within the region which separates the two Cys clusters. Furthermore, we could observe that a null-mutation in the second "finger" (Cys492 --> Ser) is resurrected upon the adjacent reduplication of a correct version of the same Cys cluster (i.e residues 471-493 duplicated). A model for a possible tertiary structure which could account for the observed sequence flexibility in the DNA binding domain of the rGR will be presented.

P2 148

BIOCHEMICAL ANALYSIS OF WILD TYPE AND MUTANT GLUCOCORTICOID RECEPTOR (GR) FRAGMENTS.

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We show that fragments corresponding to distinct portions of the rat GR expressed either in cell-free extracts, or in E.coli can be used to study protein:DNA as well as protein:protein interactions. Our data indicate that all the in vivo.tested null-mutations (Severne et al., EMBO J. 7, 2503 ff), are probably due to loss of DNA binding functions. Furthermore a novel, sensitive technique has been developed, which allows one to visualize protein:protein interactions. In this approach, either crude extracts or enriched fractions are first electrophoresed under denaturing conditions and transferred to nitrocellulose filters. The blotted proteins are renatured and the cellulose filters are subsequently reacted with radioactively labelled protein probes. In a series of preliminary experiments, we were able to visualize prominent interactions occurring between specific GR fragments and some 90-92 kd species present in different extracts. One of these interacting proteins is more abundant in extracts from heat shocked cells, thus confirming the current view of potential GR-HSP90 interactions. Little is known about the identity of the other interacting species, except that some of the other minor bands disappear from the radiograms when the GR probe is challenged with different monoclonal or polyclonal antisera. GR fragments expressed in E. coli are currently used also in an attempt to complement cell-free transcribing extracts. These studies should lead to a better understanding of the protein:-protein and protein:DNA interactions which are necessary and sufficient for transcriptional modulation of a simplified promoter/enhancer combination.

P2 149

IMMUNOCHEMICAL LOCALIZATION OF GTP CYCLOHYDROLASE I IN HUMAN MONONUCLEAR CELLS

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GTP cyclohydrolase I (GTPCH), the enzyme catalyzing the first step in the cofactor biosynthesis of aromatic amino acid hydroxylases, has been localized in situ. By the use of a monoclonal antibody (mAb) specific to human GTPCH, the enzyme has been visualized immunoenzymatically by alkaline phosphatase labeling. In routine blood smears lymphocytes, monocytes/macrophages, and granulocytes show strong cytoplasmatic staining. Erythrocytes show no staining at all. Neither is there any staining for GTPCH in the blast cells of a patient with T-cell acute lymphoblastic leukemia. It was also possible to demonstrate the absence of immunoreactivity in transformed mononuclear cell lines which lack GTPCH activity, e.g. HL-60 and U-937. GTPCH deficiency in man causes severe neurological disorders. The mAb's use as a diagnostic tool for GTPCH deficiency in newborns or prenatally is under investigation.

P2 150

SELECTIVE RESISTANCE TO INFLUENZA VIRUS OF TRANSFECTED CELLS EXPRESSING MOUSE AND HUMAN Mx PROTEINS OR DERIVED VARIANTS

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Resistance of mice to influenza virus is brought about by the action of the interferon-induced 72 kD nuclear Mx protein. We have transfected Mx protein-deficient Swiss 3T3 cells with mouse *Mx* cDNA under the control of the constitutive promoter of the murine 3-hydroxy-3-methylglutaryl coenzyme A reductase gene. Repeated subcloning permitted establishing of stable cell lines that uniformly express Mx protein. Cells accumulating high levels of Mx protein in the nuclei acquired almost complete resistance to influenza virus but remained susceptible to other viruses. A cDNA encoding a human Mx-related protein was expressed in a similar manner. Cells transfected with this cDNA construct accumulated the human Mx-related protein in the cytoplasm and showed a moderate degree of protection against influenza virus, suggesting that Mx protein located in the cytoplasm may also have some anti-influenza activity. We are currently examining the antiviral potentials of variant murine Mx proteins devoid of the nuclear transport signal. A second human Mx-related protein and some mouse/human Mx protein hybrids will also be tested in this system.

P2 151

A CYTOPLASMIC DELETION MUTANT TRANSFERRIN RECEPTOR IS INCORRECTLY SORTED TO THE APICAL SURFACE IN POLARIZED MADIN-DARBY CANINE KIDNEY CELLS

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Transferrin receptor (TR) is located at the basolateral plasma membrane of epithelial Madin-Darby canine kidney (MDCK) cells. We investigated whether the cytoplasmic domain of TR, or its phosphorylation, are required for this polarized expression. Wild-type or cytoplasmic domain mutant human TR cDNA's were transfected into MDCK cells and clonal cell lines isolated. Expression of TR at the apical and basolateral cell surfaces was examined by Scatchard analysis of 125I-transferrin binding, by surface iodination and immunoprecipitation of human TR, and by electron microscopy after immunogold labelling. Wild-type and phosphorylation site mutant TR's were located basolaterally, whereas a deletion within the cytoplasmic domain led to strong, though not exclusive expression at the apical cell surface. These results indicate that the cytoplasmic domain of TR contains information required for sorting of this protein to the basolateral surface of epithelial cells.

P2 152

A HEPATOCYTE-SPECIFIC BASOLATERAL MEMBRANE PROTEIN IS TARGETED TO THE SAME DOMAIN WHEN EXPRESSED IN MDCK CELLS

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Different mechanisms for polarized sorting of apical and basolateral plasma membrane proteins appear to be operative in different cell types. In hepatocytes, all proteins are first transported to the basolateral surface, where then sorting (probably signal-mediated) of apical proteins takes place. In contrast, in MDCK (Madin-Darby canine kidney) cells, proteins are directly transported from the trans-Golgi network to their appropriate plasma membrane domain. In order to study the differences in the sorting requirements of the two cell types, we have expressed a hepatocyte-specific basolateral membrane protein, the asialoglycoprotein receptor H1, in MDCK cells. H1 was found to be specifically transported to the basolateral domain also in this heterologous system, suggesting that either the same basolateral targeting signal is operative in both cell types, or – more likely – that basolateral transport occurs "by default", i.e. without the requirement for a sorting signal.

P2 153

IDENTIFICATION OF A VESICULAR COMPARTMENT NEAR THE CIS-GOLGI WHICH MAY MEDIATE BIOSYNTHETIC PROTEIN TRANSPORT FROM ENDOPLASMIC RETICULUM TO GOLGI

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Using a Golgi-enriched fraction of Caco-2 cells as an antigen a monoclonal antibody was produced which labels a defined vesicular-tubular compartment in close proximity to the cis-Golgi as visualized by immunofluorescence and immunoelectron microscopy. The corresponding antigen was characterized and found to be a 53 kDa membrane protein exposed on the luminal side of the vesicles. Collective indirect evidence suggests that this protein may delineate the biosynthetic transport route from the endoplasmic reticulum to the Golgi apparatus.

P2 154

Membrane association of pp60^{src} in vitro and in vivo.

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pp60^{src} is a plasma membrane-associated tyrosine kinase expressed in all cell types. A mutated form of this protein is transduced by Rous sarcoma virus and causes cell transformation. Association of pp60^{src} with membranes depends on an amino-terminal glycine-linked myristate. The cellular form of this kinase isolated from tissue culture cells can be efficiently transferred into liposomes. In vitro translated material is now used to ask the question whether myristylation is sufficient to allow insertion into liposomes or whether additional cellular factors (a membrane-bound receptor) are required. Plasma membrane fractions are assayed for an activity that may facilitate liposome association of pp60^{c-src}. A mutant protein lacking the amino-terminal glycine was constructed that is unable to associate with liposomes in vitro and does not bind to the plasma membrane in vivo in transfected cell lines. We now address the question of how membrane localization of pp60^{c-src} affects the role of this kinase in signal transduction across the membrane.

P2 155

ANALYSIS OF THY-1 EXPRESSION AND MATURATION IN THY-1 LOSS T LYMPHOMA MUTANT LINES

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Thy-1, a major constituent of mammalian neurons and mouse thymocytes, is attached to the cell surface via a phosphoglycolipid (PGL) anchor. However, in 6 complementing lymphoma mutant lines, Thy-1 is synthesized but the PLG is not attached. Any modification in the amino acid sequence necessary for the PGL addition and encoded in a single exon or in the glypiation machinery could account for the phenotype of these mutants. Analysis by Northern and T1/RNAse A protection assays of Thy-1 mRNA demonstrated that in each case the region coding for the glypiation signal was normal. This strongly suggests that these mutations affect either the biosynthesis and/or the addition of the PGL. In one of the mutants , the amount of Thy-1 mRNA was at least 10 x less than in wild type cells. This could be due to a mutation in a transcription factor affecting Thy-1 expression.

P2 156

CLONING AND SEQUENCING OF cDNA CODING FOR CONNECTIVE TISSUE ACTIVATING PEPTIDE III FROM A HUMAN PLATELET DERIVED λgt11 EXPRESSION LIBRARY.

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Connective tissue activating peptide III (CTAP-III) is a platelet α-granule specific protein, which is released upon activation, is mitogenic for connective tissue cells and is degraded to β-thromboglobulin (β-TG). A cDNA coding for CTAP-III was cloned from a λgt11 expression library prepared using mRNA isolated from human platelets. The open reading frame of the clone coded for a protein with 128 amino acid residues. Since the precursor of CTAP-III, platelet basic protein (PBP), is 94 amino acids long, the 5' translated region of the cDNA codes for a leader sequence 34 amino acids long. This leader sequence, like the sequence of mature CTAP-III, shows significant homology to the sequence of platelet factor 4 (PF4), the only other platelet specific α-granule protein cloned till now, from a human erythroleukemic (HEL) cell line derived cDNA library. Because these leader sequences are probably critical for targeting such proteins to the α-granule, the leader sequences of all known members of the β-TG family were compared. Northern blot hybridization with platelet and megakaryocyte mRNA shows a single species mRNA of approximately 0.8 kb, suggesting that the corresponding cDNA is full length.

P2 157

A SPECIFIC mRNA BINDING PROTEIN IS INVOLVED IN THE IRON-DEPENDENT REGULATION OF TRANSFERRIN RECEPTOR mRNA

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Levels of human transferrin receptor (hTR) mRNA can be increased 30-fold by treating cells with the iron chelator desferrioxamine (desf) due to stabilization of mRNA. Two domains in the 3' untranslated region (3'UTR) of hTR mRNA are responsible for this regulation. We incubated various labelled RNAs from the regulatory domains with extracts of cells grown in the presence or absence of desf. After digestion of unprotected RNA with RNAse T_1 and displacement of unspecifically bound proteins by heparin RNA-protein, complexes were analysed in non-denaturing gels. In this way we identified a protein binding with high specificity to 4 palindromes within the regulatory domains of hTR mRNA 3'UTR. The amount of binding increases 30-fold within 12 hours after desf addition and returns to the initial level 2 hours after removal of the chelator. By cross-competition with different RNAs we found the protein to be identical or similar to the one that binds in the region of ferritin mRNA that is responsible for iron-dependent translation. A model for coordinate regulation of hTR and ferritin expression is presented.

P2 158

STRUCTURE AND PROCESSING OF CHICKEN NUCLEAR LAMIN PROTEINS

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The chicken nuclear lamina is composed of three proteins called lamins A, B_1 and B_2. cDNA sequence analysis allows us to identify structural motifs that differentiate B-type lamins from A-type lamins, and they provide definitive proof for the existence of two distinct B-type lamins. *In vitro* translation of transcripts derived from lamin A and lamin B_2 cDNAs yielded proteins that comigrated precisely with the putative *in vivo* precursors of the respective lamins. However, whereas the precursor was stable, the lamin B_2 precursor was processed *in vitro*. These results confirm that two distinct activities are involved in processing of newly synthesized lamins A and B_2. Experiments to localize the site of modification are in progress.

P2 159

TENASCIN - A MODEL OF A MULTIDOMAIN PROTEIN

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Tenascin is a glycoprotein of the extracellular matrix with a unique tissue distribution during development and tumorigenesis. Electron microscope pictures of rotary shadowed molecules revealed a six-armed structure. After completing the cDNA sequencing of the three differentially spliced versions of chicken tenascin, we can assign the structural features of the homohexamers to the respective primary protein sequences of the subunits. A short stretch of amino acids near the N-terminus allows three subunits to form a triple coiled coil fixed by flanking cysteines. Two of these trimers are disulfide linked in the central globule. The six extending arms can be divided in the proximal thin part encoded by 13 EGF-like repeats, the thicker distal part built up of 8,9 or 11 fibronectin type-III repeats, respectively, for the three differentially spliced variants and the terminal globule showing a significant homology to the globular domains of fibrinogen.

P2 160

OLIGOMERIC VERSUS MONOMERIC TENASCIN: EFFECTS ON HEMAGGLUTINATION, CELL SPREADING, AND NEURITE OUTGROWTH

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Tenascin from chick embryos is an oligomeric ECM protein with disulfide-linked subunits of M_r= 190-220,000 and a three- or six-armed appearance in the EM. From adult chick gizzard, we isolated monomeric molecules with an M_r of 215,000 determined by equilibrium sedimentation and with all structural features of single tenascin arms. Hence one tenascin arm corresponds to one subunit. We compared the activities of monomeric with oligomeric tenascin. In contrast to the oligomeric form, monomeric tenascin did not agglutinate fixed sheep erythrocytes. However, monomeric tenascin still inhibited the spreading of fibroblasts on fibronectin-coated substrates. Half-maximal activity was obtained at a similar protein concentration as with oligomeric tenascin. Culture substrates coated with either oligomeric or monomeric tenascin induced slow, but significant outgrowth by embryonic sensory neurons. All described activities were blocked by mAb 68 which has a single epitope near the distal end of individual tenascin arms. Thus hemagglutination depends on the oligomeric structure of tenascin but no other functions involving cell binding.

P2 161

EXPRESSION OF GLIA-DERIVED NEXIN IN YEAST.

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Glia-derived Nexin (GDN) [Guenther et al., (1985) EMBO J. 4,1963-1966] stimulates the neurite outgrowth from both neuroblastoma cells and cultured neurons of chick sympathetic ganglion [Zurn et al., (1988) Dev.Neurosci. 10,17-24]. The cDNA sequences coding for both human and rat GDN indicate that the protein is a Nexin which belongs to the Serpin superfamily [Gloor et al.,(1986) Cell 47,687-693; Sommer et al., (1987) Biochemistry 26,6407-6410]. In vivo, GDN is predominantly found in the olfactory system, where it could be involved in the de- and regeneration processes of olfactory neurons [Reinhard et al.,(1988) Neuron 1,387-394]. We have constructed a plasmid containing the GDN cDNA under the control of the PHO5 promoter. The plasmid leads to a high level expression of the protein in yeast. We present evidence that the recombinant GDN has the same properties as GDN with respect to its protease inhibitory and neurite promoting activity.

P2 162

GLIA-DERIVED NEXIN AND COMPONENTS OF THE EXTRACELLULAR MATRIX: INFLUENCE ON NEURITE OUTGROWTH

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Glia-Derived Nexin (GDN) is a fast acting inactivator of thrombin, but it inhibits also plasminogen activators and trypsin-like proteases. *In vitro*, GDN modulates the neurite elongation in NB-2a mouse neuroblastoma cells. This effect is probably the result of the inhibition of specific protease(s). Plasminogen activator activity has been localized at the growth cone of these neurites, furthermore, this neurite outgrowth is very sensitive to thrombin. The components of the extracellular matrix can also extensively influence the neurite elongation process. Therefore, a balance between proteolytic and inhibitory activities could (i) allow a partial proteolytic degradation of the extracellular matrix and (ii) modulate the growth cone's adhesion to the substrate, and thus permit an optimal neurite growth. Here, we demonstrate that GDN, thrombin and the complex GDN-thrombin can bind both to immobilized vitronectin and laminin. Moreover, GDN and thrombin modulate the neuroblastoma's neurite outgrowth on these substrates.

P2 163

DEVELOPMENTAL PATTERN OF NEUROFILAMENT CONSTITUENT PROTEIN EXPRESSION IN CHICKEN EMBRYOS

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The NF-constituent polypeptides **NF-L, NF-M, NF-M130 and NF-H** are expressed in a "step by step" pattern during development of chicken nervous system.

These polypeptides were purified and separated from bovine spinal cords by hydroxyl-apatite, ion-exchange chromatography (DEAE and HTP) and electroelution from preparative SDS-PAGE.

A library of **monoclonal antibodies** (Mabs) raised against these proteins were screened for cross-reactivity with NFs prepared from adult chicken brain. 6 well characterized Mabs were selected to determine the developmental pattern using homogenates of brain and spinal spinal cords of chicken embryos at E4 and E7 E10, E13, E16 and E19. Crude filament fractions were obtained by centrifugation in 0.85 M sucrose.

Solid-phase binding assay (immuno-dot test) and immunoblots demonstrate a **stepwise** synthesis of NF-proteins indicating, that in certain neuronal populations NFs are at least dimeric in nature. We could not confirm the proposed **precursor role** of NF-M130. Our data obtained from binding assays with in vitro dephosphorylated NF-H and NF-M provide evidence, that one important role of **phosphorylation** of the tail domains are **changes in the conformation of the tail domain.** (Supported by EMDO-Stiftung and Hartmann-Mueller-Stiftung, Zurich).

P2 164

DNA POLYMERASES δ AND α: POSSIBLE COORDINATED ACTIONS AT THE REPLICATION FORK

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Calf thymus DNA polymerases δ and α were purified under extreme proteolysis precaution over 6000 fold in a five steps isolation procedure including: phosphocellulose, hydroxyl-apatite (where separation of the two enzymes occured), heparin-Sepharose, FPLC Mono S and velocity gradient sedimentation. A 3'- 5'exonuclease copurified with DNA polymerase δ, whereas DNA primase was tightly associated with DNA polymerase α. The DNA polymerase δ had a processivity of several thousand bases: under optimal conditions it can fully convert primed single-stranded circular DNA of 7200 bases in less than 10 min. The DNA polymerase α had a low processivity under optimal conditions, being able to synthesize DNA strands only 50-100 bases long. These facts suggested that DNA polymerase α could be a candidate for replication of the lagging strand, where replication is discontinuous. Tryptic peptide map analysis of the catalytic subunits of both the DNA polymerases indicated that these two enzymes are likely unrelated. Evidence will be presented which supports the hypothesis (Focher F. et al., 1988, FEBS lett. 229, 6-10) that DNA polymerases δ and α act coordinately at the replication fork, respectively, as leading and lagging strand replicases.

P2 165

GROWTH INHIBITION OF HUMAN MAMMARY ZR-75-1 AND MDA-MB-231 LINES BY TGF β-1: INTERACTION WITH EGF AND AMILORIDE

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Transforming growth factor beta-1 (TGF β-1) inhibited under serum free conditions the proliferation of the human mammary tumor cell line ZR-75-1 at concentrations > 10^{-11}M. The growth stimulation by EGF are inhibited by TGF β-1. S6-kinase activated by EGF is not responding to TGF β-1, and does also not alter the kinase activation induced by EGF. Amiloride, a Na^+/H^+ antiport blocker, inhibited ZR-75-1 cell growth in a dose dependent manner (ED_{50} = 50 µM). Stimulation by EGF was partially neutralized by amiloride, which on the other hand intensified the growth inhibiting effects of TGF β-1.

The growth of the estradiol- and growth factor-independent cell line MDA-MB-231 is inhibited by TGF β-1, but even at high concentrations, TGF β-1 is not very effective. Interactions with EGF are marginal. Amiloride, however, inhibited the growth of MDA-MB-231 cells in a way comparable to its effects on ZR-75-1 cells. Treatment of MDA-MB-231 cells with combinations of amiloride with TGF β-1 has only small synergistic effects.

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P2 166

NORMAL AND H-ras TRANSFECTED MASTOCYTES DIFFER IN THEIR CAPACITIES TO BIND TO STROMAL CELLS

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A mouse interleukin-3 (IL-3)-dependent mastocyte cell-line (PB-3c) has been compared to its H-ras transfected derivatives (A2 and A4), which are tumorigenic. Transfection leads to a 10-fold lower requirement for IL-3. Transfected cells also acquire the capacity to bind Swiss 3T3 fibroblasts (80% versus 30% for the PB-3c) and survival time without IL-3 is markedly increased following binding (60 hours instead of 20 hours for PB-3c). Biochemical analysis has shown that a new protein is present at the surface of H-ras transfected cells, which may be involved in binding. Our preliminary results may be interpreted as indicating a relation between the tumorigenicity of these cells and their capacity to survive that could operate via binding to stromal cells and progessive loss of IL-3 dependence.

P2 167

CHOLESTYRAMINE, AN INHIBITOR OF BILE SALT ABSORPTION, AND STIGMASTANYL-PHOSPHORYLCHOLINE(SPC), AN INHIBITOR OF CHOLESTEROL ABSORPTION, REDUCE PLASMA CHOLESTEROL LEVELS AND RETARD ATHEROGENESIS IN WHHL x BURGUNDY HYBRID RABBITS

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WHHL x Burgundy hybrid rabbits have high plasma cholesterol levels and develop severe atherosclerosis in the thoracic aorta and in the main coronary artery after 75 days of age. We used this model to evaluate the antiatherogenic potential of two cholesterol lowering drugs. Controls were fed a diet containing 12% fat (group A). In further groups the diet contained 3% cholestyramine (B) or 1.05% SPC (C). In groups B and C, as compared to A, the plasma cholesterol level was reduced by 30%. At 200 days of age, the effect on atherosclerotic lesion formation was evaluated by determining the cholesterol content in the distal thoracic aorta, and by quantitative morphometry of semithin cross-sections of the proximal and distal thoracic aorta and of the main coronary artery. In conclusion: Cholestyramine and SPC reduced cholesterol in the distal thoracic aorta and retarded the development of atherosclerotic lesions in all investigated arteries. The difference in the mechanism of action of these two drugs does not seem to be relevant for the antiatherogenic effect.

P2 168

ONCOPROTEIN AND SERUM MEDIATED INDUCTION OF A GENE WITH SEQUENCE SIMILARITY TO CARCINOEMBRYONIC ANTIGENE

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We have isolated the cDNA clone T1 which corresponds to a mRNA whose expression is strongly and transiently induced by the Ha-ras(EJ) and the v-mos oncogene products as well as by serum growth factors in a mouse cell line. Cycloheximid superinduces T1 mRNA accumulation. Oncoprotein mediated T1 expression is protein kinase C independent. The primary translation product encoded by T1 has a mol. weight of 39 kDa. In vitro transcription-translation reactions revealed a signal peptide and heavy N-linked glycosylation. A region of 174 amino acids immediately following the signal peptide sequence show sequence similarity to the human tumor marker carcino-embryonic antigen (CEA). CEA is a 180 kDa glycoprotein with three adjacent 178 amino acids long internal repeats. The extent of sequence similarity between CEA and T1 spans one repeat.

P2 169

CHARACTERIZATION OF GROWTH FACTOR REQUIREMENTS FOR HUMAN COLORECTAL CANCER CELLS

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Cells isolated from primary colorectal tumors proliferate clonally to a markedly lesser extent than cells from breast and ovarian cancer in the conditions of our 5% FCS semi-solid medium. The addition of various growth factors and hormones has no significant effect on colony formation. Similar results have been obtained with 4 colorectal cell lines two lines established in our laboratory, as well as WiDr and LISP-1 lines. We also established colorectal stromal cells cultures in the presence of EGF and hydrocortisone. Stromal cells conditionned media have revealed to be able to stimulate growth of primary tumors samples. A bioassay using colorectal cell lines is being established in order to precisely evaluate the possible stimulatory factor(s) involved.

P2 170

EXPRESSION OF THE CALCIUM-BINDING PROTEINS CALMODULIN AND ONCOMODULIN IN CHEMICALLY TRANSFORMED FIBROBLASTS

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A chemically transformed rat fibroblast cell line (T14c) has been obtained by exposure to 1-methyl-3-nitro-1-nitrosoguanidine (MNNG). Tumorigenicity was confirmed by the soft agar assay and tumor growth in nude mice. The doubling times of both cell types in culture was about 24 hours. Normal cells were contact-inhibited after about 48 hours, whereas T14c cells grew exponentially for 120 hours, when 140 mm diameter dishes were initiated with 10^6 cells. RNA extracted from both cell cultures at different degrees of confluency was analyzed by Northern blots. T14c cells produced higher levels of calmodulin mRNA as compared to normal cells. The amount of oncomodulin mRNA in T14c cells appeared to be several fold greater than that of calmodulin mRNA. Normal cells expressed no detectable oncomodulin. Levels of transcripts for calmodulin and oncomodulin were significantly higher in the log phase of growth as compared to confluent or overconfluent stages, indicating a cell cycle dependence of calmodulin as well as oncomodulin expression.

P2 171

EXPRESSION OF HUMAN-IGF II IN NIH-3T3 CELLS

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Insulin-like growth factor (IGF) I is a 7 Kd polypeptide hormone structurally related to insulin. It promotes cell growth via the type I receptor. IGF II has no known biological function. We propose to produce analogs of IGF II by site directed mutagenesis. The aim is to produce mutants which bind exclusively to the type II receptor. So far, we have expressed the hIGF II wildtype gene and two mutants. For site directed mutagenesis, the hIGF II c-DNA was subcloned into M13mp18. For expression in the NIH 3T3 cells, we used the plasmid p267-3. It contains the SV40 early promotor, a CMV enhancer and the SV40 splice and polyA signals. Using the Ca_2PO_4 coprecipitation method, we cotransfected with the pSV2neo plasmid. After selection with G418 and cloning, the serum-free supernatants were tested by RIA and western-blots. One clone expresses 0.5 pg wild type IGF II/cell in 12 h. IGF II was purified by an affinity column and HPLC. Amino acid sequencing verified the correct maturation. The study of the receptor binding of mutants will be the next step.

P2 172

SIGNAL TRANSDUCTION MEDIATED BY THE E_1 INTEGRAL MEMBRANE PROTEIN OF SEMLIKI FOREST VIRUS (SFV) DURING THE INFECTION OF SUSCEPTIBLE CELLS

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SFV infects susceptible cells by binding to the cell surface, is then taken up into endocytotic vesicles where low pH induces fusion between the viral and endosomal membranes resulting in the release of the viral genome into the cytoplasm. We had previously shown that the viral E_1 integral membrane glycoprotein is responsible for viral uptake and membrane fusion. The protein undergoes conformational changes upon binding and upon low pH exposure. In this communication, we report that virus-binding concomitantly initiates auto-proteolytic activity of the capsid protein. These results indicate that the conformational change of the E_1 protein occurring upon binding triggers transmembrane signals simultaneously across the plasma and viral membranes. The relevance of these events to the development of membrane fusion will be discussed.

P2 173

EVIDENCE FOR A PERTUSSIS TOXIN SENSITIVE G PROTEIN INVOLVED IN THE CONTROL OF MEIOTIC REINITIATION OF XENOPUS LAEVIS OCYTES

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Meiotic reinitiation of Xenopus laevis oocytes is induced in vitro by progesterone or insulin. The hormonal effect is inhibited in a dose-dependent manner by the injection of the A protomer of pertussis toxin (islet activating protein IAP) into the oocytes. This inhibition occurs only before the appearance of a maturation promoting activity in the cytoplasm. Furthermore, injection of the toxin A protomer into recipient oocytes does not inhibit the induction of maturation obtained through injection of cytoplasm containing the Maturation-Promoting Factor (MPF). The inhibition effect of the toxin A protomer is reversible with time. These results suggest that a pertussis-sensitive G-protein is involved in intracellular signalling systems leading to the induction of MPF activity.

P2 174

EXPRESSION OF INTERLEUKIN 1 AND INTERLEUKIN 6 IN PRIMARY CULTURES OF ACTIVATED ASTROCYTES

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In brain tissue astrocytes are thought to be involved in immune regulation. Astrocytes can be activated by interferon (IFN) to express major histocompatibility complex class II antigens and to secrete an interleukin 1-like factor. This allows the communication between astrocytes and T cells within the brain tissue. In the present study interleukin 1 (IL-1) mRNA was found to be expressed in astrocytes stimulated with lipopolysaccharide (LPS), tumor necrosis factor (TNF) or IFN . Interestingly, treatment of astrocytes with LPS or TNF or infection with virus induced also mRNA for interleukin 6 (IL-6). These data indicate that astrocytes may cooperate with both T and B cells for production of immunoglobulins in the central nervous system. Indeed IL-1 and IL-6 have been detected in cerebral spinal fluids in viral brain diseases.

P2 175

EFFECTS OF THE PROTEIN KINASE INHIBITOR STAUROSPORINE ON MICROFILAMENT ORGANIZATION AND MORPHOLOGY OF NEUTROPHILS

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Activation of neutrophils results in changes in cell morphology and in a concomitant reorganization of the cytoskeleton. Little is known on the molecular mechanism of these processes. Staurosporine inhibits in vitro several protein kinases, exhibiting highest affinity for protein kinase C. In an attempt to molecularly dissect these complex processes, we have studied the effect of staurosporine on actin polymerization and morphology of resting and activated neutrophils. Staurosporine (10nM-1μM) does not change the time course of actin polymerization initiated by chemotactic peptides. In resting cells the inhibitor (25-100nM) even increases cytoskeleton-associated actin by 50-100%, and induces shape changes. Front-tail polarity of cells in the presence of chemotactic peptides is markedly reduced by staurosporine (25-100nM). Our data suggest that activation of the protein kinases A, C and G is not involved in ligand-induced actin-polymerization. These kinases may however play a role in the morphological changes which correlate with directed motility.

P2 176

SIGNAL-INDUCED ASSOCIATION OF SPECIFIC CELL SURFACE RECEPTORS AND THE CYTOSKELETON

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Integrins are a superfamily of transmembrane cell surface receptors (R) including the LFA-1 complex on helper T cells (Th) and the chicken integrin complex. Members of this family are often found at sites where cells form specialized regions of adhesion to other cells. These specialized membrane regions are also the sites where actin microfilaments are attached to the membrane. In order to investigate the question whether a link between these R and cytoskeletal elements exists, antibody-induced capping of R was carried out on cloned mouse Th or on chicken peripheral blood lymphocytes. The capped cells were then examined by double immunofluorescence techniques to determine whether particular cytoskeletal proteins (α-actinin, vinculin, talin) were co-collected with the R caps. In these experiments a uniform distribution of any of the tested cytoskeletal proteins was found. However, if the cells were treated with phorbol myristate acetate (PMA), an activator of protein kinase C, talin was then found co-distributed with the integrin caps. These results indicate that in lymphocytes, R and talin are not normally associated with one another. When, and only when, an appropriate signal is received by the cell do talin and integrins become linked. PMA-treatment of cells delivers such a signal and therefore suggests that protein kinase C activation is involved.

P2 177

CHARACTERIZATION OF THE POLYSIALIC ACID CONTAINING MOLECULE IN WILMS TUMOR

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Polysialic acid (PSA) is a developmentally-regulated antigen in the mesodermally-derived kidney (P. N. A. S. **84**, 1969, 1987). Wilms tumor, a highly malignant kidney tumor, reexpressed PSA (P. N. A. S. **85**, 2999, 1988). The epitope size requirements for mab 735 binding and bacte-riophage-endosialidase digestion indicated that the long chain form of PSA of the embryonic form of N-CAM is present in both developing kidney and Wilms tumor. By combination of immunoprecipitation and Western blotting using PSA specific mab 735 and N-CAM polypep-tide specific antibodies, we now directly demonstrate that PSA present in Wilms tumor is on N-CAM. Endoneuraminidase digestion of mab 735 immunoprecipitate from Wilms tumor homogenate followed by PAGE revealed one band with an apparent m.wt. of 160 kDa, which is in contrast to rat brain showing the 180, 140, and 120 kDa polypeptides. By immuno electron microscopy, PSA labeling was present at the entire outer plasma membrane surface. Gold particle label intensity was heterogenous: weak at regions of cell-cell contact and very intense and broad on the free cell surface. Immunolabel was confined to a thick layer of amorphous material which represents the equivalent of the *E. coli* K1 and meningococci B capsular polysaccharide on mammalian cells.

P2 178

A MODEL FOR IS30-MEDIATED TRANSPOSITION IN E. COLI

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On the basis of the following experimental findings a model of transposition of the mobile genetic element IS30 is presented. First, the IS30-mediated transposon Tn2700 is unstable; it forms deletions of its internal Cm^r marker at frequencies of 0.5 - 1% per generation. Second, the main product of transposition from a plasmid donor must result from an "inverse" transposition event. Third, the ends of IS30 serve as hot spots for IS30 insertion. From these facts it is concluded that the initial step in transposition is a site-specific recombination event between the two copies of IS30 carried in Tn2700 and that this recombination results in deletion formation. The inverted repeat IS30 ends are thereby joining together to form a very active and unstable intermediate product of transposition. We have been able to detect, structurally verify and isolate this intermediate.

P2 179

TRANSFORMATION OF PROTOPLASTS OF THE MOSS *Physcomitrella patens*

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The moss P. patens might provide a very useful system to study plant development. **Mutagenesis** of spores have produced auxotrophic, morphogenic, hormonal, gravitropic and phototropic mutants. **Protoplasts** are easy to isolate and regenerate into a whole plant on a simple mineral medium. **Somatic fusions** of these protoplasts have been achieved, permitting complementation analysis. Many steps of morphogenesis have been shown to be photoregulated through phytochrome, including protoplasts' first division. But no **transformation** has been reported yet.
We present here for the first time a successful transformation of P. patens protoplasts and give phenotypical and molecular evidences of stable integration and expression of foreign genes coding for kanamycin and hygromycin resistance.

