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ANATOMY, HISTOLOGY, EMBRYOLOGY

Monoclonal antibodies against a juxtaglomerular granular cell tumor of a human kidney

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The juxtaglomerular granular cell (JEG-cell) is found at the vascular pole of the glomeruli and is strategically located to control the intrarenal hemodynamic by humoral mechanisms. The isolation of the JEG-cells and their biochemical characterization are hampered by their rarity in the normal kidney. We therefore used a renin-secreting human JEG-cells tumor to produce a library of monoclonal antibodies (McAB's). Most antibodies recognize granule or cytoplasmic components of the JEG-cells on cryostat sections of the rhesus monkey and human kidneys. Three antibodies react also with a granule antigen of mast cells. Only one out of thirty McAB's stains the JEG tumor cells in culture. These McAB's are used to characterize morphologically and biochemically the new JEG-cells molecules in order to understand their physiologic function.

Insulin increases glucose utilization in the early chick embryo

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As insulin is present in chicken eggs already before fertilization (De Pablo et al., Endocrinology *111* (1982) 1909), it could regulate carbohydrate metabolism very early in the development of the chick embryo.

Insulin effect on glucose utilization has been studied during the first 24 h of incubation. No stimulation was found at blastula stage, whereas a 40% increase was observed at gastrula and neurula stages. Both embryonic and extraembryonic regions were stimulated to the same extent. Glucose utilization was increased for concentrations as low as 1 pg/ml, whereas maximal stimulation was only obtained at about 100 ng/ml. The utilization pathways of this additional glucose consumption have been investigated. The results raise questions about the hormonal control of early embryonic development.

Dendritic reorientation in the developing isthmo-optic nucleus (ION)

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The mature ION (which projects to the retina) has its neuronal perikarya arranged in a horseshoe-shaped lamina which completely surrounds the ION neuropil; the dendrites are polarized perpendicular to the lamina, projecting into the neuropil. Golgi impregnation shows that at 10 days of incubation (E10) the dendrites usually extend ventrally or ventromedially, which for most neurons is quite different from the adult direction. At E12 (two days before the ION becomes laminated), most dendrites of neurons near the border of the ION project inwards, as in adults, but the core neurons still have ventrally polarized dendrites; the latter repolarize by about E15. Retrograde tracing experiments show that the dendrites of the 'ectopic' ION cells do not repolarize, but extend ventromedially at all ages. The changes of dendritic direction in the ION can be explained if ION dendrites attract each other during development.

Immunocytochemical distribution of the C-terminal glycopeptide of the vasopressin prohormone in rat brain

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The C-terminal glycopeptide of the vasopressin prohormone has been mapped in rat brain by immunocytochemical methods using antibodies to the sheep glycopeptide. These antibodies do not cross react with vasopressin, oxytocin or their related neurophysins.

In the hypothalamus, glycopeptide immunoreactivity was found to be identical with that of vasopressin immunoreactivity, distributed in neural perikarya, dendrites and axons. In addition, labelled fibers were consistently observed in extrahypothalamic regions known to stain for vasopressin: the frontal cortex, medial septum, amygdala, thalamic paraventricular nucleus, dorsomedian thalamus, dorsal motor nucleus of the vagus nerve and nucleus of the solitary tract. Other extrahypothalamic regions reported to contain vasopressin immunoreactivity, such as the lateral septum, substantia nigra pars compacta and locus coeruleus did not stain for the glycopeptide.

In view of the specificities of the glycopeptide antibodies used, these experiments provide an unequivocal map of the distribution of vasopressin, without interference from and distinct from that of oxytocin.

Postnatal development of the retina in the tree shrew (*Tupaia belangeri*)

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The differentiation of the retina in *Tupaia* was studied by means of light and electron microscopy on postnatal days 1, 6, 10, 14, 18 and in the adult animal. The typical layers of the retina are present already at birth. The photoreceptor cells have then only small apical protrusions, which are filled with dark mitochondria; these represent the inner segments. The light sensitive outer segments appear first as small and irregular stacks of membranes at day 10. Photoreceptor synapses are inconspicuous and rare at birth but are well developed and numerous by day 14. In contrast, synapses in the inner plexiform layer are already frequently seen at birth. Thus, the same gradient of differentiation seems to occur as in the retina of other mammals and birds, where ganglion cells mature first and photoreceptors last. An electroretinogram and a visual evoked response can be recorded already on day 16, which is 2 days before eye opening.

In vitro organogenesis from isolated hepatic cells of mouse embryo

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Hepatic cells from 15 days mouse embryos were mechanically isolated and cultivated in MEM medium supplemented with 15% calf serum and with insulin (10 µg/ml) and hydrocortisone (10 µg/ml). The cells were grown up directly on glass or on collagen-treated coverslips and filmed by time-lapse cinematography. Single-frame analysis of the films revealed the presence of histiocytic and fibroblastic moving cells. During the outgrowth of the culture these cells determine the formation in vitro of organotypic structures showing epithelial features (ducts, trabeculae).

The formative cells in embryology

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The discourse should anchor the term 'formative cells' (Gross et al., 1984) to biology. For this reason five experiments from early experimental embryology were analyzed. They furnish us with indications for the existing in the embryo of free mobile cells, which form organs by transporting other cells and differentiating them. In addition obscure results of causal embryology, chemically induced embryos in Urodele germs, e.g., can come to be intelligible by assumption of the existence of mobile inductive cells.

Direct demonstration by filming the supposed cells inside the embryo is impossible. But the cells of the amphibian blastoderm which later display inductive potencies were filmed during their changing into mobile cells and their entering the germ (IWF, 1967). Finally regarding the two movies of this meeting (Gross, 1980; Gotzos et al.) we have the very opportunity to experience whether during an organogenesis, displaced from embryo into culture, cells were found with the properties of formative cells.

Fibroblast-dependent cardiomyogenesis in vitro

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When a fibroblast, wandering in a heart cell monolayer culture, enters the sphere of influence of a myocyte, it changes its direction and heads for the myocyte. The area of the muscle cell contacted by the fibroblast darts out. The resulting protrusions, often repeatedly touched by the lamellipod of the fibroblast, develop into intercellular bridges. After contact a myocyte can also be transported to a neighboring muscle cell by a fibroblast. The accelerated initiation of spreading and the differentiation to a beating myocyte are other effects following the contact by a fibroblast. These interactions result in the development of synchronously pulsating muscle cell units.

Conclusion. For the time being no fact is known, which objects the supposition that the capabilities of fibroblasts shown in the film, do also apply to in vivo processes. In that case fibroblast activities represent the 'Vorgänge, die zwischen das Setzen irgendeines induzierenden Reizes und seine sichtbare Folge eingeschaltet sind' (Spemann, Monograph (1936) 153).

Endocytic embryonic brain cells shown to be dying neurons

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It has been reported that some, but not all, naturally dying neurons in the chick embryo's isthmo-optic nucleus (ION: source of afferents to the retina) endocytose intravascularly injected horseradish peroxidase (HRP) as they die, and that virtually all the ION neurons undergo such endocytic cell death following intraocular injection of colchicine (Clarke, Histochem. J. *16* (1984) 955). However, a trivial alternative explanation would be that the endocytosing cells were not degenerating neurons at all, but phagocytes. We can now confirm the identity of the HRP-endocytosing cells (after intraocular colchicine) as ION neurons, on three grounds. 1) They could be prelabelled by retrograde transport from the retina of Fast Blue before their death. 2) They were shown by ^{3}H-thymidine autodiagraphy to have the same 'birthdates' as ION neurons. 3) They differed morphologically at both light and electron microscopic levels from phagocytes in adjacent regions.

Plasminogen activator in mouse oocytes

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We have found that ovulated mouse oocytes contain tissue-type plasminogen-activator (t-PA), and have studied aspects of its synthesis and fate. Primary oocytes isolated from ovaries did not contain any detectable t-PA. All secondary oocytes matured in vivo or in vitro contained the enzyme. During in vitro maturation, t-PA could be detected as early as 4 h after GVBD. Induction of t-PA activity was blocked by culture of primary oocytes in presence of dBcAMP, IBMX or cycloheximide, whereas it was unaffected by actinomycin D or α-amanitin. The Ca^{++} ionophore A23187 stimulated secondary oocytes to secrete t-PA; furthermore, no t-PA could be detected in fertilized eggs denuded of their zona pellucida (ZP). These results suggest that: 1) t-PA m-RNA is present in primary oocytes; 2) enzyme production is under translational control, and is triggered upon resumption of meiotic maturation; 3) in secondary oocytes the enzyme is present in a secretory compartment, the contents of which are released upon fertilization. Possible functions for t-PA in the modifications of the ZP that follow fertilization are being investigated.

Monoclonal antibodies demonstrating GABA-like immunoreactivity

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Antisera detecting serotonin- or GABA-like immunoreactivity provide the basis for a new approach in transmitter histochemistry. Antisera are less well-defined reagents than monoclonal antibodies (MAb), for which specificity is ensured without special immunization procedures nor purification steps, but by selecting appropriate cell lines by a differential screening test. In an attempt to develop MAb against GABA, hybridomas produced by standard techniques were tested by ELISA for secretion of antibodies recognizing GABA-BSA- but not glutamate-BSA-conjugates. Specificity of MAb produced by eight selected cell lines was assessed by serial dilution experiments in ELISA using GA-treated BSA as well as various amino-acid-BSA-conjugates to test for cross-reactivity. Some cross-reactivity was found with β-alanine, less with glycine, taurine and much less with glutamate. In nervous tissue of GA-fixed rats all the MAb revealed patterns of immunoreactivity consistent with available information on the distribution of GABA-containing neurons and confirming results obtained with antisera.

Combination of the glucose oxidase method with a silver-gold intensification for demonstrating transganglionic transport of HRP in primary vagal afferents

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HRP was applied to the cut vagal nerve. After perfusion fixation according to Mesulam (J. Histochem. Cytochem. *26* (1978) 106), HRP activity was demonstrated in 70 µm vibratome slices by the cobalt-glucose oxidase method (Itoh et al., Brain Res. *175* (1979), 341), in most cases followed by a modification of the silver-gold intensification procedure of Liposits et al. (Neurosci. Lett. *31* (1982) 7). Slices were then osmicated and embedded in Epon. By using the glucose oxidase method without intensification, transganglionically transported HRP could be shown within terminals in the solitary nucleus, in semithin and ultrathin sections. The silver-gold intensification, however, led to a marked increase in the contrast of the HRP reaction product in light and electron microscopy. In the latter, even small amounts of HRP within terminals could be unequivocally detected as small, well delimited, gold loaded profiles.

By this combination and adaptation of two established methods it was possible to prove for the first time the existence of primary afferent vagal terminals within the dorsal motor nucleus of the vagus nerve.

Neural crest derivatives express myelin-associated glycoprotein (MAG) immunoreactivity during development in chicken

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Myelin-associated glycoprotein (MAG) is an integral membrane protein, which is thought to be a specific consitituent for both myelinating glia and myelin of mammalian nervous system. Light and electron microscopic immunocytochemical investigations of chicken have shown, that immunoprecipitates were associated with: class B-neurons of the dorsal root ganglion, certain cells of the cervical superior ganglion and the adrenal medulla. In B-neurons of the dorsal root ganglia immunoprecipitates were found: in the Golgi apparatus, in the endoplasmic reticulum and on the cell surface. Immunochemical techniques (immunoblots) could confirm the presence of MAG cross-reacting material in the dorsal root ganglia. The appearance of MAG in certain derivatives of neural crest could indicate a possible function of this molecule related to cell adhesion and therefore to cell membrane interactions and cell movements.

Phenotypic expression of primary sensory neurons in chicken dorsal root ganglia

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The phenotypic expression of primary sensory neurons is characterized by selected criteria. In the dorsal root ganglia (DRG), the large and pale nerve cells correspond to class A and the dark and small to class B. Within these classes, several subclasses can be distinguished by ultrastructural and cytochemical features. In the DRG of chicken, carbonic anhydrase and phosphatase acid activities failed to be correlated with a specific subclass or class of ganglion cell. In contrast, binding of ^{125}I-α-bungarotoxin is restricted to most cells of class A. The staining with anti-parvalbumine antibodies was exclusive of subclass A_2 whereas the high affinity uptake of ^{3}H-L-glutamine was characteristic of subclass A_3.
Most cells of class B reacted with anti-MAG antibodies in young chicken while antisubstance P antibodies stained only cells B_2. Since defined subclasses of sensory neurons innervate defined tissues, it is assumed that the influence exerced by the target is reflected, within certain limits, by structural and functional specialization of the neuron.

Morphometry of the lung acinus

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Paradoxical allometric relations between 0_2 consumption and pulmonary diffusing capacity suggested the existence of body size dependent P_{02} gradients within the acinus, due to varying acinar pathway length.
We therefore analyzed acinar structure on silicone rubber casts of rat (300 g) and rabbit (3 kg) by trimming off the acini at the beginning of the alveolarisation, characterizing the branching pattern and measuring on a sample length and diameters of each airway segment.
The acini of both species show a log normal volume distribution with mean 2.17 mm^3 for the rat and 3.17 mm^3 for the rabbit. Their branching pattern is nearly symmetric dichotomous with highest frequency of termination in generation 5 and 7. The mean inner and outer diameters and the length decrease with generations. The mean pathway length is proportional to the cube root of acinar volume.
We conclude that there is a size dependent difference in the length of the acinar pathway for alveolar ventilation. These morphometric data are used in computer calculations of P_{02} distribution along the acinar pathway in animals of different size.

Classes of neurons in the dorsal lateral geniculate nucleus of the tree shrew, *Tupaia belangeri*

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Light microscopic studies of relay neurons in the dorsal lateral geniculate nucleus of the tree shrew reveal three classes based on their dendritic spread in laminar and interlaminar regions: 1) Unilaminar neurons with multipolar radiate, bitufted and intermediate types of dendrites. Cell bodies as well as dendrites of these neurons are confined to one lamina. 2) Multilaminar neurons with multipolar radiate, bitufted and intermediate types of dendrites. These neurons send their dendrites to adjacent interlaminar zones and to other laminae. 3) Interlaminar neurons whose cell bodies and dendritic arbors are confined to an interlaminar zone. We have shown by retrograde transport of HRP injected into the visual cortex that these neurons are in fact relay neurons. In addition to relay neurons there are small interneurons with 'axoniform' dendrites and a clear unmyelinated axon whose arborization is confined within the limits of the dendritic spread.

Distribution of perfusion-induced edema in the rat area postrema correlates with angioarchitectonic findings

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In the area postrema (AP) of the rat the arterioles are located at its caudal tip and at the ventrolateral borders in its middle portion, whereas no arterioles are found in its largest cranial part. This distribution correlates with that of perfusion-induced edema: edema is absent from the parts containing arterioles and present in the remainder of the AP, where only capillaries are located. The venous drainage takes place from the dorsolateral border of the AP.
The earlier hypothesis – ascribing the distribution of edema to extravasation from arterial limbs of capillaries – seems unlikely on the basis of these findings. Instead, they suggest that extravasation takes place in all parts of capillaries in the AP but not in its arterioles.

Oxytocinergic-like cells and fibers in the nervous system of amphioxus (*Branchiostoma lanceolatum* Pallas)

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The neuroaxis and the apical vesicle of amphioxus, homologous to the embryonic prosencephalon of craniate vertebrates, were investigated with regard to occurrence and distribution of oxytocinergic cells and fibers by immunohistological techniques (Sternberger). Few small cells along the central canal and two pairs of fibers located laterally along the neuroaxis are specifically stained, in both sexes. No immunoreaction was observed in the previously so called neurosecretory regions (neuroporus and infundibular organ). On the other hand, we fall to demonstrate the presence of vasotocin and vasopressin.
If results confirm the presence of oxytocin, they seem to reject: 1) the earlier hypothesis that the hypothalamo-neurohypophyseal system of vertebrates should be presumely the homologous of

the infundibular organ and 2) the primitive fishes seem not to be in the direct lineage with amphioxus.

Influence of fixation methods on the ultrastructure of parathyroid cells in rats

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It is believed that parathyroid (PT) cells undergo cyclic changes during parathyroid hormone secretion. On the basis of the ultrastructure of PT cells various stages of the proposed secretory cycle may be distinguished. In a study using different fixation methods we found that light and dark chief cells, intermediate and syncytial cells occur only in PT glands fixed by immersion. Perfusion fixation prevented the occurrence of the diversity of PT cell morphology. Thus these findings suggest that the diverse morphology of rat PT cells rather depends on fixation than on the secretory activity.

BIOCHEMISTRY

Monoclonal antibodies to human galactosyltransferase (lactose synthetase a protein)

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Monoclonal antibodies (MAB) to soluble human milk galactosyltransferase (GT) have been elicited in mice by the hybridoma technique. Supernatants were first screened by RIA followed by ELISA to determine the titers. Of 14 RIA-positive supernatants, seven showed binding activity by ELISA with half-saturation dilutions between 1000 and 7800. Positive supernatants enhanced slightly GT activity while polyclonal antisera were always strongly inhibitory. Immunoblotting of purified GT from gels after SDS-PAGE confirmed results obtained by ELISA. In general, those antibodies that provided positive signals by ELISA and immunoblotting also specifically stained the Golgi apparatus in HeLa cells by immunofluorescence. Further cloning of three hybridomas produced supernatants that were monospecific as determined by SDS-PAGE/immunoblotting of human milk and purified GT. MAB will prove useful for the establishment of a sandwich-ELISA for serum GT.

The human plasma kallikrein-$\bar{C}l$-inhibitor complex possess an epitope which is not detectable on the parent molecules

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The inactivation of plasma kallikrein (K) by $\bar{C}l$-inhibitor (I) results in the formation of a KI complex and of a modified form of I (mI). To test whether neoantigens appear upon formation of KI, we studied the monoclonal antibodies (MAb) produced by clones resulting from the fusion of NS2 myeloma cells and spleen cells from a mouse immunized with KI. One clone (4C3) produced MAb specific to KI and it was grown as an ascites tumor. By RIA, 50% of 4C3 bound to KI while < 10% bound to either K or I. Furthermore, a mixture of K, I, KI, and mI was separated by SDS-PAGE and blotted onto nitrocellulose sheets, which were then studied for the binding of MAb 4C3, and polyclonal anti-K or anti-I. 4C3 reacted only with KI and mI, while polyclonal anti-K detected K and KI, and polyclonal anti-I detected I, mI, and KI. The reaction between K and I leads to the emergence of an epitope which is not detectabel on the parent molecules.

The tumor promoting phorbol ester TPA inhibits the α_1-adrenergic activation of phosphorylase in rat

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Various hormones and tumor promoting agents cause an influx of Na^+ and a stimulation of glycolysis in cultured cells. Previous own experiments have indicated that α_1-adrenergic stimulation activates (Na^+K^+)ATPase and glycolysis in perfused rat livers. Since there are similarities in the mechanism of action of Ca^{2+}-dependent hormones and phorbol esters, we have studied the influence of TPA (12-O-tetradecanoyl-phorbol-acetate) on the activation of glycogenphosphorylase by phenylephrine, vasopressin and angiotensin II.

The hormones activated phosphorylase twofold. TPA had no direct effect but inhibited the activation of phosphorylase by the α_1-agonist phenylephrine by approximately 80%. We conclude that TPA specifically acts on the transduction of the α_1-adrenergic signal at the level of the plasma membrane. This effect appears to occur before Ca^{2+} is released from internal calcium stores.

Vasopressin and glucagon activate pyruvate dehydrogenase and oxoglutarate dehydrogenase in rat liver mitochondria

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In perfused rat liver, vasopressin and glucagon produce an increase in the amount of active pyruvate dehydrogenase (PDHa). This action is still present in mitochondria isolated from these livers and is accompanied by an increase in total calcium content of these mitochondria. When Na is added to incubated mitochondria the effect of hormones is not present anymore and their total Ca^{2+} content is decreased. Another Ca^{2+}-sensitive enzyme, oxoglutarate dehydrogenase, shows the same changes after glucagon or vasopressin addition.

Oxidation of vitamin E in red cell membranes and in solution

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α-Tocopherol (vitamin E = E) is an important antioxidant of cell membranes. Oxidation of E by an enzymatic, radical-generating system was investigated. Aqueous solutions of E using deoxycholate (DOC) and serum-albumin (SA) as solubilizers were compared to E incorporated into red cell membranes. Results. In aqueous 0.2% DOC E is completely oxidized to four oxidation products separated on HPLC. One major oxidation-product of E is indistinguishable by HPLC from tocopherylchinon. A similar pattern of oxidation products was found when E was either bound to SA or incorporated into EC-membranes. Oxidation was strongly inhibited by the presence of SA. Protection is attributed to binding of E to SA and not to antioxidative capacity of SA-sulfhydryl-groups. E bound to red-cell mem-

branes is oxidized at a rate resembling E in aqueous solution (DOC) rather than protein-bound E (SA). This finding suggests that E in red cell membranes is an easily accessible to oxidation as in aqueous solution and seems not to be protected by binding to membrane proteins.

Effects of hypertension on cytoskeletal wall components of rat aortic smooth muscle cells (SMC)

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We have investigated the alterations of cytoskeletal elements of aortic SMC during hypertension produced in rats by a complete aortic constriction between the renal arteries. The level of the total proteins and DNA were determined according to Bradford (Analyt. Biochem. *72* (1976) 248) and Burton (Meth. Enzym. *12* (1968) B 163). Actin, vimentin, desmin and tropomyosin were evaluated as percentage of total proteins by densitometric scanning of one-dimensional SDS-PAGE. Protein and DNA concentrations of the artery wall were increased 7 and 40 days after aortic constriction as compared to controls but did not change between 7 and 40 days. Vimentin and tropomyosin were also increased at 7 and 40 days, whereas actin and desmin did never change. We conclude that: 1) changes in DNA and protein contents remain stable between 7 and 40 days after the beginning of hypertension; 2) the only change of cytoskeletal protein content during this type of hypertension concerns vimentin and tropomyosin.

Comparisons between trophoblastic pregnancy-specific β_1-glycoprotein and a similar immunoreacting protein in nonpregnant healthy women

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Pregnancy-specific β_1-glycoprotein (PSβG or SP-1), secreted by the trophoblast from very early pregnancy, can be detected in the maternal serum as early as hCG, i.e. when the embryo is in its early blastocyst stage. Measurement of this protein has been advocated as the powerful alternative to β-hCG determination in pregnancy testing since antisera to SP-1 do not cross-react with other protein hormones or metabolites. This is particularly important in the monitoring of patients treated for infertility with gonadotrophins, including in-vitro fertilization management. We measured SP-1 in the luteal phases of conceptive and nonconceptive cycles and found surprising 'false positives' in patients treated for infertility and in untreated controls. Early luteal positives, i.e. not ascribable to subclinical abortion, are compared biochemically to 'normal' SP-1. We suggest that there must be another source in addition to the trophoblast and neoplasms.

Kinetic studies on the interaction of Eglin c with human leukocyte elastase and cathepsin G

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The kinetic parameters k_a and K_i of the interaction of Eglin c, a naturally occurring proteinase inhibitor, with its target enzymes human leukocyte elastase and cathepsin G were studied in order to determine the likely efficacy of Eglin c in vivo. The association rate constant k_a was found to be comparable to that found with α_1PI-proteinase inhibitor (α_1PI) for both enzymes indicating that Eglin c will form complexes with the enzyme as rapidly as α_1PI. The inhibition constant K_i was found to be very low ($\sim 10^{-10}$ M) suggesting a reasonably stable complex but indicating a reversible reaction. The kinetic constants determined indicate that Eglin c is therapeutically potentially useful.

Kinetic studies on carbonyl reductase

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Carbonyl reductase, a monomeric, NADPH-dependent oxidoreductase, catalyzes the reduction of quinones, other ketones and aldehydes to the corresponding alcohols. Three molecular forms differing in size and charge were isolated from human brain. Double reciprocal plots of initial velocity with respect to NADPH and menadione yielded intersecting patterns indicating a sequential mechanism. No enzyme-dependent production of NADPH from $NADP^+$ was detectable in the presence of alcohol. At pH 7 the K_m values for NADPH and menadione were, respectively, 30 and 50 µM for the smallest and 2 and 15 µM for the medium enzyme form. Even lower values were estimated for the largest form. Product inhibition by $NADP^+$ was competitive with respect to NADPH and mixed relative to menadione. Alcohols such as 4-nitrobenzyl alcohol, benzyl alcohol and cyclohexanol, products of known substrates, did not inhibit the reduction of menadione. These findings are consistent with a mechanism in which NADPH binds to the enzyme before the quinone.

The formation of specific types of light-harvesting pigment-protein complexes from *Rhodopseudomonas acidophila* is regulated by the light intensity

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The smallest unit of the light-harvesting complexes of most purple nonsulphur bacteria consists of two apoproteins, designated as for e.g. *Rs. rubrum* B880-α and B880-β according to their NIR absorbance characteristics (Brunisholz et al., Hoppe Seylers Z. physiol. Chem. *365* (1984) 675 and 689). In *Rps. acidophila* the formation of distinct types of antenna complexes (different absorbance maxima) seems to be regulated primarily by the light intensity. In this context, the structural basis of the variable absorbance maxima of these antenna complexes is of great interest. The light-harvesting complexes B800–820 and B800–850 from *Rps. acidophila* 7750 and 7050 have been isolated by LDAO solubilization and purified by De-52 and Sepharose 4B chromatography. First results of these investigations indicate certain amino acid exchanges in the polypeptides of the antenna complexes with different absorbance maxima.

Correlation between macrophage microbicidal activity and the susceptibility/resistance phenotype of mice infected with *Leishmania* parasites

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Infection of man and animals with *Leishmania*, an intra-macrophage parasite, leads to a disease that is either self-healing or chronic, depending on the individual. In mice, cure or noncure is known to be genetically determined. In particular, the BALB/c genotype belongs to the nonhealer category, and the CBA genotype to the healer category. Studies of the leishmanicidal activity in vitro of macrophages (MØs) activated by lymphokines indicated that BALB/c MØs were less capable of destroying parasites (*L. major, L. enriettii*) than MØs of CBA origin. Defective killing in nonhealer MØs was linked to weaker excitation of

oxidative metabolism upon interaction with macrophage activating factor and/or LPS, as determined by the measurement of the hexose monophosphate shunt, and production of O_2^- and H_2O_2. In addition, presence of the intracellular parasites themselves appeared to alter the production and/or release of oxygen metabolites.

Human liver alcohol dehydrogenase: the catalytic differences between the two allelic variants $\beta_1\beta_1$ and $\beta_2\beta_2$-Bern are based on a single amino acid exchange

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Two allelic variant acohol dehydrogenase isoenzymes, $\beta_1\beta_1$ and $\beta_2\beta_2$-Bern, were isolated from human livers of Caucasian origin. They represent the 'typical' and 'atypical' phenotype, resp. $\beta_2\beta_2$-Bern has a higher specific activity and a lower pH-optimum, has a higher K_m for NAD^+, is less susceptible to inactivation by iodoacetate, and cannot be activated with chloride ions. In order to define the structural basis for these properties, we determined the amino-acid sequence difference between the β_1 and the β_2-Bern subunits. The structural analysis of tryptic and CNBr peptides showed that β_2-Bern differs at only one position from β_1: Arg-47 in β_1 is substituted by His-47 in β_2-Bern. This exchange alters the binding of the pyrophosphate group of the coenzyme NAD(H), and also that of iodoacetate, thus explaining the observed differences between $\beta_1\beta_1$ and $\beta_2\beta_2$-Bern.

Utilization of branched-chain amino acids by cultured rat skeletal muscle cells

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Cells of the L6 myogenic line were cultured in Dulbecco's MEM with fetal calf serum and 10 mM glucose. Cell fusion to myotubes started at around day 4. Protein and DNA content, as well as phosphocreatine kinase specific activity increased progressively. Branched-chain amino acids (BCAA) leucine, isoleucine and valine were readily consumed by the cultures with formation of the corresponding alphake to acids. If leucine was modulated from 0, 0.2, 0.8, 2.0 to 5.0 mM at day 8 with the other 2 BCAA at 0.8 mM, leucine disappearance from the media increased linearly, whereas valine and isoleucine were used at a constant rate at all levels. At 5.0 mM, leucine disappeared at around 2.9 µmoles/mg cell protein per 4 h with ketoleucine released at 450 nmoles/mg per 4 h and formation of CO_2 from (1-14C) leucine of 84 nmoles/mg per 4 h. With increasing media leucine, increasing amounts of alanine appeared in the media. Thus, the L6 cell line can serve as a substitute for the isolated in vitro muscle for the study of BCAA metabolism and alanine formation. Moreover, developmental aspects of muscle metabolism could be investigated.