P2 180

APPLICATION POTENTIAL OF CRYSTALLINE BACTERIAL CELL ENVELOPE LAYERS

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In the course of evolution prokaryotic cells have developed characteristic multilayered cell boundaries. One of the most remarkable features is the presence of regularly structured surface layers (S-layers) which are formed of assemblies of protein or glycoprotein subunits. S-layers completely cover the cell surface at any time during the cell cycle. Depending on the organism, S-layers lattices can exhibit oblique, square or hexagonal symmetry. The centre-to-centre spacing of the morphological units can range from 5 to 32 nm. Due to the crystalline structure, S-layers are isoporous lattices with pores showing a size of 2 nm to 6 nm. The porosity may range from 20 to 70 %.
S-layers have been used for the production of ultrafiltration membranes in which the active filtration layer is composed of coherent S-layer fragments. Recently, S-layers were found to have a high capacity for covalent attachment of foreign protein molecules. Thus, S-layer ultrafiltration membranes can also be used as supports for immobilizing molecules.

P2 181

USE OF A RECOMBINANT ECHINOCOCCUS MULTILOCULARIS ANTIGEN IN IMMUNODIAGNOSIS AND IN A SALMONELLA LIVE VACCINE VECTOR.

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The larval stage of the tapeworm E. multilocularis causes alveolar echinococcosis in man. One of us (BG) has isolated a species specific antigen (Em 2a) which was already used in sero-immunological diagnosis. Since antigen Em 2a could not be isolated in sufficient amounts we produced an appropriate antigen (antigen II-3/10) in E. coli. The immunological characteristics of the antigen in ELISA (diagnostic sensitivity = 90 % and specificity = 98 %) suggest its general usefulness in the diagnosis of human alveolar echinococcosis. In a further project antigen II/3-10 was expressed in the live attenuated Salmonella vaccine strain LT2M1C. Subcutanous and peroral administrations of this recombinant strain to mice (intermediate host) resulted in antibody synthesis and lymphocyte priming against antigen II/3-10. In dogs (final host) only a humoral immune response was observed. These results indicate that in future LT2M1C might be a suitable live carrier for presenting protective antigens to the immune system of intermediate and final hosts.

P2 182

DEFECTIVE LH, FSH, AND TSH SECRETION IN DIABETIC RAT PITUITARIES STUDIED BY REVERSE HEMOLYTIC PLAQUE ASSAY.

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To evaluate the spontaneous secretory activity of single gonadotropes and thyrotropes in vitro, we studied uncultured pituitary cells from control and 1-month streptozotocin-diabetic rats by means of a reverse hemolytic plaque assay and by morphometry. After light-microscopical immunohistochemistry for gonadotropin and thyrotropin, we recorded the ratio plaque-forming/non-plaque-forming cells. Both the area of plaques produced by the LH, FSH, and TSH cells and the area of plaque-forming cells were clearly smaller in diabetic than in control rats. The plaque area, however, was more severely reduced than the cell area. The number of plaque-forming cells percent LH-, FSH-, and TSH-immunohistochemically positive cells was highly decreased in diabetic compared to control animals. Several pathogenic mechanisms might thus be involved in the reduced gonadotropin and thyrotropin release at cellular level: a) anatomical lesions, possibly due to insulin deficiency, of organelles involved in the glycoprotein hormone synthesis and secretion; b) decreased GnRH and TRH receptors on pituitary cells; c) inadequate GnRH and TRH stimulation; d) high plasma corticosterone levels; or e) a combination of points a-d.

P2 183

STRUCTURAL AND TRANSCRIPTIONAL ANALYSIS OF AN UNUSUAL TRANSPOSON FROM A GREEN ALGA

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We have previously described the isolation and preliminary characterization of a 5.7 kb transposon (TOC1) from Chlamydomonas reinhardtii (Day et al., EMBO J. 7, 1917). TOC1 behaves like a transposon: it integrates into the OEE1 gene by illegitimate recombination, it is dispersed over a number of chromosomes, exhibits copy number variation between strains (2-30 copies) and moves during short periods of mitotic growth. As a working model we propose that TOC1 transposes via an RNA intermediate. The structure of TOC1 is unlike that of any other retroposon. The left end of TOC1 only contains a fraction of the LTR (217 bp repeat) the remainder of which (237 bp repeat) is present at its extreme right end and is separated from the complete right LTR (tandem 237/217 bp repeats) by a unique 123 bp sequence. In addition, 812 bases from its left end TOC1 contains 11 and 3/4 copies of a 76 bp repeat; such repeats are usually associated with retroposons (members of the L1 family) lacking LTRs. In addition to an unstable full length transcript whose 5' end maps within the left 217 bp repeat, in some strains the entire TOC1 element is found within large discrete sense (11.5-12 kb) and antisense (9.5-9.7 kb) transcripts. The levels of the 11.5-12 and 9.5-9.7 kb RNAs are reduced in strains expressing both RNAs relative to strains expressing only one of these RNAs.

P2 184

CAPRINE ARTHRITIS-ENCEPHALITIS (CAE)- AND MAEDI-VISNA VIRUSES DETECTED BY THE POLYMERASE CHAIN REACTION (PCR)

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It may take several months before goats and sheep infected with lentiviruses develop measurable antiviral antibodies. During this period, virus may be isolated from infected animals but current cell culture techniques for the isolation and identification of goat and sheep lentiviruses are tedious and and may take several weeks to complete. Using the polymerase chain reaction, we show here that virus can be detected in infected cell cultures. To demonstrate both CAE- and Maedi-Visna virus-specific DNA we utilized primers 20 base pairs long and framing 215 nucleotides of the gag gene (p28/p11 region) of Visna virus. Subcloning and sequencing of the amplified product confirmed the specificity of this technique. Experiments to adapt the PCR to material obtained from infected animals are under way.

P2 185

CLONING AND RECONSTRUCTION OF THE ENTIRE MEASLES VIRUS GENOME

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The genome of the measles virus (MV) Edmonston vaccine strain (≈16 kb) was reconstructed as contiguous cDNA in two plasmids allowing *in vitro* transcription of genome or of antigenome RNAs. These synthetic RNAs have 3' ends identical to natural ones, and 5' ends with 4 and 2 additional nucleotides respectively. For the reconstruction, mono- and bicistronic cDNAs of all MV genes were used, together with synthetic oligonucleotides corresponding to the genomic 5' and 3' terminal sequences. The functional integrity of all these cDNAs was tested by *in vitro* transcription and translation: mRNAs and proteins of the expected size were obtained for the six MV genes. *In vitro* synthesis of full length plus and minus strand RNAs yielded the expected ≈16 kb products. These transcripts will now be used in attempts to produce viable virus in cell lines persistently infected with defective MV genomes.

P2 186

HLA CLASS II OLIGOTYPING ON PCR-AMPLIFIED DNA: APPLICATION TO ORGAN TRANSPLANTATION.

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The remarkable polymorphism of MHC class II antigens, HLA in man, plays a crucial role in transplantation immunology. HLA class II polymorphism can now be analysed by hybridization with locus- and allele-specific oligonucleotide probes (oligotyping) derived from the variable sequences of DR, DQ and DP genes. We show that HLA typing can be performed on a large scale by direct hybridization on DNA previously amplified by the polymerase chain reaction (PCR). Oligotyping can thus replace serological as well as cellular typing and allows the detection of "hidden" alleles (e.g. splits of DRw11, DQw5 or DQw6). This methodology is now applied a) to type patients for HLA-DR for renal transplantation and b) to improve matching with unrelated donor/recipient pairs for bone marrow transplantation, by the analysis of HLA-DR and -DQ micropolymorphism.

P2 187

POPULATION GENETICS OF THE BACTERIAL GENUS LISTERIA

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205 strains of Listeria (including L. monocytogenes isolates from a Swiss epidemic caused by contaminated soft cheese) originating from human clinical, animal, and environmental sources in various countries, were analyzed for allelic variation at 16 genetic loci encoding water-soluble enzymes. 45, 14, and 4 ETs (electrophoretic types) were recognized for L. monocytogenes, L. innocua, and L. seeligeri, respectively. All the loci were polymorphic, with a mean allelic number of 6.3 for the total sample. Cluster analysis showed that the 5 species included are genetically dissimilar (genetic distance ≥ 0.67). Furthermore, the L. monocytogenes population is composed of two distinct clusters (populations I and II, separated by a genetic distance of 0.54). Antigen analysis, together with the observation that population I is genetically less diverse than population II, suggest that population I represents a lineage that derived from population II during evolution. Genetic exchanges between strains of these two populations are likely to be rare events. Surprisingly, the Swiss epidemic L. monocytogenes strain is genetically indistinguishable from the strain responsible of the 1985 California epidemic (both represented by ET1), which was also propagated by a soft cheese.

P2 188

AMPHIBIAN ALBUMIN AS A MEMBER OF THE ALBUMIN, ALPHA-FETOPROTEIN, VITAMIN D-BINDING PROTEIN MULTIGENE FAMILY. Nardelli Haefliger*, D., Moskaitis", J.E., Schoenberg", D.R. and Wahli*, W., Institut de Biologie Animale, *Université de Lausanne, CH-1015 Lausanne, Switzerland."Uniformed Service University of the Health Sciences, Bethesda MD, USA

The Xenopus laevis 68kDa albumin amino acid sequence is presented and examined with respect to its relationship to the other known members of the albumin/α-fetoprotein/vitamin D-binding protein gene family. Each of the three members of this family presents a unique pattern of conserved regions indicating a differential selective pressure related to specific functional characteristics. Furthermore, an evolutionary tree of these genes was constructed from the divergence times calculated from direct nucleotide sequence comparisons of individual gene pairs. The branch points of the tree indicate that the vitamin D-binding protein/albumin separation occurred 600-650 million years ago and the albumin/α-fetoprotein divergence 310 million years ago. This observation leads to the hypothesis according to which the amphibian/reptile phylogenetic lines separated shortly before the albumin/α-fetoprotein gene duplication. Consequently and unlike mammals, amphibians and fishes should lack an α-fetoprotein in their serum at larval stages, which is consistent with a recent analysis of serum proteins in Xenopus laevis larvae. This hypothesis will now have to be tested further in additional lower vertebrates.

P2 189

A DOUBLE-STRANDED RNA-INDUCIBLE FISH GENE WITH HOMOLOGY TO THE MURINE INFLUENZA VIRUS RESISTANCE GENE *Mx*

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Southern blot analysis revealed that presumably all mammals possess sequences in their genomes that strongly hybridize to probes derived from the murine influenza virus resistance gene *Mx*. These probes also detected specific fragments of genomic DNA from non-mammalian vertebrates such as birds and fish. To determine whether these hybridization signals were artifactual or else indicated the existence of *Mx*-like genes, we cloned and sequenced a 2.35 kb *Eco* RI fragment of genomic DNA from a fish. We find blocks of sequences that are co-linear to murine *Mx* gene exons 3 to 8 with homology values ranging from 60% to 85%. These blocks may represent exons of a bona fide fish gene because they are separated by intron sequences flanked by consensus splice acceptor and donor sites. Injection of dsRNA into the peritoneal cavity of fish resulted in 5 to 10 fold elevated levels of two liver mRNAs of about 2 to 2.5 kb length that hybridize to the cloned genomic DNA. High sequence similarity, conservation of exon structures and inducibility by dsRNA suggest that the cloned DNA fragment is derived from a fish *Mx* gene. Since influenza virus is believed not to replicate and not to cause disease in fishes, it is unclear why evolution permitted this high degree of *Mx* gene conservation.

P2 190

ARE THERE CELLULAR SEQUENCES HOMOLOGOUS TO POLYOMA EARLY GENES?

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So far two types of Polyoma viruses (Py) isolated from mice and hamster respectively have been described. Early after infection three early proteins - large, middle and small T antigen (lT, mT, sT)- are synthesized. The 3 proteins share a common aminoterminal stretch of 80 aa, and mT and sT are identical for another 110 aa.

The transforming gene product mT forms a stable complex with the cellular tyrosine kinase $pp60^{c-src}$ increasing its activity 50 to 100 fold. Only 10% to 20% of the total amount of $pp60^{c-src}$ is bound to mT and the complex is located at the plasma membrane. The role of mT in the virus life cycle is poorly understood. On the other hand it is clear that mT alters growth regulation in Py infected cells.

We speculate that the regulation of $pp60^{c-src}$ kinase activity exerted by mT is not a de novo property, but reflects a function that might be derived from a host genome. Interestingly, mouse and hamster mT show a striking homology pattern when sequence analysis is performed: conservation is restricted to aminoterminal parts of the proteins and to two short stretches at the carboxyterminal end. We designed degenerate oligonucleotide probes to highly conserved regions, that in one case include sT and mT sequences and in the other all 3 early proteins. Several sequences were isolated from lambda cDNA libaries and are now being analyzed.

P2 191

PROCESSING OF A TOBACCO GLUCANASE

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ß-1,3-Glucanase (E.C.3.2.1.39) is made as a ca 40 kD preproenzyme that is then processed to the mature, ca 33.5 kD form. Processing involves loss of a signal peptide and addition of an oligosaccharide side chain followed by removal of the oligosaccharide side chain and a C-terminal extension with a putative N-glycosylation site.

We confirmed the results of Shinshi and Kato (Agric. Biol. Chem. 47, 1455-60 (1983)) that mature ß-1,3-glucanase contains arabinose by feeding 3H-L-arabinose to tobacco cells and showing that the label is incorporated into arabinose covalently bound to the enzyme. 3H-L-arabinose is incorporated into the 33.5 kD enzyme but not into larger intermediates. Therefore, arabinosylation occurs late in processing after the enzyme has reached its mature molecular weight.

P2 192

GENETIC AND PHYSICAL ANALYSIS OF ILLEGITIMATE RECOMBINATION EVENTS IN THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE.

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We performed transformation experiments with non ars-plasmids in a yeast strain in which the homology region between the plasmid and the genome was deleted on the S. pombe genome. We obtained a total of 252 transformants from which 13 were stable. The remaining unstable transformants (5%-2o% stability) originate from the observation that non ars-plasmids are able to replicate in S. pombe. In comparison to the yeast Saccharomyces cerevisiae where autonomous replication is only possible if ars sequences are on the plasmid.
Genetic analysis of the transformants showed that the integration events are randomly distributed on the genome. We cloned eight integration events back to E. coli. We detected that duplications and deletions occured on the plasmid upon insertion. DNA sequence analysis of the junction sequences between the plasmid and the genome revealed a common five basepairs direct repeat. The duplicated motif -CTTAG- is comparable to predicted DNA motifs used in illegitimate recombination events in higher eucaryotes.

P2 193

TISSUE-SPECIFIC PAIRED-BOX GENES IN *DROSOPHILA* DEVELOPMENT

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Development is a precisely controlled process in which genes are activated and repressed in spatially and temporally ordered patterns. In an attempt to test our assumption that genes integrated into a given network contain a small number of network-specific domains, we isolated two genes, P4 and P29, that share the paired domain with the segmentation gene *paired* (*prd*). In contrast to *prd*, P4 and P29 have no homeo domain. Apart from the paired domain, the sequence of P4 exhibits particular features such as an alanine stretch and a carboxy-end enriched in acidic residues. We show that both P4 and P29 are expressed at specific stages of development and in segmentally-repeated patterns in the neurectoderm (P4) or in the mesoderm (P29) as shown by *in situ* hybridization to mRNAs and by immunostaining with antibodies raised against the corresponding proteins. We also show that the expression of P4 and P29 is affected in segmentation mutants such as *Krüppel*, *fushi tarazu* and *prd*, indicating that they belong to the network of segmentation genes. Both P4 and P29 proteins are nuclear antigens and hence, like *prd*, may be gene regulatory proteins.

P2 395

The Fate of Nucleosomes during Transcription in *Saccharomyces cerevisiae*.

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The DNA of all eukaryotic cells is densely packaged by histone proteins in a linear array of nucleosomes. Since this packaging reduces the accessibility of DNA sequences for regulatory factors and processive enzymes such as RNA polymerases, we investigate the fate of nucleosomes and their positions during transcription by using specifically designed yeast minichromosomes. The constructs contain the URA3 coding region linked to an inducible GAL1-promoter and a constitutively expressed reference gene (HIS3).
Analysis of the chromatin structures by nuclease digestion indicates precisely positioned nucleosomes and nuclease sensitive regions. Activation of the gene does neither alter the positions nor change the stability of nucleosomes on the coding region.

P2 396

TWO DIFFERENT CHROMATIN STRUCTURES COEXIST "IN VIVO" IN THE RIBOSOMAL GENES OF FRIEND CELLS THROUGHOUT THE CELL CYCLE

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The structure of ribosomal chromatin in exponentially growing Friend cells as well as in stationary cells or metaphase plates was studied by DNA-DNA and DNA-RNA photocrosslinking with trimethylpsoralen. Both, "in vivo" crosslinking in cells and "in situ" crosslinking in isolated nuclei gave the same results. The DNA which had been crosslinked in chromatin was analyzed by gel electrophoresis either after digestion with restriction enzymes or after exonuclease-S1 treatment. Complexes of nascent preribosomal RNA's crosslinked to the rDNA template were characterized by gel electrophoresis or by electron microscopy. The observations show that two distinct types of ribosomal chromatin coexist in Friend cells, one that contains nucleosomes and represents the inactive copies and one that is free of nucleosomes and corresponds to the transcribed genes. Digestion of nuclei with restriction enzymes prior to psoralen crosslinking confirms the two types of ribosomal chromatin and shows that, unlike the inactive chromatin, the active chromatin is fully accessible to the nuclease. The amounts of active and inactive chromatin remain constant throughout the cell cycle despite the fact that the run-on transcriptional activity is 6 times less in metaphase than in interphase.

P2 397

OSMIUM INDUCES DECONDENSATION OF CHROMATIN

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In dinoflagellates the chromosomes are believed to stay individually visible during the whole cell cycle. In contrast to this, de Haller et al. (1) had found decondensed chromosomes when prefixing the cells with 0.1% OsO_4 . We could now confirm this finding and furthermore show that the chromosomes are condensed when using aldehydes as fixative. That the condensed state is "normal" is corroborated by the fact that the cryofixing and cryosubstitution also leads to condensed chromosomes. By excluding an osmotic shock, which could have been arosen as a matter of the different salt concentrations between the prefixation and the fixation solution, we can now show that the osmium per se induces decondensation of chromatin. What role the concentration of OsO_4 plays will be investigated further.

(1)G. de Haller, E. Kellenberger, C. Rouiller. J. Microscopie 3, 627-642 (1964).

P2 398

BIOCHEMICAL CHARACTERIZATION AND IMMUNOLOCALIZATION OF BASIC NUCLEAR PROTEINS FROM A DINOFLAGELLATE.

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Dinoflagellates have a very unusual chromatin organized in permanently visible, arche-shaped chromosomes. No typical histone and nucleosome have been detected until now and little is known about chromatin structure. We have isolated basic nuclear proteins from the Dinoflagellate Crypthecodinium cohnii. Their electrophoretic pattern differs from that of histones in SDS- or AU-PAGE and in 2D-PAGE. Their affinity for DNA is lower than that of core histones: They are dissociated from DNA with NaCl concentrations ranging from 0.3 to 0.8M whereas core histones require 0.8 to 1.6M NaCl. The major 14kD component is composed of three related proteins resolved in 2D-PAGE and only one protein of low abundance (MW14.5kd) is immunologically related to 2 of the histones (H3 and H4). Although the majority of basic proteins is localized in the nucleoplasm and in the nucleolus, possibly involved in the control of transcription of extra-chromosomal DNA, the 14.5kD histone related protein is mainly found in the chromosomes and may play a role in their structure.

P2 399

ELECTRON MICROSCOPY OF NATIVE SPECIMEN
GLUING OF VITRIFIED *SPECIMENS* FOR CRYO-ULTRAMICROTOMY

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Native hydrated biological samples can be observed by cryo-electron microscopy of vitrified sections. The method has been used on various biological material including chromosomes and chromatine but it is still technically demanding. We present here a method for mounting the minute vitrified specimen in the cryo-ultramicrotome and show results obtained on nuclei and chomatine.

At the required temperature of less than 140K some alcohols have favorable properties for gluing vitrified specimens on the Al-pin used as specimen holder in the cryo-ultramicrotome.
-A droplet of ethanol remains viscous when applied to the specimen holder precooled around 120K. The vitrified specimen can be mounted on this soft support. After rewarming above 140K the alcohol changes into a hard milky modification which remains stiff and resistant to the cutting forces at low temperature.
-Propanol 2 and some alcoholic mixtures like Propanol 2 in Ethanol (3:1) or Butanol 2 in Ethanol (3:2) are hard enough to withstand cryosectioning below 110K. Above 130K they are soft enough for mounting the specimen. Cutting takes place after recooling when the support has become hard again.

P2 400

EM IMMUNOLABELING OF HISTONE H2B AND H3 IN MOUSE TESTIS

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The nuclear distribution of histones H2B and H3 has been studied by EM immunocytochemistry in the course of mouse spermiogenesis using specific anti-histone antibodies and colloidal gold-coupled secondary antibodies. The nuclear labeling density of histone H3 has been found to be constant during the four spermatid phases here considered. However, the nuclear labeling density of histone H2B decreases significantly in spermatid nuclei at phase C (spermatid steps 6-7) and then reaches a peak at phase D (elongating spermatids, steps 11-12). These data are discussed in the view of hypotheses considering a wave of histones synthesis at the late spermatid stages and the possibility that the anti-histone H2B antibody could also recognize some histone testis-specific variants which are known to be synthesized in the spermatid.

P2 401

COMPARISON OF IN VITRO TRANSLATION PRODUCTS OF TOTAL AND HYBRID-SELECTED RNA OF BOVINE HERPESVIRUS 1 STRAINS AND OF CAPRINE HERPESVIRUS 1

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In experiments aiming at localizing and comparing glycoprotein genes of different bovine herpesvirus 1 (BHV-1) strains we first analysed the in vitro translated RNAs of different BHV-1 strains (defined as BHV-1.1, BHV-1.2, BHV-1.3), as well as of caprine herpesvirus 1 (CapHV-1). The RNAs were isolated as total cytoplasmic RNA from cells infected with the respective virus strains or mock-infected from 0 to 5, 8, 10 and 12 hours, respectively. We found an overall number of 22-23 virus-specific polypeptides ranging in molecular weight from 15.5 Kd to 147 Kd. As could be expected from differences in polypeptide patterns found "in vivo", also in vitro produced polypeptides displayed minor differences between BHV-1.1 and BHV-1.2 and more distinct ones between BHV-1.1/-1.2 and BHV-1.3 or CapHV-1. In vitro translation products of hybrid-selected RNAs allowed a preliminary localization of single polypeptides on the genome. Hybrid-selected, in vitro translated polypeptides could not be immunoprecipitated with monospecific antisera raised to individual BHV-1 glycoproteins, probably because they were not processed.

Membrane biology (P3)

P3 194

CELL COMMUNICATION BY GAP-JUNCTIONS IN A VITRO SYSTEM USING TWO GENETIC ENZYME DEFICIENCIES OF GALACTOSE UTILIZATION

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^{14}C-galactose is incorporated into cellular (cell.) and secreted macromolecules (Mm) by human fibroblasts. Galactokinase deficient (GK-) fibroblasts incorporated 0.48 - 3.44% (mean: 1.2%) into cell. and 9.4 - 22.6% (13.1%) into extracell. Mm, while galactose-1-phosphate uridyltransferase deficient (GT-) fibroblasts incorporated 5.1 - 13.0% (8.7%) into cell. and 14.2 - 23.8% (18.7%) into extracell. Mm. Coculture of these fibroblasts at high density, corrected the macromolcular ^{14}C-galactose incorporation to 25.4 - 62.6% (49.0%) for cell. and to 39.6 - 88.0% (57.8%) for secreted Mm compared to normal. Retinoic Acid (RA) (100 μM) and the phorbolester TPA (20 ng/ml) concentrations known to totally inhibit communication through gap junctions, decreased the correction by 60.4% (RA) and by 94.5% (TPA) for cell. and by 46.2% (RA) and by 60.9% (TPA) for secreted Mm. Conclusion: 1. Correction of defective ^{14}C-galactose incorporation by coculture of GT- and GK- cells is explained by mutual exchange of sugarphosphates and nucleotides trough gap junctions. 2. This cell to cell communication is inhibited by RA and TPA, but not totally. 3. The high residual correction may suggest the existence of an other mechanism of substrate exchange.

P3 195

FUNCTIONAL IDENTIFICATION OF THE CHICK FIBRONECTIN RECEPTOR AS A HETERODIMERIC COMPONENT OF THE AVIAN INTEGRIN

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Tenascin inhibits attachment of chick embryo fibroblasts to fibronectin. We therefore wanted to isolate the chick fibronectin receptor to test whether it also interacts with tenascin. The fibronectin receptor was isolated by affinity chromatography to a cell binding fragment of chick fibronectin and was eluted with the synthetic peptide GRGDS or with EDTA. The purified receptor was a heterodimeric complex like the characterized mammalian integrins. The avian integrin has previously been reported to be a complex of at least three distinct subunits, which as an entity were claimed to bind to fibronectin as well as to other extracellular matrix molecules. Our results however indicate that the "chick integrin complex" can be separated into distinct subpopulations, among them a "classical" dimeric fibronectin receptor.

P3 196

NON-RANDOM DISTRIBUTION OF mRNA INDUCED ION CHANNELS IN THE PLASMA MEMBRANE OF XENOPUS OOCYTES

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Xenopus oocytes are asymmetrical cells. The pigmented animal hemisphere contains the giant nucleus and is distinct from the unpigmented vegetal hemisphere. We isolated poly-A^+ mRNA from chick forebrain, microinjected it into oocytes and subsequently studied the distribution of newly expressed neuronal ion channels in the surface membrane of the oocytes. The membrane current measurements were carried out by means of the two electrode voltage clamp technique. A special chamber was used which exposed only about 10% of the total surface area to the perfusion medium. We concentrated on measurements of the current responses elicited by exposure of the animal and the vegetal pole to the neurotransmitter GABA. In spite of the deposition of the mRNA in the vegetal pole we consistently found higher GABA induced signals in the animal pole. The size of the current measured 36h after injection on the vegetal pole relative to that on animal pole amounted to only 6 ± 5% (n=4). It gradually increased during the next 48h, until it reached a constant level of 38 ± 7% (n=15; day 4 to day 10). Similar results were obtained for the kainate channel.

P3 197

NON-RADIOACTIVE METHODS FOR SURFACE LABELLING OF HUMAN PLATELETS

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Biotin/streptavidin methods were investigated as replacement for radiolabelling techniques such as periodate/[^{3}H]$NaBH_4$ or lactoperoxidase/^{125}I, for platelet membrane glycoproteins, to demonstrate surface location and to identify and characterize specific components. Periodate oxidation followed by biotin hydrazide or biotinamidocaproyl hydrazide was used as a carbohydrate specific method and photobiotin as a protein-specific method. After gel electrophoresis and Western blotting the biotin labelled components were detected with streptavidin -phosphatase. All methods were non-permeant and specific as neither cytoplasmic nor granule components were labelled and all labelled major glycoproteins, particularly IIb and IIIa. Photobiotin gave the most general labelling which closely resembled the results with 125I. GPIb blots poorly and was weakly detected. GPIIIb, a highly glycosylated, very hydrophobic component labelled only with biotin hydrazide.

P3 198

A MEMBRANE-ASSOCIATED HOMOLOGUE OF THE AXONALLY SECRETED PROTEIN AXONIN-1: III. QUALIFICATION AS AN INTEGRAL MEMBRANE PROTEIN AND CHARACTERIZATION OF ITS STRUCTURAL RELATIONSHIP TO SECRETED AXONIN-1

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Recent investigations of the axonally secreted glycoprotein axonin-1 had raised evidence for a membrane-associated homologue involved in neurite fasciculation (Stoeckli et al., and Ruegg et al, accompanying abstract). To elucidate the nature of the membrane-association of the axonin-1 immunoreactive material, brain membranes were prepared from 14-day-old chicken embryos and a single axonin-1 immunoreactive protein was isolated by immunoaffinity chromatography. By its resistance to high salt and high pH it qualified as an integral membrane protein. Membrane-bound axonin-1 appeared identical to soluble axonin-1 with respect to the pI of its isoelectric variants, but its apparent molecular weight was about 2 kDa lower. The homology of this protein to secreted axonin-1 was corroborated by identical peptide maps and crossreactivity with monoclonal anti-axonin-1 antibodies. In view of the lower apparent molecular weight of the membrane-bound form, lipid-mediated membrane anchorage is conceivable.

P3 199

A MEMBRANE-ASSOCIATED HOMOLOGUE OF THE AXONALLY SECRETED PROTEIN AXONIN-1: II. CHARACTERIZATION AS A NOVEL CELL ADHESION MOLECULE INVOLVED IN NEURITE FASCICULATION

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The location of axonin-1 immunoreactivity at the surface of developing nerve fiber tracts (Stoeckli et al., accompanying abstract) closely parallels the tissue distribution of cell adhesion molecules involved in neurite fasciculation. Indeed, in the presence of anti-axonin-1 Fab-fragments during axon outgrowth from cultured dorsal root ganglia, fasciculation was markedly reduced. At the end of the perturbation experiment the Fab-fragments were localized at the axonal surface by immunoelectron microscopy. The membrane-associated axonin-1 immunoreactive material was found represented by a single protein. It is immunologically distinct from the cell adhesion molecules reported thus far. Radioactive pulse-chase and double-labeling experiments revealed distinct biosynthetic pathways for the secreted and the membrane-bound form. Especially, the secreted form was shown not to be derived from the membrane-bound form, but from an intracellular pool.

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A MEMBRANE-ASSOCIATED HOMOLOGUE OF THE AXONALLY SECRETED PROTEIN AXONIN-1: I. IMMUNOHISTOCHEMICAL LOCALIZATION IN DEVELOPING NERVE FIBER TRACTS

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Axonin-1, a 132-140 kDa glycoprotein, is secreted from axons of cultured embryonic dorsal root ganglion neurons and a number of other neuronal subpopulations (Stoeckli et al, Neuroscience 22, 593, 1987). It has recently been purified from the ocular vitreous fluid of the chicken embryo (Rüegg et al., EMBO J., in press). Axonin-1 is probably neuron-specific and prevalently expressed in the embryo. In the present study, the expression of axonin-1 in the developing nervous system of the chick was investigated immunohistochemically at the light and electron microscopic level. In both the developing retina and spinal cord, axonin-1 was found mainly associated with nerve fiber tracts. In the spinal cord, the staining disappeared after hatching. Immunoelectron microscopy of the optic fiber layer of the retina located the axonin-1 immunoreactivity of axon bundles to the surface of the axonal membrane. The specific location in bundled axons and the early disappearance after birth suggests implication in the formation of nerve fiber tracts.

P3 201

CLONING, EXPRESSION ANALYSIS AND REGULATION OF THE MURINE 4F2 HEAVY CHAIN ANTIGEN

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A cDNA coding for the murine 4F2 heavy chain antigen was isolated using human 4F2 cDNA as a probe. This antigen, whose function is still unknown, is one of the earliest surface markers appearing after lymphocyte stimulation. Sequence analysis shows that the murine heavy chain antigen has a high homology with the human protein and is also a type II transmembrane glycoprotein. We used this cDNA as a probe to analyse the expression of the gene in murine tissues, cell lines and in "in vitro" stimulated lymphocytes. Mouse 4F2 heavy chain mRNA is present in all tissues analysed, although it is expressed at different levels. In NIH 3T3 and 21tb cell lines we did not detect any significant difference in its expression after growth stimulation. In lymphocytes 4F2 mRNA levels are strongly induced after activation but we found no corresponding increase in transcription rates as mesured by nuclear run on assays, suggesting postranscriptional regulation.

P3 202

Interaction between antigenic peptides and MHC class I molecules.

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The murine H2Kd restricted CTL clone CW3/10.1 is specific for the human antigen HLA-CW3. This clone can kill H2Kd positive cells incubated with a short synthetic peptide corresponding to amino acids 170-182 of the HLA-CW3 molecule. We derived an L-cell line, 1LHD13, transfected with the H2Kd cDNA under the control of the LTR promoter of MMTV. After induction with hydrocortisone the 1LHD13 cells express the H2Kd molecule on the cell surface and can be lysed in the presence of the HLA-CW3 peptide. However, no lysis was obtained when 1LHD13 cells were pulsed with the antigenic peptide and extensively washed before hydrocortisone induction. Control cells induced to express H2Kd before antigen pulsing were lysed. One interpretation of these results is that only class I molecules present on the cell surface can interact with exogenously added synthetic peptides.

P3 203

MONOCLONAL ANTIBODIES AGAINST HUMAN EMBRYONIC SPINAL CORD

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We attempted to prepare monoclonal antibodies specific for anterior horn cells of human embryonic spinal cord. To increase the specificity of the immune response towards the anterior horn, mice were first injected with antigens from the posterior part of the spinal cord and then immunosuppressed before receiving the anterior horn as immunogen. The hybridoma supernatants were screened using formalin-fixed cryostat sections of human embryonic spinal cord. One of them labelled a small group of cells in the anterior horn. This antibody is species-specific, as it does not stain the rat and chick embryonic spinal cord. The labelling appears in a transitory fashion; at 7-9 weeks there is no labelling, at 9-10 weeks the anterior horn cells are marked, and at later stages there is very little labelling. A short (5 min) pre-permeabilisation of the sections in 0.2% triton does not change the staining pattern; however, when the sections are incubated overnight with supernatant containing 0.2% Triton X-100, the labelling becomes more general, but is still more intense in the anterior horn. Further investigation is needed to assess the specificity of this antibody.