Diacylglycerol and fatty acids are found in large alpha-actinin-actin complexes in vitro and in the cytoskeleton of activated platelets

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Different types of attachment sites between the plasma membrane and cytoskeleton have been described. Each site may be built up of more than one type of linker protein, among which α-actinin will have to be considered as well.
The results show that in the presence of diacylglycerol and palmitic acid a supramolecular complex between α-actinin and actin is formed in vitro. In the electron microscope this complex displays substructures similar to those of microfilament bundles seen in vivo. Further more, such α-actinin-lipid complexes are now also shown to be formed in situ, during the stimulation of blood platelet aggregation. Thus, α-actinin may be one of the proteins that is directly involved in structures which associate the cytoskeleton with membranes.

The cytosolic-free calcium, $[Ca^{2+}]_i$, transient induced in adrenal glomerulosa cells by angiotensin II

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The $[Ca^{2+}]_i$ rises induced by angiotensin II (AII) in isolated bovine glomerulosa cells were measured with the fluorescent indicator quin 2 and correlated with aldosterone production. AII and analogs displayed similar potency ratios in raising $[Ca^{2+}]_i$ and stimulating steroidogenesis. Induction of a $[Ca^{2+}]_i$ rise with AII fully desensitized the glomerulosa cells to a subsequent identical challenge. AII was still able to raise $[Ca^{2+}]_i$ in Ca^{2+}-free medium. Nifedipine lowered basal $[Ca^{2+}]_i$ levels, did not prevent AII-induced $[Ca^{2+}]_i$ rises, but inhibited the functional response. (Sar^1,Ala^8)-AII suppressed AII-induced $[Ca^{2+}]_i$ rises and function. Addition of the antagonist after AII, when $[Ca^{2+}]_i$ was back to basal levels, blocked steroidogenesis without affecting $[Ca^{2+}]_i$. Our data demonstrate not only that a critical threshold $[Ca^{2+}]_i$ rise due in part to mobilization from intracellular pools is necessary to trigger steroidogenesis, but also that other intracellular signals are required to maintain this response.

Fatty acid β-oxidation in arterial smooth muscle cells

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Pig coronary media exhibits a high degree of fatty acid β-oxidation activity which is made up of both mitochondrial and peroxisomal β-oxidation. These two components differ from each other in sensitivity to inhibitors of the oxidative chain, to bovine serum albumin and in their capacity for palmitoyl CoA-dependent H_2O_2 production. β-Oxidation activity was also found to be high in rat aorta media as well as in pig coronary smooth muscle cells in culture. The effect of a lack of insulin on β-oxidation of arterial media was also studied, both in vivo and in vitro. The capacity of arterial smooth muscle cells to degrade fatty acids might play a role in preventing the in situ accumulation of glycerolipids and cholesterol esters that are implicated in the development of arteriosclerosis.

Anion recognition site in cytochrome *c*

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Recent studies in this laboratory have suggested a role for Arginine 91 in the binding of anionic ligands to cytochrome *c*. Such binding is proposed to be of significance in the biological function of the protein. This residue could serve as part of a positively charged recognition site for physiologically important anions such as ATP, ADP, P_i. Any reagent that specifically reacts with guanidinium groups should, therefore, be useful in probing such a putative role.
We have examined the ability of certain anions to protect Arginine 91 in cytochrome *c* from the action of specific guanidinium reagents.

Calmodulin-model peptides interaction

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Calmodulin (CaM) interacts in a calcium-dependent manner with a variety of enzymes, peptides and psychotropic drugs. Our attempts to elucidate the structural features responsible for these interactions involved the design of peptides which mimicked the structure, hydrophobicity and charge distribution of a group of naturally occurring CaM-binding peptides. Although the latter display little exact sequence homology to one another, they all contain a sequence capable of forming a basic amphiphilic α-helix. The importance of this structural feature was tested by synthesizing the peptide FMOC(LKKLLKL)$_2$, the quintessence of a basic amphiphilic α-helix. This peptide binds CaM competitively with phosphodiesterase in a Ca^{2+}-dependent manner with a 3 nM K_d. FMOC(LKKLLKL), which has a much lower helical potential due to its decreased length, binds CaM with reduced affinity (0.15 µM). Also, FMOC(LLELLEL)$_2$ which forms acidic amphiphilic α-helices fails to bind CaM. These results suggested that basic, amphiphilic peptides bind to an acidic, hydrophobic region on CaM. To identify this region, a threee-dimensional model of CaM was constructed by interactive computer graphics at Du Pont de Nemours & Co. The model for CaM contains two shallow hydrophobic crevices flanked by a region of highly negative charge. On the hypothesis that these sites are the target-binding sites, new peptides were modelized: two of them display for CaM affinities about 10-fold higher than any reported peptide.

Changes in rat serum MCF (IL-1) following acute inflammation

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Following localized injury, the rat hepatocyte greatly increases the synthesis of several plasma proteins. A monocyte mediator (IL-1) is thought to be responsible in triggering such an event. IL-1, as defined by mononuclear cell factor (MCF) activity, induces PGE_2 and collagenase release from human cultured synovial cells. Rat serum isolated following s.c. injection of 0.3 ml turpentine showed a 50-fold increase in PGE_2-stimulating activity by 6 h. At 12 h, the PGE_2-stimulating activity dramatically decreased to 85% of the 6 h level and gradually approached 70% of the 6 h value at 48 h. Column fractionated 6 h rat serum showed a $M_r \sim 18$ kd, similar to the reported IL-1 and MCF values, while fractionated control serum was negative. Furthermore, the 6 h serum from injured rats was highly active in cell proliferation assays. Collagenase-stimulating activity was undetected in all experiments. The separation of PGE_2 and collagenase activities and the biphasic PGE_2 response may indicate that the acute-phase response is triggered by different monocyte factors from different gene products, previously thought to be regulated by a single IL-1 molecule.

Processing of cytochrome *c* and derived peptides by bone marrow macrophages

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Antigen specific T cell proliferation and lymphokines production is a process triggered by interaction between T helper cells and antigen presenting cells (APC). Little is known about the biochemical events involved in the antigen presentation by APC to specific T helper cells. One of our goal is to determine the chemical nature of the presented antigen and the biochemical events which take place in the antigen presentation. For this purpose we have analyzed the fate of differently radiolabeled cytochrome *c* and derived peptides taken up by macrophages. Lysates of macrophages pulsed with different peptides preparations have been submitted to reverse phase HPLC analysis. Some cytochrome *c* fragments have shown a striking increase in hydrophobicity. Comparisons between different peptides preparations and different radiolabeling procedures (125-iodine vs tritium) would indicate that hydrophobicity of peptide 1–38 is mainly affected by the interaction with the macrophage. Hydroxylamine or alkaline treatment deeply modifies HPLC elution profile of macrophages lysates by increasing the amount of radioactivity associated with hydrophilic fractions. These results support the idea that some cytochrome *c* peptides generated by macrophages become more hydrophobic either by covalent linkage of a lipid or a phospholipid material or by a tight association with the latter ones.

Depolarization induced release of endogenous sulfur containing amino acids in rat brain slices

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In order to screen and select the neuroactive substances possibly involved in neurotransmission, we took advantage of the fact that they are released from nerve terminals upon depolarization in a Ca^{++}-dependent manner. Slices of various rat brain regions were depolarized either by 50 mM [K^+] or by veratrine (33 µg/ml). The perfusates were derivatized with DABITC (4-dimethylaminoazobenzene-4′-isothiocyanate) and analyzed with reversed-phase HPLC. Substances more polar than Glu and Asp, among them sulfur containing amino acids (cysteic acid, cysteine sulfinic acid, homocysteic acid and homocysteine sulfinic acid) were increased in perfusates by K^+ depolarization in a Ca^{++}-dependent manner or under veratrine stimulation. At rest, these compounds are present in the perfusate in amounts 10 to 50 times smaller than that of the major aa. K^+ depolarization increased their rate by a factor of 2 to 4. This release was high in neocortex, hippocampus and medulla and low in striatum and spinal cord. The release of endogenous sulfur containing aa supports the proposal, made by others, that they play a role as neurotransmitters.

Characterization of iron-requiring hydrolyases

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Hydrolyases acting on di- or tricarboxyllic acids (aconitase, malease, tartrase, etc.) are inactive upon purification and require activation with Fe^{2+} ions. The metal requirement is associated with the presence of a 3Fe-XS cluster in the inactive enzyme, undergoing conversion to a Fe-4S core upon activation with Fe^{2+}. Sulphide ions are required in addition to Fe^{2+} in certain cases (e.g. maleate hydratase) to rebuild the Fe-S cluster.
Mannonate and altronate hydratases are also Fe-requiriquing enzymes acting on aldonic acids and have been purified and characterized. One Fe per mole is incorporated into the enzyme upon activation; by contrast to other Fe-hydratases, they do not bear an Fe-S cluster and Co^{2+} can replace Fe^{2+} in the activation process. These enzymes and other aldonic-acid hydratases (fuconase, gluconate hydratase, etc.) with similar properties in crude preparations constitute a different subclass of Fe-requiring hydratases.

Evidences for common epitopes on the PRL receptor molecules originating from rabbit mammary gland and from toad kidney, *Bufo marinus*

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Specific binding sites for prolactin (PRL) were identified on toad kidney plasma membranes. Solubilization of this receptor with 1% triton X-100 and its purification was achieved as for the PRL receptor of the rabbit mammary gland. The solubilized toad receptor eluted from sepharose 6B at a mol. wt. of approx. 200,000. Further purification was obtained by affinity chromatography using biotinylated human growth hormone and streptavidine-coupled Affi-gel 10.

In order to determine structural homologies between these two PRL receptors, three antisera raised against the PRL receptor of the rabbit mammary gland were used to screen the solubilized toad PRL receptor. None of these antisera recognized the PRL binding site but one of them identified epitopes located on the envelop of the receptor. This antiserum is used presently for purifying the toad PRL receptor by affinity chromatography. In summary, high affinity PRL receptors could be identified in toad kidney. It appears that part of the PRL receptor structure has been maintained during evolution.

Interaction of C1q with heparin

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C1q, a subcomponent of the first component of complement, is the first reactant of complement activation by complexed IgG or IgM. This property of C1q to bind to complexed IgG is used in the C1q-binding assay (C1q-BA) to detect circulating immune complexes in pathological sera. However, C1q is also able to bind to heparin in normal human sera, giving raise to false-positive results using the C1q-BA when heparin is present. This study was undertaken to further elucidate the interaction of C1q with heparin.

In displacement experiments with aggregated human gammaglobulin (AHG) or heparin and different C1q preparations the following results were obtained: as expected, decreasing binding activities of labelled C1q to AHG in presence of increasing concentrations of 'cold' C1q were found. However, the experiments with heparin gave unexpected results; increased binding activities of radiolabelled C1q to heparin resulted with increasing concentrations of 'cold' C1q. Possible molecular mechanisms for this effect will be discussed.

Up-regulation by interleukin-2 (IL-2) and interleukin-1 (IL-1) of high and low affinity IL-2 receptors on a T-cell hybridoma

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PC60 is a hybrid between a rat thymoma and an IL-2 dependent murine cytolytic T-lymphocyte line. The hybrids grow independently of IL-2 and do not express cytolytic activity or IL-2 receptors when cultured in normal medium, but a combination of IL-2 and IL-1 induces both. Using a monoclonal anti IL-2 receptor antibody and ^{3}H-IL-2 we have explored the kinetics of receptor appearance and their affinity. IL-1 alone induces a small number (1000/cell) of receptors. Exposure to IL-1 and IL-2-induces 20- to 50-fold more receptors of two affinity classes, the proportions being similar to that among the receptors on the parental CTL-line. Maximal receptor expression requires 3 days induction. We are now investigating the control of IL-2 receptor expression on PC60 cells by immunoprecipitation of biosynthetically labelled receptor molecules. These results show that IL-2 is acting not only as a lymphocyte specific growth factor but also regulates the expression of its own receptors in an unusual way.

Sugar transport in bacteria: molecular cloning of *ptsM* and characterization of its gene product, enzyme II^{man}

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The bacterial phosphotransferase system mediates active transport and concomitant phosphorylation of sugars. It comprises two cytoplasmic proteins (EnzI, HPr) and a number of different sugar-specific transmembrane permeases (enzymes II) which also serve as receptors in chemotaxis. The mannose-specific enzyme II (II^{man}) is required for penetration of λ DNA through the cytoplasmic membrane of *E. coli*.

We have cloned *ptsM* into a mini-Mu bacteriophage containing a plasmid replicon and hence into pBR322, and we have purified active II^{man} from an overproducing strain. II^{man} comprises four proteins of apparent mol. wt 60 kD, 35 kD, 28 kD and 23 kD. The 60-kD protein can be dissociated into 35-kD proteins, indicating a dimeric structure of II^{man}. Both bands are phosphorylated by enzyme I and HPr in the presence of phosphoenolpyruvate. The 28-kD and 23-kD bands are nonphosphorylated hydrophobic proteins. It will be discussed whether the 35-kD and the 28-kD proteins are products of a single larger precursor protein or whether they are encoded in a polycistronic operon.

Purification and structural comparison of the major surface glycoproteins of Old World *Leishmania* promastigotes

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Promastigotes of *Leishmania donovani*, *L. major* and *L. tropica* were surface radioiodinated. A single major iodinated glycoprotein of approximately 63 kDa (p63) was identified by SDS-PAGE in each species. These glycoproteins were partially purified by phase separation in Triton X-114 nonionic detergent solution, demonstrating that the p63 of each species is an integral membrane protein. Peptide maps were prepared by partial proteolysis followed by SDS-PAGE. Maps of *L. donovani* and *L. major* p63's are practically identical, while only partially homologous to the map of *L. tropica* p63. p63 of *L. major* was purified by ion exchange chromatography.

The data show that promastigotes of Old World *Leishmania* express on their surfaces a single major integral membrane glycoprotein. These glycoproteins are immunologically and structurally related among the three species of *Leishmania* that we have examined.

Binding characteristics of naturally occurring antibodies to band 3 protein of human red blood cells

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Naturally occurring anti-band 3 antibodies (J. Immun. *133* (1984) 2610) were ^{125}I-iodinated by two different techniques. Their binding to blotted and dotted antigen was studied. Binding was up to eight times higher to previously denaturated than to native band 3 protein, as expected from the cryptic nature of most of the antigenic regions. Specific binding was detected at concentrations of 100 ng/ml and higher. Binding increased rapidly up to a concentration of 500 ng/ml and approached saturation at 1.2 µg/ml. Binding was not significantly different

for antibodies labeled by either chloramin T or Bolton Hunter technique. Affinity constants derived from these binding assays were in the range of 1 to 10×10^9 M^{-1}. These findings are relevant in view of the low concentration of these antibodies in normal sera ($1–2 \times 10^{-9}$ M) and their physiological role. The concentration of these antibodies is suboptimal for binding and binding is greatly influenced by small variations in the titer.