P3 204

FUSION AND LIPID EXCHANGE BETWEEN INFLUENZA VIRUSES AND LIPOSOMES MEASURED WITH THE R18 MEMBRANE MARKER

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PR8 influenza viruses and ganglioside GD1a-containing liposomes were incubated under various conditions, to differentiate between fusion and lipid exchange. Octadecyl Rhodamine B chloride (R18) was incorporated into liposomes at a quenched concentration, and membrane interactions, leading to dilution of R18, were monitored with a fluorescence spectrometer. At pH5.3 (37°C), conditions under which fusion takes place, a rapid dequenching (DQ) occurs ($t_{1/2} \sim 1$ min) with a plateau at 75-85% DQ. A much slower rate ($t_{1/2} = 40$ min) is found at pH7.4, however, the same plateau is finally reached. Inactivation (acid treatment) or removal (bromelain digestion) of the virus hemagglutinin both abolish the fast DQ reaction, whereas slow DQ is still measured at pH5.3 and pH7.4. Kinetic analysis at various temperatures (Arrhenius plot) yields a higher change in free energy for the pH5.3 than for the pH7.4 reaction. Two distinct membrane interactions can thus be distinguished indicating that both fusion and lipid exchange occur. The consequences in view of the use of liposomes as drug carriers will be discussed.

P3 205

PHOSPHORYLATION OF GPIIIa BY PROTEIN KINASE C AND FIBRINOGEN BINDING TO PLATELETS

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Glycoproteins IIb/IIIa form the major binding site on platelets for proteins such as fibrinogen containing an RGD sequence and are essential for normal haemostasis. They are missing in the bleeding disorder Glanzmann's thrombasthenia. Activation of protein kinase C (PKC) is necessary for fibrinogen binding. We have shown that GPIIIa but not GPIIb is phosphorylated in resting platelets and that this is increased 2-5 fold by platelet activators like thrombin or 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and decreased slightly by agents that raise cAMP. GP IIIa could be phosphorylated with [^{32}P]-ATP in vitro by PKC in the presence of phosphatidylserine. Since resting platelets in contrast to activated platelets do not bind fibrinogen, increased phosphorylation of GPIIIa may be related to conformational changes in the GPIIb/IIIa complex exposing the fibrinogen binding site.

P3 206

CLONING AND SEQUENCING OF A γ-IFN INDUCED MELANOMA ANTIGEN

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A γ-IFN inducible melanoma antigen expressed also on B lymphoblastoid cell lines has been characterized as a non covalently bound glycoprotein dimer of 33-38 kD. Using genomic gene transfer and screening with a monoclonal antibody (Me14/D12) specific for this antigen, we isolated a genomic probe. The probe identifies two mRNA species of 3.5 and 2.2 kb in melanomas and stable transfectants. γ-IFN induces a comparable increase in expression of the antigen at the mRNA and protein level. A λgt10 cDNA library from Raji cell line was screened with the genomic probe. Upon transfection of a full length 3.0 kb cDNA under the control of the human transferrin receptor promoter in mouse L cells, a surface antigen recognized by Me14/D12 is expressed. The 3.0 kb cDNA sequence predicts a 57 kD protein suggesting that it could be processed into the melanoma-associated antigen of 33-38 kD. Analysis of the protein sequence did not reveal homology with any other known sequence.

P3 207

INTERFERON-GAMMA ENHANCES THE EXPRESSION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX H-2K IN MOUSE FIBROSARCOMA CELLS

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The influence of recombinant murine interferon-gamma# (INF-γ) on the expression of the major histocompatibility complex (MHC) of class I (H-2K) was studied in cells from the mouse fibrosarcoma cell line MC57. The lymphokine was added to cultures at different concentrations. Two days later, the cells were harvested and the H-2K antigen presenting cells were stained by immunofluorescence. The degree of expression was quantified by a two parameter (volume/fluorescence) flow-cytometry analysis. The presence of H-2K antigen was already detectable in unstimulated cultures. 0.2 U/ml of IFN-γ had no effect. Two, 20 and 50 U/ml IFN-γ produced a 2, 18 and 25 fold increase of this expression, respectively. IFN-γ therefore induces in vitro a strong and dose-dependent amplification of the H-2K antigen on cells from the MC57 cell line.
#gift from Dr. G.B. Adolf, Ernst-Boehringer, Wien.

P3 208

EFFECTS OF VASOPRESSIN ON (Na^+/K^+)TRANSPORT BY HEPATIC PLASMA MEMBRANE VESICLES

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Arginine vasopressin (AVP) has previously been shown to rapidly stimulate ($Na^+ + K^+$)ATPase of intact rat hepatocytes. With the present experiments the effects of the hormone on (Na^+/K^+)transport by inside-out plasma membrane vesicles isolated from rat livers were studied. Vanadate sensitive ^{22}Na uptake was measured at different ATP concentrations in the presence of either high (100 mM/60 mM) or low (1 mM/1 mM) concentrations of Na^+/K^+ respectively. The K0.5 (ATP) was 100 ± 25 µM at high and 15 ± 1 uM at low Na^+/K^+ concentrations in the absence of AVP and 60 ± 6 µM and 62 ± 9 µM in the presence of the hormone. AVP increased Vmax by 90% and 300% respectively. The results demonstrate that isolated plasma membrane vesicles can be used to study the effects of AVP on the kinetics of (Na^+/K^+)transport.

P3 209

ACTIN-ASSOCIATED PROTEINS IN HUMAN NEUTROPHILS: IDENTIFICATION AND REORGANIZATION UPON CELL ACTIVATION

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A functional cytoskeleton is essential for neutrophil chemotaxis. During cell activation by chemotactic peptides a dynamic reorganization of the cytoskeleton, especially of the microfilaments, takes place. The role of actin-associated proteins in this process has not yet been clarified. We have identified α-actinin (an actin-cross-linking protein) and vinculin (a putative actin-membrane linker) in human neutrophils using specific antibodies against these proteins. Talin (another putative actin-membrane linker) was barely detectable in neutrophils, whereas human platelets and lymphocytes could be shown to contain sizable amounts of this protein. We also studied the association of α-actinin with the Triton X-100-insoluble cytoskeletal fraction of neutrophils during their activation by chemotactic peptides. A rapid increase in α-actinin association with the cytoskeleton could be measured upon activation, and its time course parallels that of actin association. α-Actinin may thus play an important role in determining the structure of actin networks in motile cells.

P3 210

CHEMICALLY STABILIZED MEMBRANES

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For membrane stabilization purposes two butadiene phospholipids have been synthesized (C_{18}-DENPC: 1,2 bis (octadeca)-2,4-dienoyl-sn-glycero-3-phosphocholine and its C_{16} analogue) and purified by reverse phase FPLC. Stabilized membranes are produced by polymerisation of the diene function leading to intermolecular links at a defined level within the hydrophobic core of the bilayer. The phase transition of C_{18}-DENPC membranes occurs at 19°C. Polymerisation broadens the phase transition. Mixtures of DENPC and saturated PC reveal heterogeneous phase transitions at intermediate temperatures. This indicates strong interaction between the diene lipid and saturated PC. It allows modulation of the fluidity and transition temperature of the membrane for protein reconstitution purposes. The kinetic of DENPC-photopolymerisation is 25 times faster with vesicles than with the monomer in organic solvents. This evidences the topological proximity of the butadiene between neighbouring lipids in the bilayer. Phospholipase A_2 hydrolyses non polymerized butadiene phospholipids. Partially polymerised DENPC or mixed lipid vesicles can thus be permeabilized for delivery of their contents.

P3 211

RECONSTITUTION OF A FUNCTIONAL AMILORIDE SENSITIVE Na-CHANNEL INTO XENOPUS OOCYTES

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An amiloride-sensitive Na^+ channel is localized to the apical membrane of high resistance Na^+ transporting epithelia such as the amphibian kidney cell line A6. Antibodies directed against the fusion protein of a cDNA clone from an A6 expression library recognize the Na^+ channel purified from A6cells. The cDNA clone hybridizes to a 5.7 kb mRNA from A6 cells. To assist with cDNA cloning we developped a ^{22}Na transport assay to detect functional expression of the Na^+channel in Xenopus Oocytes microinjected with A6 mRNA. RNA injected oocytes exhibited a 15fold greater ^{22}Na uptake which was inhibited by amiloride (IC_{50} = 60 mM). Amiloride-sensitive ^{22}Na was maximally expressed by oocytes injected with size-fractionated mRNA sedimenting between 18 S and 28 S. The amiloride sensitive Na^+ channel can be functionally expressed in Xenopus oocytes microinjected with total and size-fractionated A6 mRNA. Such assays will serve to screen for full length cDNA coding for the Na^+ channel.

P3 212

Lipid composition and membrane fluidity of fibroblasts grown at hypothermic and hyperthermic temperatures.

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Hibernating animals decrease cell membrane anisotropy by changes in their membrane phospholipids. We investigated whether human fibroblast cultures grown at 30, 37 and 40°C for 10 days would exhibit similar mechanisms. Total cell phospholipids (PL), cholesterol, DNA and protein were determined in trypsinized cells by routine methods. Individual PL were separated by HPTLC and quantified by reflectiondensitometry. Fluorescence anisotropies (rG) of fibroblasts grown on glass coverlips were determined at 30, 37 and 40°C using diphenylhexatriene (DHP) for the deeper layers of the membrane and trimethylammonium-DPH (TMA-DPH) for the superficial layers of the membrane. Hypothermia as well as hyperthermia changed the lipid composition and phospholipid pattern. In cells grown at 30°C, apparent rG of deeper layers (DPH) was lower than in cells at 37°C, while at 40°C cells exhibited intermediate values. However at superficial membrane layers (TMA-DPH) apparent rG was highest in cells at 30°C and lowest at 40°C. There appears to be a relation between membrane fluidity and culture temperature depending of the depts of the layers.

P3 213

IDENTIFICATION OF PARATHYROID HORMONE (PTH)-REGULATED PLASMA-MEMBRANE PROTEINS OF THE OK CELL

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There is evidence that PTH-dependent regulation of Na-phosphate cotransport in established Opossum kidney (OK) cells involves irreversible inactivation of the transport system (or part of it) possibly realted to internalization. Accordingly, the abundance as well as the rate of synthesis of specific membrane proteins should depend on PTH addition/removal. This hypothesis was tested by an analysis of (apical) plasmamembrane proteins by 2-dim gelelectrophoresis. i) The relative abundance (analyzed by silver staining) of four proteins (28,35,45 and 48 kD) was reduced by 40 to 60 % after exposing cells to 10^{-10} M PTH for 3 hours (60 % inhibition of P_i-transport. ii) Similarly, labelling experiments indicated a 2 to 3-fold enhanced incorporation of ^{35}S-L-methionine into the same four proteins during the recovery phase after PTH removal.
We conclude that the identified proteins represent possible candidates for PTH regulated proteins of the plasmamembrane of the OK cell. Furthermore, these proteins might represent candidates for the Na/P_i-cotransporter.(supported by SNF 3.854.0.88 and 3.854.1.88)

P3 214

EFFECTS OF 2,3-BUTANEDIONE MONOXIME ON THE ELECTRICAL PROPERTIES OF GUINEA PIG MYOCYTES

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2,3-butanedione monoxime (BDM) has been proposed to act as an electro-mechanical uncoupler which does not interfere with the cellular $Ca2^+$ cycle (Mulieri and Alpert, Biophys. J. 45: 48a, 1984). In the present study we explored the effects of BDM on the electrical properties of myocytes isolated from adult guinea pig ventricles utilizing patch pipettes in the whole cell, thight seal recording mode. Exposure to 20 mM BDM produced a 50-75% decrease in $Ca2^+$ inward current which does not account fully for the loss in contractility. The steady-state current-voltage relationship became more inward at negative potentials and more outward at positive potentials. Experiments aimed at blocking the K^+ currents (bath: 1 mM Ba^+, 2 mM Cs^+; pipette: 120 mM Cs^+, 10 mM TEA^+) revealed that these effects are not caused by an increase of the inward rectifier or the delayed rectifier. Experiments with glibenclamide (1-5 µM) suggest that an activation of the ATP-dependent K^+ current is not involved either.
Supported by SNSF (grant 3.253-0.85).

P3 215

EXPRESSION OF THE CHICK CARDIAC Na^+-Ca^{2+} EXCHANGER IN XENOPUS OOCYTES

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Total mRNA was prepared from hearts of 1-day-old chicks and microinjected into Xenopus oocytes. After five days in culture the oocytes were freed from follicular cell layers and loaded with Na^+ by incubation in hypertonic EGTA solution. Subsequently, the Na^+-loaded oocytes were placed in a Na^+-free medium containing $^{45}Ca^{2+}$ and the rate of uptake of the isotope into oocytes was determined. mRNA-injected oocytes accumulated $^{45}Ca^{2+}$ at a rate 3 to 18-fold higher than oocytes injected with control buffer not containing mRNA. Na^+-loaded oocytes assayed in Na^+ containing medium showed a strongly reduced mRNA dependent $^{45}Ca^{2+}$ uptake. No mRNA dependent uptake was observed in oocytes not subjected to the Na^+-loading procedure. Total mRNA was fractionated according to size using sucrose density gradient centrifugation. A 25 S fraction was found to be competent for the expression of the Na^+-Ca^{2+} exchanger. This mRNA fraction represents a suitable starting point for the expression cloning of the DNA coding for the Na^+-Ca^{2+} exchanger.

P3 216

DYE AND ELECTRICAL COUPLING BETWEEN ENDOTHELIAL CELLS IN SITU: EFFECT OF BRADYKININ.

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Electron microscopy studies show that endothelial cells of pig coronary arteries are linked by gap junctions. We investigated the dye and electrical coupling of these junctions in a strip of pig coronary artery in vitro.The membrane potential of two neighbouring (0,1 mm) endothelial cells were simultaneously recorded with two microelectrodes. The fluorescent dye lucifer yellow was microiontophoretically injected through one of the microelectrodes. We showed that the membrane potential of endothelial cells in situ was -40 ± 4 mV (n=9). Bradykinin (BK) transiently hyperpolarized these cells by 14 ± 2 mV (n=9). The endothelial cells in situ were dye and electrically coupled. The dye coupling extended parallel to the longitudinal axis of the arteries. The endothelium was never coupled to the underlying smooth muscle cells. We conclude that BK elicits an electrical message in the endothelial cells and that such an information can be conveyed electrotonically by the endothelium along the longitudinal axis of arteries. This is important in the context of the role played by the endothelium in the local control of vascular tone.

P3 217

EFFECTS OF DIPHENYLAMINE-2-CARBOXYLATE (DPC) ON ION TRANSPORT IN MONOLAYERS OF BOVINE TRACHEAL EPITHELIUM.

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DPC has been reported to act as a blocker of Cl^- channels in various Cl^--secreting epithelia, such as shark rectal gland and dog trachea. In the present study, I have found that the effect of DPC is not restricted to Cl^- channels, since Na^+ transport is also inhibited by the drug. Cow tracheal epithelial cells in primary culture form confluent monolayers. The electrical values were: short-circuit current (I_{sc}) 12 ± 2 μAcm^{-2}, resistance 570 ± 84 Ωcm^2 (n = 28); amiloride (2µM, mucosal side) reduced I_{sc} from 11 ± 3 to 6 ± 2 μAcm^{-2} ($p < .01$; n = 5); prostaglandin E2 (PGE2, 1 µM) induced a 3-fold increase in I_{sc} (n = 7); in PGE2-stimulated monolayers, bumetanide (50 µM, serosal side) decreased I_{sc} from 34 ± 6 to 17 ± 3 μAcm^{-2} ($p < .01$; n = 7). These data show that monolayers behave like native tissue. DPC (10 µM to 10 mM, mucosal side) caused a dose-dependent, reversible inhibition of I_{sc} (97 ± 4 % recovery at 10 mM, n = 8). In PGE2-stimulated monolayers, 3 mM DPC diminished I_{sc} from 28 ± 6 to 1 ± 1 μAcm^{-2} (n = 5); in Cl^--free solution and in the presence of PGE2, DPC reduced I_{sc} from 16 ± 3 to 2 ± 1 μAcm^{-2} (n = 4). Dose-response curves for DPC in Na^+-free or in Cl^--free solutions revealed that the potency of DPC as a blocker of Na^+ transport was not different from that exerted on Cl^- channels.

P3 218

CELLULAR MECHANISMS IN PARATHYROID HORMONE (PTH) CONTROL OF Na/H EXCHANGE IN CULTURED KIDNEY (OK) CELLS

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Opossum kidney (OK) cells contain proximal tubular transport functions (eg. Na/H exchange and Na/phosphate cotransport) regulated by PTH. Using BCECF, Na/H exchange was measured as the Na-dependent recovery of cytosolic pH (pH_i) from acid loads. Using cells grown on filters, the pH_i-recovery measured in single cells could be attributed to Na/H exchange (apparent K_t (Na)= 35 mM) localized exclusively in the apical membrane. PTH (10^{-8} M) inhibited pH_i-recovery, with apical PTH having a larger effect than basolateral PTH. In suspended OK cells, net Na/H exchange ceased at pH_i values above pH 7.1 (a 'set point'). PTH inhibited Na/H exchange, but had no effect on the set point value. Both forskolin and 8-Br cAMP also inhibited Na/H exchange. Phorbol esters were shown to inhibit Na/H exchange, also without effect on the set point. Neither additiv (nor synergistic) effects were observed between maximal (or submaximal) doses of phorbol esters with either forskolin or PTH. It is concluded that apical Na/H exchange is inhibited by PTH without alteration in set point, and that activation of kinase A and/or kinase C may mediate independently this response (supported by SNF 3.854.0.88).

P3 219

ELECTRICAL PROPERTIES OF GAP JUNCTION CHANNELS IN GUINEA PIG VENTRICULAR CELL PAIRS.

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Cell pairs isolated from adult guinea pig ventricles were used to study the properties of gap junction channels. The experimental approach adopted involved a double voltage-clamp method and whole-cell, tight-seal recording. Single channel activity was resolved after reducing the intercellular current flow by exposure to 3 mM heptanol. The current-voltage relationship of the channels was linear over the voltage range examined (+/- 95 mV). The conductance of a single channel turned out to be insensitiv to the non-junctional membrane potential (range tested: - 90 to +10 mV) and to a moderate elevation of the intracellular Ca concentration. The transition times to open or close a channel were slow compared to non-junctional membrane channels and ranged from 2 to 45 ms (mean value for opening : 13 ms). With pipette solutions containing Cs aspartate, single channel conductances ranged from 18 to 88 pS (mean value : 35 pS, modal value : 27 pS); with CsCl pipette solution, the mean value increased 1.4 -fold. Addition of cAMP (0.1 mM) to the pipette solution led to a small increase in channel conductance, addition of ATP (3 or 6 mM) produced no change. Supported by SNSF (grant 3.253 -0.85) .

P3 220

INJECTIONS OF Na OR ADP+Pi CAUSE AN INCREASE OF O_2 CONSUMPTION IN THE PHOTORECEPTOR CELLS OF BARNACLE

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In photoreceptor cells of invertebrates, a brief flash of light induces a transient increase in the rate of O_2 consumption (ΔQO_2). In the lateral ocellus of the barnacle, a crustacean, experimental evidence supports the hypothesis that the light-induced ΔQO_2 is controlled by the rate of Na pumping in the photoreceptor cells. Further support to this hypothesis is provided by the following results: 1) injection of Na into one of the photoreceptor cells induced a hyperpolarization of the cell membrane and a transient drop of the PO_2 near the cell (ΔPO_2), indicating a ΔQO_2; 2) injection of ADP+Pi (i.e. the products of ATP hydrolysis by the Na pump) induced also a ΔPO_2, without affecting the membrane potential of the cell. We conclude that, in the barnacle, all the results so far are consistent with the hypothesis that the light-induced ΔQO_2 is controlled by the rate of Na pumping.

P3 221

PHOTOTRANSDUCTION AND CONTROL OF OXIDATIVE METABOLISM IN INVERTEBRATE PHOTORECEPTORS

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Absorption of light by the visual pigment triggers a serie of chemical reactions terminating in the opening of sodium channels in the cytoplasmic membrane. This leads to a transient rise of the cytoplasmic concentration of Na and secondarily to Ca ions. The rise of Na+ activates the sodium pump and the Na^+ is extruded again to the extracellular space. The rise of Ca (several folds) transitorily desensitizes the photoreceptor (light adaptation). Then the Ca is sequestered again in the endoplasmic reticulum and the cell dark adapts. These processes need metabolic energy. The dark adapted photoreceptor contains (variation among species) 1.5-6 mM of ATP and O_2 consumption (QO_2) triples in 2-3 sec following a flash. Thus the availability of ATP is more than sufficient. Direct evidence showed that it is not the activation of the sodium pump by Na^+ ions that triggers QO_2. In the contrary Ca ions must play a key role in the control of the activated QO_2 since intracellular injection of Ca ions mimics the effect of light.

P3 222

DIPHENYLAMINE-2-CARBOXYLATE (DPC) STIMULATES SODIUM ION TRANSPORT IN FROG SKIN EPITHELIUM

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DPC is a well known blocker of the Cl^- conductance in different tissues, e.g. the thick ascending limb of Henle's loop and the shark rectal gland. This study examined the effect of DPC on Na^+ transport in frog skin. All experiments were performed in $SO_4^{=}$ Ringer. The following observations were made: (1) DPC, added to the mucosal side, induced a dose-related stimulation of the short-circuit current (I_{sc}). (2) This effect was reversible (recovery 89 ± 8 %, n=10) and (3) it was blocked by amiloride. (4) An analysis of the dose-response curve of DPC according to Lineweaver-Burk yielded a K_m value (concentration producing a half maximal effect) of $2.6 \cdot 10^{-4}$ M (n=7) and the maximal stimulation of I_{sc} was 140 % of the control value (9.0 ± 1.3 μAcm^{-2}, n=23). (5) The natriferic response to DPC was additive to that of oxytocin. (6) DPC caused no appreciable shift in the dose-response curve of amiloride. We conclude that DPC is not only a blocker of the Cl^- conductance, but it stimulates also the amiloride-sensitive mucosal sodium entry in frog skin epithelium.

Poster Session, Friday

Anatomy, Histology, Embryology (P AHE)

P AHE 223

Sandor KASAS, Prof. G. CONTI, Institut d'Histologie et d'Embryologie générale, Université, Fribourg

Semi-automatic 3-dimensional reconstruction of serial histological sections

The program presented here reconstructs 3D pictures from series of histological sections. Important structures are selected and automatically digitized resulting in considerable gain of time and precision compared to manual procedures.

The apparatus used includes a monochromatic light source to illuminate the section, a CCD camera coupled to a microscope for the image acquisition and an IBM AT compatible computer.

P AHE 224

CHONDROCLASTS STRUCTURE AND FUNCTION IN OSTEONEOGENETIC IMPLANTS OF DEMINERALIZED BONE POWDER (DBP).

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Rat DBP implanted in the rectus abd. muscle of adolescent rats induces the formation of bone (Urist, 1965). The primarily invading mesenchyme cells differentiate into chondrocytes forming cartilage which readily calcifies. From day 10 to 20 after implantation, the cartilage is invaded by capillaries. Osteoblasts and bone marrow appear, leading to the formation of a shell shaped ossicle containing bone marrow.
In this study we have implanted DBP enriched as to the active principle in order to obtain more cartilage. The obtained "pellets" were retrieved and studied by histological, histochemical and EM techniques. From the obtained results, we infer that as soon as cartilage is calcified, it is invaded and resorbed by chondroclasts. These cells contain multiple nuclei, they give a positive reaction for tartrate resistant acid phosphatase. By EM it can be shown that they contain many vacuoles and display ruffled borders. Hence chondroclasts seem to make way for the blood vessels invading the implant.

P AHE 225

ACTION OF DIFFERENT CYTOKINES ON ACTIN ISOFORMS EXPRESSION FIBROBLASTS IN VITRO

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Myofibroblasts are known to appear in granulation tissue during wound healing; these cells are characterized by ultrastructural features intermediate between fibroblasts and smooth muscle cells (Gabbiani et al 1971 Experientia 27:549). α-smooth muscle (α-SM) actin isoform, a differentiation marker of smooth muscle cells, is transitorily expressed in myofibroblasts of rat skin wound; in humans, α-SM actin has been shown to be particularly abundant in hypertrophic scars, fibromatoses, and stromal reaction to tumors (Skalli et al 1989 Lab Invest in press). Mononucleated cells are present locally at the site of the inflammation or fibrotic processes and may play a role in the modulation of these different fibroblast phenotypes. We have studied the action of different cytokines on α-SM actin expression in cultured rat fibroblasts by means of immunocytochemistry, densitometric analysis of two-dimensional gel electrophoresis, Western blots and Northern blot hybridization. γ-interferon inhibits fibroblast proliferation and selectively decreases a-SM actin content at the level of the protein and of the mRNA. Tumor necrosis factor and interleukin-1β have the same effects. Granulocyte-macrophage-colony stimulating factor and transforming growth factor-β increase α-SM actin isoform expression. Our observation may help in the inderstanding of normal and pathological wound healing.

P AHE 226

FORMATION OF ORGANOTYPIC STRUCTURES BY TUMOR CELL LINES CULTIVATED IN A CLONOGENIC SYSTEM (methylcellulose).

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Cells from human tumor cell lines (WiDr: colon adenoCa;Adams: ovarian adenoCa; IMR-32: neuroblastoma; MeF7 breast adenoCa and primary breast adenoCa) were trypsinized and cultivated in semi-solid medium (methylcellulose:synthetic medium,1:1) at 10^4 cells/ml. After 21 to 44 days some of the colonies were transferred in liquid medium and the remaining colonies were immediately fixed and examined for morphology. Monoclonal antibodies were used for the identification of cells. The results show that the tumor cells of a colony express some of their organotypic capacities with formation of ducts, vesicles, clusters, canaliculi, accompanied or not by a basal lamina. These organotypic structures are specific of each tumor and the colonies are formed by the same histomorphologic type of cells. The colonies keep their morphology after the transfer to the liquid medium till 5 to 10 days. Our results suggest that some of the organotypic capacities express themself regardless of classic factors as the presence of mesenchyme, collagen, or connective tissue cell interactions.

P AHE 227

DETECTION OF PARVOVIRUS NUCLEIC ACID IN INFECTED TISSUE BY IN SITU HYBRIDIZATION

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Infection by Human Parvovirus B19 is very frequent and causes erythema infectiosum, arthropathy and occasionally aplastic anemia. Hematogenous, transplacentar infection in the human may lead to hydrops fetalis with consecutive intrauterine death of the fetus. In such cases, dot blot hybridization studies have shown all examined organs to contain parvoviral nucleic acid without predominant involvement of any organ. Histology often reveals inclusion bodies in the immature hematopoetic cells which are markedly increased in number. To further elucidate this question, we performed *in situ* hybridization with a biotinylated cDNA probe to all major organ systems. We found a strongly positive signal in the nuclei of immature hematopoetic cells within blood vessels of all organs. This accounts for the presence of viral nucleic acid in all organs as seen by filter hybridization. The cell specificity can be explained by the fact that parvoviruses replicate only in cells in the S-phase.

P AHE 228

DOES SPEMANN'S ORGANISER CREATE A FIELD OR DOES IT EMIT THE INDUCING AGENT IN THE FORM OF FORMATIVE CELLS?(A FILM CONTRIBUTION TO THE QUESTION.)

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The film shows that in the embryo there are formative cells. Then in the upper blastopore-lip they must be crowded and in SPEMANN's and H. MANGOLD's famous experiment they have been transferred from the Cristatus to the Taeniatus germ where they formed the axial organs of a second embryo partly out of the material of the host. Influences by the host and the formation of embryos by dead "organisers" are due to the host's own formative cells. They are attracted by those inocula partly in a relay mechanism through the cytolysis products of injured cells. The gradient field theory in this explication has no place.

Biochemistry (P BIO)

P BIO 229

NEW ANTI-GD2 MONOCLONAL ANTIBODIES PRODUCED FROM GAMMA-INTERFERON TREATED NEUROBLASTOMA CELLS

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Three monoclonal antibodies have been produced by hybridomas obtained by fusion of mouse myeloma cells and spleen cells of mice hyperimmunized with γ-Interferon treated neuroblastoma cells. The 3 Mabs detected an antigen present on neuroblastoma tumors and cell lines. All 3 clones were shown to react with an epitope of the disialoganglioside GD2 molecules highly expressed by some neuroectoderm-derived tumors, mainly neuroblastoma. Whereas Mab 1G8 specificity was restricted to GD2 and its o'acylated form, Mab 2A6 and 7A4 were also able to detect GD3 at high concentration of antibody as shown by TLC analysis and immunodetection. The 3 Mabs were able to lyse 100% neuroblastoma cells in the presence of rabbit or human complement. The high proportion of anti-GD2 Mabs obtained by our fusion and the increased binding of anti-GD2 Mabs on γ-IFN-treated neuroblastoma cells suggests a modulation of the exposure and an increase in the immunogenicity of GD2 induced by the lymphokine γ-IFN.

P BIO 230

A NOVEL DERIVATIVE OF THE CHELON DESFERRIOXAMINE FOR SITE-SPECIFIC CONJUGATION TO ANTIBODIES

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We describe the preparation of the modified chelator aminooxyacetyl-ferrioxamine, and the replacement of its iron atom by 67-Ga at high specific activity. The aminooxy function of this compound was allowed to react with the aldehyde groups generated by the periodate oxidation of the oligosaccharide of either a mouse monoclonal antibody directed against carcinoembrionic antigen, or a non-immune IgG. The use of the aminooxy group allowed the formation of a stable bond between the chelon and the antibody without the need of any reduction. The labelled antibody was injected into nude mice bearing a human colon carcinoma having the appropriate antigenicity. Unoxidized antibody, labelled with 125I by conventional methods, was co-injected as an internal control. The in-vivo distribution of the modified antibodies was evaluated by γ-camera imaging and γ-counting of the various organs, with satisfactory results.

P BIO 231

THE REACTIVITY OF A PHOTOGENERATED CARBENE WITH AMINO ACIDS

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The selectivity of a diazirine-photogenerated carbene toward amino acid side chains is investigated by analysing amino acid retention following photocoupling with an immobilized carbene precursor. The heterobifunctional photocrosslinker 3-trifluoromehtyl-3-(m-isothiocyanophenyl)diazirine has been synthesized and coupled to fiber glass. Photoinduced amino acid binding to the solid support is analyzed. The immobilized diazirine-photogenerated carbene preferably binds to cysteine and to aromatic amino acids. Amino acids carrying sulfur or oxygen as side chain heteroatoms are, in general, more carbene-philic than amino acids with aliphatic side chains. Marginal carbene insertion was obtained with glycine. Based on the empirically determined photocoupling capacities of the applied amino acids, a carbene philicity scale has been established. Among homologous amino acids carbene selectivity partly correlates with their hydrophobicity and the number of chemical bonds. Diazirine-derived glass filters are used for amino acid sequence analysis of peptides photocoupled to the solid supports.

P BIO 232

SUBUNIT II FROM THERMOPHILIC BACTERIUM PS3: COPPER CONTENT AND SPECTRAL ANALYSIS.

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Cytochrome c oxidase from thermophilic bacterium PS3 was isolated as a three-subunit enzyme containing the hemes c and a. By trypsin treatment subunits (SU) I and III were almost completely digested. Purification of the undigested SU II was achieved by passing the protein mixture through an anion- followed by a cation-exchanger resins. The final purification was by hydroxylapatite. Trypsin traetment reduced the M_r of SU II by ca. 10,000. The cytochrome c and the postulated copper binding domaines were not attacked by trypsin. The two membrane-spanning segments were digested as established by N-terminal amino acid sequence analysis. The polypeptide originated from SU II (Mr = 28,000) had a copper content of about 1 mol Cu/ mol heme c. The elimination of the interferring hemes a absorption in the visible region allowed the characterisation of the copper absorption of SU II. The visible air-oxidized spectrum showed a cytochrome c maximum at 409 nm and maxima assigned to the copper absorption at 623 and 800 nm, which disappeared upon addition of ascorbate. The behaviour of the copper absorption was typical of a blue-copper type.

P BIO 233

SOLUTION STRUCTURE OF THE ANTENNAPEDIA HOMEODOMAIN BY NMR

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The three-dimensional structure of the homeodomain encoded by the Antennapedia (Antp) gene of Drosophila was studied in aqueous solution by nuclear magnetic resonance (NMR). The polypeptide studied comprises the amino acid residues 297 to 363 of the Antp protein, with an additional methionine residue at the N-terminus. The secondary structure determined from nuclear Overhauser effects (NOE) and information about slowly exchanging amide protons includes three helical segments consisting of the residues 10-21, 28-38 and 42-52, respectively. Computer modeling based on preliminary additional NMR data indicated the presence of a helix-turn motif. Near the turn, this supersecondary structure is very similar to the DNA binding sites in the 434 and P22 c2 repressors, but both helices in the homeodomain include 2 to 3 additional residues when compared with these procaryotic DNA-binding proteins. The determination of the complete three-dimensional structure of the homeodomain is in progress, and will be the main subject of the poster.

P BIO 234

Immunological and Biochemical Studies on the Heterogeneity of Cytochrome P-450IIB

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Four monoclonal antibodies (mca) against the major phenobarbital inducible rat liver microsomal P-450 were tested for their ability to distinguish between six subfractions of P-450IIB purified by isoelectric focusing (Oertle et al. in "Cytochrome P-450, Biochemistry and Biophysics", Schuster, I., ed., Taylor and Francis, Philadelphia, in press). Each mca exhibits a characteristic recognition pattern with the six subfractions, in support of specific structural differences between the subfractions. To minimize the amount of antigen used in the ELISA, preformed mouse mca-antigen complexes were bound to anti-mouse IgG-coated plastic plates. The P-450IIB subfractions were further characterized by their ability to react with cytochrome b_5: stimulation by cytochrome b_5 of the P-450 catalyzed oxidation of p-nitroanisol is different for individual P-450IIB-subfractions.