Rabbit secretory components: structural and biological studies

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High and low mol. wt secretory components (HMW SC and LMW SC, respectively) which are noncovalently bound to the secretory IgA (sIgA) in rabbit milk, were subjected to structural studies and used for determining their relative affinities for SC-depleted IgA dimers. HMW and LMW SC have identical N- and C-terminal amino acid sequences which suggest that the LMW SC results from an internal deletion of 25 kD from the HMW SC. LMW SC exhibited a 5- to 10-fold lower affinity for SC-depleted IgA dimers as compared to the HMW SC. Tryptic digestion of the HMW SC produced two major fragments of ~ 40 kD and ~ 20 kD. The 40-kD fragment is the C-terminal half of the HMW SC and did not bind to SC-depleted IgA dimers whereas the 20 kD-fragment did. Tryptic digestion of the LMW SC generated two major peptides of ~ 30 kD and ~ 18 kD. The 30-kD fragment did not bind to SC-depleted IgA dimers whereas the 18 kD peptide did. Full characterization of the sIgA binding fragments is under way. Preliminary peptide mapping data suggest that the third Ig-like domain (Mostov et al., Nature *308* (1984) 37) is deleted in the LMW SC.

Regulation of the synthesis of betalains in red beet (*Beta vulgaris* ssp.) cells in culture

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Red beet cells able to synthesize the red pigment betanin, are used as a model system to study the mechanisms which control the production of secondary compounds (alcaloids) by plant cells.

Cell suspensions have been obtained from seedlings and propagated on defined media. Habituated cell lines were isolated from which we have selected low or highly pigmented clones. No clones were found which were totally stables (relatively to alcaloid-pigment content). Nevertheless, it was found possible, using dark to light transition to modulate pigment synthesis. When a dark grown nonpigmented culture was transfered to light, pigment synthesis and accumulation was induced. The resulting population is not homogenous: some cell do not respond to the transition, other cells do synthetize yellow (betaxanthines!!!) pigments.

HPLC analysis of pigments and precursors have been made. In competent cells, betanine synthesis start immediately after the transfert to light. An other set of pigments (red and yellow) appears after a lag-period of 40 h. It remain to be shown wether this represent the behavior of specific cell lineages or wether the complet set of pigments is present in the majority of the cells. SDSPAGE analysis indicate that new specific proteins are rapidly synthetized during this transition.

Transverse localization of galactolipids in oat thylakoid membranes during greening

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The lipase from *Rhizopus arrhizus* has been used to determine the transmembrane distribution of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) in prothylakoids and mature thylakoids from oat. The hydrolysis kinetics of MGDG and DGDG were characterized by several pools of different reactivity. The number and the rate of these pools were dependent on the temperature, ionic conditions and the presence of bovine serum albumin. The amplitude of each of these pools was different for MGDG and DGDG but similar in both prothylakoids and thylakoids. These results suggest that as for phosphatidylglycerol, the transverse distribution of galactolipids is not modified during greening, i.e. by the incorporation of chlorophyll-protein complexes.

Acetoacetate-mediated energy production by rat liver mitochondria in anaerobiosis

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Submitting rat liver mitochondria to anoxia, in vitro, impairs respiration-linked functions subsequently measured. Energy depletion during the anoxic period may be responsible for the impairment. Since energy production by substrate-level phosphorylation is limited by NAD^+ availability in anoxia, we evaluated the influence of NAD^+-generating reactions in the presence of 2-oxoglutarate as substrate. Mitochondria were incubated at 25 °C in a KCl-medium, pH 7.4, and loaded with 5–20 µg-ion Ca^{2+}/g protein. Deenergization resulting from O_2 deprivation was reflected by Ca^{2+}-release from mitochondria, as shown by pCa measurement in the medium. Providing acetoacetate (0.3–1.2 mM) to deenergized mitochondria elicited a larger Ca^{2+} uptake than obtained by providing malate (1.2 mM). The effect of acetoacetate was inhibited by β-hydroxybutyrate, and that of malate was suppressed by rotenone. The effects of both agents were abolished by oligomycin. The data indicate that acetoacetate may promote energy supply by mitochondria in anoxia.

Genetic polymorphism of debrisoquine/sparteine oxidation: two functionally different cytochrome P450 isozymes purified from human liver catalyze bufuralol hydroxylation

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The common deficiency of the matabolism of debrisoquine, bufuralol (Bu) and other drugs has remained unexplained. Two cytochrome P450 (P450) isozymes (*P450 buf I* and *P450 buf II*) purified from human liver microsomes catalyzed the 1'-hydroxylation of Bu to carbinol (Ca). However, *P450 buf I* metabolized (−) and (+)-Bu in a highly stereoselective fashion ((−)/(+) ratio: 0.13) as compared to *P450 buf II* ratio: 0.9). The apparent Km for Bu was 4–5 times higher with *P450 buf II* than with *P450 buf I*. Metabolism of Bu by *P450 buf II* consistently resulted in the generation of additional metabolites. Quinidine inhibited Bu-1'-hydroxylation with an apparent Ki for *P450 buf I* of ~ 60 nM as opposed to a Ki of ~ 80 µM for *P450 buf II*. Conclusion. The collective evidence from the functional differences allows a distinction of the two isozymes. Data in liver microsomes from so-called 'poor metabolizers' suggest that a deficiency of the 'high affinity' *P450 buf I* alone that a deficiency of the 'high affinity' *P450 buf I* alone causes this common genetic polymorphism.

Z-DNA-binding proteins in mammalian tissues

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Z-DNA is a left-handed form of natural and synthetic double-stranded polydeoxynucleotides. Its physiological significance is not clear but Z-DNA may serve as a recognition signal for the attachment of gene regulatory proteins. We have undertaken a search for Z-DNA-binding proteins in mammalian tissues. Tissue extracts were fractionated by various techniques including affinity chromatography on immobilized Z-DNA. Fractions were analyzed by filter-binding and blotting assays using ^{32}P-labeled Z- and B-DNA as probes. Evidence is presented for the occurrence of putative Z-DNA-binding proteins in mammalian tissues.

The regulation of early development in *Dictyostelium discoideum*

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Unlike wild type cells of *Dictyostelium discoideum*, the mutant HG 302 develops normally in the presence of adenosine 3',5'-cyclic phosphorothioate (cAMP-S), a phosphodiesterase resistant derivative of cAMP. Nonaggregating mutants were obtained by further mutagenizing HG 302. These mutants were screened for the presence of 3 developmentally regulated products (adenylate cyclase, extracellular phosphodiesterase and contact site A). In addition, the expression at the mRNA level of 20 developmentally regulated genes was measured by hybridization on northern blots. The genes could be attributed to two classes according to their expression in the mutants. One of these classes showed the same pattern of expression as adenylate cyclase and contact site A, but not phosphodiesterase.

Primary structure of *Streptomyces glaucescens* tyrosinase

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The amino-acid sequence of *Streptomyces glaucescens* tyrosinase has been determined. The molecule contains 273 amino acids and has a mol. wt of 30,900 including two copper atoms. The primary structure was established by a combination of DNA and protein sequence analysis. The tyrosinase gene shows a strong preference for codons ending in G or C (97%). The enzyme contains no leader peptide despite the fact that it is secreted into the culture medium. The sequence homology of *Streptomyces* tyrosinase to the enzyme from *Neurospora crassa* is only 23.8%. However, the sequence homology is higher than 50% in the regions thought to be involved in metal binding of the binuclear active site copper.

Nonenzymatic protein glycosylation: the browning reaction proceeds without intermolecular cross-linking

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Browning and dimerization as a consequence of nonenzymatic protein glycosylation seem to be of relevance in diabetic long-term complications. Evidence for intermolecular cross-linking is based on the dimerization of albumin and RNase A when incubated with glucose. Albumin was isolated on Blue-Trisacryl, freed of dimers by gel filtration, and incubated with excess glucose for 20 days. Browning was detected by absorbance at 292 nm and fluorescence at 430 nm. Cross-linking was measured by SDS-PAGE or gel filtration. Although browning increased with time cross-linking was not observed. Our results do not support published data and show that dimerization is not essential for browning. However, intramolecular cross-linking may have contributed to browning. Browning is also caused by acetaldehyde and the essential feature for its occurrence appears to be the Schiff's Base adduct. The chemical structure of the chromophore remains to be established.

Purification of human interleukin-1 by high performance reverse phase chromatography

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Supernatants from LPS-stimulated human blood leukocytes were concentrated and chromatographed on Biogel P-100. The biological activity of interleukin-1 (IL-1) migrated in a single peak of 15 kD mol. wt. Subsequent chromatofocusing resulted in two IL-1 fractions (pI 5.2 and 6.6). Both were subjected separately to HPLC (reverse phase) using a Biorad RP-304, C-4 column in two subsequent steps, first at pH 3.2 (0.1% H_3PO_4, gradient of CH_3CN) and then at pH 2.1 (0.1% TFA, gradient of CH_3CN). The two forms differing in IEP eluted from HPLC in sharp peaks exactly at the same CH_3CN concentration. These results suggest that the two peaks of activity represent the same protein with different degrees of glycosilation. The highly purified material obtained is rather unstable and tends to bind to surfaces. This can be partially prevented by the addition of PEG or protein.

Possible regulation of glycogen synthesis by a purine derivative in hepatocytes from fasted rats

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Amino acids such as glutamine, glycine and alanine strongly stimulate glycogen synthesis from glucose and lactate/pyruvate. Since these stimulatory amino acids are also direct or indirect precursors of purine synthesis, we looked for a possible correlation between the two processes. Stimulated glycogen synthesis is strongly inhibited by 6-mercaptopurine and 6-methylmercaptopurineriboside, two purine synthesis inhibitors. This inhibition is correlated with a decrease of the formation of adenine compounds indicating that an adenine derivative may be a stimulator of glycogenesis. Addition of adenosine and glucose has been reported to lead to a synergistic stimulation of glycogen synthase phosphatase in a glycogen particle preparation from rat liver. In our system, addition of adenosine which is rapidly metabolized did not result in a stimulation of glycogenesis, whereas 3-deoxyadenosine showed a small activation. Stimulation of glycogen deposition was also achieved with 5-iodotubercidin, an inhibitor of adenosine kinase.

Regulation of gluconeogenesis by glucose, role of fructose 2,6-P_2

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In hepatocytes from starved rats, we had shown earlier that 5 to 10 mM glucose (G) suppressed gluconeogenesis (GN) from 2 mM alanine but not from 9 mM lactate/1 mM pyruvate (Biochem J. *192* (1980) 377) and as now shown also not from 2 mM lactate/0.2 mM pyruvate. In similar experiments, fructose 2,6-P_2 levels strongly increased upon addition of G alone. This increase was only little affected by alanine, but was significantly reduced

by lactate. This suppression was dependent on the lactate/pyruvate levels and occurred in the physiological range of G and of lactate/pyruvate concentrations. It appears therefore that GN in the alanine system is inhibited by G as a result of the increase in fructose 2,6-P_2 formation. With lactate, however, GN is much less influenced by added G because lactate, by a so far unknown mechanism, prevents the increase in fructose 2,6-P_2 due to added G. These results are in agreement with the finding observed postprandially in vivo that GN in the liver continues despite the increasing level of portal G.

Semisynthetic [^{3}H]human proinsulin

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Like insulin, proinsulin exerts a hypoglycaemic effect, though with a somewhat lower specific activity. A means of following its fate in the circulation is therefore of interest. Biosynthetic human proinsulin (obtained by recombinant DNA methods) has been used as the starting material for the preparation of [^{3}H]proinsulin by semisynthetic methods, with the label at the amino-terminal phenylalanine. The labelled protein was characterized by its elution volume on reverse-phase h.p.l.c.; by polyacrylamide gel electrophoresis; by the time course of its enzymatic conversion to insulin and by chromatographic analysis after extensive proteolytic degradation. The labelled protein can be used as a tracer for proinsulin in the rat, and may be suitable for use in man.

Nematocysts of *Hydra:* biological and biochemical characteristics of their venom

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The injection of a crude nematocyst extract from *Hydra attenuate* (cp. Weber et al., same symposium) into larvae of *Drosophila melanogaster* causes the same symptoms that appear when larvae are contacted by the tentacles of living polyps. Injections or tentacle contact leads to the contraction of the larvae. Depending on the amount injected, the larvae is partially or totally paralyzed. In this condition unidirectional, wave-like contractions of individual muscles can be observed. Adult *Drosophila* show similar reactions to extract administration. These observations suggest that at least one of the active components acts as a muscle- or neuromuscular toxin. Depending on the extract concentration neither the larvae nor the flies survived injection. In addition these extracts are highly hemolytic to human erythrocytes. This activity is inhibited by Concanavalin-A.

Alteration of the plasma membrane's lipid composition in Semliki Forest Virus (SFV) induced cell fusion of *Aedes albopictus* cells

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Membrane fusion involves a rearrangement of membrane components in its transition from two bilayers to a single one. We would therefore expect considerable rearrangements of membrane lipids during the process. Therefore the lipid composition of 1) SFV infected cells (pH 7), 2) fused cells (pH 6) and 3) uninfected cells was analyzed. Plasma membranes were isolated according to Butters and Hughes (BBA *640* (1981) 655) and lipids were extracted and analyzed by TLC. Phospholipid analysis showed no difference between plasma membranes derived from infected (1) or infected and fused (2) cells but rendered one spot less than in uninfected (3) cells. This suggests that one component is lost during the infection. The composition of neutral lipids was qualitatively identical for control (3) and infected (1) cells. After fusion (2) free fatty acids could not be detected by simple TLC. Thus, these preliminary results suggest that during fusion the lipid composition of the membranes is altered.

Ca^{2+}-induced hepatic K^+ uptake is inhibited by the calcium channel blocker verapamil

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Perfused livers from fed rats respond to exogenous Ca^{2+} with an activation of glycogenolysis and an uptake of K^+ either if phenylephrine is present or after pretreatment with EGTA, followed by perfusion with Ca^{2+}- and Mg^{2+}-free medium. By studying the influence of verapamil on the response to exogenous Ca^{2+} we have attempted to answer the question whether Ca^{2+} influx into the cell mimicks all the effects of the α_1-adrenergic agonist phenylephrine. Verapamil (10 and 100 μM) strongly inhibited Ca^{2+}-induced K^+-uptake but had little or no effect on the glycogenolytic response, indicating that an inhibition of Ca^{2+} influx was not the primary effect. Ca^{2+}-induced K^+ uptake and glycogenolysis appear to be independent and verapamil may act on K^+ transport across the plasma membrane, rather than on Ca^{2+} influx under these conditions.