P BIO 235

MOLECULAR CLONING OF CHICKEN TYPE VI COLLAGEN

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Type VI collagen is a transformation-sensitive glycoprotein of the extracellular matrix of mesenchymal cells. We have isolated and sequenced several overlapping cDNA clones which encode the entire α2 subunit of chicken type VI collagen. The clones hybridize specifically to a 4.2 kb mRNA abundant in chicken fibroblasts. Virally transformed fibroblasts contain reduced levels of this mRNA indicating that the synthesis of type VI collagen might be blocked at the transcriptional level. The cDNA clones cover a total of 4153 base pairs. The deduced amino acid sequence predicts that the α2(VI) polypeptide consists of 1015 amino acid residues which are arranged in four distinct domains: a hydrophobic signal peptide of 20 residues, an amino terminal globular domain of 228 residues, a collagenous segment of 335 residues and a carboxy terminal globular domain of 432 residues. The collagenous domain contains one interruption in the repetitive Gly-X-Y sequence. In this domain there are seven Arg-Gly-Asp sequences some of which are likely to be used as cell-binding sites. Type VI collagen might therefore belong to the family of extracellular multidomain proteins that play an active role in cell adhesion.

P BIO 236

PURIFICATION OF CHOLINE ACETYLTRANSFERASE (CHAT) FROM THE ELECTRIC ORGAN OF TORPEDO MARMORATA

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Choline Acetyltransferase (ChAT) is the enzyme responsible for the synthesis of the neurotransmitter acetylcholine. The method of purification used in these studies included affinity chromatography with a new derived ligand of the ChAT inhibitor (2-Benzoylethyl)trimethylammonium chloride (BETA)(Räber et al. Experientia 44:A38, 1987) which was covalently linked to Sepharose 4B. Using electric organs of ***Torpedo marmorata*** as biological material, ChAT was first purified by ammonium sulfate fractionation, gel filtration, affinity chromatography on Sepharose-hexane-coenzymA and finally in the second affinity chromatography step on Sepharose-hexane-BETA. Double-affinity-purified ChAT was subjected to SDS-PAGE and detected by staining with silver ions. One major band of the isolated protein had an apparent M_r of 67000.

P BIO 237

Immunochemical Characterization of Two NADPH-linked Aldehyde Reductases which Reduce Succinic Semialdehyde to 4-Hydroxybutyrate in Human Brain.

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The neurotransmitter GABA is metabolised via succinic semialdehyde (SSA) to succinate or to the reduced metabolite 4-hydroxybutyrate. Two enzymes, aldehyde reductase (EC 1.1.1.2) and SSA reductase,which catalyze SSA in vitro, exist in human brain.We report an improved method of purification of SSA reductase from human brain, including ion exchange chromatography,gel filtration and affinity chromatography (Cibacron blue Sepharose).Analysis of the purified enzyme by SDS-Page under reducing conditions yielded a single band (MW 4o'ooo).In contrast,isoelectric focusing in the pH range 5,5-6,5 resolved 4 to 5 narrowly spaced protein band (IEP = o,1 pH units) which all showed enzyme activity. Antibodies were produced in rabbits against the affinity purified enzyme. In the Ouchterlony test,the antibodies selectively recongnized SSA reductase. Similarly, antibodies against aldehyde reductase recongizes aldehyde reductase, but not SSA reductase.The antibodies provide the means to localize the two enzymes in brain tissue and possibly obtain information about their function.

P BIO 238

RETINYLISOTHIOCYANATE, A COVALENT PROBE FOR RETINYLIDENE PROTEINS

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The chromophore of bacterial rhodopsins and visual pigments contain a retinal which is covalently Schiff base linked to the protein moiety (opsin). Both, retinal and the apoprotein are required for the conversion of photon absorption into protein conformational changes effecting the transfer of ions and the regulation of ionic conductance respectively. Retinylisothiocyanate has been prepared and chemically characterized for the selective modification of retinal binding sites. Covalent binding of the probe to bacterio-opsin yields a retinyl-thiocarbamoylated protein with the Schiff base replaced by a thiourea function. All-trans retinylisothiocyanate is converted to its cis-isomere by **irradiation with light. Insertion of the probe into the** retinal pocket occurs as documented by the formation of a chromophore with an absorption band at 550nm. Covalent binding of retinylisothiocyanate to bacterio-opsin is ascertained. The probe is recovered in the proteolytically generated V-2 fragment which includes the two C-terminal rods of bacteriorhodopsin and the retinal binding site.

P BIO 239

PREPARATIVE ISOELECTRICFOCUSING FOR FUTURE MICROSEQUENCING OF PROTEINS SEPARATED BY 2DGE

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High resolution, two dimensional gel electrophoresis (2DGE) is the most powerful biochemical method available for analysis of complex biological mixtures. When coupled with the electrotransfer of separated proteins to PVDF membranes, microsequencing of proteins at the pmolar level can be achieved. Here we suggest an additional step whereby, prior to 2DGE, a preparative IEF is performed in liquid phase by means of a Bio-Rad Rotofor. The advantages of the modification are three-fold. i) It allows particularly dilute protein solutions to be concentrated for subsequent separation by 2DGE, thus greatly facilitating the microsequencing procedure. ii) Fractionation of the Rotofor gradient means that only proteins within narrow, predefined pI ranges need be carried through to the 2DGE phase. Proteins present in such concentrations that they could interfere with 2DGE separation can be eliminated. iii) It has preparative applications in that proteins visuallised by appropriate staining procedures can be electroeluted from excised regions of the (2nd dimension) polyacrylamide gel. The potential of this approach is demonstrated by its application to studies of newly-identified plasma apolipoproteins.

P BIO 240

A NOVEL PROTEIN ASSOCIATED WITH RAT APOLIPOPROTEINS

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Plasma lipids are transported by lipoproteins, macromolecular complexes of lipids and speciallized proteins termed apolipoproteins (apo). Due to the functions associated with specific apos they are responsible for metabolic channelling of lipoproteins. Identification and characterisation of apos is thus important for understanding lipoprotein metabolism. Rat high density lipoproteins contain several apos (AI, AII, AIV, C, E) with a large degree of structural homology, but little immunological cross-reactivity with their human counterparts. Whilst raising monoclonal antibodies against rat HDL, a particularly immunogenic protein, present in apparently low concentrations, was detected. SDSPAGE and immunoblot studies showed it to be a 20Mr protein which did not correspond to any known rat (or human) apo. Competitive ELISA and immunoblots demonstrated its presence in rat HDL and VLDL. Cross-reactivity was also observed with human plasma, suggesting that the protein may have a human counterpart. Given that the majority of rat anti-apo antibodies do not cross-react with human apos, retention of this epitope indi ates that it is of particular importance, perhaps with respect to lipoprotein metabolism which is essentially controlled by apos.

P BIO 241

SYNTHESIS OF ALPHA1-ACID GLYCOPROTEIN BY HUMAN T LYMPHOCYTES

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Alphal-acid glycoprotein (AAG) belongs to the acute phase "reactant" plasma proteins: its concentration rises several fold following inflammation. Numerous in vitro studies indicate an interaction of AAG with the immune system as an immunomodulator. AAG is synthesized by the liver, but some contradictory reports have been published on the synthesis of AAG by lymphocytes . In order to confirm these findings, the culture medium of lymphocytes has been checked for the presence of AAG. To exclude artefacts, analyses were performed with clonal populations of single isolated T cells activated by PHA and cultivated at least 1 or 2 months in vitro in the absence of AAG. The results clearly show production of AAG by human T lymphocytes.

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P BIO 242

Copper and PQQ are the cofactors of two amine oxidases from the yeast *Pichia pastoris.*

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Methylamine oxidase and benzylamineoxidase from *P. pastoris* are both inactivated by the addition of 2,4-dinitrophenylhydrazine (DNPH). The occurrence of an absorption band at 360 nm indicates that the hydrazone adduct of pyrolloquinoline quinone (PQQ) and DNPH is formed. The copper content of the two amine oxidases has been determined by atomic absorption spectroscopy. In both enzymes the copper to PQQ ratio appears to be one.

P BIO 243

MOLECULAR MODELLING OF COLLAGEN: ONE SINGLE MUTATION PRODUCES A KINK IN THE TRIPLE HELIX

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In previous work on the disease *Osteogenesis Imperfecta*, a single-base mutation was identified which converted the glycine at position 748 of the α1 chain of type I collagen to a cysteine. The conformation-specific enzyme procollagen N-proteinase cleaved very slowly only at the aminoterminus of the protein, which is 225 nm away from the mutation. Other patients have been analyzed and it was found that single-base mutations at different positions also slow down the enzymatic cleavage.
The results were explained with a phase-shift within the triple helical molecule leading to a structural "bulge" of one or two chains at the mutation site. Models were built which predicted a flexible kink of about 45°. We carried out rotary shadowing electron microscopy of samples containing the mutated and the normal procollagen in a ratio of 3:1. About 25% of the molecules had a kink at the site of the cysteine substitution.
One of the implications of a kinked collagen molecule for the functionality of collagen is that no intact collagen fibrils may be formed. This prediction was verified by *de novo* fibril formation experiments (K.E.Kadler, 1988). It is suggested that the kinked structure is present in any collagen molecule which is cleaved slowly with procollagen N-proteinase. The result may explain one of the reasons for the lethality of *Osteogenesis Imperfecta.*

P BIO 244

PREGNANCY-ASSOCIATED PLASMA PROTEIN B: IS THIS HIGH-MW PROTEIN MADE UP OF SMALL UNITS BELOW 100 kD? UPS AND DOWNS IN THE PURIFICATION AND CHARACTERISATION OF PAPP-B.

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The presence of a protein with an unusually high molecular weight (1,000 kD) in the serum of pregnant women has been reported in 1973 by Lin et al. The concentration of this protein called pregnancy-associated plasma protein B (PAPP-B), in the serum, was highest in the third trimester of pregnancy. The protein has a pI of 4.6-5.0, it is glycosylated and heat-labile. Our purification strategy is based on the precipitation of late pregnancy serum with ammonium sulphate (30% saturation), pressure-driven (1.5 bar) ultrafiltration with a cutoff of 300 kD followed by antibody affinity chromatography and gel filtration using HPLC. The isolated fraction presented four bands on SDS-PAGE, with apparent molecular weights of 75, 47.5, 29.2, and 25.7 kD. An albumin contamination could also be identified. Two precipitin lines lines are obtained on two-dimensional crossed immunoelectrophoresis.

P BIO 245

The BChl c/e-Binding Polypeptides from Chlorosomes of Green Photosynthetic Bacteria

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Compared to purple bacteria, green photosynthetic bacteria contain an additional, extramembrane, light harvesting system located in the chlorosomes, which are oblong bodies attached to the cytoplasmic membrane. The light absorbing pigment of the chlorosomes is BChl c, d or e, which is proposed to be specifically bound by a protein, the so-called BChl c, d, e-binding protein. A 6.3 kDa polypeptide has been isolated from chlorosomes of the green photosynthetic bacterium *Pelodictyon luteolum*, and its complete amino acid sequence has been determined. It exhibits an overall homology of 30% to the BChl c-binding protein of *Chloroflexus aurantiacus*. Preliminary results from the N-terminal sequence analyses of the analogous polypeptides isolated from *Chlorobium limicola*, *Prosthecochloris aestuarii* and *Chlorobium phaeovibrioides* revealed a highly conserved sequence. This protein is suggested to be the BChl c/e-binding polypeptide in the family of the Chlorobiaceae.

P BIO 246

EVOLUTIONARY RELATIONSHIPS AMONG AMINOTRANSFERASES

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Extensive sequence information on aminotransferases, particularly on aspartate aminotransferases (AspATs), is now available. In order to compare the structural relatedness of these enzymes, a data base was compiled containing 20 amino acid sequences (12 AspATs and 8 others) . A multiple sequence editor (MSE) was developed to display the whole set of amino acid sequences in parallel on a single worksheet. MSE can combine editing and analysis of individual or a set of sequences and allows the introduction of gaps, permitting their alignment. Conventional computer programs that allow pair-wise comparison only, did reveal the well-established homology of all eukaryotic AspATs and considerable sequence similarity (40 - 42%) of aspartate and tyrosine aminotransferases of *E. coli* with eukaryotic AspATs. They failed, however, to detect any significant similarity with the rest of the aminotransferases. A more rigorous search for similarity was conducted by comparing (1) hydropathy profiles and (2) elements of secondary structure as predicted from the amino acid sequences. This combined approach yielded convincing evidence for homology of rat tyrosine aminotransferase and *E. coli* imidazolylacetolphosphate:L-glutamate aminotransferase with AspATs despite their low degree of amino acid sequence similarity (12 and 16%, respectively). A similar detailed comparison of other aminotransferases is underway.

P BIO 247

SITE-DIRECTED MUTAGENESIS OF ASPARTATE AMINOTRANSFERASE: Lys 258 His

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Lys 258 at the active site of AspAT apparently fulfills a dual role: It binds pyridoxal 5'-P covalently via an aldimine linkage and on structural grounds seems also to function as the H^+ donor/acceptor in the aldimine-ketimine tautomerization step. Mutagenesis of Lys 258 to Ala resulted in inactivation of the enzyme (Malcolm, B.A. and Kirsch, J.F., BBRC 132, 915, 1985). The side chain of Ala 258, however, cannot act as an acid/base group, thus we decided to replace Lys 258 by an ionizable amino acid residue such as His. The cloned cDNA of mitochondrial AspAT from chicken was mutagenized and expressed in E. coli. The product was purified to homogeneity. The mutant protein reacts with antibodies raised against the wild type enzyme and shows the expected M_r on SDS-polyacrylamide gel electrophoresis. Low UV circular dichroism spectra do not indicate a difference in the folding of the wild type and the mutant enzyme. The latter has no detectable activity when measured by the standard transaminase assay at pH 6.0, 7.5 and 9.0. However, on addition of a keto acid substrate to the pyridoxamine 5'-P form a spectral shift indicates that the enzyme is very slowly converted to the pyridoxal 5'-P form. The reaction is 7×10^5 times slower than that of the wild type enzyme and is reversible on addition of an amino acid substrate.

P BIO 248

MECHANISM OF THE ENANTIOMERIC ERROR IN ASPARTATE AMINOTRANSFERASE

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During the reverse half-reaction of enzymic transamination, protonation at C_α of the quinonoid intermediate from the si or the re side will yield the L- or D-amino acid product, respectively. In the aspartate aminotransferase, protonation from the si side is efficiently mediated by active site Lys 258. Protonation from the re side is a very rare event: enantiomeric error frequency is only 1.5×10^{-7} (JBC 175, 433, 1988). The present study elucidates the mechanism of protonation from the wrong side. The enzyme undergoes a syncatalytic conformational change from the "open" form to the "closed" form, the shift in conformational equilibrium being about 3 times more pronounced with C_4 substrates than with C_5 substrates (JBC 253, 3158, 1978; JBC 260, 11414, 1985). A 5fold lower error frequency in the case of the C_4 substrates compared to C_5 substrates suggested that the conformational equilibrium between the "open" and the "closed" form might be a determinant of the frequency of the enantiomeric error. The conformational shift to the "closed" form is markedly less pronounced in aspartate aminotransferase 27/32-410 (JBC 255, 10284, 1980). This truncated enzyme showed a 13fold increase in the error frequency. The pH and temperature dependence of the racemization was almost identical to that of the transamination reaction, indicating that the fraction of the quinonoid intermediate undergoing protonation from the wrong side is independent of pH and temperature. The data are consistent with the notion that a water molecule diffuses into the "open" active site to protonate the quinonoid intermediate from the wrong side.

P BIO 249

SYNTHESIS OF ACTIVE SPINACH THIOREDOXIN F IN TRANSFORMED E. COLI

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Thioredoxin f is involved in the light catalyzed activation of fructose 1,6-bisphosphatase [Schürmann, Maeda & Tsugita (1981) E.J.B. 116:37]. The complete nuclear gene from spinach was recently cloned and sequenced and the aminoacid sequence of thioredoxin was determined. Based on these results we subcloned the gene in a modified pKK233-2 expression vector and used it for transformation of E. coli BNN 103 containing the lac I^q plasmid (gift from Dr. Müller, Bern). Transformants were screened with anti-thioredoxin f antibodies and positive clones were tested by monitoring the protein profiles (FPLC). We identified a protein fraction containing a 12 kD protein strongly interacting with the antibodies and specifically catalysing in vitro the reduction of FBPase, indicating that the E. coli synthesized protein is identical with thioredoxin f. This has been confirmed by purification to homogeneity of the E. coli produced protein and its characterization.

P BIO 250

STRUCTURE DETERMINATION OF HUMAN METALLOTHIONEIN-2 IN SOLUTION

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The solution structure of the protein human metallothionein-2 (MT-2) was determined using nuclear magnetic resonance (NMR) spectroscopy. The protein was obtained from human livers, and used as the native zinc compound $[Zn_7]$-MT-2, or reconstituted from the apoprotein with either $^{112}Cd^{2+}$ or $^{113}Cd^{2+}$. Sequence-specific assignments of the ^1H NMR spectrum were obtained, and the coordinative bonds between the 7 metal ions and the 20 cysteinyl residues of the polypeptide chain were identified using NMR data recorded at 600 MHz and 500 MHz. With the information on the metal binding sites and additional conformational constraints from nuclear Overhauser effects as the input, the three-dimensional structures of the α- and β-domains were computed using the distance geometry program DISMAN. Corresponding results were previously obtained for rat MT-2 and rabbit MT-2. The molecular structure in solution determined by NMR is very similar for all three species, but there are major differences between these structures and a crystal structure reported for rat MT-2.

P BIO 251

PERIODIC CRYSTALLIZATION AND CRYSTALLIZATION OF BIOLOGICAL MACROMOLECULES IN GELS

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While studying the crystallization of reconstituted nucleosome-core particles, a 206 kD DNA-histone complex, we have observed the occurrence of periodic crystallization in modified vapour-diffusion assays. We found that crystals grew only on two discrete and continuous rings separated by approximately 0.3 mm. When the salt gradient was decreased the two rings were found to merge with growth of crystals strikingly turning into amorphous precipitation. These results are reminiscent of crystallization experiments on small molecules.

The feasibility of crystallizing quite distinct biological macromolecules in gels has been recently demonstrated (1). Analogous to vapour-diffusion experiments, as described above, we observed in gel assays that upon decreasing the salt gradient growth of crystals would border on amorphous precipitation. However, these problems were overcome and high quality crystals of three biological macromolecules could be grown in Sephadex. We note that the gel method may be an alternative to space-shuttle experiments.

References:
1. Turnell, W.G. and Struck, M.-M., in preparation.

P BIO 252

A FRAGMENT OF LAMININ COMPRISING THE ENTIRE LONG ARM

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We have investigated the effects of Ca^{2+} ions on the proteolytic digestion of the laminin-nidogen complex by the neutral protease, Cathepsin G. As revealed by the time-course of the proteolytic digestion, we obtained larger fragments in the presence of Ca^{2+} than in its absence. This was interpreted as being due to an increased stability of the laminin structure in the presence of Ca^{2+}. We used this property to produce a fragment of laminin comprising the long arm. According to electron microscopy the new fragment has the shape and dimensions of the long arm of laminin. The rod like region with a high alpha helix content reveals a kink probably at the N-terminus of fragment E8. In previous proteolytic fragmentations of laminin only E8 was isolated and the region between its N-teminus and the cross was lost. The new fragment may be used in exploring the properties and functions of the upper long arm region which so far was not available in a fragment.

P BIO 253

LUNG SURFACTANT PROTEIN SP-A EXHIBITS A STRUCTURAL HOMOLOGY WITH THE COMPLEMENT FACTOR C1Q

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For SP-A isolated from dog lung lavage, a flower bouquet-like hexameric structure with six globular domains connected by short stalks to a common stem was revealed by electron microscopy, using the rotary shadowing technique. This structure with six globular domains connected by short stalks to a common stem was revealed by electron microscopy, using the rotary shadowing technique. This structure is very similar to that published for the subcomponent C1q of the first component of complement C1. For recombinant SP-A mostly smaller aggregates like di-, tri- and tetramers as well as very high aggregates were observed. Mild reduction of the recombinant material revealed the lollipop-shaped monomers composed of a globular domain and a tail with a discrete kink in the middle portion. The collagenous nature of the tail was demonstrated by circular dichroism spectroscopy. This implies that the mammalian expression system assembles the monomeric subunits correctly. Assembly into the hexameric structures, however, does not proceed quantitatively. It is currently investigated whether the assembly can be facilitated by protein disulfide isomerase.

P BIO 255

INTESTINAL CADMIUM-BINDING PROTEINS OF LUMBRICUS TERRESTRIS AS INDICATORS OF SOIL CADMIUM CONTAMINATION.

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In our previous work, we demonstrated that cadmium was readily bioaccumulated by the worm Lumbricus terrestris. More than 90 % of total cadmium mass is found in the intestinal compartment of the worm. The concentration ratio of intestinal cadmium to soil cadmium content ranged from 40 to 120, depending on soil cadmium content. Polyacrylamide gel electrophoresis has been used for separating the different proteins of the worms intestines. Slicing the gels and analysing the slices allowed us to determine the cadmium location. The cadmium bound to the proteins was also exchanged with labelled cadmium; then electrophoresis was performed and radioactive proteins were detected using autoradiography; discrets bands were observed. Cadmium binds to the intestinal proteins induced when soil cadmium concentration is sufficiently high. These proteins could be related to metallothionein such as those found in other worms species.

P BIO 256

TRIPLE COILED-COIL REGIONS OF LAMININ: SPECIFICITY AND CHAIN STABILITY

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Laminin is a large extracellular glycoprotein, which is a ubiquitous component of basement membranes. It consists of three different poly-peptide chains, linked by disulphide bonds to form a characteristic asymmetric cross structure. The mechanism of laminin chain assembly is unknown, but interactions within the α-helical long arm may be important. To test this hypothesis, we have examined the specificity of chain assembly of an α-helical fragment of laminin, E8, after chain separation and α-helix denaturation. Chain re-assembly in the E8 fragment is not a random process. When all three chains are urea denatured, they specifically renature into structures which in their α-helix content, chain composition and ultrastructural appearance are indistinguishable from native E8. These data indicate the presence of a triple helical coiled-coil in the long arm of laminin and suggest that sequences within this region alone can direct laminin chain assembly. Interestingly, native E8 has a low urea stability, and it may be that other interactions eg. disulphide bond formation are important in the stabilisation of the laminin structure.

P BIO 257

Purification of a catalytically active cytochrome P-450 from rat oesophagus by sequential immunopurification with two antipeptide antibodies.

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N-Nitrosomethylamylamine and related asymmetric nitrosamines selectively induce oesophageal carcinomas in laboratory rodents. There is evidence that this effect is due to organ-specific bioactivation involving a specific P-450 dependent monooxygenase. Antipeptide antibodies were used to purify by affinity chromatography. Two peptides corresponding in sequence to highly conserved P-450 segments present in all rodent species and selected by hydrophilicity indices were chemically synthesized, used to prepare site-specific rabbit and mouse antipeptide antibodies and employed successively to purify P-450 from solubilized microsomal extracts. This procedure yielded a SDS-PAGE homogeneous protein with a M_r of 52K. In a reconstituted monooxygenase system, the enzyme showed significant activity for 7-ethoxycoumarin O-deethylation and metabolized asymmetric nitrosamines. The electrophoretically homogeneous protein could be separated into at least four subisozymes by isoelectric focusing. These four subisozymes were immunologically indistinguishable by our antibodies. The reason for this heterogeneity is not known.

P BIO 258

STRUCTURAL REQUIREMENTS FOR VITAMIN E INHIBITION OF PROTEIN KINASE C ACTIVITY.

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Gamma- and delta- tocopherol, which only differ from alpha-tocopherol in the number and position of methyl groups on the chroman, showed similar concentration dependent inhibition profiles as α-tocopherol (IC_{50} = 768, 559, 450 μM). In contrast, phytol (0-1 mM), the hydrophobic tail of α-tocopherol, and Trolox (0-1 mM), a negatively charged water soluble derivative of α-tocopherol, had little effect on the kinase activity. The oxidized product of α-tocopherol, α-tocopherylquinone (IC_{50} = 624 μM), and Trolox-amide (IC_{50} = 701 μM), had a similar concentration dependent inhibition profile as vitamin E. These data indicate that the inhibition of kinase activity is 1) not mediated by the anti-oxidant properties of vitamin E, 2) the essential structural features required for inhibition are a neutral chroman ring, and 3) that the number and positions of methyl substituents on the chroman ring are not critical for inhibition.

P BIO 259

RAT ALKALINE PHOSPHATASE : STRUCTURAL DIFFERENCIES BETWEEN THE INTESTINAL AND RENAL ISOENZYMES

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We have isolated the rat intestinal (IAP) and renal (RAP) phosphatases to apparent homogeneity using a partly modified purification method reported for the calf intestinal enzyme by Portmann et al, 1982 (Helv. Chim. A. 65, 2688). We replaced the CM-cellulose chromatography by an affinity chromatography on a phosphonic acid resine. This allowed to increase the yield of pure IAP (58%) and permitted to isolate the less stable RAP with a yield of about 4%. The specific activities obtained in this way were : 1860 U/mg (IAP) and 1100 U/mg (RAP) measured at 25°C. On the SDS-PAGE the IAP showed 2 bands (about 62 and 85 kDa) the RAP only one (72 kDa). Without SDS the M.W. corresponded to the sum (IAP) resp. twice the subunit values (RAP). This results agree with 2 identical subunits in the RAP and with either 2 different chains in the IAP or the presence of 2 different IAP-isoenzymes. Despite the structural differencies the IAP and the RAP showed in the Ief each one 2 bands with the pI-values 4.3 and 4.5 (Coomassie, silver and specific staining). The IAP and RAP differred in the sugar and the amino acid contents. The N-terminal amino acid is Leu in IAP, but in RAP it seems to be Ile or Leu but not Val nor the Phe reported in the lit.

P BIO 260

REGULATION OF ASSIMILATORY SULFATE REDUCTION AND GLUTATHIONE SYNTHESIS BY CADMIUM IN PEA PLANTS (PISUM SATIVUM L.)

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In higher plants, cadmium is known to induce the formation of heavy metal chelating peptides called phytochelatins with the general structure $(\gamma\text{-glu-cys})_n$gly n=2-11. Because of their extraordinary high content of cysteine and the similarity of their structure with glutathione we measured the activity of adenosine 5'-phosphate sulfotransferase (APSSTase), the key enzyme of sulfate assimilation, and of glutathione synthetase in 7 to 10 days old plants treated for 1 to 3 days with 5 and 50 μM Cd (added to the nutrient solution as $CdCl_2$). In roots, APSSTase activity increased to a maximal level after 1 day with Cd (490% and 850% of control with 5 and 50 μM Cd, respectively). GSH-synthetase activity was maximal after 2 days (132% and 570% of control with 5 and 50 μM Cd, respectively). In shoots, only 50 μM Cd had a clearly stimulating effect with a maximum after 2 days: 202% and 166% of control activity for APSSTase and GSH-synthetase, respectively. These facts lead to the conclusion, that phytochelatin synthesis induces an increase in assimilatory sulfate reduction.

P BIO 261

STRUCTURE AND EXPRESSION OF THE CHICKEN CDC2 PROTEIN KINASE

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The cdc2 protein kinase is thought to play a key role in the regulation of the eukaryotic cell cycle. Using as a probe a human cdc2 cDNA (kindly provided by Dr. P. Nurse, Oxford), we have cloned and sequenced the chicken homolog of this kinase. Chicken cdc2 (cdc-GG) consists of 303 amino acids, has a perdicted molecular mass of 34'688, and is highly homologous to the human and fission yeast proteins. By northern analysis we detected a single transcript of 1.8 kb. Interestingly, cdc2-GG transcript levels decreased significantly during embryonic development. Moreover, mRNA levels were much higher in embryonic liver than in brain. So far, we have not detected significant levels of cdc2-GG mRNA in adult tissues. These results suggest that there is a correlation between the abundance of cdc2-GG mRNA and the proliferative state of a tissue.

P BIO 262

Induction and cloning of L-amino acid oxidase from *Neurospora crassa*.

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L-amino acid oxidase (EC 1.4.3.2) from *N.crassa* is a flavoenzyme with a molecular weight of 68 kDal. It catalyzes the oxidative deamination of many L-amino acids, either proteinogenic, non-proteinogenic or synthetic. Different kinetic patterns are observed on induction of L-amino acid oxidase by ATP and D-phenylalanine. In order to elucidate the number of L-amino acid oxidase genes in *N. crassa* a cDNA-bank synthesized from a mRNA of an ATP-induced culture was screened by hybrid-released translation. A 375bp fragment from a positive clone was then used to screen a genomic library of *N.crassa*. Only one gene encoding for L-amino acid oxidase was found. Its structural organization will be presented.

P BIO 263

SUBCELLULAR LOCALIZATION OF GLYOXYLATE CYCLE ENZYMES DURING GERMINATION OF SOYBEAN SEEDS

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The glyoxylate pathway is essential to the initiation of net gluconeogenesis in germinating oleaginous seeds. The activity evolutions of the specific enzymes used as glyoxysomal and mitochondrial markers were investigated (soybean cotyledons). *De novo* synthesis can be postulated for malate synthase and isocitrate lyase, whereas malate dehydrogenase and citrate synthase were found to be constitutive. Purified mitochondrial and glyoxysomal fractions were prepared, in order to ascertain the localization of all glyoxylate cycle enzymes. Glyoxysomal fractions were characterized by low malate dehydrogenase and citrate synthase activities (in contrast to published results pertaining to castor bean and cucumber seeds), suggesting that citrate and oxaloacetate synthesis is essentially restricted to mitochondria. As a consequence, a new interpretation of the glyoxylic cycle of soybean is proposed.

P BIO 264

REACTION RATES OF CEREBRAL CREATINE KINASE, MEASURED BY 31P NMR SATURATION TRANSFER, CORRELATE WITH BRAIN ACTIVITY

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The levels of ATP and PCr in different organs have frequently been measured in order to gain insight into normal and pathological function. However, it turns out that these levels are poorly responsive indicitators, since they seem to be well regulated. In the heart e.g., as the performance is varied considerably, the levels of ATP and PCr remain unchanged. In contrast, the rate constants of the creatine kinase reaction (k[CK]), determined by 31P NMR saturation transfer, are increased with cardiac performance and O2 consumption (Bittl et al, JBC 260:3512 (1985)). In order to see if this holds true also for the brain, we have determined by 31P NMR saturation transfer, using a surface coil placed on the intact skull of the rat, the k[CK] following treatment with pentobarbital (PB) or bicuculline (BC), two drugs known to alter brain activity. PB reduces and BC increases the k[CK] from 0.67/s (controls) to 0.53/s and 0.97/s respectively, while the levels of ATP and PCr remain unchanged. It is concluded that the k[CK] in the brain can be measured non-invasively by 31P NMR and that it is a responsive indicator also of cerebral function.

P BIO 265

ZINC SIGNATURE IN THE PROMASTIGOTE SURFACE PROTEASE OF LEISHMANIA

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Most species of Leishmania express a very abundant surface membrane glycoprotein of approximately 63 kDa. This glycoprotein has an endopeptidase activity and is anchored in the membrane of the parasite by a glycosyl phosphatidylinositol. Recent investigations have shown that the protease can be biosynthetically labeled with radioactive zinc. The presence of zinc in the active site of the enzyme is confirmed by the homology existing between a sequence of amino acids present in the active site of thermolysin (a zinc protease with a known 3D structure) and a short sequence of PSP. The design and synthesis of zinc chelating oligopeptides analogous to substrates of the protease has permitted the identification of inhibitors of the enzyme.

P BIO 266

GLYCOSYL-PHOSPHATIDYLINOSITOL ANCHORED AND COLLAGEN TAILED CHOLINESTERASES FROM FLOUNDER MUSCLE

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Flounder muscle contains two types of cholinesterases (ChEs), that differ in molecular forms and in substrate specificity. About 8% of ChE activity could be attributed to collagen tailed acetylcholinesterase (AChE) that showed catalytic properties of a true AChE, whereas 92% of ChE activity corresponded to a membrane bound dimeric enzyme. Treatment of the latter enzyme with phospholipase C yielded a hydrophilic form and uncovered an epitope called cross-reacting determinant, which is found in the hydrophilic form of glycosyl-phosphatidylinositol (G-PI) anchored proteins. This enzyme showed catalytic properties intermediate to those of AChE and butyrylcholinesterase (BuChE). It hydrolyzed acetyl-, propionyl-, butyryl- and benzoylthiocholine. The K_m and the maximal velocity decreased with the length and hydrophobicity of the acyl chain. At high substrate concentrations the enzyme was inhibited. The pI_{50}-values for BW284C51 (specific AChE inhibitor) and ethopropazine (specific BuChE inhibitor) were between those found for AChE and BuChE. Taken together these results show that flounder muscle contains collagen tailed AChE and G-PI anchored ChE with intermediate substrate specificity. The parallel occurence of both true AChE and intermediate ChE in flounder muscle does not sustain the hypothesis that the presumed gene duplication leading to AChE and BuChE occurred subsequent to the point at which the vertebrate and invertebrate lines diverge.

P BIO 267

INTRACELLULAR PROCESSING OF p62 PRECURSOR OF SEMLIKI FOREST VIRUS (SFV) IN C6/36 CELLS

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Cleavage reactions involved in the biosynthesis of α-viruses have been extensively investigated in higher eukaryotes. Structural proteins are translated as polyproteins which are cleaved to yield the core (C-) protein and two membrane glycoproteins, p62 and E_1. p62 undergoes a final cleavage,in or near the plasma membrane, (30-35 min post-protein synthesis (pps)), to yield the virion glycoproteins E_2 and E_3. As virus formation takes place at the plasma membrane, this cleavage is considered to be a morphogenetic cleavage. In C6/36 cells intracellular virus formation is detected. Therefore, transport and cleavage of SFV membrane proteins were investigated in these cells. Our results show that the cleavage of p62 is an early phenomenon starting at 5-10 min pps and probably continues along the transport pathway.

P BIO 268

PHOSPHOLIPID ANCHORING OF SURFACE GLYCOPROTEINS IN S. CEREVISIAE.

Conzelmann, A. and Desponds, C.

Many eukaryotic membrane proteins are anchored in the lipid bilayer via covalently attached glycophospholipid anchors.[1]/ In S. cerevisiae, several of these proteins exist: they all are glycoproteins. We isolated the ^{3}H-myoinositol-labeled anchor of such proteins. The anchors contain mostly PI since they are sensitive to mild alkaline hydrolysis. In contrast to mammalian and trypanosomal anchors, they are resistant to degradation by nitrous acid at pH 3.75, indicating that they donot contain nonacetylated aminosugars. We analysed a number of mutant cells defective in the biosynthesis of the core of N-linked carbohydrates. Among these, only alg4=sec53 was deficient in anchor addition at the restrictive temperature. In sec53 anchor addition stops within minutes after the shift to the restrictive temperature, indicating that the pool size of anchor precursors is small.

[1]/Low, H.G. and Saltiel, A.R. Science 239, 1988, p.268.

P BIO 269

CELL TYPE SPECIFIC TRANSPORT OF AN ER RESIDENT GLYCOPROTEIN INTO POST-GOLGI APPARATUS COMPARTMENS

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Glucosidase II, an asparagine-linked oligosaccharide processing enzyme, is a typical endoplasmic reticulum resident glycoprotein. In kidney tubular cells, in contrast to previous findings on hepatocytes, we found immunoreactivity for glucosidase II not only in the endoplasmic reticulum but also in the apical and basolateral plasma membrane and endocytic tubulo-vesicular profiles. Immunoprecipitation confirmed presence of the glucosidase II subunit in purified brush border preparations. Kidney glucosidase II contained species carrying endo H sensitive, high mannose as well as endo H resistant, sialylated oligosaccharide chains. Sialylated species of glucosidase II were enzymatically active. This study provides a unique example that a resident glycoprotein of the endoplasmic reticulum is not completely retained within this compartment but can reach post-Golgi apparatus compartments in certain cell types.

P BIO 270

Isolation and characterization of the tyrosinase gene from *Neurospora crassa*.

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A precursor form of *N. crassa* tyrosinase has been identified by Western transfer from crude protein extracts and by immunoprecipitation of *in vitro* translated tyrosinase mRNA. The molecular weight of pretyrosinase (75 kDal) exceeds that of mature tyrosinase (46 kDal) by more than 50%. In order to deduce the primary structure and the nature of the extension, the tyrosinase gene was cloned. The deduced amino acid sequence of pretyrosinase revealed the presence of a carboxyl terminal extension of 201 amino acids.

P BIO 271

A CELL-FREE SYSTEM TO STUDY THE CONTROL OF CELL DIVISION

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Incubation of chicken embryonic nuclei in extracts prepared from metaphase-arrested chicken hepatoma cells resulted in prophase-like nuclear envelope breakdown, lamin depolymerization and chromatin condensation in an ATP-dependent manner. Subsequent depletion of ATP and simultaneous addition of calcium to this cell-free system induced telophase-like reformation of the nuclear membrane and reassembly of the nuclear lamina around condensed chromatin. Metaphase extracts showed activity for specific hyperphosphorylation of several proteins including lamins A, B1 and B2, nucleolin, B23 and histon H1. Interestingly, by ammonium sulfate fractionation of the extract, we were able to separate activities required for nuclear membrane breakdown and lamin depolymerization from those essential for chromatin condensation.

P BIO 272

A MONOCLONAL ANTIBODY TO RAT LIVER ALPHA2,6 SIALYLTRANSFERASE

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Beta-galactoside alpha(2-6) sialyltransferase (ST) is a glycosyltransferase catalyzing the transfer of sialic acid from CMP-sialic acid to the terminal galactose residues of N-linked glycans. We raised mouse monoclonal antibodies to rat liver ST. One of them was selected (MAB 74/18, IgM) which recognized by immunoprecipitation of ^{35}S-methionine labeled rat embryo fibroblasts a 43 kD protein corresponding to the soluble from of the enzyme. This protein was shown to be ST by the following criteria: 1) Upon addition of the antigen the intensity of the 43 kD band was significantly reduced; 2) the 43 kD band co-migrated on SDS-PAGE with one of the proteins precipitated by a polyclonal antiserum to rat liver ST (kindly provided by J.C. Paulson) and an antiserum to bovine colostrum ST as described previously; 3) purified rat ST in solution could be precipitated by MAB 74/18 as shown by immunoblotting and reduction of ST activity in the supernatant; 4) ST activity as measured in homogenates of rat embryo fibroblasts was precipitated to the extent of 60% by MAB 74/18.
Further work is aimed at understanding intracellular transport and maturation of ST in comparison with galactosyltransferase.
Supported by grant 3.136.0.88 of SNSF.

P BIO 273

IN VITRO ATTACHMENT OF GLYCOSYL-INOSITOLPHOSPHOLIPID ANCHOR STRUCTURES TO MOUSE THY-1 ANTIGEN AND HUMAN DECAY-ACCELERATING FACTOR

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Glycosyl-inositolphospholipid (GPL) anchoring structures are incorporated into GPL-anchored proteins (glypiation) posttranslationally, but the biochemical and cellular constituents involved in this processing are unknown. To establish if glypiation can be achieved in vitro, mRNAs encoding two GPL-anchored proteins, murine Thy-1 antigen and human decay-accelerating factor (DAF), and a conventionally-anchored control protein, polymeric immunoglobulin receptor (poly IgR), were translated in a reticulocyte lysate. Upon addition of rough microsomes nascent polypeptides generated from the three mRNAs translocated into vesicles. The selective incorporation of phospholipase-sensitive anchoring moieties into Thy-1 and DAF but not poly IgR translation products during in vitro translocation indicates that rough microsomes are able to support and regulate glypiation. This system is now used to define signals for glypiation.

P BIO 274

POLY(ADP-RIBOSE)POLYMERASE MODULATES HISTONE H2B-DNA INTERACTIONS.

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The poly ADP-ribosylation system of higher eukaryotes is involved in DNA excision repair. We have examined the possibility that this posttranslational protein modification in chromatin could affect DNA - protein interactions. Using mobility shift gel electrophoresis we studied the binding of histone H2B (purified by RP-HPLC) to 5'-endlabeled core DNA fragments in the presence of the enzyme poly(ADP-ribose) polymerase. Following activation of the polymerase, we observed dissociation of the histone H2B-core DNA complex. This dissociation was directly dependent on the number of protein-bound ADP-ribosyl polymers formed in the reaction. Dissociation of the H2B-DNA complex did not require covalent modification of H2B, suggesting non-covalent interactions between H2B and ADP-ribosyl polymers. This was confirmed by competition experiments showing higher binding affinity of H2B to polymerase-bound ADP-ribosyl polymers than to DNA.
(Supported by NF 3.354.086 & 3.161.088).

P BIO 275

SIZE DISTRIBUTION ANALYSIS OF ADP-RIBOSE POLYMERS PRODUCED IN VIVO DURING DNA EXCISION REPAIR

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De novo poly ADP-ribosylation of nuclear proteins is an obligatory postincisional event in DNA excision repair. A 27-fold increase in protein-bound ADP-ribose polymer levels was observed in cultured hepatocytes treated with the carcinogen MNNG. The molecular properties of these polymers were determined as follows: radiolabeling in vivo using [^{3}H]adenosine, release of polymers from the acceptor protein, purification by boronate affinity chromatography, eletrophoretic size separation using TBE-20%PAGE, quantification of the relative frequency of individual size classes of polymer molecules. Results: Carcinogen treatment increases the number of polymers and the proportion of specific polymer size classes, the average polymer size increased only slightly from 3.64 to 4.15. Conclusion: The poly ADP-ribosylation system reacts to postincisional DNA strand break formation by an adaptation of polymer numbers and specific size classes rather than by random elongation of preexisting polymers.
(Supported by NF 3.354.086 and 3.161.088.)

P BIO 276

MOLECULAR FACTORS DETERMINING COMPLEX ADP-RIBOSE POLYMER PATTERNS ON NUCLEAR PROTEINS

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We have set up a reconstituted in vitro system to study the molecular signal involved in the modulation of chromatin functions by poly(ADP-ribose). Using methodology described previously (cf. Naegeli, H. and Althaus, F.R., this issue) we determined that the nuclear enzyme poly-(ADP-ribose)polymerase generates a typical ADP-ribose polymer pattern, which is strictly maintained throughout the linear portion of the reaction kinetics. Surprisingly, the polymerase specifically adapts to the presence of other DNA-binding proteins (i.e. purified histones H1, H2A, H2B, H3, and H4) by producing different polymer numbers and size patterns for each histone species. Conclusion: The enzyme poly(ADP-ribose)polymerase can specifically adapt its function to the presence of other DNA-binding proteins on a given DNA template. (Supported by NF 3.354.086 & 3.161.088).

P BIO 277

POLY ADP-RIBOSYLATION OF PROTEINS: A PROCESSIVE OR DISTRIBUTIVE REACTION MECHANISM ?

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We have studied the molecular mode of operation of the nuclear enzyme poly(ADP-ribose)polymerase, which catalyzes the de novo poly ADP-ribosylation of chromatin proteins in DNA excision repair. For this purpose,we have reconstituted purified poly(ADP-ribose)polymerase with 146 bp core DNA. Alternatively, we have examined the ADP-ribose polymer patterns produced by endogenous polymerase in nucleosomal core particles and isolated nuclei. The reaction products were analyzed by boronate affinity chromatography, SAX-HPLC and TBE-PAGE. Results: Under all conditions, the number of polymers and the proportion of modified acceptor proteins increased concomitantly with ADP-ribose polymer concentrations, while both the average sizes and the relative size distributions of polymers were strictly maintained. Conclusion: The poly ADP-ribosylation of proteins follows a processive reaction mechanism. (Supported by NF 3.354.086 and 3.161.088)

P BIO 278

POLY(ADP-RIBOSE)POLYMERASE ALTERS THE SUSCEPTIBILITY OF DNA-H2B COMPLEXES TO MICROCOCCAL NUCLEASE AND DNase I.

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We have studied the interaction of micrococcal nuclease and DNase I with 5'-endlabeled core DNA fragments in the presence of poly(ADP-ribose)polymerase, a polyanion generating enzyme of chromatin which automodifies itself. Results: Hydrolysis of core DNA by micrococcal nuclease, but not DNase I, was inhibited in the presence of active poly-(ADP-ribose)polymerase. The degree of inhibition was dependent on the polymer number covalently bound to the polymerase. By contrast, hydrolysis of DNA by both enzymes was greatly enhanced in DNA-histone H2B complexes reconstituted with active poly(ADP-ribose)polymerase. Conclusions: These findings suggest a molecular mechanism by which the enzyme poly(ADP-ribose)polymerase facilitates access of DNA repair enzymes to damaged DNA templates. (Supported by NF 3.354.086 and 3.161.088.)

P BIO 279

USE OF 3-AMINOBENZAMIDE-AFFIGEL FOR THE PURIFICATION OF POLY(ADP-RIBOSE)POLYMERASE AND SEPARATION OF AUTOMODIFIED FROM UNMODIFIED ENZYME MOLECULES.

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The nuclear enzyme poly(ADP-ribose)polymerase catalyzes the polymerization of the ADP-ribose moiety of NAD to form poly(ADP-ribosyl)ated proteins. We have purified the enzyme protein from calf thymus using ammonium sulfate precipitation, gel filtration, ion exchange chromatography, and 3-aminobenzamide-Affigel affinity chromatography. The purified protein yielded one band at 116 kD on SDS-PAGE. In addition, we have been able to completely separate the poly ADP-ribosylated form of the polymerase from unmodified enzyme protein on 3-aminobenzamide-Affigel columns. The separation results were independently confirmed using boronate affinity chromatography in conjunction with immunoblot analysis. Thus, 3-aminobenzamide-Affigel chromatography allows for the specific isolation of unmodified enzyme from a complex mixture of variously automodified polymerase. (Supported by NF 3.354.086 and 3.161.088).

P BIO 280

EFFECT OF INSULIN ON THE ACTIVITY OF PHOSPHO-FRUCTO-2-KINASE (PFK_2) AND PYRUVATE KINASE (PK) DURING A EUGLYCEMIC HYPERINSULINEMIC CLAMP.

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In the normal rat during a euglycemic hyperinsulinemic clamp, insulin has been shown to increase the level of fructose 2,6-bisphosphate and of lactate plus pyruvate. This could be due to changes in the activity of PFK_2 and PK due either to modification of allosteric effectors or to dephosphorylation of the enzymes. For this reason, the effect of insulin on the activity of the two enzymes was studied in rats during a euglycemic hyperinsulinemic clamp and correlated to the effect of the hormone on hepatic glucose production (HGP), metabolite and cAMP level in the liver and counterregulatory hormones levels. The data show that insulin without modifying the level of cAMP, increases the activity of PFK_2 and pyruvate kinase by modifying the phosphorylation state of the two enzymes, but without modification in the level of cAMP.

P BIO 281

A NOVEL GLYCAN INVOLVED IN SPONGE CELL RECOGNITION

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Species-specific cell aggregation of the marine sponge Microciona prolifera is mediated by a proteoglycan-like aggregation factor (MAF) of $M_r=2x10^7$ via a cell-binding and a self-interaction domain. The localization of the cell-binding site in the highly polyvalent glycan of $M_r=6x10^3$ (G-6) is shown by: 1) decrease of the specificity and affinity of MAF binding to cells upon release of G-6 glycan by PNGase, 2) quantification of the amount of G-6 obtained by pronase digestion of MAF, which revealed that this glycan is represented by 1200 copies per one MAF molecule, and 3) recovery of the specificity and affinity of binding achieved by reconstitution of the G-6 polyvalence using cross-linking with glutaraldehyde. Chemical analyses showed that G-6 is composed of 3 fucoses, 7 glucuronic acids, 2 mannoses, 5 galactoses, 14 N-acetyl glucosamines, 2 sulfates and 1 asparagine. Such unusual carbohydrate composition indicated a novel type of structure involved in sponge cell recognition.

P BIO 282

CELLULAR LOCALIZATION OF THE MAJOR PHOSPHOLIPID ANCHORED MEMBRANE PROTEIN OF SACCHAROMYCES CEREVISIAE.

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Numerous integral membrane glycoproteins of eukaryotic cells are anchored in the plasma membrane by glycophospholipid anchors.[1] Here we study a 125 kDa glycoprotein of S. cerevisiae which is anchored in this way.[2] We used the nonpermeant reagent trinitrobenzene sulfonic acid to tag the periplasmic and plasma-membrane forms of this protein in $^{35}SO_4$-labeled cells. We found that ~40% of the cell-associated protein can be precipitated by TNP antibodies. The remainder seems to be localized intracellularly since it is not destroyed by removing the cell walls by lyticase and subsequent protease treatment. Most of the newly-made protein (~75%), however, gets secreted and ends up in the culture medium in a form which cannot be immunoprecipitated by a rabbit antiserum raised against the intracellular form of the protein unless the secreted form is first boiled in SDS. Further experiments address the question of whether the secreted material is membrane-anchored during biosynthesis.

1/ Low, H.G. and Saltiel, A.R., Science 239, 1988, p.268.
2/ Conzelmann A. et al., EMBO J. 7, 1988, p.2233.

P BIO 283

ANALYSIS OF PANCREATIC ZYMOGEN GRANULE MEMBRANES BY 2D-PAGE: THE PROBLEM OF SOLUBILIZATION

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Many membrane proteins are very hydrophobic and therefore difficult to separate by isoelectric focusing. A procedure has been developped which aimes at quantitative solubilization of pancreatic zymogen granule membrane (ZGM) proteins and at removing excess phospholipids. ZGM (1 mg protein/ml) were dissolved in 2% SDS by heating to 90°C and were subsequently cooled down to room temperature. Phospholipids precipitated and were removed by filtration. No loss of protein was noted. The filtrate was dialyzed against water to remove unbound SDS. CHAPS (2%) was then added to replace bound SDS and after reheating and redialyzing the sample was lyophilized. The sample was mixed with rehydration buffer (8M urea, 10% glycerol, 1% CHAPS, 2% ampholytes 3-10) and applied to the alkaline half of an Immobiline PAG plate. Electrofocusing was performed for 6 hours at 20°C, 4000 V, 1 mA, 4 W. After silver staining the gel showed sharp bands over the whole pH range (3.6-9.3). No collapse of the pH-gradient occurred near the cathodic end, a finding often observed with phospholipid containing samples. The 2D-PAGE analyses of pancreatic zymogen granule membranes of various species are presented.

P BIO 284

MUTATION IN THE CYTOPLASMIC TAIL OF THE HUMAN ASIALOGLYCOPROTEIN RECEPTOR AFFECTS ENDOCYTOSIS AND CELLULAR DISTRIBUTION

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The cytoplasmic domain and particularly a tyrosine residue are implicated in endocytosis of several plasma membrane receptors. To study this phenomenon in the asialoglycoprotein receptor system we introduced an alanine in place of the single tyrosine in the cytoplasmic tail of subunit H1 and in place of phenylalanine at the corresponding position of H2. The mutant H1 alone or both mutated subunits together were expressed in 3T3 fibroblasts and the cellular distribution of these proteins was examined by surface digestion at 4°C followed by immunoblot analysis. Both cell lines show an increased ratio of surface to internal receptors as compared to the corresponding wild-types. To analyze these findings in more detail, the cells were surface labeled at 4°C with ^{125}I and then shifted to 37°C for increasing times. Internalized receptor was analysed by its resistance to externally added protease followed by immunoprecipitation. Both mutated H1 alone and together with mutated H2 get internalized to a significantly lesser degree than the wild-type receptors. These results indicate that tyrosine and the corresponding phenylalanine play crucial roles in the endocytosis of the human asialoglycoprotein receptor.

P BIO 285

ANTI-MMTV SECRETORY IGA AND THEIR PROTECTIVE ROLE AGAINST VIRALLY-INDUCED MAMMARY CARCINOMA

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Mouse mammary tumor virus (MMTV) induces mammary tumors in female mice of certain strains. The infection is transmitted from infected mother to newborns via milk. In order to test whether mucosal antibodies have protective properties and prevent the appearence of mammary tumors, we have raised IgA monoclonal antibodies against MMTV and have designed strategies to make them resistant against proteolysis.One of the monoclonal antibodies of the IgA isotype (MB 2.2) recognizes a continuous epitope on the envelope protein gp52. A strategy using fusion proteins has allowed to map this epitope. Restriction fragments of a cDNA coding for gp52 were inserted in an expression vector (pATH) in frame with a bacterial protein (TrpE of E. Coli). MB 2.2 specifically recognizes the N-terminal part of gp52. Stabilization of the antibodies can be obtained by combining them to secretory component (SC), the ectodomain of the poly Ig receptor. We transfected myeloma cells with a cDNA coding for the rabbit poly Ig receptor and tested expression and secretion of SC. Transformants did express poly Ig receptor mRNA but there was no receptor nor SC production. Strategies have been designed to overcome this problem.

P BIO 286

CHARACTERIZATION OF HUMAN GRANZYMES A AND B ISOLATED FROM GRANULES OF CLONED HUMAN CYTOLYTIC T LYMPHOCYTES

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A human CD8+ CTL clone with cytolytic potential was shown to express two serine proteases, a 50 kDa homodimer and a 27 kDa monomer, which were purified from cytoplasmic granules. N-terminal sequencing and Western blotting of the purified proteins, as well as Northern blotting, revealed that the 50 kDa homodimer is the gene product of the HuHF cDNA clone and that it represents the human homologue to granzyme A. The 27 kDa protein was shown to be the serine esterase encoded by the HLP cDNA clone and corresponds to granzyme B. There was no evidence for the presence of other granzymes, in particular for the human homologues to murine granzymes C, D, E and F. Upon triggering of the TCR-CD3 complex with a monoclonal anti-CD3 antibody, granzyme A was released into the culture medium. Granule-associated hemolytic activity was detected after salt extraction and partial purification of granule proteins.

P BIO 287

USE OF RADIATION SUICIDE TO ISOLATE CONDITIONAL (TEMPERATURE-SENSITIVE) AND NON-CONDITIONAL CHO MUTANTS WITH DEFECTS IN THE ENDOCYTOSIS OF LDL.

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Mutagen-treated Chinese hamster ovary (CHO) cells were maintained in culture at 34°, shifted to 39.5° (nonpermissive temperature) for 16 hrs, and incubated for 6 hrs at 39.5° with r[^{3}H-cholesteryl linoleate]LDL. The rLDL with high specific activity (500 cpm/ng LDL protein) was prepared by replacing the endogenous cholesteryl esters of native LDL with [^{3}H]-cholesteryl linoleate. Ten million treated cells were harvested and stored in a liquid nitrogen freezer for 2.5 months to permit radiolytic killing of >99.9% of those cells which internalized the radiolabeled LDL normally. Surviving cells were screened by a replica-plate/autoradiographic assay to identify cells that expressed reduced ability to accumulate ^{125}I-LDL. Eleven putative mutants were isolated, cloned and shown to express 1-30% of wild-type LDL receptor activity (^{125}I-LDL degradation assay). Six of these exhibited a marked temperature dependence of LDL receptor activity. Characterization of these LDL receptor-defective cells, including genetic complementation analysis, is currently in progress. K.M. was supported by fellowships from Kanton Zurich and Schweizerische Stiftung fur Medizin und Biologie.

P BIO 288

EXPRESSION OF GRANZYME GENES IN T LYMPHOCYTE SUBSETS

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The secretory granules of murine cytolytic T cell lines contain a family of higly related serine proteases (granzymes A, B, C, D, E, F, and G). Their physiological role is unknown.

Using RNase protection assays, we have studied the expression of these genes in subsets of normal T lymphocytes. We found that granzyme A is present after stimulation in both CD4^{+} and CD8^{+} T cell subsets, whereas the other granzymes (B, C, D, E, F and G) are only expressed in stimulated CD8^{+} cells.

P BIO 289

IN VITRO TRANSCRIPTION FROM THE XENOPUS VITELLOGENIN B1 PROMOTER IN HOMOLOGOUS LIVER EXTRACTS: EFFECTS OF PROMOTER SEQUENCES.

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Transfection experiments have led to the identification of a DNA palindromic sequence (estrogen responsive element: ERE) that is responsible for the hormonal induction of the Xenopus vitellogenin genes. As a first step toward characterizing the biochemical and molecular mechanisms of this regulation, we reported the development of a hormone-dependent *in vitro* transcription system in nuclear extracts from Xenopus liver. In these extracts, transcription of the vitellogenin promoter is correctly initiated and dependent on the presence of both the ERE and estrogen. By testing a battery of 5'-deletion mutants, we demonstrate that the upstream domain of the vitellogenin gene can be divided into three functional regions comprising a basal promoter element (BPE), a down-regulation element (DRE) and the ERE. The two first regions are responsible for tissue-specific expression of the gene.

P BIO 290

ISOLATION AND CHARACTERIZATION OF PROLIFERATING CELL NUCLEAR ANTIGEN FROM CALF THYMUS

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Proliferating cell nuclear antigen (PCNA), also known as cyclin or DNA polymerase δ auxiliary protein, is a cell-cycle regulated protein with a Mr of 36kDa. PCNA is expressed at high levels in transformed and tumor cell lines and has been described to stimulate DNA polymerase δ but not DNA polymerase α. In order to study the effects of PCNA on calf thymus DNA polymerase δ in a homologous system, we have isolated PCNA from this tissue. PCNA in the crude extract was separated from DNA polymerases α and δ on a phosphocellulose column. Subsequent purification to apparent homogeneity included chromatography on DEAE cellulose, ammonium sulfate precipitation and chromatography on Phenyl-Sepharose and FPLC (Mono Q). The purified protein had a Mr of 36kDa and was identified as PCNA by immunoblotting using a rabbit antiserum against human PCNA. The purified PCNA had no in vitro effect on DNA polymerase δ. The polymerase δ itself was able to replicate a 7200 base single-stranded circular DNA in less than 10 min and hat processivity of several thousand bases. Thus we postulate a regulatory protein which could link DNA polymerase δ to the cell cycle regulated PCNA.

P BIO 291

MITOTIC PHOSPHORYLATION OF TWO MAJOR NUCLEOLAR PROTEINS (NUCLEOLIN/C23 AND NO38/B23)

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The two nucleolar proteins nucleolin/C23 (92kd) and No38/B23 (38kd) are thought to play important roles in ribosome biogenesis. Both proteins are phosphorylated in vivo, presumably by casein-type II kinases. Using monoclonal antibodies for immunoprecipitation of chicken nucleolin/C23 and No38/B23, respectively, we found that both proteins become hyperphosphorylated when embyronic nuclei are incubated in extracts prepared from metaphase-arrested chicken hepatoma cells. Similarly, they are hyperphosphorylated when isolated directly from metaphase-arrested ^{32}P-labeled hepatoma cells. Tryptic phospho-peptide maps and analyses of phospho-amino acids show that mitotic phosphorylation involves novel sites and occurs on threonine residues, whereas interphase cells contain phospho-serine only.

P BIO 292

A novel human T cell activation antigen gp33-38.

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Monoclonal antibody (mAb) Me14/D12 recognizes a structure present on activated but not on resting T lymphocytes. This novel molecule was identified as a dimeric glycoprotein composed of two non-covalently linked subunits of 33 and 38 KDa. The expression of gp33-38 was detectable as early as 24 hours after activation of T cells with either PHA, PMA, Con A or anti-CD3 mAb, and was maximal between 48 and 96 hours. The appearance of gp33-38 at the cell surface was concomitant with the appearance of IL-2 receptor. Northern blot analysis, using a specific probe for gp33-38, showed that m-RNA was detectable as early as 2 hours after activation.

P BIO 293

BIOCHEMICAL ANALYSIS OF HUMAN PLACENTAL EXTRACT

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The healing and antiarthritic activities of "cold" placental extracts, probably linked to the contents of these extracts, are well documented, pharmacologically as well as clinically.

Systematically investigating the contents of such a preparation, we have identified significant amounts of diverse agents bearing established biological activities.

Steroids, like estradiol, estriol, progesterone and DHEA-S, linked to a high molecular weight carrier.
Other hormonal factors : hCG, hGH, β-endorphin, β-lipotropin.
Inter- and intra-cellular messangers : calmodulin, cAMP, prostaglandin E2.
And plasma proteins related or unrelated to pregnancy : SP1β, PAPP-A, transferrin, ferritin,ceruloplasmin, IgG, IgA and IgM.

New components are identified every month, contributing to the clear up of clinical activity of placental extracts.

P BIO 294

ESTABLISHMENT OF A NEW HUMAN MAMMARY EPITHELIAL CELL LINE BY MICROINJECTION OF TRANSFORMING GENES

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We have established short-term cultures of mammary epithelial cells collected from human milk. To establish human epithelial cell lines with a differentiated phenotype we microinjected a series of transforming genes including SV40, SV40 + c-Ha-ras, v-myc, and v-myc + c-Ha-ras. An increased proliferation rate was observed in primary cultured milk cells after microinjection of more than one transforming gene. Only cells injected with SV40 DNA were stably maintained in culture for more than ten months. One immortalized human mammary epithelial cell line has been established and passaged over 60 times. This cell line is of epithelial morphology, contains cytokeratin intermediate filaments and expresses the SV40 T-antigen. Cells are diploid and at passages 10 to 15 showed minor chromosomal changes. Colony formation in semi-solid medium (methylcellulose) was not observed in early passages but was seen after passage 50 with enhancement by EGF. This cell line is used as a homologous recipient to study the oncogenic potential of transfected breast cancer DNA, selection by hygromycin resistance genes and semi -solid transformation assays.

P BIO 295

MODIFICATIONS OF GLUCOSE METABOLISM IN COLD ADAPTED LEAN AND OBESE ZUCKER RATS.

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Glucose tolerance, insulin secretion and glucose utilization by peripheral tissues were tested before and after cold adaptation in lean and obese Zucker rats. Cold-adapted lean rats showed no modifications of glucose tolerance and insulin secretion while glucose utilization was largely increased in brown adipose tissue (BAT) ; obese rats after cold exposure reduced both glucose tolerance and insulin secretion ; glucose utilization remained low in muscles, as it was already at room temperature, and it was only slightly increased in BAT.
It is concluded that cold exposure failed to correct insulin resistance in muscle of obese rats and that BAT metabolic activation in answer to cold is impaired in these animals.

P BIO 296

PRODUCTION OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) BY CLONED OSTEOBLASTIC CELLS.

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Previously we have shown that mouse calvaria produce GM-CSF (R. Felix et al.: J. Bone Min. Res. 3, 27, 1988). Since calvaria contain a variety of cell types, there is no proof that the activity was produced by cells of the osteoblastic lineage. We therefore investigated the CSF production by clonal rat cell lines of the osteoblastic phenotype. The clone CRP 10/30 and the immortalized cell line HGR1/c-myc1 which both show osteoblastic properties (high alkaline phosphatase, c-AMP production in response to PTH, identification of osteocalcin m-RNA by Nothern blot) produced hemopoietic growth activity on stimulation with mouse rTNFα or $1,25(OH)_2D_3$. This activity showed similar properties to mouse GM-CSF with respect to stimulation of various hemopoietic cell lines and chromatographic behaviour under various conditions. In semisolid medium GM colonies were induced. Finally, antiserum against rat GM-CSF (a gift of Dr. Ming-chi Wu, Denton, Texas, USA) inhibited the growth activity. Thus, through the production of GM-CSF, osteoblasts might regulate osteoclast formation, influence hemopoiesis and/or participate in immunological defense in bone.

P BIO 297

ABNORMAL BRAIN DEVELOPMENT INDUCED BY HYPERPHENYLALANINEMIA: BIOCHEMICAL AND IN VIVO 31-P NMRS STUDIES

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Hyperphenylalaninemia (hyper-Phe) in rats, induced by injections of alpha-methylphenylalanine (MP) and phenylalanine (Phe), reduces myelinogenesis strongly. We compared the brain damage and regeneration of MP/Phe treated rats to normal development. MP/Phe injections during the period of most active myelin formation (postnatal day 3-17) caused significant increases in serum and brain Phe concentrations at day 17. Compared to control rats there were decreases in body weights (-37.5%), brain weights (-35.7%), galactocerebrosides (-62.9%), -sulfatides (-46.7%), and in the activities of cerebroside sulfotransferase (-42.1%) and 2`, 3`-cyclic nucleotide 3`-phosphohydrolase (-32.7%) (CNP). No differences in proteins, total lipids, phospholipids and phosphatidylethanolamine were found. Brain in vivo 31-P nuclear magnetic resonance spectroscopy (NMRS) of 17-day-old MP/Phe treated rats showed a normal age-dependent pattern. Compared to controls no changes in the phosphomonoesters and -diesters, which were related to brain phospholipids, were found. This correlated well with the normal biochemical values for phospholipids. To follow brain development after stopping the MP/Phe injections, 17-day-old treated rats were let to recover for 7 weeks (59-day-old). Phe levels and all measured brain compounds of recovered animals were normal. Brain weights were 22.1% lower than in controls. In conclusion: Hyper-Phe induced defective brain myelinogenesis. This could not be detected by in vivo 31-P NMRS. A recovery of the myelin formation was observed. (Supported by SNF Grants 3.156-0.88, 3.262-0.85)

P BIO 298

LIPOPROTEIN SUBFRACTIONS MODULATE ACYL CoA CHOLESTEROL TRANSFERASE (ACAT) ACTIVITY IN THE MACROPHAGE IC-21 CELL LINE

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Uncontrolled cholesterol accumulation can transform macrophages into foam cells, which are prominent in atherosclerotic lesions. Mechanisms which remove cellular cholesterol thus have important implications *in vivo* for limiting potentially this process. Here, we describe preliminary studies in which we have developed an *in vitro* model system which can be used to explore mechanisms (as yet poorly defined) implicated in the control of cholesterol accumulation. It focuses on the activity of acyl CoA acyl transferase (ACAT) the key enzyme of intracellular cholesterol storage. In the first series of experiments, IC-21 macrophages were incubated with various human lipoprotein subfractions, and their influence on ACAT activity measured. Three increased ACAT activity (suggesting accumulation of cholesteryl esters) in the order vortexed LDL > LDL > ApoE-free HDL-2. Vortexed LDL induced a 5-fold increase in ACAT activity. The tested lipoproteins were also found to increase acyl CoA glycerophosphate acyltransferase (AGAT) activity in the order LDL > ApoE free HDL-2 > vortexed LDL. A second series of studies examined the ability of HDL subfractions to modulate ACAT and AGAT activities previously upregulated by incubation with vortexed LDL.