90 kD disulfide-bonded dimeric and 45 kD T cell receptors are precipitated from human T lymphocytes and HPB-ALL by the clone 9.3 antibody (9.3 moab)

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The effects of the 9.3 moab in cell culture suggest that the 9.3 T cell antigen functions in growth control. With normal T cells the 9.3 moab precipitates 90 and 45 kD receptors from cell lysates; by S-S reduction the 90 kD species is cleaved into subunits of 40–50 kD. T cells carry a further 45 kD receptor identified by Triton-insolubility and in isolated membrane fractions, which by peptide fragment analysis is closely related to the 9.3 antigens. With one of several T cell lines tested, HPB-ALL, the 9.3 moab precipitates analogous 90 and 45 kD receptors from cell lysates, but the Triton-insoluble 45 kD species is not detected. Tryptic/chymotryptic fragments of the 9.3 moab precipitate and of T_i were found identical. It is concluded that biochemically 9.3 antigen and T_i are closely related. The 45 kD species are probably free (small) monomers; their different expression in HPB-ALL is of interest in view of tumor cell growth control.

Biosynthesis of two surface membrane glycoproteins identified by monoclonal antibodies which stimulate interleukin-2 production by the murine T lymphoma EL-4

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In an effort to derive monoclonal antibodies (mAb) with lymphocyte-activating properties, spleen cells from rats immunized with the mouse T lymphoma EL-4 have been fused with the mouse myeloma X63-Ag8.653 and supernatants from growing hybrids were assayed for their ability to stimulate interleukin 2 production by the EL-4 cell line. Two such reagents were obtained which recognized two different surface membrane glycoproteins of mouse lymphoma cell lines and activated lymphocytes. The first mAb detected a disulfide-linked heterodimer complex composed of a glycosylated heavy subunit (Mr = 85,000) and a non glycosylated light subunit (Mr = 40,000). The second mAb recognized a heavily glycosy-

lated polypeptide (Mr = 52,000). The biosynthesis, the identification of the precursor forms and the maturation of both components have been studied.

Human transferrin receptor: genomic organization and protein sequence

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The cell surface receptor for the iron-carrying serumprotein transferrin is composed of two identical glycosylated subunits of 95 kD. The expression of this receptor is essential to cellular iron uptake and is regulated in cell proliferation. We have isolated genomic DNA clones and a cDNA clone encoding the receptor. Heteroduplex analysis reveals that the gene contains at least 19 exons distributed over 33 kb of genomic DNA, specifying a 4.9 kb mRNA. The nucleotide sequence derived from the cDNA shows a single complete open reading frame of 2277 bases, that is preceeded by a short 5'-nontranslated region and followed by 2.5 kb of 3'-nontranslated sequence. The deduced protein sequence of 759 amino acids lacks an N-terminal signal peptide and contains a single hydrophobic transmembrane region, located 60 amino acids from the N-terminus. Based on the sequence and published biochemical data we predict a transmembrane orientation with a cytoplasmic N-terminus. The receptor has no homology to any known proteins.

A cAMP-dependent phosphorylation of both spectrin bands in vitro as well as in intact human red blood cells

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Purified spectrin dimer can be phosphorylated with [γ^{32}P]-ATP by a cAMP-dependent protein kinase or its catalytic subunit (FEBS Lett. *169* (1984) 323). A cAMP-dependent phosphoform of spectrin is also generated in intact human red cells in the presence of cAMP and ^{32}P-phosphate. This also results in phosphorylation of both spectrin bands. The consequences are: 1) The extractability of spectrin (low ionic strength) from membranes was reduced by 20%. 2) The extractable portion of spectrin revealed the same phosphopeptide map irrespective of whether cAMP was added or not. 3) The retention of high mol. wt proteins (region of spectrin/ankyrin) by inside-out vesicles (IOV) was enhanced correspondingly, implying that a cAMP-dependent phosphoform of spectrin remained – like ankyrin – ionically bound to IOV. Similarly, IOVs obtained from resealed ghosts loaded with an ^{125}I-iodinated, cAMP-dependent phosphoform of spectrin retained four times as much label than those IOVs derived from ghosts loaded with autophosphorylated spectrin.

Synthetic peptides as substrates for the characterization of hormone precursor (proenkephalins) processing enzymes

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The tetrapeptide Ala-Lys-Arg-Tyr and other longer synthetic peptides containing a pair of the basic amino acids lysine and arginine, were used as substrates. Fractions of lysed granules from bovine adrenal medulla were shown to hydrolyse these peptides in vitro. The fragments produced were analyzed by HPLC after derivatization according to Chang, which led to high sensitivity (picomolar range) and to a dispay of all degradation fragments. The splitting seems to occur in two steps possibly catalyzed by two different proteases. Two populations of granules could be separated with highly different content in processing enzymes. Degradation was impaired by EDTA showing a dependency upon a divalent cation. The method is a progress towards the understanding of the time dependent processing in vivo and the isolation of the processing enzymes.

Identification of the self interaction site of a sponge aggregation factor

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Reaggregation of dissociated cells from the sponge *Microciona prolifera* is mediated by an aggregation factor (AF) via two functional domains: a highly polyvalent AF cell binding domain and an AF-AF self interaction site. Monoclonal antibodies were produced against AF to identify the AF-AF self interaction site. Fab fragments from two clones inhibited the AF-AF interaction. Binding of these Fab fragments to AF showed that they were directed against two different determinants, both of which occur in more than 2000 copies in the AF molecule. After pronase digestion of AF three classes of oligosaccharides were isolated by gel filtration and they could be immunoprecipitated by the inhibitory antibodies. The AF-AF binding is proposed to be based on multiple interactions of oligosaccharide chains of AF. The polyvalency of the AF domains necessary for cell aggregation is in contrast with most examples of cell adhesion molecules which involve single high affinity interactions.

Binding of ADP to a cytosolic fraction from rat liver with low adenylate kinase activity: effect of Mn^{2+} and polyethyleneglycol (PEG)

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Adenylate kinase (AK) interferes with studies on ADP-binding to cytosolic proteins. Liver cytosol of fed rats was therefore chromatographed on $diAp_5A$-Sepharose thereby removing 94% of AK activity. The resulting fraction with low AK activity contained 79 ± 1% of the cytosolic proteins, > 80% of pyruvate kinase and phosphoenolpyruvate carboxykinase and > 60% of hexokinase. It was concentrated and supplemented with 10 mM (+)-catechin to inhibit residual AK activity. Binding of 2×10^{-6} M ^{3}H-ADP was determined by rate of dialysis measurements. In the absence of further additions, binding of ADP, normalized to 10 mg protein/ml, was 22%. This value was increased to 32% by 5% PEG, to 29% by 10 μM Mn^{2+} and to 42% by 5% PEG + 10 μM Mn^{2+}. 10 μM Mg^{2+} had no effect on ADP binding. These results suggest a role for Mn^{2+} in the control of free and bound cytosolic ADP and therefore of cytosolic enzymes regulated by adenine nucleotides.

The degradation of [Gly^{A1}-^{3}H]insulin and[Phe^{B1}-^{3}H]insulin by insulin protease

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Previous studies have identified an intermediate in the degradation of [Phe^{B1}-^{3}H]insulin by the enzyme insulin protease [EC 3.4.22.11]. The position of the label allowed the characterization by electrophoresis of the remaining labelled portion of the B chain in this intermediate. After oxidation in performic acid a peptide corresponding to B1 to B7–9 was the major labelled species. The chemical form of the A chain, however, remained uncertain.

We have now obtained an intermediate in the degradation of [Gly^{A1}-^{3}H]insulin which corresponds to that obtained from [Phe^{B1}-^{3}H]insulin. Performic acid treatment of this intermediate

gives a labelled A-chain peptide which elutes from Sephadex G25 after the intact A chain. The electrophoretic mobility on paper in 30% formic-acid of this A chain peptide is, however, the same as the intact A chain. These two results together indicate that the A chain may have been cleaved around A13 or A14. Further experiments are in progress to test this hypothesis.

Receptor-dependent binding of C-reactive protein to human neutrophils

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C-reactive protein (CRP) has been considered an acute inflammation response protein, but despite functional similarities with immunoglobulins its role in host defence against bacterial infections remains unclear. Therefore, we tested whether CRP has any effect on human neutrophils. By using highly purified CRP we could demonstrate a saturable cellular binding with half-saturation of specific binding occuring at $10 \pm 5.7 \times 10^{-8}$ M ($n = 6$). This binding was Fc-receptor dependent and induced adherence of cells to plastic surfaces when assayed in heat inactivated plasma. However, stimulation of cells measured by the release of secondary granulate constituents, superoxide production and activation of the hexose monophosphate pathway did not occur. Therefore, the CRP-mediated adherence of neutrophils might be without cellular stimulation an important step in localizing an inflammatory focus, because CRP was found deposited at sites of tissue injury.

Differences in the response of human neutrophils to complement fragments C5a and C5adesArg

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In the course of complement activation in plasma, C5a, a cleavage product of the fifth component of complement (C5), is released into fluid phase and thereby converted rapidly to C5adesArg by cleaving off an arginyl residue. Both, C5a and C5adesArg act as mediators in inflammatory reactions of human neutrophils. The highly purified C5 fragments were examined for binding to receptors and for some cellular responses of neutrophils. Specific half-maximal binding occurred at concentrations of 3–5 nM for C5a and 30 nM for C5adesArg. As a biological activity following receptor-binding of C5 fragments, chemotactic responses of neutrophils were assessed: C5adesArg was 1.5-fold more active than C5a. However, adherence to plastic surfaces was 2-fold higher with C5a when compared with C5adesArg. Furthermore, competition experiments implied the existence of two distinct receptors on neutrophils: One binding the in vivo unstable C5a and thereby mediating adherence, and the other binding C5adesArg and inducing chemotactic activity of human neutrophils.

Labeling of cytochrome c oxidase with fluorescent probes

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Cytochrome c oxidase is a protein complex composed of 13 different polypeptides. Subunits 1, 3 and 6a contain each a reactive SH-group. We have tested the covalent binding of N-(iodoacetaminoethyl)-1-naphthylamine-5-sulfonic acid, N-(4-anilino-1-naphthyl)-maleimide and eosin-5-maleimide (EMA) to the above mentioned subunits. In addition, the glutamyl residue 90 of subunit 3 was shown to be specifically labeled by N,N'-dicyclohexylcarbodiimide (Casey, R.P., Thelen, M., and Azzi, A., J. biol. Chem. *255* (1980) 3994), and the binding to the same residue of a fluorescent carbodiimide, i.e. N-cyclohexyl-N'-(4-dimethylamino-1-naphthyl)carbodiimide, is presently being tested. The most selective SH-group reagent was found to be EMA, which bound to the cysteinyl residue 115 of subunit 3. Under appropriates conditions approximately 1 mole of EMA bound to 1 mole of enzyme.

The role of the peroxidase (EPO) in the formation of the 6-trans-LTB_4's in eosinophil leukocytes

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Horse eosinophils stimulated with the Ionophore A23187, in the absence of exogenous arachidonic acid, generate predominantly LTC_4, LTD_4, their 11-trans-stereoisomers, 6-t-LTB_4, 6-t-12-epi-LTB_4 and a small quantity of LTB_4. The 6-t-LTB_4's can be formed by nonenzymatic hydrolysis of LTA_4 or by the interaction of released EPO, H_2O_2 and Cl^- to form hypochlorus acid which generates a chlorosulfonium ion intermediate from each sulfidopeptide leukotriene and subsequently the 6-t-LTB_4's. The EPO inhibitor azide (2 mM) slightly reduces the formation of all leukotrienes without changing the relative proportions of the 6-t-LTB_4's versus the peptide LT's. The HOCl scavengers serine and glycine (20 mM) have almost no effect on the LT-formation. Also the omission of chloride in the medium did not inflence the LT-generation. These results and the time course studies of the LT-formation suggest that the EPO, if any, play a minor role in the formation of the 6-t-LTB_4's.

Antibodies to myelin associated glycoprotein reveal new antigenic structures

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A number of patients with IgM gammopathy and associated peripheral neuropathy have been found to have circulating monoclonal antibodies which react with myelin associated glycoprotein (MAG). Studies with these human M-IgM antibodies have revealed that the determinant of MAG recognized is carbohydrate in nature and that this determinant is shared by a peripheral nerve ganglioside. We now show that a mouse monoclonal anti-idiotype antibody which reacts with the variable region of one such human M-IgM antibody inhibits the binding of the M-IgM not only to MAG but also to the ganglioside, thus proving that the monoclonal IgM reacts with the same epitope on both antigens. It is of interest that this ganglioside and the carbohydrate portion of MAG have also been found to share an epitope or epitopes with a surface antigen of human NK cells (HNK 1 antigen) and with the neuronal cell adhesion molecule (N-CAM). It is suggested that the shared carbohydrate moiety implies a common functional role for each of these membrane surface molecules.

Biosynthesis of the brush border membrane enzymes of the human small intestine

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The biosynthesis of the intestinal brush border membrane enzymes has been studied in organ cultures of human intestinal biopsies using monoclonal antibodies directed against the sucrase-isomaltase complex (SI), maltase-glucoamylase (MGA), lactase-phlorizin hydrolase (LPH), aminopeptidase N (APN) and dipeptidyl peptidase IV (DPPIV). Long pulse experiments using ^{35}S-methionine followed by immunoprecipitation and electrophoretic analysis of the precipitates by SDS-PAGE under reducing and nonreducing conditions revealed polypeptides of

apparent Mr of 245 kD, 350 kD, 160 kD and 128 kD corresponding to the mature forms of SI, MGA, APN and DPPIV. The LPH molecule consisted of two polypeptides which are not linked by disulfide bridges of Mr 215 kD and 160 kD. Whether these are the mature forms of two different precursors or products of post-translational processing of a single precursor is currently under investigation.

Monomeric and dimeric cytochrome *C* oxidase and bc_1-complex: structural and functional analysis

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Monomers and dimers of cytochrome *c* oxidase, dispersed in dodecyl maltoside, could be separated by gel filtration under low ionic strength conditions. Mol. ws of 400,000 and 170,000–200,000, for the dimeric and monomeric enzyme (corrected for the nonprotein components) respectively, were estimated by sedimentation analysis in sucrose gradients. Steady state kinetics of the cytochrome *c* oxidase activity showed a monphasic Eadie-Hofstee plot for the monomer and a biphasic behavior for the dimer. The subunit III depleted enzymes from bovine heart and *Paracoccus denitrificans* monomerized easer and gave always monophasic kinetics. Under the same conditions as for cytochrome *c* oxidase also the bc_1-complex could be split into monomers. At low but not at high quinol concentrations the kinetics of cytochrome *c* reduction were monophasic for both, dimers and monomers with similar K_m and V_{max}.