P BIO 299

URINARY EXCRETION OF ^{13}C-SUBERIC ACID IN PREMATURE INFANTS AFTER THE ORAL ADMINISTRATION OF ^{13}C-MCT

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The partial replacement of cow's milk fat by MCT is used in adapted preterm infant formulas and has been shown to improve fat absorption. Experiments in premature infants using ^{13}C-MCT showed that no more than 50% of the MCT dose was oxidized and expired as $^{13}CO_2$. From these results it was concluded that more complete ^{13}C balance studies were necessary. To this end, quantitative analysis of the dicarboxylic acids: adipic, suberic and sebacic acid excreted in the urine of 7 premature infants fed ^{13}C-trioctanoyl-glycerol and their ^{13}C enrichment, was performed. The urinary concentrations of adipic, suberic and sebacic acids varied from 1.5-12, 2-8 and 3-17.5 mmol/mol creatinine respectively. Significant ^{13}C enrichment of suberic acid was observed in only 4 of the 7 infants studied. No enrich-ment could be demonstrated for sebacic and adipic acid. These results demonstrate that dicarboxylic acid formation in premature infants is a minor metabolic pathway and that octanoic acid can be the direct precursor of suberic acid.

P BIO 300

STRUCTURE OF THE HUMAN BLOOD PLATELET MEMBRANE GLYCOPROTEIN Ibα GENE

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Human blood platelet glycoprotein (GP) Ib is a platelet receptor for von Willebrand factor and thrombin. It consists of two subunits, α and β, connected by a disulphide bond, and is missing in the bleeding disorder Bernard-Soulier syndrome. The gene for GPIb α-chain has been cloned from a genomic cosmid library using a partial cDNA clone as probe. A 6,5 kb EcoRI fragment, corresponding to the EcoRI fragment detected by genomic Southern blotting, was subcloned in M13 Bluescript™. 3530 bp were sequenced including the entire transcribed part, as well as additional 5' and 3' regions. A single intron was found 6 bp upstream of the ATG initiation codon. An exceptionally long exon (2354 bp) was identical to the recently published cDNA sequence. The 5' upstream promotor region is atypical for eukaryotic genes with only a weak homology to the characteristic promoter consensus sequences. A comparison with the promoter regions of other platelet specific proteins will elucidate the mechanism of megakaryocyte specific expression. The 3' region contains two repetitive Alu elements, belonging to distinct subfamilies, connected by an oligo(dA) linker. Several Alu repeats were also found in the 5' region upstream of the promoter sequence.

P BIO 301

ANTITHROMBIN BINDING TO ENDOTHELIAL CELLS GROWN IN VITRO.

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Cloned rat epididymal fat pad endothelial cells (RFPEC) synthesize anticoagulantly active heparan sulfate proteoglycans (HSPG) that bind antithrombin (AT). We have developed a direct binding of ^{125}I-AT to cells grown as monolayers or in suspension. The binding of AT to RFPEC was documented by biochemical and electron-microscopic studies. ^{125}I-AT binding is competed by either AT or heparin, and the apparent K_{diss} for AT is 30 nM. In RFPEC monolayers, the number of binding sites for AT increases linearly with time. In suspension, AT binding to RFPEC is restored within 6 hours after trypsinization, and plateaus at about half the value observed for monolayers. These data suggest that the AT binding sites are secreted by RFPEC rather than bound to the cell surface. Electron-microscopic and light autoradiographic studies have shown the incorporation of AT into the extracellular matrix and in the surface-connected vesicles of the RFPEC. Our results suggest that the anticoagulant HSPG synthesized by RFPEC is secreted into the extracellular space where it binds to the cell matrix.

P BIO 302

MICROTUBULE-ASSOCIATED PROTEINS IN TRYPANOSOMA BRUCEI

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Because of the simplicity of their cytoskeleton, Trypanosomes represent a suitable system for studying cytoskeletal structures. A dense cage of parallel single microtubules (MTs) underlying the cell membrane forms the major cytoskeletal component of the cell body. Different MT-associated proteins (MAPs) are believed to confer the structural and functional properties to different subsets of MTs. In Trypanosomes, MAPs are likely to mediate the links between neighbouring MTs of the membrane skeleton as well as those to the cell membrane. Two large and highly repetitive MAPs associated with the MT-membrane complex of the cell body have been analyzed. Although the strongly conserved repeats of each protein are identical in length (38 amino acids), their sequences share only 50% identity.

Further investigations deal with potential connections of the cytoskeleton to the outside of the cell. Such components might offer a possible target for immunological attack.

P BIO 303

The Genes and Proteins of the Paraflagellar Rod of Trypanosoma brucei

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The flagellum of the parasitic hemoflagellate T.brucei contains, besides the microtubular axoneme, a second structure, the paraflagellar rod (PFR). This flexible, filamentous network runs in parallel to the axoneme along almost the entire length of the flagellum. It consists predominantely of two very similar proteins of 69 kDa and 73 kDa, which are present in equimolar amounts. Using polyclonal antibodies, the corresponding genes have been isolated, via gt 11 , from a genomic library in an EMBL phage. We will present the data on the genomic arrangement and the sequence analyses of the two proteins, αPFR and βPFR. The interaction of these major structural proteins with other components of the PFR structure is currently being investigated using biotinylated probes. Recent work in this laboratory has shown, that the PFR contains proteins which are immunologically and structurally related to spectrin. To further characterize this relationship, we are investigating these spectrin-like proteins for their interaction with calmodulin, which is also a component of the PFR structure.

P BIO 304

PERIODIC FLUCTUATIONS IN THE F-ACTIN CONTENT OF HUMAN NEUTROPHILS CORRELATE WITH OSCILLATIONS IN CELL SHAPE

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Neutrophils treated with 17-hydroxywortmannin (HWT) or phorbol 12-myristate 13-acetate and subsequently stimulated with fMLP undergo periodic changes in shape as monitored by turbidity. Electron micrographs showed the typical extension of lamellopodia, necessary for the crawling or swimming motions associated with migration. The cytoskeletal content of filamentous actin (F-actin, detected by fluorescein-phalloidin staining) of HWT-treated, fMLP-stimulated neutrophils closely followed the changes in cell shape, and exhibited the same periodicity and relative oscillation amplitudes. Fitting the experimental curves to an A→B→C series reaction model extended by an oscillatory state B allowed the kinetic comparison of the rates (0.15+-0.04/s for turbidity, 0.11+-0.02/s for actin polymerisation) and the frequencies (0.095+-0.007 Hz and 0.092+-0.006 Hz) of the two neutrophil responses. This close correlation suggests that variations in actin polymerisation are the source of the oscillations in neutrophil shape and that this type of response may give new insights in cell activation and its feedback controls.

P BIO 305

SPECIFIC LOCALIZATION OF INOSITOL LIPID ENZYMES IN LECTIN-STIMULATED PERIPHERAL BLOOD MONONUCLEAR CELLS

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The effect of the two mitogenic lectins Phytohemagglutinin (PHA) and Concanavalin A (Con A) in the inositol lipid enzymes was analyzed in human peripheral blood mononuclear cells (PBMN). After incubations with either PHA or Con A during 5 or 30 min, the activity of phosphatidylinositol 4,5-bisphosphate phospholipase C and inositol 1,4,5-trisphosphate monophosphatase was measured in various subcellular fractions When compared with resting cells, PHA-stimulated PBMN displayed an increased activity of both enzymes especially in the nuclear fraction with a corresponding reduction in the soluble fraction, whereas, in cells stimulated with Con A, the enzyme activities were raised in the microsomal fraction. Our findings suggest that the mitogenic signal induces specific response pathways, i. e. a partial translocation of inositol lipid enzymes from the soluble compartment either to the nucleus or to the plasma membrane depending whether lectins activate helper or suppressor lymphocytes.

P BIO 306

Plasma membranes from bovine adrenal medulla contain Diglyceride-Kinase activity.

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Stimulation of chromaffin cells with acetylcholine result in catecholeamines secretion and simultanious liberation of arachidonic acid (AA). Our laboratory has demonstrated that isolated plasma membranes (PM) from bovine adrenal medulla generate AA from diacylglycerol (DG) by DG-lipase (DGL) followed by monoglyceride lipase. However, DG is known to be also phosphorylated by DG-kinase (DGK) resulting in phosphatidic acid (PA) formation, thus restoring DG into the PI-cycle. Since both pathways utilize DG as substrate, we were intrested in the control at this bifurcation point. This new presentation shows that DGK activity is associated with the isolated PM. MgATP (optimal at 10 mM) is obligatory for DGK . DGK and DGL operate simultaneously (37^{0}C, pH above 6). However, during the first 10 min DGK is more rapid than DGL. Specific inhibitors were used to resolve better between DGK and DGL. RHC - 80267 (25 uM) inhibits up to 80% of the AA release, with no effect on PA production. Similarly, R 59022 (250 uM) inhibits more than 60% of PA production but hardly affects AA release. The results indicate that DGK and DGL pathways operate independently one from each other. Therefore, DG may exist in two distinct pools at the PM and thus segregate between these two pathways.

P BIO 307

PURIFICATION OF A PROTEIN KINASE C/ PHOSPHATIDYLSERINE-BINDING PROTEIN FROM HUMAN PLATELETS.

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We searched for a PKC-binding protein which could be involved in PKC translocation during platelet activation. PKC-binding proteins were detected in blots of SDS-PAGE by probing with PKC, followed by antiPKC antibody and second antibody coupled to alkaline phosphatase. No PKC binding was observed in the absence of phosphatidylserine (PS). In the presence of PS, several PKC-binding proteins were identified; the dominant species with Mr 72 kD. This protein was purified from platelet extracts by ammonium sulfate precipitation, gel filtration, anion exchange chromatography on Q-Sepharose and affinity chromatography on PS-Sepharose. The purified protein functioned as a PKC substrate. The binding of this protein to PS vesicles was inhibited by mM Mg^{++} and Ca^{++}, and competitively inhibited by phosphatidic acid, platelet activating factor and phosphatidyl inositol. The protein was not found in Western blots of neutrophil or monocyte extracts. The role of this protein in signal transduction merits further investigation.

P BIO 308

ALTERED EXPRESSION OF PHOSPHOINOSITIDE ENZYMES IN LEUKEMIC CELLS

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The proliferative capacity of human leukemic cells was investigated in connection with the phosphoinositide degrading enzymes, phosphatidylinositol 4,5-bisphosphate phospholipase C (PIP_2-PLC) and inositol 1,4,5-trisphosphate monophosphatase (IP_3-MP). Both enzymes measured in whole homogenate were found generally diminished in leukemic cells with respect to resting and lectin-stimulated peripheral blood mononuclears (PBMN). PIP_2-PLC and IP_3-MP activities increased from very low level toward values found in PBMN along with the stage of cell differentiation in both lymphoid and mieloid leukemias.
These preliminary findings suggest that phosphoinositide enzymes might be depressed as a consequence of an impaired inositol lipid turnover or alternatively affected by different membrane lipids, likely abnormal, associated with the neoplastic proliferation.

P BIO 309

INOSITOL PHOSPHATES IN TRYPANOSOMA BRUCEI RESPOND TO LITHIUM AND BIOGENIC AMINES

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The role of inositol phosphates has been investigated in procyclic Trypanosoma brucei cells. HPLC analysis has revealed peaks of glycerylphosphoryl inositol and mono-, bis- and traces of tris-phosphate after in vivo labeling with radioactive inositol. The ratio between the peaks of phosphorylated inositol is modified upon addition of lithium ion. The lithium effect is consistent with the inhibition of inositol phosphate phosphatase observed in other systems. Changes in inositol containing lipids were also investigated by TLC and found to be correlated to the phosphates variations induced by lithium.
An effect of biogenic amines is also observed suggesting that these monocellular organisms respond to some external stimuli by modifications of inositol phosphate concentrations.

P BIO 310

COMPARISON OF SHAPE CHANGES, RESPIRATORY BURST AND RISES IN CYTOPLASMIC FREE CALCIUM IN HUMAN NEUTROPHILS

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Neutrophils respond to chemotactic receptor agonists by extending pseudopods, which are essential for motility, and by producing O_2^- (respiratory burst). The transient kinetics of these responses were studied, along with the rapid raise in intracellular free calcium (Ca^{2+}_i). Shape changes (detected by turbidimetry) were found to take place even when protein kinase C (PKC) was inhibited by staurosporine or Ca^{2+}-depletion, both of which blocked burst activity (H_2O_2/luminol dependent chemiluminesence). Both response curves decayed rapidly when receptor antagonists were added after stimulation. The decay kinetics showed long term agonist-dependence of shape changes and respiratory burst, while the (Ca^{2+}_i)-decay was less influenced by the disruption of the ligand-receptor complex. While one portion of the agonist dependent signal transduction appears to be common for burst and shape changes, the pathway involving phospholipase C and PKC seems to be necessary only for O_2^--production.

P BIO 311

EXPRESION, INTRACELLULAR SORTING AND SELECTIVE CELL SURFACE TARGETING OF Thy-1, THE POLYMERIC Ig RECEPTOR (pIgR) AND Thy-1-pIgR CHIMERAS IN MDCK CELLS.

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cDNA encoding Thy-1, pIgR, mutated pIgR and Thy-1-pIgR chimeras were inserted in expression vectors and introduced in high resistant MDCK cells. In MDCK cells, Thy-1 is glycosylated and glypiated. The mature protein is selectively directed to the apical cell surface, where it can be recovered in the medium following treatment with ip-phospholipase C. No Thy-1 is associated with the basolateral cell surface, in contrast to pIgR which is initially targeted to this cell surface domain. These data suggest that a signal associated with the cytoplasmic tail of the pIgR is required for sorting and selective targeting. To identify such a signal, the phosphorylated Ser residues on the cytoplasmic tail of the pIgR have been mutagenized. Thy-1-pIgR chimeras were constructed to test which element of the pIgR's tail is able to redirect Thy-1 from the apical to the basolateral plasma membrane.

P BIO 312

Activation of $CD4^+$ peripheral blood T lymphocytes induced by an anti-CD4 monoclonal antibody.

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The CD4 molecule is a glycoprotein found on the surface of a subset of T lymphocytes which recognize antigen in association with major histocompatibility complex (MHC) class II molecules. While it is generally accepted that direct binding of CD4 to MHC class II molecules may facilitate the interaction between a T cell and an antigen-presenting cell, little is known about the possible role of CD4 in signal transduction. We have found that the anti-CD4 monoclonal antibody (mAb) B66, in contrast to other anti-CD4 mAbs, can activate directly human $CD4^+$ T lymphocytes in the absence of other stimuli. Activation results in cell proliferation and production of lymphokines such as IL-2. The binding of mAb B66 to CD4 induces mobilization of cytoplasmic free Ca^{2+}. These results suggest that CD4 may play an active role in the signal transduction that follows recognition of an antigen-MHC complex by $CD4^+$ T lymphocytes.

Genetics (P GEN)

P GEN 313

DNA FINGERPRINTING IN ANIMALS: IDENTITY AND PEDIGREE TESTINGS

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Based on the discovery of variable numbers of tandemly repeated DNA sequences (VNTRs) or "minisatellites" in the non-coding part of the genome, Jeffreys et al. developed a method to reveal the DNA polymorphism among different individuals (Nature 314, 67-73, 1985). Appearing as hypervariable bar code like band patterns, the set of an individual's DNA fragments produced is so unique (except in monozygous siblings) as to be termed a DNA "fingerprint" and moreover, provides a powerful tool in human genetics, forensic medicine and animal breeding. In the present study identity and relationship testings were done in several animal species. In particular, DNA fingerprinting was performed in different mouse inbred strains (C57BL/6, C57 BL/10, Balb/c, A/J and DBA/2J) and crossmatings between some of them in order to test its reliability to control the genetic quality of inbred mice. The band patterns obtained were first, strain specific, although slight variations within a strain was observed, and second, they displayed band patterns distinct from those of the parental strains. In conclusion, due to its potential to detect genetically contaminated individuals, the DNA fingerprinting technique represents a novel kind of monitoring the authenticity of the mouse inbred strains tested.

P GEN 314

DNA FINGERPRINTING AND THE USE OF NON-ISOTOPIC DETECTION

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The routine use of techniques like DNA fingerprinting would become more convenient and safer if the radioactive detection methods could be replaced by non-radioactive ones. The question whether there are non-isotopic detection methods sensitive enough for the detection of a DNA fingerprint has been answered positively, although they are not yet sensitive enough to be considered a true alternative to the radioactivity. The attempts made to use the different commercially available kits also allowed a crude comparison of the performances of these methods.

P GEN 315

GENETIC MAPPING OF A MUTATION CAUSING POLYDACTYLY (PD) IN XENOPUS LAEVIS.

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A linkage map of the Xenopus laevis genome is being established using isozyme markers and RFLP's (restriction fragment length polymorphisms). A recessive mutation inducing polydactyly (pd) and showing variable expressivity has been introduced into a highly heterozygous background generated by inter-subspecies hybridization. Progeny derived from a hybrid victorianus/laevis (pd) backcross were utilized to map pd with respect to established linkage groups. The pd locus was found to segregate with Linkage Group III (PepB, MPI-1, SORD, mIDH-2), and appeared to map very close to the mIDH-2 (mitochondrial isocitrate dehydrogenase) locus. The mapping of pd should facilitate further genetic and molecular analyses of polydactyly in Xenopus.
Supported by SNF, Grant 3.596-0.87.

P GEN 316

GENETIC RELATIONSHIP OF BORRELIA BURGDORFERI STRAINS

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Borrelia burgdorferi, a tick-transmitted spirochete and etiologic agent of Lyme disease, has been identified by fluorescent antibodies directed against outer membrane and flagellar proteins. This method has revealed antigenic heterogeneity among European isolates but it is unsatisfactory. We studied relationships among B. burgdorferi strains isolated from ticks in Switzerland at the DNA level: 1) Randomly cloned chromosomal DNA fragments were used as probes in Southern hybridizations. 2) Profiles of supercoiled plasmids and homologies between these plasmids were analysed. 3) B. burgdorferi cells contain double-stranded, linear plasmids of 5 to ≥50 kb with covalently-closed ends - quite an unusual DNA structure among prokaryotes. DNA preparations enriched for these linear plasmids were compared. One of the Swiss strains is very similar to the type strain B31 (Shelter Island, USA) while the others are quite different. We noticed plasmid loss during cultivation.

P GEN 317

THE GENOTOXIC EFFECTS OF DNA SYNTHESIS INHIBITORS IN SOMATIC CELLS OF THE WING OF DROSOPHILA MELANOGASTER

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In the somatic cells of the wing primordia of Drosophila larvae, genetic changes due to mutation, deletion or mitotic recombination can be induced. On the wings of adults which are trans-heterozygous for the recessive wing cell markers mwh and flr, such events become visible as mutant spots on a wildtype background. The following DNA synthesis inhibitors had genotoxic effects in this assay system: (1) the dihydrofolate reductase inhibitor aminopterin, (2) the ribonucleotide reductase inhibitor hydroxyurea, (3,4,5,6) topoisomerase-II inhibitors such as ellipticine and mitoxantrone which intercalate into DNA, or teniposide and etoposide, which do not intercalate, (7) azauracil as a nucleotide synthesis inhibitor, as well as (8,9) the anti-viral drugs azidothymidine and dideoxycytidine that act as polymerase associated inhibitors of DNA chain elongation. Quantitatively, aminopterin as the most effective compound was over 1000x more effective than hydroxyurea, the weakest compound tested.

P GEN 318

INFLUENCE OF X-RAYS AND INCORPORATED IODINE-125 ON INDUCTION OF DNA STRAND-BREAKS AND PROLIFERATION IN SINGLE MAMMALIAN CELLS

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Chinese hamster cells (M3-1) irradiated with 2 Gy (X-rays) show a reversible G2-block (colony forming capacity at this dose about 70%).Cell populations with incorporation of iodine -125 as iododeoxyuridine in the DNA show a G2-block at the levels of 0.32,0.5 and 1.0 µCi/ml medium.Higher levels as 3.0 and 5.0 µCi/ml iodine-125 induce an S-phase block (colony forming capacity at 5.0 µCi/ml about 1.5%).DNA strand-breaks were estimated by using a flow cytometric method (DNA alkaline-unwinding and acridine orange staining, Rydberg 1985).Cells irradiated with X-rays show a dose dependent induction of DNA strand-breaks.After repair incubation at 37[o]C for 30 min,the number of breaks almos reaches control values.Iodine-125 decays by electron capture,comparable to a local (range 2 mm) microexplosion.Contrary to X-rays,DNA breaks induced by DNA incorporated iodine-125,tend to show up a slower and poorer repair (also observed by other investigators).This is supposed to be due to a relatively high quantity of double strand-breaks compared to single strand-breaks.

P GEN 319

MOLECULAR CLONING AND CHARACTERIZATION OF A HOST MEDIATED FACTOR CONTROLLING METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS.

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A trans-acting factor that is essential for the expression of methicillin resistance in Staphylococcus aureus was cloned and partially characterized. This factor is host mediated and maps in chromosomal linkage group 18. It is not linked to the methicillin resistance determinant (mec), which is thought to reside on a transposable element that integrates into chromosomal linkage group 10. When this factor is inactivated by a Tn551 insertion, methicillin resistance is reduced, both in strains carrying mec and in Mc[r] strains obtained by in-vitro selection for growth at higher methicillin concentrations. The DNA coding for this factor carried an open reading frame of 1302 nt. A protein of an estimated size of 46 kD was produced by the appropriate DNA fragment in maxicells. It might correspond to the product coded for by the open reading frame. It's function is not known yet.

Pharmacology, Toxicology (P PHA)

P PHA 320

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As the choice of medication may depend on the pharmacogenetic status of the patient, a rapid test procedure would be welcome. In this study, the question was examined whether subjects could also be separated into extensive and poor metabolisers in calculating the metabolic ratio with the concentrations of unconjugated dextromethorphan and dextrorphan only. About sixty healthy volunteers and patients (the latter tested both after a wash-out period and during a treatment with various psychotropic drugs) were phenotyped with 25 mg dextromethorphan (and 100 mg mephenytoin). The usefulness of this procedure for rapid phenotyping will be discussed on the basis of the data obtained with this small group of EM's and PM'S.
Financed by the FNSRS (3.962.0.85)

P PHA 321

ACETYLATION AND DEACETYLATION OF XENOBIOTICS IN HUMAN LIVER

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Arylamine N-acetyltransferase (NAT) is the target of one of the most common genetic polymorphisms of drug metabolism in the liver.It is involved in the acetylation of a variety of clinically important drugs and several potential carcinogens. Recent observations (Carcinogenesis, 8, 1939, 1987) suggest however, that acetylated metabolites may be substrates for a microsomal deacetylase (DAC) and be reconverted to the original substrate. The combined action of NAT and DAC thus determines the fate or toxicity of a given compound. Human and rabbit NAT have recently been purified in this laboratory based on a sensitive functional assay for this enzyme (see abstract Grant et al.). Similarly, a HPLC-assay for DAC based on the deacetylation of the carcinogen 2-acetylaminofluorene (AAF) to 2-aminofluorene (AF) was developed. This assay is used to purify DAC from human liver and to evaluate the role of this enzyme in modifying the consequences of slow or rapid acetylation.

P PHA 322

GENETIC POLYMORPHISM OF RABBIT AND HUMAN N-ACETYLTRANSFERASE: MOLECULAR MECHANISM

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Arylamine N-Acetyltransferase (NAT) is the target of one of the most common genetic polymorphisms. Phenotypically slow acetylators have been shown to be homozygous for an autosomal recessive gene. The NAT polymorphism confers marked interindividual variation in the disposition of numerous drugs but also potential carcinogens.
Due to the very low concentration of NAT in human liver we first purified the enzyme from rabbit liver. Using an antibody raised against the purified rabbit protein we isolated and functionally expressed a full length cDNA clone from a λgt11 library, which was constructed from liver mRNA of an in vivo phenotyped homozygous rapid acetylator rabbit. In Western and Northern type experiments we could show that both protein and mRNA were below the detection level in liver of a homozygous slow acetylator rabbit. Data from Southern blot analysis are consistent with a deletion mechanism causing the NAT defect. Internal amino acid sequence information from the purified human NAT is presently used to clone the human NAT gene. Initial experiments with the rabbit probes indicate a similar mechanism for the human NAT-defect.

P PHA 323

UNIQUE PATTERN OF DRUG METABOLIZING ENZYMES IN BILE DUCTULAR EPITHELIAL CELLS

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We recently demonstrated that pure preparations of intrahepatic biliary epithelial cells contain high activities of the conjugating drug metabolizing enzymes, glutathione transferase and UDP-glucuronyl transferase (Mathis et al.,Hepatology,in press). In continuation of these studies we found that the third major conjugating activity, sulfotransferase, was also present in these cells. However, microsomal preparations of bile ductular epithelial cells did not contain spectroscopically measurable amounts of cytochrome P-450. In addition using Western and Northern blot techniques, we were unable to detect in these cells apoprotein or messenger RNA of the major isozyme cytochrome P-450p. To our knowledge there is no other tissue known which contains conjugating drug metabolizing enzymes but does not express cytochrome P-450-dependent oxidases. If the present findings can be confirmed with other cytochrome P-450 isozymes, bile ductular epithelial cell cultures might be a unique model system for the study of conjugating drug metabolizing enzymes by themselves.

P PHA 324

EXTRAHEPATIC ALDRIN EPOXIDATION IN FRESHLY ISOLATED CELLS AND TISSUE PIECES FROM RATS

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The oxidation of lipophilic chemicals is an important step in the formation of genotoxic metabolites in animals and man. This pathway is often attributed to cytochrom P-450 dependent oxygenases or alternatively to an hydroperoxide-dependent oxidation, triggered by the prostaglandin H (PHS) synthase. The epoxidation of aldrin to dieldrin was used to characterize the metabolizing capacity of lung, stomach, urinary bladder, liver and subcutaneous granulation tissue from rats. Tissue pieces or freshly isolated cells were exposed to aldrin in order to mimic most closely the in vivo situation. The contribution of the P-450 and PHS dependent aldrin epoxidation was examined by the addition of indomethacin (IM) or/and arachidonic acid (AA). In the liver the epoxidation activity was not influenced by the two agents. In bladder, granulation tissue and stomach the epoxidation rate (5-30 fmol/min x mg tissue) can be stimulated by AA 2, 3 and 2 fold respectively. IM reduced the activity in lung only. Therefore in extrahepatic tissues the two oxidation pathways contribute in individual organs to a different extent to the total xenobiotic activation capacity.

P PHA 325

COMPARISON OF THE GENOTOXIC EFFECTS OF THE STRUCTURE-RELATED COMPOUNDS DEN AND ENU IN TWO DROSOPHILA MELANOGASTER STRAINS WITH DIFFERENT P-450 METABOLISM

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Drosophila melanogaster larvae and adults have been found to be useful genotoxicity test systems capable of detecting effects of promutagens requiring different activation pathways. In the Somatic Mutation and Recombination Test (SMART) larvae trans-heterozygot for two recessive wing markers on chromosome 3 are exposed to test compounds. Genotoxic effects are indicated by spots on the adult wing surface. In order to improve the metabolic efficency of our tester strains with respect to promutagens chromosomes 1 and 2 have been substituted by those of a strain with a constitutively increased P-450 metabolism determined by a locus on chromosome 2. The effects of the chromosomal substitution on the sensitivity for the structure-related compounds diethylnitrosamine (DEN) as a model promutagen requiring metabolic activation and the directly acting mutagen ethylnitrosourea (ENU) are compared. Work supported by Swiss Cancer League.

P PHA 326

PHARMACOKINETICS AND PLACENTAL TRANSFER OF DIAZEPAM IN PREGNANT RATS

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The pharmacokinetics and the placental transfer of diazepam (DZ) and its major metabolite desmethyldiazepam (DMDZ) were studied in time-pregnant Long Evans rats at gestational day (GD) 21 and in non-pregnant controls. Sixty and 180 min after a single dose of DZ (3 mg/kg i.v.), the concentrations of DZ and DMDZ were determined in maternal plasma, brain, adipose tissue, fetal brain and fetal liver by reversed phase HPLC with UV-detection.

In maternal tissues, 60 min levels of DZ were higher but elimination half-lives were shorter in pregnant rats vs controls. While in pregnant rats DMDZ was detectable at 60 min, in controls the metabolite became measurable only at 180 min. DZ and DMDZ were present at both times in fetal tissues. Preliminary results indicate that DZ is more rapidly eliminated from fetal than from maternal tissues, while DMDZ elimination appears to be slower in fetus. Fetal and maternal tissues are currently beeing studied after chronic administration of DZ to the mother from GD 14 to GD 20.

P PHA 327

ANTAGONIST Ro 15-3505 PRECIPITATES A WITHDRAWAL REACTION IN SQUIRREL MONKEYS TREATED SUBCHRONICALLY WITH DIAZEPAM

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Monkeys received 11 daily oral administrations of diazepam (DZ; 30 mg/kg) or vehicle (VEH). Ro 15-3505 was given iv at the doses 0.25 (N=10), 0.5 (N=9) or 3 mg/kg (N=7) 5 hr after the eleventh treatment injection and observations made for 2 or more hr. Ro 15-3505 induced precipitated withdrawal (e.g. tremor, emesis, and/or convulsions) in all of the DZ-treated monkeys but no appreciable effect in the VEH-treated animals (except for 1 VEH-treated monkey which exhibited vomiting 88 min after injection of 3 mg/kg Ro 15-3505). Not only was the withdrawal reaction precipitated by 3 mg/kg Ro 15-3505 very pronounced in the majority of DZ-treated monkeys, but severe symptoms reoccurred sporadically over several hours. The two lower doses of Ro 15-3505 rarely induced any convulsions in DZ-treated monkeys more than about 40 min post-injection. Thus, 0.25 mg/kg Ro 15-3505 (iv) induces a short, but clear and consistent, precipitated withdrawal reaction in squirrel monkeys treated subchronically with DZ, but no observable effect in VEH-treated monkeys, making this dose appropriate for use in evaluating the potential dependence liability of benzodiazepine receptor ligands.

P PHA 328

IN VITRO FORECASTING OF DRUG INTERACTIONS WITH MIDAZOLAM

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Midazolam (MDZ) is a short acting imidazobenzodiazepine used as sleep inducer. Its biotransformation (1' and 4-hydroxylations) is mediated by cytochrome P-450 isozyme-NF (Kronbach, 1987). To identify factors influencing its activity (e.g. drug-drug interactions), MDZ was incubated in human liver microsomes. Metabolites production was monitored in incubates by direct HPLC-UV detection (220 nm) and other drugs were screened for possible interference with MDZ oxidation. For 1' and 4-hydroxylations Km were 6-4 μM and 15-18 μM, and respective Vmax 26-30 and 18 nmol x mg P^{-1} x h, both reactions being catalyzed by the same or coregulated isozymes. MDZ hydroxylations were inhibited in vitro by phenothiazine-type neuroleptics, calcium channel blockers, ergot alcaloids, cyclosporine, erythromycin, amiodarone, cimetidine. The example of a 61 years old man treated with amiodarone, erythromycin and MDZ (300 mg iv during electrical cardioversions) illustrates the clinical relevance of such forecasting. Despite an expected elimination $t_{1/2}$ of 1.5-2.5 h, MDZ-induced sleep lasted 6 days. Due to inhibition of MDZ biotransformation, measured $t_{1/2}$ was 24.8 h.

P PHA 329

CORRELATIONS BETWEEN CHRONIC EFFECTS OF ANTIDEPRESSANT DRUGS ON PLASMAMEMBRANES OF CULTURED CELLS

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We have recently shown that chronic exposures of various cultured cell types to antidepressant drugs induced a number of cellular changes including receptor functions, lipid contents, phospholipid composition and membrane fluidity. In order to focus the investigations on changes in the plasma membrane as the relevant compartment new and modified analytical methods had to be introduced. Highly purified plasma membranes were isolated by vesiculation of control and drug exposed cells with 25 mM formaldehyde and 2 mM dithiothreitol. The determination of the phospholipid (PL)-composition was achieved by onedimensional chromatography of the membrane PL extracts on HPTLC-silicagel plates. The spots of the separated individual PL were quantified by reflection densitometry. Correlations were elaborated between the drug induced changes in receptor functions, PL-compositions, cholesterol contents and membrane fluidity of cells exposed to antidepressants. In order to show the specificity of the effects the findings were compared to membrane properties of cells exposed to drugs of other therapeutic classes.

P PHA 330

BINDING OF THE VOLATILE ANAESTHETIC HALOTHANE TO SOME HUMAN PROTEINS

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There is some evidence in the literature that halothane (H) may cause conformational changes of proteins. In order to investigate the mechanism of this interaction the binding of H to human serum albumin (HSA), hemoglobin (Hb) and γ-globulin (gG) was studied with a new method (based on equilibrium dialysis) which allows very precise and reproducible binding studies with volatile compounds. In two closed systems phosphate buffer (PB) and protein dissolved in PB were pumped through a dialyzer. PB circulated through an "oxygenator" whose gas supply came from an H vaporizer; the entire system could be equilibrated at each desired H concentration. At steady state samples of both systems were analyzed for H by HPLC. From the difference of the H concentrations in the two systems the binding of H could be evaluated. The binding of H to HSA and Hb was characterized by two classes of binding sites: one class with high affinity but low capacity (= number of binding sites) and a second class with low affinity but high capacity. The corresponding two affinity constants for H were similar for HSA and Hb, whereas the binding capacity was much greater for HSA. Human gG did not bind H at all.

P PHA 331

BEHAVIOR EFFECTS IN RATS FOLLOWING LONG ANAESTHESIA

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Surgical patients often complain about impairment of memory and learning ability after longtime anaesthesia. For the investigation of this phenomenon adult Wistar rats were tested with respect to long-term memory in a structured environment (wheel-shaped activity monitor) and to short-term memory and learning ability in two operant conditioning paradigms before and after anaesthesia. In three to four pre-anaesthesia and two to three post-anaesthesia sessions of 90 minutes, storage and retrieval of spatial long-term memory was determined by the increase of path-iteration frequencies and the decrease of exploration activity. The short-term memory was assayed by a go-no go alternation schedule in Skinnerboxes, training before and testing after anaesthesia. Effects on learning ability was tested in the same rats after anaesthesia by training of a DRL20 schedule. Two different techniques were applied for anaesthesia of 4 hours duration: one inhalation anaesthesia (halothane/N_2O/O_2) and a fentanyl-oroperidole-neuroleptanalgesia combined with N_2O/O_2 inhalation. The anasthesia was monitored by ECG, blood pressure and body temperature and the recovery by home-cage activity measures. Effects of these anaesthetic procedures on all measured variables will be discussed.

P PHA 332

EFFECTS OF ANTIMYCOTIC COMPOUNDS ON SPERM FUNCTION, ON FERTILIZATION, AND ON PREIMPLANTATION EMBRYOS IN VITRO

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Ketoconazole (K), naftifine (N) and terbinafine (T) were tested in vitro for their potential to affect sperm function, fertilization and subsequent preimplantation embryo development. Mouse sperm was incubated together with the test substances (1-100 µg/ml) during capacitation; ova were then added. Acrosomal integrity, sperm motility, fertilization rates and subsequent embryonic development were assessed. All compounds caused acrosomal losses after 30 and 90 min incubation. Both T (10 and 100 µg/ml) and N (100 µg/ml) reduced sperm motility at the end of the capacitation and fertilization phases; K caused no decrease. K (all concentrations) and N (100 µg/ml) reduced the fertilization rates; T caused no effects. All compounds arrested embryonic development in drug-free medium at the morula/blastocyst stage. The azole compound caused effects different from those of the allylamines, suggesting different mechanisms of toxic action. All three compounds, however, had an 'all or nothing' effect in vitro, i.e., exposed embryos either die before implantation or develop normally.

P PHA 333

THE EFFECTS OF TIME AND DURATION OF EXPOSURE TO QA 208-199 ON EMBRYOTOXICITY, METABOLISM AND ACCUMULATION IN VITRO.

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In a previous study we have shown that the rat conceptus metabolizes the lipoxygenase inhibitor QA 208-199 (QAB) in vitro. In the present study the time and exposure dependency of the embryonic capacity to metabolize QAB and accumulate its main metabolites QA-acid (QAA) and the unknowns M5 and M6 were determined. 9.5 day old embryos were cultured for 48 h. The compound was added at various times and for various periods. Medium and tissue samples were analyzed by HPLC for the presence of the parent compound and metabolites. Morphological abnormalities were only observed when the embryos, already for at least 15 h in culture, were exposed to QAB (115uM). QAA and M6 proved to be the main metabolites in serum, whereas M5 was a breakdown product. QAB could not be detected in tissues, but tissue accumulation of QAA, M5 and M6 started after 24 h. Tissue levels were higher in the yolk sac than in the embryo or ectoplacental cone. QAA itself showed no embryotoxicity, metabolism or accumulation in embryonic tissues. It can be concluded that QAB metabolism starts within the embryonic unit, leading to high levels of tissue metabolites, when the embryo is 10.5 days old.

P PHA 334

CHARACTERIZATION OF CADMIUM UPTAKE AND BINDING USING LLC-PK_1 CELLS AND BRUSH BORDER MEMBRANE VESICLES

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An established epithelial cell line (LLC-PK_1) with proximal tubule properties, and renal brush-border membrane vesicles (BBMV) were used to study cadmium ($CdCl_2$) uptake. 1). Cells grown at confluence on plastic dishes were exposed (apical side) to Cd (1 or 5 µM in serum-free culture medium) during 1-6 h, at 37 or 4°C. Cells were then trypsinized, sonicated and ultracentrifuged (110'000 g, 75 min). Cd accumulation in the cytosolic fraction was found to be time and concentration dependent. Uptake was nearly abolished at 4°C and was inhibited in presence of Zn^{+2}. 2). Similar experiments were carried out with LLC-PK_1 cells grown at confluence on Transwell-collagen filters, allowing separate apical or basolateral access. Uptake of Cd had the same characteristics as described above, and was found to occur also from the basolateral side of the cells. Transepithelial movement of Cd was extremely low but was further reduced at 4°C. 3). In preliminary experiments, BBMV from rabbit kidney were found to take up Cd, most likely through binding, in a time- and concentration dependent manner.

P PHA 335

TOXICITY OF TRYPTOPHAN IN ADRENALECTOMISED RATS

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Tryptophan (Trp) is becoming widely used as a mild sedative, an analgesic and antidepressant. Much of the excess Trp is metabolised in the liver by the enzyme Trp pyrrolase. This enzyme is induced by Trp and adrenal steroids. It is posssible that in adrenal insufficiency Trp breakdown via this pathway might be impaired. Trulson and Ulissey (*Life Sci* **41**, 349, 1987) have reported dramatically increased toxicity of Trp (LD_{50} = 11.4 mg/kg) in adrenalectomised rats (LD_{50} > 1 g/kg in normal rats), and have suggested that the increase in toxicity is due to accumulation of tryptamine. We have repeated these experiments in normal and adrenalectomised rats. The oral LD_{50} was > 850 mg/kg in all groups. In addition, 2 hours after an oral dose of 1 g/kg, plasma and tissue Trp levels were similarly elevated in all groups but tryptamine was undetectable (ie. < 10 ng/g) in all tissues except kidney where post-treatment tryptamine levels reached ~ 280 ng/g in both normal and adrenalectomised animals. In conclusion, we could not confirm the dramatic increase in toxicity of Trp in adrenalectomised rats.

P PHA 336

COMPARISON OF IN VITRO CELL TOXICITY WITH IN VIVO EYE IRRITATION

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The effects of 26 different cosmetic ingredients (e.g. permanent wave and hair dye compounds, emulsifiers, resins and detergents such as quats) were assessed by four endpoints indicative for qualitatively and quantitatively different cytotoxicity:
1. Neutral red uptake (reduction to 90% and 50% of controls)
2. Cell detachment from culture dish (CD50)
3. Growth inhibition (GI50)
4. Membrane permeability (dye retention and exclusion).
The cytotoxicity potentials of the test agents were ranked for each in vitro test and compared to the in vivo eye irration in guinea pigs determined according to Draize (application of 2.5% and 5 % w/v solutions of the same test batches).
Strong irritants could be easiliy detected by all in vitro tests. Slightly irritating test agents were distinguished from strong irritants as well as from non-irritants only by assay nr.1. The other cell tests had to be combined to allow an adequate interpretation of in vitro cytotoxicity.

P PHA 337

NEUROTOXICITY TESTING IN VITRO: THE CHICK BRAIN AND RETINA CELL AGGREGATION MODEL

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Mechanically dissociated brain and retina cells from 8 d old chick embryos were allowed to aggregate under gyratory movements in analogy to embryonic rat brain cells (Honegger 1985). Culture vessels and cell culture conditions were selected on the basis of easy to use for large scale series of test substances. Based on morphological criteria it was observed that brain cells aggregate more homogeneously than retina cells, probably due to their slower aggregation mechanism at the stage of cell dissociation. Most culture vessels including multi-well dishes could be used for the formation of proper aggregates. Endpoints for cytotoxicity and critical differentiation steps are used in order to evaluate the significance of this in vitro model for in vivo teratogenicity and neurotoxicity screening.

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P PHA 338

THE ERGATT/FRAME DATA BANK OF IN VITRO TECHNIQUES IN TOXICOLOGY

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This data bank aims at a supply of state-of-the-art information on the in vitro techniques in use and under development. The system has been designed on the basis of easy-to-use and fast access of literature, in vitro data, endpoints and applications, as well as the state of validation procedures. Within a few months more than 120 european users have registered and with this presentation further potential users are informed in order to increase support, collaboration, development and acceptance of in vitro toxicity tests.

Supported by **FRAME** (Fund for the Replacement of Animals in Medical Experiments), **ERGATT** (European Research Group for Alternatives in Toxicity Testing) and **WWF** Schweiz)

P PHA 339

LIPOSOMES AS CARRIER FOR RADIOACTIVE RHENIUM-COMPLEX

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Rhenium 186 and Rhenium 188 (Re) are strong β-emitters (2.1 MeV) and therefore potentially useful for therapy against cancer. Liposomes are used as carrier for various molecules to the liver. We loaded liposomes with a high dosis of radioactive Re required for therapy (about 10 mCi) and investigated their stability.

We synthesized the very lipophilic Re-complex Oxodichloroethoxy-bis(triphenylphosphine)rhenium(V), that can be easily introduced into the bilayer by gelfiltration of the mixed micelle of egglecithin with deoxycholate as detergent on a Sephadex-G25M-column (PD10). The diameter of the liposomes is 68±16 nm measured by dynamic laser light scattering. The activation of the Re-complex is done by neutronirradiation in the reactor Saphir at the PSI ($4.32 \cdot 10^{18}$ n·v·t) and yielded 38 mCi/mg Re-complex.

Within 2 hours 46-54 % of the radioactive Re-complex is incorporated into the liposomes (5 mg lipid per ml). The stability of these liposomes was investigated by dialysis and 45 % of the radioactivity remained in the liposomes after 8 days. The decomposition of the complex was caused by the oxidation of the Re(V) and can partially be prevented by antioxidants.

P PHA 340

BETALACTAM ANTIBIOTICS AFFECT DNA REPLICATION

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Proliferation of eukaryotic cells was found to be S-phase specifically and dose-dependently inhibited by degradation products of betalactam derivatives. Derivatives seem to possess, beside the open betalactam-ring, structures that are important for inhibiting DNA polymerase alpha and delta as well as viral DNA polymerases. In addition reverse transcriptases of HIV and FTLV were susceptible to betalactams. Inhibition of DNA polymerases alpha and HIV-reverse transcriptase was found to be competitive with dTTP and DNA. We found amino acid sequence homologies between the Penicillin binding proteins 1A, 1B, 3, 5, and 6, the bacterial target for betalactams, and human DNA polymerase alpha and HIV-reverse transcriptase. They were located in functional domaines of both enzymes. We isolate these degradation products by using its inhibitory effect on DNA polymerases, try to define the structure and to elucidate if there is a common binding pattern of betalactam derivatives to either Penicillin binding proteins and polymerases.

P PHA 341

EFFECTS OF DIFFERENT CYTOKINES ON PROSTAGLANDIN E2 (PGE2) AND PHOSPHOLIPASE A2 (PLA2) RELEASE FROM RAT GLOMERULAR MESANGIAL CELLS (MC).

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Treatment of MC with recombinant human interleukin 1α (rIL-1α), recombinant human interleukin 1β (rIL -1β) or recombinant human tumour necrosis factor (rTNF) induces PGE2 synthesis and the release of a PLA2 activity. Recombinant human interleukin 6(rIL -6) has no effect on PGE2 and PLA2 release and recombinant rat interferon γ (rINFγ) only stimulates PLA2 release from MC. rIL-1β is significantly more potent than rIL-1α or rTNF in stimulating PGE2 as well as PLA2 release. When given together, rTNF interacts in a synergistic fashion with rIL-1α and rIL-1β to enhance both PGE2 and PLA2 secretion. Likewise rINFγ interacts in a synergistic fashion with rIL-1β and rTNF to amplify PGE2 and PLA2 release from MC. rIL-6 has no effect on prostaglandin and enzyme release induced by the other cytokines.

P PHA 342

INTERLEUKIN-1 STIMULATES THE SYNTHESIS AND RELEASE OF NERVE GROWTH FACTOR (NGF) IN CULTURED ARTERIAL SMOOTH MUSCLE CELLS

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NGF is a protein produced in limited amounts by targets of NGF-responsive neurons and functions as a retrograde trophic messenger. To investigate specific signals which regulate expression of this trophic molecule in peripheral targets, we have examined cultured mouse aortic smooth muscle cells. NGF protein was determined by a sensitive ELISA and NGF mRNA by an optimized Northern blot technique. We have found that among a variety of inflammatory mediator substances, interleukin-1 (IL-1) is a potent stimulator of NGF synthesis. Treatment of smooth muscle cells with human recombinant IL-1 elicited a significant increase in NGF mRNA and NGF protein release in a dose-dependent manner. This effect was abolished by 10^{-7}M dexamethasone. We postulate that NGF could be involved in immunological reactions leading to chronic inflammatory processes including atherosclerosis.

P PHA 343

EFFECTS OF CHOLINERGIC AGENTS ON ACh- (ACh-M) AND PHOSPHOINOSITIDE- (PI-M) METABOLISM IN DIFFERENT RAT BRAIN REGIONS

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Cholinergic neurotransmission plays a role in the processing of recent memories, and abnormalities of this system may underlie some of the symptoms in Alzheimer's disease (AD), in which a marked decrease of presynaptic cholinergic markers has been found. For a symptomatic treatment of AD, direct postsynaptically acting muscarinic agonists are therefore of interest. The effects of different cholinergic agonists on ACh-M and PI-M, two possibly specific markers for M2 and M1 receptor interactions, were determined in different rat brain regions after sacrification by micro-wave irradiation. All agonists tested dose-dependently increased the steady-state levels of ACh and the accumulation of inositol-1-phosphate (I1P), following LiCl pretreatment, indicating a decreased ACh-M and an increased PI-M respectively. The maximal accumulation of I1P, after p.o. administration of SDZ 210-086 (45 umol/kg), was seen in the hippocampus (9 fold), while in the cortex and striatum it was only 2-3 fold. ACh-M and PI-M might therefore be useful for the in-vivo characterization of muscarinic agents with respect to their putative receptor-subtype and brain-regional specificities.

P PHA 344

STIMULATION OF MEPHENYTOIN HYDROXYLASE ACTIVITY UNDER VARIOUS ISOLATION AND INCUBATION CONDITIONS

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For the improvement of the radiometric mephenytoin hydroxylase (MH) determination in vitro, we have studied varying experimental parameters. MH activity was measured at 0.05mM incubation concentrations using tritiated R,S-mephenytoin. Ethoxycoumarin deethylase activity served as a reference for cytochrome P-450 activity. In stability experiments with microsomes (100'000xg pellet), the MH activity decreased with a half-life of 2-3h; full stability of the enzyme was obtained upon the addition of EDTA 1mM to all purification steps. MH activity was inhibited by most organic solvents at 0.1% final concentrations. By contrast, MH activity was stimulated by 250% after replacement of phosphate buffer 0.1M with 1.0M and by 130% after addition of 5% serum to microsomes. Repeated washings of the 100'000 x g pellet with KCl 0.15M yielded progressively more active enzyme preparations. Thus, for reliable MH activity determinations, appropriate incubation parameters are mandatory and the presence of an endogenous inhibitor is suspected.

P PHA 345

MECHANISM OF [ARG^8]VASOPRESSIN (AVP) INDUCED CALCIUM ENTRY IN VASCULAR SMOOTH MUSCLE CELLS

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We investigated cytosolic [Ca^{2+}] in vascular smooth muscle cells after stimulation with AVP using fura-2. In the presence of extracellular Ca^{2+}, AVP induced a rise in cytosolic [Ca^{2+}] at lower concentrations than it did in Ca^{2+}-free medium. Measuring (1,4,5)-IP_3 revealed a threshold between 10 and 100 nM AVP for the second messenger production in these cells. AVP stimulation of Ca^{2+} entry begins at a concentration about 10 times lower; thus we hypothesise another mechanism unrelated to IP_3 or its metabolites underlying AVP stimulated Ca^{2+} entry. Protein kinase C activation with phorbol esters resulted in a down regulation of the Ca^{2+} entry, excluding kinase C as an activator of Ca^{2+} influx. Ca^{2+} entry after AVP stimulation was not inhibited by dihydro-pyridines is therefore distinct from the voltage-sensitive Ca^{2+} channels. Thus, the opening of the hypothetical receptor-operated Ca^{2+} channel seems to occur either by diacylglycerol itself or by a direct link between the receptors and the channel.

P PHA 346

EFFECTS OF VARIOUS CHEMICALS ON SERUM-FREE CULTURES OF FETAL CHICK BRAIN, NEURONAL RETINA AND MENINGUS.

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In order to study the effect of various chemicals on nerve tissue differentiation and proliferation fetal chick brain and neuronal retina cells were cultured. The effects were compared with data on fibroblast proliferation, using primary serum-free cell cultures of the meningus. In brain cell cultures it was found, that nerve cells and astroglia cells maturate, as indicated by the presence and developmental pattern of the amount of MAP2 or GFAP antigens, respectively. It was found that the maturation of the nerve- and astroglia cells are not linked with each other. In cultures of the neuronal retina photoreceptors showed to maturate, as indicated by the presence and developmental pattern of the binding of peanut(PNA) lectin. Various concentrations of the test substances were added after one day of incubation. 7 days later differentiation and various viability parameters were measured. The ranking order of the LC50 values for the substances tested corresponds to that observed in toxicity studies in mammals. Minor but significant effects on the differentiation could be seen at much lower concentrations.

Physiology (P PHY)

P PHY 347

MAPPING OF IONIC CURRENTS IN THE CHICK BLASTODERM

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The distribution of extracellular electrical currents in the gastrulating chick blastoderms has been mapped using a computer-controlled scanning vibrating electrode. The extraembryonic ring-shaped area opaca (AO) generates two families of current loops. The external loops leave the ventral side of AP, turn around the edge of blastoderm and reenter the dorsal side of AP. These currents are strong, radially symmetrical and periodically oscillating. The internal loops converge ventrally towards the anterolateral limits of the embryonic area pellucida, pass trough the embryonal cavity, traverse the ectoderm ventrodorsally and close on the dorsal side of AO. These currents are weak and bilaterally symmetrical. All currents are sodium-dependent and inhibited by ouabain. Comparisons of these organized currents with regionally differentiated cellular activities and fibronectin distribution pattern in the blastoderm (J.Cell.Sci.,Sup. 8:415-31,1987) suggest the role of ionic currents in information transfer within the embryonic disc.

P PHY 348

FIBRONECTIN IN THE BLASTULATING CHICK EMBRYO

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The distribution of fibronectin immunoreactivity (FN) in blastulating chick embryo has been studied using the electron-microscopical immunogold staining technique. During the first 10 hours of post-laying development, FN was present ventrally in both embryonic area pellucida (AP) and extraembryonic area opaca (AO). In the center of AP, FN was associated with the basal lamina of epiblast, present at contacts between the epiblastic and hypoblastic cells, and, occasionally, internalized in the hypoblastic cells. The highest density of FN was found at the periphery of AP (marginal zone) where the basal lamina is incomplete and where occur intense cell movements. In the AO, FN was sporadically found at contacts between medially located cells but was absent in the edge cells of the blastoderm. The results suggest that fibronectin might participate in the modulation of the early epithelization-migration processes leading to axialization and formation of the primary embryonic "milieu interieur".

P PHY 349

REDUCED 24 HOUR ENERGY EXPENDITURE AND BASAL METABOLIC RATE IN GAMBIAN MEN

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Using a respiration chamber both in The Gambia (Africa) and in Lausanne, 24 hour energy expenditure (EE), diet induced thermogenesis (DIT), basal and sleeping EE and the energy cost of walking on a treadmil (at 3.2 km/h, 0 and 10% slope) were measured in 20 young rural Gambian (G) men during the "hungry"season (weight: 60.8±1.1kg; body fat 9.8±0.5%) and in 16 European (E) men matched for body composition (weight: 66.9±1.9kg; body fat 10.8±0.6%). The 24 hour EE was significantly lower in G than in E men (2047 ± 46 vs 2635 ± 74 kcal/day, $p<0.001$ respectively) even after allowing for differences in body weight and fat free mass ($p<0.001$ in both instances). Basal EE and sleeping EE were also lower in G than in E men (1.05±0.02 vs 1.25±0.02 kcal/min, $p<0.001$, and 1.0±0.01 vs 1.18±0.02 kcal/min, $p<0.001$ respectively). DIT was blunted in G compared to E men(6.3±0.6% vs 12.1±0.5%, $p<0.001$ respectively). The net efficiency of walking was greater in G than in E men (23.2±0.3 vs 20.1±0.4%, $p<0.001$ respectively). It is concluded that a low basal and sleeping EE, a reduced DIT as well as a high work efficiency are energy sparing mechanisms in G men which allow them to cope with a marginal level of dietary intake during the "hungry" season.

P PHY 350

GLUCOSE PRODUCTION FROM GLYCEROL AND ALANINE IN LIVER OF GENETICALLY OBESE (fa/fa) RATS IN VIVO.

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Increased hepatic glucose production has been reported to occur in the insulin-resistant genetically obese fa/fa rats. The aim of the present study was to investigate the possibility of an increased gluconeogenesis, studying the metabolic fate of glycerol and alanine in liver of fed, anesthetized lean and genetically obese (fa/fa) rats. Glycerol turnover rate was three times higher in obese animals compared to the lean (2.8±0.4 versus 0.9±0.1 umol/min). This increase in glycerol turnover rate was associated with an increase in blood glycerol levels in obese animals. The rate of conversion of glycerol to glucose was significantly incresed in obese animals (2.0±0.4 compared to 0.8±0.1 umol/min in lean rats). In contrast, the rate of conversion of alanine to glucose was not different between lean and obese animals. It is concluded that in fed genetically obese (fa/fa) rats, the high blood glycerol concentration is the major driving force for the increased hepatic conversion to glucose of this substrate.

P PHY 351

BRIGHT LIGHT SUPPRESSES CARBOHYDRATE INTAKE IN SEASONAL AFFECTIVE DISORDER (SAD)

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SAD patients report marked "carbohydrate (CHO) craving" during their depressive phase in winter. Using food frequency questionnaires (FFQ) at each season we have demonstrated a seasonal rhythm in CHOstarch (st) intake, but not in protein. SAD ate more CHOst than controls in all seasons except summer.

A daily FFQ was applied during weekly periods at the beginning of the winter depressive phase, light therapy (1h am or pm, 2500 lux), withdrawal, and during summer when not depressed. Both am or pm light were equally effective in improving clinical depression (N=36 SAD, using observer and subjective rating scales). Light (am or pm) reduced CHO-rich food intake, especially sweets and in the second half of the day, and this continued during withdrawal, with results comparable to summer. Protein-rich foods were not modified by light or season.

The "CHO craving" in SAD can be interpreted as an unsuccessful "self- healing" attempt. During improvement with light and in summer, "self- healing" with CHO is no longer necessary. There is evidence that a biological basis of this psychological interpretation can be found in peripheral and central processes involving neurons (e.g. serotoninergic, noradrenergic) in the medial hypothalamus.

P PHY 352

HYPERINSULINEMIA IN RATS PRODUCES MUSCLE INSULIN RESISTANCE WHILE OVERSTIMULATING ADIPOSE TISSUE.

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The problem was to decide whether hyperinsulinemia may be causative in bringing about insulin resistance. Normal rats were infused with saline (controls) or 2U human insulin per day for 4 days (insulinized rats). Saline-or insulin-treated rats were then clamped at euglycemia. Insulinization increased in vivo labelled 2-deoxy-D-glucose uptake by white adipose tissue only (control : 1.7 ± 0.2; insulinized 10.2 ± 0.2 ng/mg), while it halved that of all muscle types studied except soleus. Analogous data were obtained in adrenomedullectomized or propranolol-infused, insulinized rats, ruling out an implication of catecholamines in the effects observed. Chronic insulinization of normal rats results in the establishments of an insulin resistance state at the level of most muscles, while overstimulating adipose tissue

P PHY 353

PRIMAPTERIN, ANAPTERIN, AND 6-OXO-PRIMAPTERIN, THREE NEW 7-SUBSTITUTED PTERINS EXCRETED IN PATIENTS WITH HYPERPHENYL-ALANINEMIA

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Three unknown compounds present in the urine of a patient with mild hyperphenylalaninemia were identified by HPLC and GC-MS to be L-erythro-7-isobiopterin, D-erythro-7-isoneopterin, and L-erythro-6-oxo-7-isobiopterin. The newly identified pterins were named primapterin, anapterin, and 6-oxo-primapterin, respectively. Primapterin and anapterin are present in very low concentration in every human urine, whereas 6-oxo-primapterin was detected in the patient's urine only. Substantial amounts of primapterin were excreted in two patients and in the healthy brother of one of the patients. Differential oxidation of urines showed that the urinary primapterin was excreted in tetrahydro form. Following oral tetrahydrobiopterin administration (2 mg/kg/d) neopterin normalized while biopterin and primapterin increased about 8-fold in one of the patients. This finding suggests that primapterin can be formed from biopterin by an isomerization reaction. However, the exact metabolic origin of primapterin and anapterin is still obscure.

P PHY 354

CREATINE KINASE ISOENZYMES IN BOVINE RETINA AND THEIR POSSIBLE FUNCTION IN PHOTOTRANSDUCTION

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Brain-type (B-CK) and mitochondrial-type creatine kinase (Mi-CK) were identified by immunoblotting and localized by indirect immuno-fluorescence in bovine retina. Cryosections of retina reveal that two isoenzymes are distributed differently: Mi-CK is predominantly located in inner segments of the photoreceptor cells, whereas B-CK is distributed in a more homogeneous pattern, comparable to previous evidence in chicken retina (Wallimann et al. PNAS 83: 3816, 1986). However, in bovine retina due to better structural preservation B-CK could be shown to be associated to the plasma membrane of rod outer segments (ROS). In addition, by CK activity measurements on isolated ROS it was shown that a significant amount (1-2%) of the total retinal CK activity is in ROS. This represents a minimal value since B-CK is a mainly soluble enzyme and some leakage out of the isolated ROS cannot be prevented. The fact that the remaining B-CK was bound tightly in ROS in combination with the strong immunofluorescence signal observed along the plasma membrane is indicative for specific membrane-association of some B-CK , possibly involving one of the B-CK subspecies identified recently (Quest et al. in preparation). The possible roles of the two isoenzymes in energy regeneration of phototransduction will be discussed.

P PHY 355

PREPONTINE KNIFE CUT - INDUCED HYPERTHERMIA IS ABSENT IN HYPOTHYROID RAT

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The mechanism of the altered regulatory thermogenesis in hypothyroid state is still not fully understood. Brown adipose tissue (BAT) the main effector of this thermogenesis in small mammals is affected in different ways by hypothyroidism: decreased sensitivity to noradrenaline (NA), reduction of the uncoupling protein level, increased lipogenesis and glycolytic flux and an almost normal maximal thermogenic capacity. Thus, the defective thermogenesis is not entirely explained by alterations of BAT metabolism. In control rat, a stereotaxically micro-knife cut placed in the prepontine region is followed by a marked stimulation of BAT leading to a sustained hyperthermia of 3-4°C. It had no effect in hypothyroid rat which contrasts with NA infusion which induced an almost normal hyperthermia, albeit at higher concentrations. Concentrations of T_4 or T_3 that produce a normal metabolic response in hypothyroid rats exposed to cold, were ineffective in restoring the thermic response to the cut. It can therefore be inferred that the lack of response of hypothyroid rat is due to alterations in the neural input to BAT rather than to defective tissue response.

P PHY 357

ESTIMATION OF BROWN ADIPOSE TISSUE CONTRIBUTION TO THE PREPONTINE KNIFE CUT-INDUCED HYPERTHERMIA IN THE RAT BY MEANS OF MODELING

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In the urethane-anesthetized rat, a prepontine knife cut-induced a lasting increase in colonic temperature (Tc) of 3-4°C. Brown adipose tissue (BAT) was found to be the only organ to which the fractional blood flow increased markedly during the development of hyperthermia. BAT temperature rose faster, with a shorter latency, and attained a higher steady state value than Tc. In adrenalectomized rats, the temperature gradient between BAT and colon was 4 times higher following the cut than in control rats with a similar BAT blood flow. Tc, however rose less and for a shorter period than in control rats, which suggests the existence of other effector than BAT responsible for the hyperthermia. By means of a mathematical model, estimation of the contribution of BAT to the total heat production shows that it amounts to 45% in the control, whereas in adrenalectomized rats it amounts to 60% and 100%, during the first and second hour of hyperthermia, respectively. The model predicts that BAT is not the only effector of regulatory thermogenesis. A sympathetically mediated extra BAT source of heat, of still unknown nature, remains to be determined.

P PHY 358

AGE DIFFERENCES IN RENAL RESPONSIVENESS TO ALPHA-HUMAN-ATRIAL NATRIURETIC PEPTIDE (α-hANP) IN RABBIT

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ANP is a hormone with natriuretic, diuretic and vasodilating properties. Elevated levels of ANP at birth have been demonstrated, suggesting a difference in the response to the hormone at different maturational ages. Plasma levels of ANP and renal function have consequently been studied in newborn and adult rabbits. Plasma basal ANP levels were similar in 18 newborn and in 19 adult rabbits (50±23 and 51±19 pmol/l). Eleven newborn (4 to 8 day-old) and 11 adult rabbits were then anaesthetized and mechanically ventilated. After a control period, each animal received an α-hANP loading dose (3 µg/kg i.v.), followed by an infusion of 0.3 µg/kg·min. Blood gases remained stable throughout the experiment in both groups. Mean blood pressure decreased in immature (28±1 to 26±1 mm Hg) and mature (92±3 to 84±3 mm Hg) animals. ANP-induced changes in renal functions in newborn and adult rabbits were respectively: diuresis: -20% and +55%; urine Na excretion: -5% and +75%; inulin clearance: -19% and -5%; PAH clearance: -22% and -12%. As expected, diuresis and natriuresis increased in adult rabbits. Failure of ANP to increase natriuresis and diuresis in newborn rabbits could be related to the marked decrease in GFR. Unusual response to ANP in immature animals could be due to receptor immaturity, lack of ANP-activating factor or interactions with other hormonal systems.

P PHY 360

P-AMINOHIPPURATE TRANSPORT ACROSS BRUSH BORDER AND BASOLATERAL MEMBRANES FROM THE PROXIMAL TUBULE OF PIG KIDNEY.

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Previous data on brush border membrane vesicles indicated that the luminal step of p-aminohippurate (PAH) secretion in pig proximal tubule occurred by a saturable, potential-driven mechanism. The present study suggests that this potential-stimulated transport mechanism might be a channel, rather than a carrier, as no trans-stimulation of ^{3}H-PAH uptake was observed when an outwardly directed PAH gradient of 30-fold was imposed across the membrane. To investigate the basolateral step of PAH secretion, basolateral membrane vesicles were prepared from pig kidney. Compared to the initial homogenate, the enrichment factors in the membrane vesicles were 17.4 ± 2.4 and 0.87 ± 0.13 (n = 8) for Na^+/K^+-ATPase and leucine aminopeptidase respectively. An inwardly directed Na^+ gradient (out: 150 mM; in: 0 mM) was unable to enhance PAH uptake. When 10 µM 2-oxoglutarate was added to the inwardly directed Na^+ gradient condition, 1 min PAH uptake was stimulated more than 2-fold and a transient overshoot was observed. This suggests that in the pig, as in the rat, PAH is transported across the basolateral membrane by a mechanism indirectly coupled to Na^+, i.e. Na^+/dicarboxylate cotransport coupled to PAH/dicarboxylate exchange.

P PHY 361

PASSIVE REABSORPTION OF ^{14}C-SALICYLIC ACID IN THE RABBIT PROXIMAL TUBULE (S_2 segment)

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Micropuncture studies showed that salicylate (Sal) can undergo net reabsorption in the proximal tubule. This transport could occur by simple nonionic diffusion as well as by a carrier mediated process. To estimate the importance of nonionic diffusion we measured the Sal flux at 19°C and in the presence of 0.1 mM probenecid in the bath to abolish the active transport. Tubule perfusate containing 0.1 mM ^{14}C-Sal was buffered with MES 20 mM at three different pH, bath pH being kept constant at pH 7.4 (20 mM HEPES). Sal apparent permeability (10^{-5} cm/s) was 22.1 ± 3.5 (n=5), 8.1 ± 1.4 (n=6) and 4.4 ± 1.2 (n=4) respectively at luminal pH of 6.0, 6.5 and 7.0. When the permeability was calculated using the concentration of the nonionized Sal at each pH, it averaged 0.22 ± 0.03 cm/s and was not pH dependent. Thus, in our experimental conditions, all the reabsorptive flux can be attributed to nonionic diffusion. This high permeability, about 50-fold higher than in the cortical collecting duct, could be explained by the large area of the apical membrane of the proximal tubule.

P PHY 362

ELECTROPHYSIOLOGY ON CULTURED EPITHELIAL CELLS: TECHNIQUE AND CHARACTERIZATION OF THE TBM CELL LINE.

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Cultured cell lines present numerous advantages (homogeneity, single cell type, growth in defined medium) over whole organ or ex vivo isolated epithelium for the physiological and biochemical study of epithelial transport. We have developed a new technique allowing for simultaneous intracellular and transepithelial electrophysiological measurements in the epithelium formed by a cultured cell line grown on thin collagen membranes. This technique was applied to the TBM (toad bladder origin) cell line. The transepithelial and basolateral potential were -32 ± 3 and -70 ± 3 mV, respectively. Using the effect of amiloride, which partially blocks the apical membrane conductance, and circuit analysis, the following values were estimated for the apical and basolateral membrane conductances : 0.35±0.03 and 0.90±0.11 $mS.cm^{-2}$, respectively. By ionic substitution and addition of transport blockers a Na-selective conductance was demonstrated in the apical membrane, while a barium-sensitive K-selective conductance and an ouabain-sensitive electrogenic Na-K-pump were shown to be present in the basolateral membrane. The TBM cell line appears to be a convenient model to study the regulation of membrane ion transport.