Cloning of cDNAs encoding eukaryotic protein synthesis initiation factors

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Regulation of gene expression at the level of initiation of translation plays an important role in eukaryotic cells. To learn more about the biochemical reactions involved we are studying the mechanism and regulation of mRNA binding to yeast *(S. cerevisiae)* and mammalian ribosomes. We purified eukaryotic initiation factors (eIF) involved in the mRNA binding step (eIF-3, eIF-4A, eIF-4E) and prepared polyclonal and monoclonal antibodies against them. These antibodies were then used 1) to study their effects on initiation of translation in cell-free systems and 2) to isolate cDNA clones encoding eukaryotic initiation factor sequences in the expression vector λgtll. We present data which show that the initiation factors eIF-4A and eIF-4E exist in two different mol. wt forms and that the two forms of eIF-4A have different biological function(s).

Interaction of the cytoskeletal component vinculin with bilayer structures in a model system

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Vinculin, a protein component of the cytoskeleton, present in focal contacts with the substrate and in regions of cell-cell contact, has been postulated to function as an actin-plasma membrane linker. As yet however very little direct evidence has been presented to substantiate this claim. We have now investigated the interaction of purified vinculin (from chicken gizzard) with lipid vesicles. Vinculin was either incorporated into liposomes by adding it to lipids (soybean phospholipids) solubilized in cholate, followed by dialysis to remove the detergent, or was added to preformed liposomes.

Both experimental conditions result in an association of vinculin with the liposomes, as shown by sucrose density gradient centrifugation. Bovine serum albumin, as control, did not interact with the liposomes under these conditions. Lipid specificity and the nature of this interaction are currently under investigation.

The involvement of disulfide-sulfhydryl exchange in Semliki Forest Virus membrane fusion induced by low pH

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Aedes albopictus cells infected with Semliki Forest Virus fuse when exposed to low pH. Several reagents were applied for 1) reduction of disulfide bonds and 2) modification of thiols, to determine the role of these functional groups in the fusion process. Fusion was inhibited if the reactions were performed during or after, but not before pH 6 exposure suggesting that a disulfide-sulfhydryl exchange is involved in the conformational change earlier described of the putative fusogenic viral E1 protein located in the plasma membrane. This rearrangement may entail the exprimation of a hydrophobic peptide segment which triggers the fusion by penetration into the lipid bilayer of the neighboring cell.

Clone 9.3 antibody blocks antigen response of *M. leprae*-specific cell clone, but the human 9.3 T cell antigen may differ from T_i

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Previous data (Gmünder and Lesslauer, Eur. J. Biochem. *142* (1984) 153) suggested that the 9.3 monoclonal antibody (moab) binds to a common site of receptors related to the T cell antigen receptor T_i. To test this hypothesis the response to antigen of a *M. leprae*-specific cell clone was measured (^{3}H-thymidine incorporation, 10^4 cells cocultured with 5×10^4 irradiated autologous antigen presenting cells). A 9.3 moab-concentration dependent inhibition was found. However, by FACS of normal T cells no comodulation of 9.3 and T3 antigens was found. It is concluded that the 9.3 antigen is located in the T3/T_i site of the cell surface; the identity of 9.3 antigen and T_i cannot be excluded, but more likely the 9.3 antigen together with T3/T_i are integrated into a receptor complex which functions as major mitogenic triggering site.

Development of an enzyme-linked immunoassay (ELISA) to quantitate human apolipoprotein A-I

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The inverse relationship between the risk of atherosclerosis and the plasma levels of high density lipoprotein cholesterol (HDL-C) has been firmly established. More recent studies have suggested that apolipoprotein A-I (apo A-I), the major protein component of HDL, may be a more precise indicator than cholesterol for the risk of atherosclerotic disease. As no single technique has been universally adopted for the routine clinical analysis of apo A-I, we have investigated the potential of the ELISA system. In the preliminary studies reported here, an ELISA for purified human apo A-I has been developed. It can detect as little as 0.1 ng of apo A-I, with a working range of 0.2 to 2.0 ng. Intra- and interassay coefficients of variation were 7.25 ($n = 24$) and 7.3 ($n = 4$) respectively. No reactivity with apolipoproteins A-II, C, and E, or with albumin, was observed. Parallelism of the dose-response curves for purified apo A-I and whole plasma suggests that the system can be applied to measuring serum levels of apo A-I.

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Cell surface sialic acid binding fimbriae in *Escherichia coli* strains from newborn meningitis

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Binding to specific receptors on the host epithelial cells is recognized as an important virulence factor for pathogenic bacteria. We have shown that several *E. Coli* strains bind to sialic acid containing structures on human erythrocytes. Hemagglutination inhibition studies with purified sialyloligosaccharides indicated that the binding activity has specificity for the sialyl (α2–3) galactoside sequence. The bacterial structures mediating binding to sialyl galactosides were identified as fimbriae. These S-fimbriae were morphologically similar but immunologically non-crossreactive with other fimbriae of *E. coli*. S-fimbriae were associated with *E. coli* strains isolated from neonatal sepsis (29%) and meningitis (36%) as compared to *E. coli* strains isolated from other infections (4–7%). The possible pathophysiological role of S-fimbriae in septic neonatal infections is under study.

Aortic collagen induces aggregation of washed fixed platelets in the presence of factor VIII/von Willebrand factor

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Adhesion of human blood platelets onto deendothelialized blood vessels is mediated by factor VIII/von Willebrand factor (FVIII/vWF). The exact vascular structures responsible for platelet adhesion are unknown; both collagen and non-collagenous microfibrils have previously been implicated. We have succeeded in isolating a fibrous protein from bovine aorta which is insoluble under physiologic conditions. In the presence of human FVIII/vWF washed fixed human platelets adhere onto isolated fibrous protein. Molecular size of subunits, amino-acid composition and binding of fibronectin indicate that the fibrous protein is collagen. Suspended aortic collagen is stable in chaotropic salt (e.g. guanidinium chloride solutions). It can be used as a reagent for measuring FVIII/vWF-dependent platelet adhesion onto collagen in a simple turbidimeter. Factor VIII-collagen cofactor activity, determined by this turbidimetric assay, is reduced in plasma from patients with von Willebrand's disease.

Isolation of the two functional domains of PRA isomerase IGP synthase from *E. coli*

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Bifunctional enzymes may have evolved by gene fusion from single function enzymes. We have attempted to reverse the putative gene fusion event by expressing the functional domains of a bifunctional enzyme separately. To separate the N-terminal protein fragment of PRA isomerase: IGP synthase a stop codon was introduced by site-directed mutagenesis at the predicted gene fusion site. To get the C-terminal protein fragment the proximal part of the *trp*C(F) gene was deleted by Bal 31 digestion and religation of the first few base pairs of the *trp*C(F) gene with the predicted gene fusion site. Desired mutants were selected by in vivo complementation of a *trp*C$^+$(F$^-$) host. Both mutations were confirmed by restriction enzyme analysis and DNA sequence determination. Their purified gene products were stable and showed the expected enzyme activities.

Semisynthesis of cytochrome *c*-T analogues

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Cytochrome *c*-T, formed by the noncovalent association of the two fragments produced by limited tryptic cleavage between Arginine 38 and Lysine 39, is a useful model for structure-function studies in this region of cytochrome *c*. Modifications at the carboxyl terminus of fragment 1–38 and at the amino terminus of fragment 39–104 have been performed, and the functional consequences investigated.
Reformation of the 38–39 bond has been attempted through activation of the α-carboxyl group of fragment 1–38 by an enzymatic route. This technique has been extended to creating activated carboxyl groups on fragments 1–37 and 1–39.

Functionally defective fibrinogen resulting from replacement of aspartic acid by valine in position γ-330

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The molecular defect of a new congenitally abnormal fibrinogen, fibrinogen Milano I, has been characterized. Following thrombin treatment, fibrinopeptide release from fibrinogen Milano I was normal but the subsequent fibrin polymerization delayed. A charge anomaly was detected in the carboxy-terminal portion of the γ-chain. After cyanogen bromide cleavage of either intact fibrinogen Milano I or of the reduced γ-chain, an abnormal peptide was isolated by HPLC. Sequencing of this γ-chain fragment showed an amino-acid substitution Asp→Val at the position 330. This is the first molecular defect identified in the QFST-fragment of the γ-chain comprising presumably binding sites for calcium and/or for the N-terminal sequence of the fibrin α-chain.

Selective uptake of neuroactive amino acids by oligodendrocytes in primary culture

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It has been suggested that glial cells may modulate the level of neuronal excitability in the CNS by the uptake and metabolism of neuroactive amino acids. We have shown previously, using autoradiography and immunocytochemistry, that oligodendrocytes in primary culture accumulate ^{3}H-GABA via a sodium dependent, ouabain sensitive, transport system. We now show that, in contrast to GABA, oligodendrocytes show virtually no labelling with ^{3}H-L-glutamate and L-aspartate, which label almost exclusively astrocytes. When the cultures are incubated with ^{3}H-D-aspartate, a nonmetabolized analogue of glutamate, oligodendrocytes become labeled after short incubation periods. This suggests that the excitatory amino acids may be transported but are rapidly metabolized and the metabolites released. Oligodendroglial uptake of β-alanine, glycine, or taurine was not observed under any conditions. Thus we think that oligodendrocytes, in addition to astrocytes, may be involved in the control of the extracellular concentration of neuroactive amino acids.

Endocytosis in yeast

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Uptake of fluid by endocytosis can be followed in *Saccharomyces cerevisiae* cells using a fluorescent dye, lucifer yellow CH.

Uptake of the dye is time, temperature and energy dependent and is not saturable. Internalized lucifer yellow is accumulated in the vacuole. Many of the yeast mutants conditionally defective in secretion (Novick, P., Fields, C., and Schekman, R., Cell *21* (1980) 205) are also defective in endocytosis. Analysis of these mutants leads to the following conclusions. Efficient transport of proteins from the endoplasmic reticulum to the Golgi apparatus and from the Golgi apparatus to secretory vesicles is not necessary for endocytosis. Part of the endocytic pathway may be obligatorily coupled to the late stages of secretion. We are isolating mutants which are defective in endocytosis but not in secretion. At least one such mutant will be described.

Towards purification of the outershell polypeptide (VP7) specific for the newly identified human serotype 4 rotavirus

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Differential centrifugation methods used to purify Hochi serotype 4 rotavirus from infected MA104 cells resulted in a poor production of outershell containing particles. However SDS-PAGE analysis of the unreduced virus revealed a 32 kD protein identified as VP7 and, by treating the virus with 1 mg/ml digitonine plus 2% NP40, the main component to be solubilized and isolated by isoelectric focusing was VP7 (pI = 4.9–5.1). This technique can be envisaged to purify VP7. In addition, it was observed that, when Hochi virus was extracted by trichlorotrifluoroethane (freon), VP7 was 'lost' and recovered at the interphase between the freon and the aqueous phase.

Inactivation and stabilization of glucose-6-phosphate dehydrogenase in extracts from germinating wheat

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Glucose-6-phosphate dehydrogenase was considerably more stable in endosperm extracts (pH 5.4) from ungerminated than from germinated wheat seeds. The stability in vitro decreased after germination for 2 days at 24 °C. Aminopeptidase and pyrophosphatase were affected in these extracts in a similar manner, becoming more labile during germination. A simultaneous increase in endopeptidase activity suggested an inactivation of these enzymes by proteolysis. This hypothesis was supported by the fact that added casein was an effective protectant. The inactivation of glucose-6-phosphate dehydrogenase was delayed in presence of inorganic phosphate, NADPH, NADP or NADH. A stabilization by pyridine nucleotides was also observed when the rapid inactivation of this enzyme in extracts from ungerminated seeds was initiated by the addition of purified trypsin or chymotrypsin.

Simultaneous determination of SST, SST-28 and LHRH in rat median eminence (ME) by HPLC with electrochemical detection (ECD)

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The sensitivity of an HPLC assay depends equally on detector performance and peak shape: e.g. with increasing retention time (RT), peaks become undetectable. On a RP-18 column that adequately separates LHRH (RT < 5 min), SST in ME extracts are eluted as broad, flat, ECD-undetectable peaks (RT > 60 min). Since ECD, at high gain, does not tolerate solvent or temperature gradients, this problem had to be solved with a two-column-switching technique: a short RP-18 column (SC) was connected over a valve to a long RP-18 column (LC). In valve position (VP1), the sample was injected and flowed to the SC, the LC and the ECD. LHRH was detected, after having passed over the SC and the LC, at 3.5 min RT. Then, the valve was switched to VP 2 (flow: pump-LC-SC-ECD) allowing the detection of SSTs as they elute from the SC only (RTs: SST-28 5.5, SST 8.0 min). After a total run time of less than 10 min the valve was switched back to VP1 and the next sample injected. The following peptide levels were found: LHRH 2, SST 12 and SST-28 20 ng/ME.

A rapid and sensitive method to determine N-formyl-methionyl, N-acetyl-methionyl and methionyl residues at the N-terminus of proteins and polypeptides

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When polypeptides and proteins are produced by means of recombinant DNA techniques, it is occasionally found that an initiator methionyl residue, formylated or not, remains attached to the target polypeptide chain. Particularly in the case of the formylated species, it has been difficult to quantitate such modifications, which are potentially antigenic. We have developed a method, based on combined gas liquid chromatography – mass spectrometry, for the determination of methionyl and acyl-methionyl species. The method is compatible with the micropermethylation procedure (Rose et al., Biochem. J. *215*, 261–272; *217*, 253–257), which may be performed on the same sample to permit a more general analysis.

The primary structure of the heavy chain of porcine plasminogen. Comparison with human and bovine plasminogen

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The heavy chain of porcine plasminogen (560 residues, Mr 58,049) was cleaved with CNBr, elastase, V8-protease and clostripain. The fragments were separated by chromatographic methods and their sequence determined with a Beckman sequencer. Comparison with the known primary structures of human and bovine plasminogen shows an overall homology of 71.5%. The five kringles have with 77.6% a higher degree of homology than the connecting strands between the kringles (54.1%). In all three species ASN 289 (kringle 3) is only partially glycosylated to various extents. The O-glycosidic site in human plasminogen in Thr_{345} and in bovine plasminogen Ser_{339}, both located in the connecting strand between kringles 3 and 4. In porcine plasminogen, however, the O-glycosidic site is shifted to Thr_{249} in the connecting strand between kringles 2 hand 3.