P PHY 363

INTRODUCTION OF EPITHELIAL Na CHANNELS IN OOCYTES OF Xenopus laevis BY INJECTION OF mRNA FROM A6 CELLS

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RNA was extracted from cultured epithelial cells (A6 grown on plastic culture dishes) and enriched in poly A^+ RNA by Oligo-dT chromatography. Amiloride-sensitive conductance (G_A) and current (I_A) were measured under voltage-clamp conditions to assay for Na channel activity. G_A was not detectable in oocytes not injected with RNA, but was measurable at increasing levels (30 to 1000 nS) with increasing quantities of RNA injected (3 to 80 ng) and increasing times after injection (2 to 4 days). G_A was observed with Na^+ or Li^+ in the bath but not with K^+ or $NMDG^+$. Current-voltage analysis of I_A showed Goldman-type rectification with reversal potentials at membrane voltages (Vm) more positive than +60mV. I_A measured at Vm=-100mV was a saturable function of extracellular Na; half-maximal current was obtained at ~ 10mM. At Vm=-100mV, I_A was inhibited by amiloride with an apparent K_I of 100nM. Conductances with properties expected of epithelial Na channels can be induced in Xenopus oocytes by injection of mRNA from epithelial cells.

P PHY 364

THE RESPIRATORY SYSTEM AS AN ENDURANCE LIMITING FACTOR

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Respiration is not considered a factor limiting exercise capacity, because maximal voluntary ventilation exceeds ventilation during maximal exercise. This argumentation ignores the duration of maximal voluntary ventilation (20 s) and of maximal exercise (20 min). To reconsider the role of the respiratory system as a exercise limiting factor, we measured submaximal endurance capacity on a bicycle ergometer in 4 sedentary subjects before and after a training period of 4 weeks during which the subjects breathed daily about 90 l/min for half an hour but remained physically inactive. Throughout the respiratory training CO_2 was added during inspiration to keep isocapnic conditions. Whereas a work load of 129 ± 51 Watt was tolerated for 26 min 51 s ± 5 min 52 s before the respiratory training, the same work load could be sustained for 40 min 14 s ± 9 min 10 s thereafter. The corresponding increase in lactate amounted to 5.4 ± 0.3 before and 2.9 ± 1.0 mmol/l after. Therefore we conclude, that respiratory muscle training can considerably increase endurance and that respiration has to be taken into serious consideration discussing exercise limiting factors.

(Supported by Hartmann-Müller foundation)

P PHY 365

ANALYSIS OF COORDINATION BETWEEN BREATHING AND RUNNING RHYTHM

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The degree and type of coordination between breathing and leg movement were investigated in 40 untrained volunteers during running on a treadmill at two work loads (60% and 80% of subject's work capacity 170) and compared with the degree of coordination during cycling. Both work loads were applied in three different modes, i.e., running 1) with arm movements, 2) without arm movements, and 3) with paced breathing controlled by an acoustical signal which was triggered by the leg movement. The sequence of these runs was randomized. Respiratory parameters and leg movements were continuously recorded and on-line evaluated. From the results we conclude, that (a) there are three different patterns of coordination between breathing and running rhythm, (b) this coordination can partially be increased by controlled breathing, (c) the degree of coordination between leg movement and breathing is higher during running than during cycling, and (d) the difference between running and cycling is unlikely due to failing arm movement during cycling since the degree of coordination during running with and without arm movement was the same.

P PHY 366

A FACTOR DETERMINING RED CELL VOLUME : RESISTANCE TO HYPERTONIC ENVIRONMENT

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To answer the question whether red cell volume is determined by some physiological constraint we tested the hypothesis that the fragility of erythrocytes bathing in hypertonic solutions is a function of their volume. Mammalian red cells of 6 species (25 to 95 μm^3) were exposed to 10 hypertonic NaCl solutions of increasing strength. After 24 hours at 5°C the amount of hemoglobin in solution was measured. By interpolation we calculated the osmolality y (in osmol) that yielded a threshold concentration of 1.2 g/L of free hemoglobin. This concentration showed a negative linear relation with red cell volume x (in μm^3). The corresponding equation was y= -0.036x + 4.92. The same type of relation could be found between maximal urine osmolality and red cell size (y = -0.034x + 4.56). Since the maximal urine osmolality corresponds to the highest plasma osmolalities to which a red cell is exposed as it passes through the renal medulla, we can speculate that a link between red cell volume

P PHY 367

PATTERNED GROWTH OF NEONATAL RAT HEART CELLS IN CULTURE

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Analysis of impulse propagation in syncytial myocardium is limited by technical difficulties to recognize the exact branching patterns of myocardial cells at a microscopic level, and by the three-dimensional arrangement of the myocytes. We developed a new technique which allows one to define a two-dimensional arrangement of cardiac cells in culture. The method used is derived from microelectronic and photolithographic techniques. Plastic coverslips are coated with a photoresist, which inhibits the attachment of cells without exerting a toxic effect as judged from morphological criteria. After coating, the coverslips are exposed to a high resolution film which carries the desired patterns. Subsequently, they are developed and etched. Conventional culture techniques are used to prepare and seed cells on this substrate which now possesses two different adhesive properties. The method allows the fabrication of two-dimensional patterns for cell growth ranging from single cable-like structures to networks with complex branching patterns.

P PHY 368

INDOMETHACIN INHIBITS THE TONIC PHASE OF ACETYLCHOLINE CONTRACTION OF CORONARY ARTERY: ROLE OF DIACYLGLYCEROL LIPASE.

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Acetylcholine (ACh) receptors of pig coronary smooth muscle are coupled through a G protein complex to a phospholipase C that hydrolyzes phosphatidylinositol 4,5-bisphosphate and leads to the formation of inositol 1,4,5-trisphosphate (1,4,5 IP_3) and diacylglycerol. 1,4,5 IP_3 releases Ca^{++} from the sarcoplasmic reticulum and causes a rapid Ca^{++} dependent phasic contraction which is followed by a lower tonic contraction, characterized by a low cytosolic Ca^{++}. We observed on a strip of artery that indomethacin inhibited the tonic contraction. We investigated the mechanism of action of this cyclooxygenase inhibitor. The effect of indomethacin implicates that prostaglandins (PG) participated to this contraction. PGE_2 and $PGF_{2\alpha}$ indeed caused a tonic contraction and did not influence the cytosolic Ca^{++} shown by the intracellular probe Fura 2. The specific inhibitor of diacylglycerol lipase (RG 80267) diminished the tonic contraction. We concluded that this contraction was partly activated by the generation of arachidonic acid through the phospholipase C-diacylglycerol lipase pathway. Indomethacin thus interfers at the level of this particular arachidonic acid cascade.

P PHY 369

SERUM TRANSIENTLY INCREASES THE BEAT RATE OF CULTURED NEONATAL RAT HEART CELLS

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In regularly beating cultured heart cells every exchange of medium containing 5% serum led to a transient increase (+50%) in beat rate with a peak response 2-3 hrs after the exchange and a subsequent decay over 24 hrs to control values. Omitting serum from the exchange medium transiently decreased rather than increased the beat rate. Freshly drawn human plasma and serum, neonatal calf serum and horse serum all had similar effects. The dose-response relationship for this effect showed saturation at serum concentrations > 10% and was not influenced by small shifts of pH and osmolality induced by the addition of serum to the medium. The molecular weight of the stimulating component(s) was > 10'000. The effect was abolished after boiling the serum for 10 min or by the presence of 17 µM Cycloheximide (inhibition of protein synthesis) in the medium. Neither propranolol nor phentolamine (10^{-5}M) had any influence on the increase in beat rate.

P PHY 370

EFFECT OF COORDINATION BETWEEN BREATHING AND RUNNING RHYTHM ON OXYGEN UPTAKE

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The effect of coordination between breathing and running rhythm on oxygen uptake ($\dot{V}O_2$) was investigated in 20 untrained volunteers running on a treadmill at work loads corresponding to 60% and 80% of their work capacity 170. Both work loads were repeated in different runs at random order with three modifications: 1) with arm movements, 2) without arm movements, and 3) with paced breathing controlled by an acoustical signal triggered by leg movement. $\dot{V}O_2$ as well as other respiratory parameters, heart rate and leg movements were continuously recorded and on-line evaluated. Comparisons between the different runs revealed a general tendency to $\dot{V}O_2$-reduction with increasing degree of coordination. This tendency was more pronounced at 60% than in 80% of work capacity. In addition, in 20 subjects 28 single runs were found in which two phases largely differing in the degree of coordination occurred. During the phases with better coordination, $\dot{V}O_2$ was significantly lower. From these results we conclude that an optimal coordination between breathing and leg movement can reduce $\dot{V}O_2$ during running. However, in situations with high efficiency, as e.g. at the higher work load in the present experiments, $\dot{V}O_2$ cannot be further reduced by an increased degree of coordination.

P PHY 371

ON THE INFLUENCE OF BDM ON CONTRACTILE PROPERTIES OF SINGLE FROG SKELETAL MUSCLE FIBRES

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2,3-Butanedionemonoxime (BDM) depresses tension production in smooth, cardiac and skeletal muscles. The mechanism of action at the subcellular level is still controversial. Tension development in single twitches, tetanus and double stimulation experiments was studied in presence of 0 to 20 mM BDM at sarcomere lengths (SL) from 2.5 to 3.3µm. BDM reduces single twitch, tetanus amplitude and tension build-up in a dose dependent manner. The relative reduction in amplitude is larger in single twitches than in tetani. At concentrations >15 mM intermittent twitch failure is observed after a series of stimulations at 1/s. At 20 mM only tetanic stimulation elicits tension. Records of tetani (duration .3 s) show that the fibre is not able to hold tension during the whole stimulation period at BDM concentrations higher than 7.5 mM. The results are consistent with the assumption that BDM acts on the level of the contractile proteins and that there is additional influence either on excitability or calcium release at concentrations >5 mM.

P PHY 372

INFLUENCE OF BDM ON LATENCY RELAXATION OF SINGLE FROG SKELETAL MUSCLE FIBRES

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Butanedionemonoxime (BDM) is a substance which reduces tension development in muscles, supposedly mainly by interfering with the contractile proteins. To further check this hypothesis it's effect on latency relaxation (LR) was studied at different (2.5 to 3.3µm) sarcomere lengths (SL). LR amplitude (A_{LR}) and the three temporal parameters begin (t_1), time to peak (t_2) and time to end of LR (t_3) were measured. The bell-shaped correlation between SL and A_{LR} is preserved up to 10mM BDM with a maximum at ~2.9 µm. t_1 remained unchanged, t_2 and t_3 increased up to 10 mM BDM. At BDM concentrations above 10 mM a significant increase of t_1 and a depression of A_{LR} occurred suggesting an additional effect of the drug. Considering LR as the addition of two independent processes as proposed by Sandow (1944), "relaxation tension" and "contraction tension", the above results are consistent with the idea that BDM up to 10 mM mainly acts on contraction tension, while at higher concentrations an additional effect on relaxation tension (probably related to the process of calcium release) must be considered.

P PHY 374

DOES HYPOXIA INFLUENCE THE RESPIRATORY RESPONSE TO INDUCED BRONCHOSPASM ?

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In order to investigate whether hypoxia modulates the respiratory response to acute bronchospasm, we compared mouth occlusion pressure ($P_{0.1}$), tidal volume (V_T), respiratory frequency (f) and inspiratory time (T_I) obtained in normoxia (N) and in hypoxia (H, FIO2=.13-.14) in response to methylcholine inhalation (M) in 11 normal subjects. As compared to control conditions (C), the percent decrease in FEV_1 (±SEM) after M was the same in N [17.8(2.1)] and in H [19.0(2.4)]. Mean results (±SEM) were:

	C(N)	M(N)	C(H)	M(H)
SaO2 (%)	96.6 (.5)	94.0 (.8)	83.8 (.4)	83.7 (.3)
PO.1 (mmH20)	14.4 (1.9)	* 24.7 (2.5)	15.3 (2.3)	* 25.9 (3.2)
VT (ml)	782 (41)	762 (39)	863 (33)	* 785 (23)
f (min-1)	15.6 (1.0)	16.0 (1.0)	15.2 (.8)	16.0 (.6)
TI (sec)	1.64 (.15)	1.64 (.11)	1.65 (.12)	1.57 (.08)

* different from C (P<0.05)

Only V_T was not the same in C(N) and C(H). After M, the same increase in $P_{0.1}$ was observed in N and H, while f and T_I remained unchanged in both conditions. M induced a decrease in V_T in H only.
These results suggest that mild hypoxia does not modulate respiratory drive ($P_{0.1}$) and breathing pattern (f,T_I) in induced bronchospasm.

P PHY 375

INCREASE IN CARBONDIOXIDE PRESSURE PROMOTES CELLULAR POTASSIUM LOSS DURING EARLY MYOCARDIAL ISCHEMIA.

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This study was undertaken to better understand the mechanisms underlying extracellular potassium ($[K^+]_o$) accumulation during ischemia in ventricular myocardium. The experimental preparation consisted of an arterially perfused isolated right ventricular papillary muscle suspended in a H_2O-saturated atmosphere. During myocardial ischemia miniature ion-selective electrodes were utilized to measure $[K^+]_o$ and extra- cellular pH (pH_e) in 15 preparations under three different experimental conditions. At the onset of ischemia the atmosphere of the recording chamber was made anoxic; however, the pCO_2 was altered in one of three different ways. Condition 1: The pCO2 of the atmosphere was held constant at 55mmHg (n=6). Condition 2: The atmosphere was increased at a constant rate beginning after 4-5min of ischemia from 55 to 315mmHg (n=6). Condition 3: The pCO2 of the atmosphere was abruptly increased from 55 to 315mmHg after 5min of ischemia.

Results: Values obtained after 10min of ischemia.	$[K^+]_o$ mM	pH_e units
Condition 1 (pCO2 =55mmHg)	7.6 ± 0.8	6.6±0.2
Condition 2 (pCO2 =315mmHg, slowly increased)	11.5 ± 0.9	6.7±0.2
Condition 3 (pCO2 =315mmHg, abruptly increased)	8.8 ± 1.2	6.5±0.1

Conclusion: The results show that the magnitude of the extracellular potassium accumulation in ventricular myocardium during ischemia is dependent on the extracellular pCO_2 but not the pH_e. A hypothesis which relates the findings are related to differences in buffer capacity of the extracellular space. The results are strongly supportive for a direct relationship between lactate efflux and cellular K^+ loss.

P PHY 376

ATRIAL NATRIURETIC PEPTIDE (α-hANP) AMELIORATES HYPOXAEMIA-INDUCED RENAL INSUFFICIENCY IN ANESTHETIZED ADULT RABBITS.

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Hypoxaemia promotes renal vasoconstriction, with a concommitant decrease in diuresis, glomerular filtration rate (GFR) and renal blood flow (RBF) in adult rabbits. Taking into account the diuretic, natriuretic and vasodilating properties of ANP, a beneficial effect of this hormone could be expected in hypoxemia-induced renal insufficiency. Renal functions of the right control kidney and left saline-infused kidney were separately assessed in 7 anesthetized and mechanically-ventilated adult rabbits. Inulin and PAH clearances were used as indices of GFR and RBF, respectively. The functions of the right and left kidney were similar during a 20 min control (PaO_2=131±12mm Hg) period. The subsequent 20 min hypoxic period (PaO_2=30±1mm Hg) was associated with the following changes in the right and left kidneys: diuresis (-38±5% and -36±6%), GFR (-25±5% and -29±6%), RBF (-16±9% and -29±7%) and an increase in renal vascular resistance (RVR) (+28±16% and +59±30%). ANP infusion (100ng/min) during the third subsequent 20 min hypoxic period (PaO_2=30±2mm Hg) was associated with a marked improvement in diuresis (+52±11%), GFR (+6±12%), RBF (-17±6%) and RVR (+18±13%) in the left ANP-infused kidney, whereas the right control kidney remained impaired. The beneficial effect of ANP in renal insufficiency remained to be demonstrated in clinical conditions.

P PHY 377

PROTECTION BY ANTIOXIDANTS AGAINST AN O_2- AND Pi-DEPENDENT DAMAGE OF Ca^{2+} -LOADED RAT LIVER MITOCHONDRIA

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Rat liver mitochondria loaded with small amounts of Ca^{2+} were incubated under conditions resembling those present in the cytosol of O_2-deprived tissue and submitted to O_2 lack and repletion. At high Pi levels (4-6 mM) in the presence of NAD^+-linked substrates, acetoacetate addition promoted Ca^{2+} retention under anoxia but elicited Ca^{2+} release from mitochondria at reoxygenation. Ca^{2+} retention under anoxia was paralleled by succinate production from 2-oxoglutarate and presumably supported by ATP produced by substrate-level phosphorylation at the succinylthiokinase step. Ca^{2+} release at reoxygenation was opposed by α -tocopherol, next to an initial activation, and by other antioxidants (retinol, ubiquinone). When triggered by Pi under aerobic conditions. Ca^{2+} release from mitochondria was also antioxidant-sensitive. Similar data were obtained at higher Ca^{2+} loads in the absence of acetoacetate. Our data indicate that the rise of cytosolic Pi known to occur in O_2-deprived tissue predisposes mitochondria to damage by Ca^{2+} entering the cell at reoxygenation. Damage involves generation of active O_2 species by mitochondria, membrane peroxidation injury and failure to resume oxidative phosphorylation.

P PLA 380

ELEMENTAL SULFUR (S^o) METABOLIZATION INTO SULFUR AMINO ACIDS AND GLUTATHIONE, FROM BACTERIA TO MAMMALIAN CELLS.

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S^o at non toxic levels ($\leq$10μM) was metabolized into the sulfur amino acids cystein, methionin and glutathione by bacteria (E. coli, Bacillus subtilis), fungi (Phomopsis viticola, Neurospora crassa, Aspergillus nidulans, Allomyces arbuscula, Physarum polycephalum), yeast (Saccharomyces cerevisiae), algae (Chlorella vulgaris, Scenedesmus communis), higher plants (spinach, beet). Mammalian cell lines (3T3, HeLa) were also able to metabolize S^o (1μM) into cystein and glutathione, but not into methionin. Incorporation studies were performed with radiolabelled (^{35}S)-S^o. The free and protein amino acids were purified by column- and thin layer chromatograhies.

During the first minutes of germination of P. viticola spores, the S^o was metabolized essentially into cystein and glutathione. The methionin synthesis occurs after 1 hour of incubation. The azide (2mM), inhibitor of the mitochondrial respiratory chain, strongly inhibited the S^o incorporation. In contrast, the uncoupler 2,4-dinitrophenol (15μM) and sulfates (1mM) did not affect the synthesis of sulfur amino acids and glutathione from S^o.

Plant Physiology (P PLA)

P PLA 378

A SINGLE ASSAY FOR MEASURING SEVERAL DIFFERENT LIPASE ACTIVITIES

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We have developed a simple, continuous spectrophotometric assay which is applicable to almost any type of lipolytic enzymes. It is based on the metachromatic properties of various dyes (e.g. safranine), and makes use of the fact that a suitable combination of a given lipase and one of its substrates can lead to a change in the net negative charge at the lipid/water interface, which can be monitored as an absorbance change. The main advantages of this assay are (1) high sensitivity (lipase amounts can be detected in the picomole range or even lower); (2) low cost (it does not require any radiolabelled lipids or lipid analogues); (3) rapidity (determination of initial rates can be achieved in about 1 min). In this respect, this assay will find its main use in monitoring column effluents during lipase purification steps. Examples will be provided to show how phospholipase A2, triacylglycerol lipase, phospholipase D, phospholipase C (either general or phosphatidylinositol-specific) activities can be detected.

P PLA 379

STABILITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN EXTRACTS FROM WHEAT SEEDS: EFFECTS OF SOLUTES

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As reported earlier, the inactivation of glucose-6-phosphate dehydrogenase in extracts from ungerminated wheat seeds is accelerated by the addition of extract from germinated seeds or of endopeptidases. Under such conditions the enzyme is stabilized by some solutes (e. g. inorganic phosphate or NADP). At pH 7.5 sulfate protected the enzyme in the same concentration range as phosphate (2 mM or higher). At pH 5.4, phosphate was less effective than sulfate, which may depend on the loss of negative charge. Much more effective than any single compound was the combined application of NADP (0.1 mM or higher) and phosphate. The stabilization of the enzyme could depend on the occupation of different substrate binding sites. In a model experiment with purified glucose-6-phosphate dehydrogenase from yeast and purified endopeptidases, the degradation of the intact enzyme subunit was delayed in presence of NADP, phosphate or - most effectively - their combination (SDS-PAGE). In this case protection of the enzyme activity by solutes was associated with reduced proteolysis of the subunit.

P PLA 381

REGULATORY EFFECTS ON SULFATE ASSIMILATION OF ZEA MAYS BY HERBICIDE ANTIDOTES

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Effects of the herbicide antidotes, R25788 and CGA154281 on ATP-Sulfurylase and adenosine 5'-phosphosulfate sulfotransferase (APSSTase) were analyzed in roots and leaves of maize seedlings. R25788 is known to enhance the glutathione (GSH) level in maize, permitting the plant to detoxify herbicides by conjugation. The increase in GSH suggested an increase in sulfate reduction. 1 mM R25788 applied to the roots increased the activity of APSSTase about 30fold compared to the control. CGA154282 caused a 10fold increase when applied at a concentration of 10 μM. In leaves the same enzyme increased 2fold. Under the same conditions ATP-Sulfurylase showed a 2fold increase in the roots with both herbicide antidotes. GSH and cysteine content were increased 4- and 3fold, respectively. This suggests that there was no end product control by cysteine in maize in the presence of both antidotes. Herbicide antidotes seem to affect sulfate assimilation as well as the glutathione pathway. The highest effect was detected with APSSTase suggesting a key function of this enzyme in assimilatory sulfate reduction.

P PLA 382

EFFECT OF CADMIUM ON ADENOSINE 5'-PHOSPHOSULFATE SULFOTRANSFERASE FORM ISOLATED PEA ROOTS

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Isolated pea roots cultivated in the presence of Cadmium produced phytochelatins and contained increased levels of adenosine 5'-phosphosulfate sulfotransferase (APSSTase). Since these roots also synthesized increased levels of ethylene, we hypothesized that this plant hormone could be involved in the cadmium effect. Indeed, ethrel, a substance producing ethylene, caused an increase in APSSTase activity. The high levels of ethylene induced by cadmium could be decreased by L-α-(2-aminoethoxyvinyl)-glycine (AVG). Since an increased activity of APSSTase was still detected under these conditions, it seems very unlikely that ethylene is involved in the mechanism causing an increase in this enzyme activity and in phytochelatin synthesis.

P PLA 383

EFFECT OF SODIUM CHLORIDE, OSMOTIC AND WATER STRESSES ON THE IONIC AND SUGAR CONTENTS OF CARROT AND TOMATO CELL SUSPENSIONS.

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The stress induced by salinity has chemical, osmotic and waterstress components. The osmotic component can be studied separatly using mannitol and the water stress by externally applying a solution of a high molecular polyetylene glycol. The results obtained with carrot, a salt sensitive plant where compared with those of tomato, a semi tolerant plant. In carrot, sodium competes with potassium and calcium; chloride competes with nitrate and phosphate, in the uptake. In plant cells of both species, the sugar contents are elevated by all stresses. Under NaCl stress the organic acids are lowered only in carrots, this could indicate a metabolic block and partly explain that this plant is less tolerant than tomato.

P PLA 384

ISOLATION AND ANALYSIS OF PLANT GENES CONTAINING STRETCHES OF SINGLE REPEATING AMINOACIDS

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Oligonucleotide probes were made to isolate plant sequences coding for proteins which contain poly Gln and more general stretches of the same repeated aminoacid. A cDNA bank made from young seedlings of Arabidopsis was screened with these probes and 15 cDNA clones were isolated. By comparison with Drosophila it is hoped that such sequences may be present in domains of proteins involved in gene regulation. Sequence analysis has shown that these clones can be grouped into 10 groups. All contain repeated DNA sequences, some of which are capable of coding for poly amino acid stretches of up to 24 residues. Analysis of the expression and possible function of these genes is in progress.

P PLA 385

THIOREDOXINS IN EUGLENA GRACILIS

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Thioredoxins are low molecular weight hydrogen carrier proteins present in multiple forms in many different organisms and implicated in numerous redox reactions. In photosynthetic cells, thioredoxins exist in the cytoplasm and in chloroplasts. Chloroplast thioredoxins are implicated in the light-dependent regulation of selected enzymes and two forms can be distinguished by their specific target enzymes. In order to approach the problem of multiple forms of thioredoxins and to evaluate their importance in vivo we began a protein and genomic characterization of thioredoxins in the protista Euglena gracilis, capable of an autotrophic and heterotrophic form of life. - Thioredoxin purification by FPLC from mixotrophic cultures showed the presence of two thioredoxin activities that differ largely in their charge as evidenced by their behaviour in ionic exchange chromatography. Both thioredoxins showed nearly the same molecular weight of 12'000, characteristic of thioredoxins from other organisms and both are able to activate NADP-malate dehydrogenase of higher plants, but not fructose 1,6-bisphosphatase.

P PLA 386

THE CULTURE OF COTTON OVULE CALLUS CELLS IN ORDER TO STUDY THE FORMATION OF SECONDARY CELL WALL CELLULOSE

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When ovules from young cotton fruits are cultured *in vitro*, in a defined culture medium containing an auxin and gibberellic acid as plant growth substances, the epidermis cells differentiate to give cellulose-rich cotton fibres which resemble those grown *in vivo*. If gibberellic acid and ethylene are present in such a culture medium, callus formation is induced instead of fibres. The cells of this callus tissue are long and resemble young fibre cells. Suspension cultures of the callus tissue were initiated with the aim of studying their eventual further differentiation to fibre cells. The cells were analysed for cellulose formation by polarisation microscopy and for vitality. Many different factors, which could influence the differentiation process (elongation and cellulose deposition), were varied. The cell wall composition was examined by GLC, but no conditions were found where cellulose synthesis was markedly increased. The composition was always typical of that of a dicotyledonous primary wall. The influence and role of ethylene during callus formation was studied and the development of the callus was followed by light microscopy.

P PLA 387

PHOTOACTIVATION OF GRAVITROPISM IN GROWING AND NON-GROWING MAIZE ROOTS

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Maize roots (var. LG 11) require light and gravity for positive gravitropism which is expressed by asymmetrical growth in the elongation zone of the root. In order to characterize the different steps of gravitropism in a better way, root growth was stopped in some cases by a cold treatment during the photoactivation and restored to allow gravireaction.
When one minute of white light was applied directly or 3 hours before gravipresentation, the non growing roots (5°C) and the control (without cold treatment) exhibited the same gravireaction.When light was applied at 20°C, the reactivity of the root to gravity decreased, as time before gravipresentation was increased. This reactivity was lost almost totally 8 hours after light application. Most of on growing roots (5°C) were still reactive, even 96 hours after photoactivation when they were gravipresented at 20°C, their growth being then restored at the same level.
These results suggest that the photoinduction is independant from metabolic expression of gravitropism. These experiments indicate also that the cold treatment could be a great tool for sensing system studies.

P PLA 388

NUCLEAR DNA CONTENT OF MAIZE ROOT CELLS

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Changes in the nuclear DNA content of plants is a controversial subject. Correlations between the DNA level and specific physiological events have been postulated. However, these results are often considered to be artefacts or due to randomly disturbed cell cycles. The aim of the present report was to measure the DNA of Feulgen stained nuclei of the maize root (histological sections), using a scanning microdensitometer. Large changes in DNA content were detected in some tissues, especially in differentiating vascular cells. One question is still open: are the observed changes in DNA level a signal for cell differentiation or rather a consequence of it?

P PLA 389

SOLUBILISATION AND PROPERTIES OF THE NPA BINDING SITE PREPARED FROM MAIZE ROOTS

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The efflux of IAA (indol-3yl-acetic acid) from the cell is mediated by a carrier which can be blocked by NPA (N-1-naphthylphthalamic acid). A study of the binding of (^{3}H)NPA to a membrane fraction (5000g supernatant; 48000g pellet) prepared from maize roots is described. Comparing such preparations from different parts of the root, binding activity was found to be preferentially localised in the elongating zone. When membranes were fractionated on sucrose density gradients, (^{3}H) NPA binding coincided with plasmalemma markers. Binding sites were solubilised from the total membrane preparation and from plasmalemma-enriched fractions using Triton X-100 or Nonidet NP40 and solubilised activity was assayed using polyethyleneimine (PEI) treated filters. Similar results were obtained with Sephadex PD10 columns; however, the advantage of the PEI method is its rapidity. The characteristics of (^{3}H)NPA binding to the 5000-48000g fraction and to the solubilised site are compared.

P PLA 390

ADVENTITIOUS ROOTING IN CUTTINGS OF *POPULUS TREMULA*: A COMPARISON OF THE EFFECTS OF EXOGENOUS IAA AND IBA

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Cuttings of trembling aspen (*Populus tremula* L.) are of the hard-to-root type. Adventitious rooting of green cuttings is stimulated by exogenous auxins applied at the stem base. It has been shown that IBA (indolylbutyric acid) is already active at 5 µM, whereas 25 µM IAA (indolylacetic acid) is necessary to obtain the same effect. In general up to 500 µM IBA, IBA is about 5x more effective than IAA. In the upper internode segments of stems treated with IBA at the base, basipetal transport (agar block technique) of exogenous IAA is more rapid so that the better stimulation may be due to increased basipetal transport of endogenous IAA. IAA applied to the base of the cuttings is conjugated to form IAAsp which is then rapidly (in 8-10 h) metabolised to oxIAAsp. Pulse chase experiments with ^{14}C-IAA, applied to petioles, show that oxIAAsp is already formed after 2h and that metabolism to oxIAAsp in leaves as well as in the stem is quasi immediate. The oxIAAsp is then metabolised to give an unknown low-molecular weight substance which is then transformed to give a stable metabolite. Exogenous IBA, applied to the stem base, is also conjugated, but the metabolic pathway appears different to that of IAA.

P PLA 391

Callus induction and morphogenesis in Portulaca grandiflora (PGJf).

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We have tested the effect of various auxins and cytokinins combinatins on Portulaca grandiflora (PGJf). 2,4 dihydrophenoxyaceticacid (2,4D) associated to 6-furfurylamino-purin (kinetin) at concentrations ranging from 0,5 mg/l to 1 mg/l, induce the formation of white and friable callusses, while naphtalaceticacid (NAA) and 6-benzylamino-purin (BAP) produce compact green callusses. Kinetin or BAP alone induce buds formation at a high percentage (+60%) on leaf and stem explants. Roots formation occure in stem and root explants when 2,4D or NAA alone are used. Buds formation in callus hapened with the use of a high concentration (> 2mg/l), when those buds, at two leafs stage, are transfered on free-hormone medium, roots are formed.

P PLA 392

GENETIC OF DOUBLE RESISTANCE IN TRANSFORMED TOBACCO PLANTS

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In 1984 Paszkowski et al. demonstrated the feasibility of direct gene transfer to plants; many laboratory have since applied this method to various plants. We did so and are rising in this work the question of the segregation behaviour of transfered genes. Starting with haploid protoplasts of Nicotiana tabacum ssp. atropurpurea transformed with kanamycin (KM) or hygromycin (HG) resistance plasmids, we regenerated plants on selective medium. Part of them were sterile haploids and the other were fertile diploids. For further experiments we selected two KM and one HG diploid plants. Using specific crosses (backcross, selfcross, intraspecific cross) we hope to point out to the following problem: inserting a foreign gene on a plant chromosome create a new locus, could this be source of problems during chromosome duplication and pairing? Our diploid transformed regenerants TORO are homozygous for resistance gene. Crossing KM resistant with HG resistant plants give double resistant TOR1 heterozygotes KM - / HG -. We are now testing self and backcrosses from this lines and will present results of this experiments.

P PLA 393

UNDERSTANDING THE INTERACTION OF GROUNDNUT PLANTS WITH THE PATHOGEN *CERCOSPORA ARACHIDICOLA*.

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Cercospora arachidicola is an important pathogen of groundnut plants (*Arachis hypogaea*) causing the early leaf spot disease. The symptoms appear about 2 weeks after inoculation and there is no apparent hypersensitive reaction. Interactions between all known cultivars are compatible with all known races of the pathogen. Typical defense mechanisms, e.g. the synthesis of stilbene type phytoalexins and PR-proteins have been recognised, but the exact nature of the elicitors of such reactions is unknown. The cell walls of the pathogen contain, *inter alia*, a (1→3)-β-linked glucan and the primary cell walls of the plant are rich in galacturonan, which are possible sources of carbohydrate elicitors. When the pathogen is cultured in the presence of groundnut cell walls, (1→3)-β-glucanase activity can be detected. *Cercospora beticola* causes a similar leaf spot disease in sugar beet plants, but is not a groundnut pathogen. Comparative studies are now being carried out by infecting suspension cultures of groundnut cells with *C. arachidicola and C. beticola* in order to discover the defense mechanisms involved with the aim of increasing disease resistance in groundnut plants.

P PLA 394

INDUCTION OF SECONDARY METABOLISM BY BIOTIC ELICITORS IN COMPACT GLOBULAR STRUCTURES (CGS) CULTURES OF CINCHONA ROBUSTA

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From a non-producing fine cell suspension culture of *Cinchona robusta*, a CGS culture was obtained. This culture contained indole and quinoline alkaloids (100-200 µg/g DW) among them quinine and quinidine. Moreover, this culture was found to produce high levels of anthraquinones (until 6% DW) after elicitation with a crude homogenate of the fungus *Phytophthora cinnamomi*. Most of them were recovered from the medium, 1-hydroxy-2-hydroxymethylanthraquinone being the major compound (> 50%). The competition between the two pathways which share common precursors will be discussed.

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