Antiserum against a synthetic peptide duplicating a polymorphic determinant of HLA-B_7 recognizes HLA

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In order to define segments of HLB-B_7 and A_2 involved in target cell recognition of cytotoxic T-cells , peptides duplicating polymorphic stretches of HLA have been synthesized. Peptides I (HLA-A_2 143–160), II (HLA-B_7 143–160), III (HLA-A_2 143–152), IV (HLA-B_7 143–152) and V (HLA-A_2, B_7 153–160) have been synthesized by the solid phase method. Peptides II and IV have been coupled to KLH and antibodies have been raised in rabbits. In ELISA assays, anti-II was found to bind to peptides I, II and V but not to III and IV. That implicates that anti-II is directed against the C-terminal segment of peptide II. Anti-IV recognizes IV but not III. In immunoblots of cell membranes

from a lymphoblast line anti-II labels specifically a band of 43 kD.

Microheterogeneity of trypanosomal tubulins

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Total tubulin, isolated from *Trypanosoma brucei* by taxol induced polymerization, was analyzed by isoelectric focusing. Alpha and betasubtypes were identified by western blot analysis with alpha and beta specific antibodies and by tyrosinolation of the C-terminus of alpha-tubulin. At least five major alpha and 5 major beta-isotubulins could be distinguished. The isoelectric points and the extent of the microheterogeneity were different from mammalian tubulins.

The microheterogeneity of the alpha-tubulin was not due to posttranslational tyrosinolation.

In *T. brucei* there are three different pools of tubulin: cytoplasmic, pellicular and flagellar tubulin. Preliminary experiments show that an alpha-isotubulin is enriched in isolated flagella when compared to total trypanosomal tubulin.

Complete amino-acid sequence of m-type thioredoxins from spinach chloroplasts

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The complete primary structure of thioredoxin mc from spinach chloroplasts has been sequenced by Edman degradation and carboxypeptidase digestion yielding 103 residues which add up to a mol. wt of 11,425. Only 19 common amino acids are present. As already reported (Tsugita, A., Maeda, K., and Schürmann, P., Biochem. biophys. Res. Commun. *115* (1983) 1) thioredoxin mc contains the same active site sequence as thioredoxins from other sources and reveals a high degree of homology around the active site. Compared with the thioredoxin from *E. coli* the overall homology however is 44% mainly due to differences in the N-terminal and C-terminal regions of the protein. A molar absorption coefficient at 280 nm of 19,300 $M^{-1}\,cm^{-1}$ has been determined. In contrast to other thioredoxins there is no Trp fluorescence increase upon reduction indicating a possibly different spacial configuration near the active site.

Determination of LHRH in rat median eminence (ME) by HPLC with electrochemical detection (ECD)

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Peptides containing oxidizable amino acids can be directly quantified in biological material by HPLC-ECD. Although this approach is less sensitive than RIA, it has certain distinct advantages such as simplicity and specifity. Single rat ME's were homogenized in 25 µl 1 M acetic acid, centrifuged, the supernatant neutralized with 25 µl column buffer; 20 µl were injected. LHRH eluted after 7 min, was detected and quantified by ECD. Chromatographic conditions: column: RP-18, 2.1 × 100 mm; buffer: phosphate 300 mM, pH 5.5, containing 1-propanol 7% (vol); flow: 0.4 ml/min; detector: glassy carbon (BAS) at 1.0V vs Ag/Cl. The specifity of the method was verified: 1) No peak at 7 min when brain tissue other than ME was processed. 2) Spiking of ME extract with LHRH resulted in a single, higher peak with no distortion. 3) When the LHRH peak was collected and subsequently assayed with RIA, identical values were obtained; fractions adjacent to the peak gave the RIA blank.

Intramolecular equilibria in binary and ternary complexes of adenine-nucleotides

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As substrates for many enzymic reactions adenine-nucleotides are receiving much attention. Their self-association via the base moieties is promoted by metal ions (Scheller and Sigel, JACS *105* (1983) 5891), but below concentrations of 1 mM the monomeric complexes dominate and macrochelates are formed by intramolecular coordination of M^{2+} to the phosphate moiety and to N-7, leading to equilibria between 'open' and 'macrochelated' isomers: e.g. 38% of $Mn(ATP)^{2-}$ and 62% of $Zn(ATP)^{2-}$ are present in the closed form. The extent of macrochelation varies: usually $\%M(AMP)_{cl} < \%M(ADP)^{-}_{cl} > \%M(ATP)^{2-}_{cl}$ (Sigel und Scheller, EJB *138* (1984) 291). In ternary M(ATP) (amino acid)$^{3-}$ complexes with tryptophanate or leucinate the stacking or hydrophobic interactions between the adenine moiety and the indole or isopropyl residues give also ris to intramolecular equilibria (Sigel et al., Inorg. Chem. *22* (1983) 925). Preliminary studies with Cu^{2+}/ATP/2,2'-bipyridyl indicate that these equilibria are not very solvent dependent.

Mercury-bacteriorhodopsin: selective heavy metal modification of the transmembrane protein

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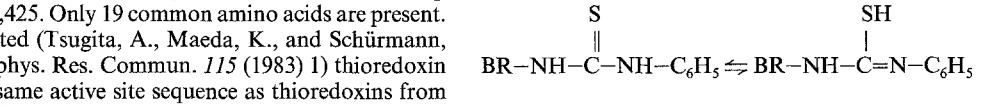
Selective heavy metal modification of bacteriorhodopsin (BR) is achieved by treatment of the phenylthiocarbamoylated protein with ethyl mercury chloride. The phenylthiocarbamoyl derivative of the modified lysine-41 ε-amino group shows considerable tautomerism.



As a consequence it was possible to prepare a heavy atom derivative of BR, arranged crystalline in the purple membrane. Optical investigations including circular dichroism document invariable chromophore-protein and protein-protein interactions in mercury-labeled purple membranes. The data indicate that the incorporation of mercury is as specific as the phenylthiocarbamoylation reaction which is directed to lysine 41 (Sigrist et al., J. molec. Biol. *173* (1984) 93). By this approach an electron dense reference can selectively be appointed in the tertiary structure of the protein.

Partial sequence analysis of the human $\bar{C}ls$ heavy chain. Sequence comparison with the α^2-chain of human haptoglobin

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Partial amino-acid sequence of human Cls heavy chain (M_r 57,000) was determined with fragments produced with CNBr, BNPS-skatole and trypsin. CNBr cleavage gave at least five fragments (M_r between approx. 470 and 22,000). The three smaller ones, including the C-terminal part of the heavy chain (145 amino acids) consisting of two CNBr fragments (CB3: 101 residues, C-terminal fragment CB4: 44 residues) were completely sequenced except for two positions. The two larger ones were characterized by their N-terminal sequences.

The C-terminal part of Cls heavy chain (145 residues) shows 30% sequence identity with the α^2-chain of human haptoglobin. The cysteine residues involved in disulfide bonds found in both parts of the molecules are located at identical positions.

The two larger CNBr fragments show intrachain sequence homology. 2 N-glycosylated asparagines were identified, one of them localized in the C-terminal CNBr fragment.

Location of a structural probe on the transmembrane peptides of the human erythrocyte band 3 protein

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The anion transport protein of the human erythrocyte membrane, band 3, possesses distinct membrane-integrated domains. Considerable interest has focussed on two segments of band 3, both of which are known to span the membrane and to partake in the anion transport function. These segments (apparent mol. wt 17,000 and 10,000 D), are selectively modified by 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate (DABITC). Generated by thermolytic digestion of DABITC-treated erythrocyte ghosts, the labelled peptides are purified by chromatographic procedures. Both peptides are characterized with respect to DABITC-binding and identified by their N-terminal amino-acid sequence. The location of DABITC within the protein is essential with regard to the investigation of the transmembrane protein structure by means of the physical and chemical properties of this reagent.

PABA-peptide hydrolase (PPH): a brush border membrane-bound, divalent metalion-dependent endopeptidase which hydrolyses angiotensins, bradikinin and substance P

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PPH has been purified by immunoaffinity chromatography using a monoclonal antibody and further characterized. SDS-PAGE under reducing or nonreducing conditions yielded polypeptides with apparent Mrs of 150 kD and 200 kD respectively suggesting that the native enzyme is a dimer. PPH acitivity was not inhibited by P_i, pepstatin, jodoacetate, PMSF, phosphoramidon and captopril (1 mmole/l). Inhibitors were DTT and EDTA; the EDTA-inhibited enzyme could be reactivated by metalions, particularly Mg^{2+}. The pH optimum for the enzyme was between 7.5 and 8.0. The K_m determined with N-Bz-L-Tyr-PABA was 16.7 mmoles/l. PPH hydrolyzed biologically active peptides including β-chain of insulin, angiotensin, bradikinin and substance P. Thus the enzyme is a Mg^{++}-dependent neutral endopeptidase located in the brush border membrane of the enterocytes.

Development of a method for the isolation of brush border membranes (BBM) from rabbit large intestine

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In order to elucidate the physiological functions of the colonic epithelium on a subcellular level a procedure was developed for the isolation of colonic BBM. The method is based on the purification of intact brush border caps (monitored by phase contrast microscopy) of isolated colonocytes by Percoll gradient centrifugation followed by Tris-disruption. The vesiculated BBM were further subfractionated on sucrose density gradients. Since no accepted marker for the colonic BBM is known the validity of our approach was tested and confirmed by its application to the small intestine. The results show that the distal but not the proximal colonic BBM possess ouabain-insensitive K^+-ATPase activity. However, they cast doubts on the usefulness of alkaline phosphatase as a marker enzyme for this membrane.

Gluconeogenesis in vitro: formation of hexose-6-phosphates from lactate + glutamate in a cell-free system derived from rat liver

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Few attempts have been made to study the regulation of the whole gluconeogenic chain in vitro due to the lack of a suitable system. In our cell-free system containing rat liver cytosol and mitochondria plus a number of cofactors and gluconeogenic intermediates at near physiological concentrations, we found a production of hexose-6-phosphates of 0.82 ± 0.05 μmoles $\times$ min^{-1} $\times$ g $liver^{-1}$ (mean $\pm$ SEM, n = 8, 37 °C) from lactate + glutamate. This rate was measured between 20 and 60 min incubation time when the system was near steady state. Elevation of the redox ratio by addition of increasing amounts of lactate + pyruvate (10:1) or of ethanol resulted in an inhibition of the gluconeogenic rate because of a drop in the oxaloacetate concentration. Furthermore, the gluconeogenic rate was found to be regulated by the nucleotide ratio and by the nutritional state of the rats used for the preparation of the cytosols.

Sequence and topology of human complement component C9

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A partial nucleotide sequence of human complement component C9 cDNA representing 94% of the coding region of the mature protein is presented. The amino-acid sequence predicted from the open reading frame of this cDNA concurs with the amino-acid sequence at the amino terminal end of three proteolytic fragments of purified C9 protein. No long stretches of hydrophobic residues are present, even in the C9b domain which reacts with lipid soluble photoaffinity probes. Secondary structure predictions within this region however support the possibility of amphiphilic helices with a high hydrophobic moment which could interact with membrane lipid molecules. The C9a domain has an unusual arrangement of cysteine residues which might correlate with the inert nature of C9 once incorporated into the membrane. The binding of various monoclonal antibodies to hybrid beta-galactosidase proteins containing domains of the C9 protein, and to proteolytically or chemically cleaved fragments of C9 aligned on the sequence by amino-acid sequencing, has been used to study the topology of monomeric and poly-C9.

Specific ^{3}H-DSIP binding sites in the rat brain

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A binding assay for detection of specific DSIP binding sites in the CNS of the rat has been developed. The degradation of ^{3}H-DSIP (exclusively labeled at the N-terminal Trp) by rat-brain membranes could be fully prevented in the presence of the inhibitors O-Phenanthroline (0.5 mM) and PCMBA (0.03 mM). However, in this system DSIP binding wàs also impaired.

Finally, specific DSIP binding could be detected after elimination of the main degradative process to Trp by using DSIP (2–9) as displacer. Optimalization of this system in a fresh P2 membrane pellet of rat brain minus cerebellum revealed one binding site for DSIP (2–9) with a Kd of 14 μM and a Bmax of 260 pmoles/mg protein.

In the presence of 160 mM NaCl, a sevenfold reduction occurred in DSIP (2–9) affinity without a change in the Bmax. The affinity spectrum of several smaller DSIP fragments indicated a crucial

role for position 5 (Asp) of DSIP (2–9) in its interaction with the ^{3}H-DSIP binding site. A primary estimation of the brain distribution pattern revealed highest specific DSIP binding in the cortex and significantly less in cerebellum and medulla-pons regions.

Regulation of ferredoxin-sulfite reductase from *Pisum sativum* L.

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Since ferredoxin-dependent sulfite reductase (Fd-SiR; EC 1.8.7.1) can catalyze the reduction of SO_3^{2-} and NO_2^-, physiological and biochemical evidence is needed for properly naming this enzyme activity. It is inhibited by 100% with 30 µM sulfide produced by itself during the assay. 100 µM sulfide added to the assay mixture completely inhibited SO_3^{2-} reduction in the crude extract, the 30,000 × g pellet and its respective supernatant. In the second leaf of 7 days old dark grown pea plants Fd-SiR and ATP-sulfurylase (ATPase; EC 2.7.7.4) were at 24 and 14% of their maximal activity. After 11 and 12 days, respectively, both enzymes were no longer detectable. The inhibition by sulfide and the changes in activity during ontogenesis similar to ATPase represent biochemical and physiological evidence for the correct naming of Fd-SiR in *Pisum sativum* L.

Fructose-6-phosphate/fructose-1,6-P_2 cycle in rat liver cytosol

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Glycolytic and gluconeogenic fluxes in a rat liver cytosolic system were measured at the site of fructose-6-phosphate/fructose-1,6-P_2 cycle. The activities of phosphofructokinase (PFK) and of fructose 1,6-bisphosphatase (FBPase) were determined by metabolite formation measured after 10 min at 30 °C. Cycling was determined by measurement of PFK dependent ADP production. In the presence of the respective substrate for either PFK or FBPase, a significant cycling was found between pH 7.7 and 8.5. Liver cytosols from 48 h starved rats showed a higher recycling of fructose-6-phosphate than those from fed rats. When the substrates for both enzymes were added, the fluxes through PFK and FBPase were about the same as when either substrate was added alone e.g. both fluxes occurred simultaneously. Furthermore, the inhibition by citrate of PFK was partially released after addition of glyceraldehyde 3-phosphate. The results show that this cytosolic system allows the determination of simultaneous fluxes through PFK and FBPase by metabolite measurement.

Incorporation of totally synthetic peptides in the semisynthesis of cytochrome *c*

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In protein semisynthesis, fragments of the native protein are prepared by chemical or enzymatic cleavage. After appropriate modification, the fragments are recombined by chemical (or enzymatic) condensation to form a specific analogue.

In the case of vertebrate cytochromes *c*, two such fragments obtained by CNBr cleavage will spontaneously recombine in aqueous buffers. This phenomenon has been used to advantage in semisynthesis of cytochrome *c* analogues and can be employed to make interspecific hybrids.

We report the preparation, by total synthesis, of fragments of sequence corresponding to residues 67–108, 70–108 and 71–108 of the *saccharomyces* protein. Recombination of these fragments and the native 1–69 haem fragment of yeast cytochrome *c*, or the corresponding 1–65 fragments of cow or horse cytochrome *c* was attempted. The functional properties of the products of successful couplings were examined.

Nematocyst of *Hydra:* isolation and general properties of toxic and other components

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Nematocysts are the highly specialized organelles of stinging cells typically to and exclusively found in most Coelenterates (corals, jellyfishes, sea anemones). With one representative of this phylum, the freshwater polyp *Hydra*, we have developed a new, rapid and efficient method for the isolation and preparation of pure undischarged cysts. They contain a high amount of protein and peptides and a total magnesium-calcium concentration exceeding one molar. On the other hand cysts contain only small amounts of sugar. The soluble components of these capsules have rapid lethal effects on *Drosophila* (cp. Klug et al., same symposium) and exert strong hemolytic action upon human erythrocytes. Furthermore phospholipase and low protease activities are detectabel. Gel-filtration chromatography permitted a rough fractionation of the nematocyst extract without loss of the toxic property.

Age-related changes in liver specific gene expression

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The effect of aging on tyrosine aminotransferase (TAT) and tryptophan oxygenase (TO) gene expression was studied. In aging rodents, TAT induction is delayed during short term exposure to cold and fasting. Aged rats show no impairment of adenocortical function during physiological stress, as measured by corticosterone levels. We showed that pharmacological levels of glucocorticoid induced TAT enzyme activity and mRNA levels to the same extend in both age groups, implying no impairment in the maximal ability to synthesize them in aged rats. However, under physiological conditions, TAT mRNA levels paralleled changes in enzyme activity. Both were lower before and after cold stress as compared with young animals. TO mRNA induction showed no such age related difference after cold stress. No differences in transcription rate were observed between adult and old rats for both genes. These results suggest an age-related post-transcriptional difference under physiological conditions which is specific for TAT.

Effects of leukocyte elastase on structure and function of human platelet membrane glycoprotein Ib

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Human blood platelets, either simply washed or suface-labelled by the periodic acid/NaB^3H_4 technique were incubated with purified human leukocyte elastase for various times. They rapidly lost their ability to aggregate with bovine von Willebrand factor (bvWf) or human vWf/ristocetin, showed an increasingly slower shape change and, after prolonged treatment, a delayed and decreased aggregation response to thrombin. PAGE analysis revealed that concomitantly with the loss of bvWf-aggregation a 45-kD piece is cut off from the extracellular end of GPIbα. Polyclonal rabbit-antibodies against the purified 45-kD glycopeptide had the same effect on the action of bvWf and thrombin on platelets as cleavage of GPIbα by elastase. This strongly indicates that the binding sites for bvWf and thrombin

on GPIbα are in th e 45-kD globular end region. This thrombin binding site, while not essential for platelet activation, has a strong influence on the kinetics of the activation process.

Retinal benzodiazepine receptor binding

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Dopamine synthesis in the amacrine cells of the mammalian retina shows circadian rhythmicity, is stimulated by light, and this stimulation is suppressed by GABA agonists and benzodiazepines. There are 25% more specific binding sites for flunitrazepam (Bmax: 490 ± 16 fmoles/retina, N = 6) than for the antagonist Ro 15-1788 (Bmax: 396 ± 20 fmoles/retina, N = 6). We attempted to characterize a circadian rhythm in retinal benzodiazepine receptors throughout 24 h under 12:12 light:dark (LD) conditions, in continuous darkness (DD) and continuous light (LL), and after a 5-h phase advance or phase delay of the LD cycle. No clear circadian rhythm could be demonstrated, although light-dependent changes were observed. Chronic imipramine treatment induced a rhythm in the Bmax of both ligands that followed a parallel time course: maximal binding at the end of the subjective day and minimal binding at the end of the subjective night (DD). Imipramine significantly decreased mean flunitrazepam and Ro 15-1788 binding by 23% and 16% respecitively; this decrease was manifested only in the subjective night.

Actin-binding proteins from brain cytosol

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The bovine brain extract was chromatographed on DEAE- and CM-cellulose and assayed by viscometry for its ability to change the state of actin. Two peaks of calcium-independent actin gelation activity and three peaks of actin restricting activity were obtained. Following molecular exclusion chromatography, the major component of gelation activity appeared as a homogeneous, monomeric protein of about 55,000 mol. wt. The most abundant component of actin restricting activity was related to complexes of actin and several actin-binding proteins recently isolated from brain in other laboratories, including the 90,000 D Ca^{2+}-dependent protein. The two other components of actin restricting activity were free of actin and further chromatographed on hydroxylapatite and Sephacryl S-200. One of them was reminiscent of the 19,000 D actin depolymerizing protein originally isolated from chick-embryo brain. The second component of actin restricting activity coincided with a polypeptide of about 40,000 D. The latter protein preparation reduced the low-shear apparent viscosity of actin filaments and inhibited the rate of actin polymerization provided that Ca^{2+} was present.

Effects of stimulators and inhibitors of human platelet activation on phosphorylation of GPIb and other proteins

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Changes in phosphorylation of receptor structures are often related to cellular activation mechanisms. Platelet GPIb is the receptor for von Willebrand factor and a receptor for thrombin and is the only phosphorylated membrane glycoprotein. Washed human blood platelets were incubated for 60 min with [^{32}P]-orthophosphate. After washing they were treated with thrombin, forskolin and a stable prostacyclin analogue. The various platelet preparations were solubilized and separated by 2D PAGE (O'Farrell or nonreduced-reduced) alkaline treatment of the gels gave a marked decrease in the ^{32}P content of the spot identified as GPIbβ, indicating that it is phosphorylated predominantly on serine or threonine residues. This was confirmed by hydrolysis of GPIbβ and analysis by 2D thin-layer electrophoresis/chromatography. Tryptic phosphopeptide mapping of GPIbβ from unactivated and thrombin activated platelets were both similar. Forskolin and the prostacyclin analogue both raised phosphorylation of P22/24, a hydrophobic membrane protein not detected by surface-labelling techniques.

CELLULAR AND MOLECULAR BIOLOGY

Transposon-like elements in the chromatin eliminating nematode *Ascaris lumbricoides*

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A clone has been isolated from the gene library of germ line DNA of *A. lumbricoides* which contains a transposon-like element of about 7 kb length immediately adjacent to satellite DNA sequences. This element (TAS) proved to be structurally very similar to the transposons Ty in yeast and Copia in *Drosophila*. TAS is present in 50–100 copies per haploid germ line genome and exists in at least 2 different molecular forms. TAS 1 occurs at many different chromosomal sites. Whereas our results indicate that the flanking sequences on the left side of TAS 1 are stable, those adjacent to the right side have undergone rearrangements and/or excisions during development. However, copy number and structure of TAS 1 remained unchanged. In contrast to TAS 1, TAS 2 becomes eliminated from the somatic genome during embryonic development, thus indicating that it may be active in somatic cells. We are currently testing whether this transposable-like element is directly or indirectly involved in the process of chromatin elimination.

Structure and regulation of genes involved in the nitrogen fixing *Rhizobium japonicum* soybean symbiosis

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A total of eight *Rhizobium japonicum* genes involved in symbiotic nitrogen fixation were cloned and characterized. They are organized in two clusters. One cluster contains the genes *nifDK-nifB-nifH-fixBC* whereas the other one contains the genes *fixA-nifA-nod*. *NifDK* and *nifH* are the nitrogenase structural genes. *NifB* is presumably involved in MoFe cofactor incorporation. *NifA* appears to be a regulatory protein. *Nod* is responsable for nodulation. The functions of *fixA* and *fixBC* are unknown.

These gene clusters are surrounded by a large number of repeated sequences. So far, three different RS elements were detected: RSRjα is present in 12 copies per genome, RSRjβ with six copies, and RSRjγ with 6 to 7 copies. The promoters of *nifDK*, *nifH*, and *fixA* were sequenced and located by nuclease Sl mapping. They share homology with each other and with *nif* promoters from other nitrogen fixing bacteria. In *E. coli* the *R. japonicum nifDk* and *nifH* promoters are activated by the gene products of *nifA* plus *ntrA*, but not by the gene products of *ntrC* plus *ntrA*.

Characterization of the prosome from *Drosophila* and its similarity to the cytoplasmic structures formed by the low MW heat shock proteins

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We have identified and characterized a ribonucleoprotein structure from the cytoplasm of *Drosophila melanogaster* tissue culture cells which is equivalent to the prosome a recently described ribonucleoprotein particle of duck and mouse cells (Schmid et al., EMBO J. *3* (1984) 29). Small RNAs species are associated with the *Drosophila* prosome. One of them has a strong sequence homology with the U6 mammalian small nuclear RNA. During the recovery period following a heat shock, the low mol. wt heat shock proteins form cytoplasmic ribonucleoprotein particles which share several structural properties with the prosome.

Characterization of measles virus (MV) gene expression defects in cases of subacute sclerosing panencephalitis (SSPE)

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SSPE is a slow, fatal disease, associated with MV persistance, affecting the human central nervous system. To investigate differences distinguishing SSPE from lytic MV infections, sequences with intergenic regions of MV nucleocapsid RNA obtained from lytically infected MA160 or HeLa cells and from brain RNA of a SSPE case were cloned by using synthetic oligonucleotides as primers. Furthermore, MV-specific RNA species from 5 SSPE-cases were characterized a) by Northern blotting, using 6 probes specific for each of the viral genes and b) by in vitro translation followed by electrophoretic resolution of the resulting polypeptides immunoprecipitated with antibodies specific for single viral proteins. In each case different specific defects in mRNA and protein synthesis were found and no common denominator for all cases such as lack of matrix protein synthesis could be defined.

Actin mRNA levels in normal and proliferating rat aortic smooth muscle cells (SMC)

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Previous work has shown that normal rat and human aortic SMC express α, β and γ-actin isoforms in decreasing order of quantity; however, in intimal thickening after endothelial injury or in atheromatous plaques, β-actin is predominant. We have investigated the level of actin mRNA in SMC of normal rat aortic media, intimal thickening and media under thickening 15 days after balloon induced endothelial injury. Translation products were detected on 2-D gels prepared from reticulocyte lysates primed with RNA. Normal media produced a pattern of actin isoforms with predominance of β-actin. Intimal thickening SMC showed a decrease of α-actin translation compared to SMC of normal media and of media under thickening. We conclude that: 1) aortic SMC show a discrepancy between the pattern of actin isoforms translated by mRNA using a reticulocyte lysate and the expression of actin seen in 2-D gels of total cell extracts; and 2) α-actin mRNA is decreased in SMC proliferating after endothelial injury compared to normal SMC.

Characterization of peptide-containing rat sensory neurons in culture

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Sensory neurons from trigeminal and dorsal root ganglia of neonatal rats were grown in dissociated cell cultures. Non-neuronal cell growth was inhibited by cytosine arabinoside. The neurons contain immunoreactive peptides such as substance P (SP), somatostatin and vasoactive intestinal polypeptide. Peptide content of cultured neurons increases with age with a time course similar to that observe in vivo. The peptides can be released by depolarizing stimuli such as, high potassium (47 mM) or veratridine (10 µM). In addition, the neurons are sensitive to capsaicin (8 methyl-N-vanillyl-6-nonenamide). In concentrations as low as 10^{-8} M, capsaicin selectively releases SP. We conclude that neurons in culture express the characteristic properties of sensory neurons in vivo and are a valuable tool to study the biochemical mechanisms involved in the mediation of pain and inflammation.

Regulation of the promoters in SV40 chromosomes and construction of a substituted SV40 containing a *Drosophila* hsp70 promoter

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We have studied the chromosome structural elements and transacting factors which together regulate the late and early promoters of SV40. Isolated SV40 chromosomes were bound to hydroxylapatite and the chromosomal proteins recovered by washing with increasing concentrations of salt and urea. A protein fraction was obtained which, when added to in vitro transcription reactions with naked SV40 DNA templates, markedly stimulated the late promoter. Experiments using an electrophoretic band-shift assay suggested that protein(s) in the active fraction bound specifically to an SV40 DNA fragment containing the late promoter, giving rise to two distinct DNA-protein complexes of different electrophoretic mobility.

To study the role of chromatin structure in regulating the *Drosophila* hsp70 promoter we are inserting a DNA fragment containing this promoter into SV40. The hsp70 sequences replace a similarly sized stretch from the SV40 early region, giving a defective virus able to replicate in T-antigen-containing COS cells.

Cloning of telomeric DNA sequences of *D. hydei* with a modified microdissection and microcloning procedure

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Telomere specific DNA sequences differ between *Drosophila* species. To clone such sequences from *D. hydei*, a telomere specific clone from D. melanogaster was first used as a probe. Several clones with homology to this sequence were isolated from a *D. hydei* cosmid library. In situ hybridizations revealed

that these sequences do not hybridize to the chromosome ends of *D. hydei*, but label a few scattered sites on several chromosomes. To continue the characterization of telomere sequences in this species, we used a modified microdissection and microcloning procedure which will be described in detail together with the results on the clones analysed so far.

Isolation of a rat parvalbumin genomic clone

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Parvalbumin (PV) is a high affinity Ca^{2+}-binding protein. It is suggested to function as a soluble relaxing factor in fast contracting/relaxing muscle. Recently we have isolated PV cDNA clones and used them to study tissue distribution and developmental regulation of PV transcripts (Berchtold, M.W., and Means, A.R., PNAS, in press). One clone, 9d, contains 65 bp 5′ noncoding and 94% of the coding sequence. It was used to screen a Charon 4-A rat genomic library. A 2.8 Kb *Eco* RI fragment from the positive clone λCh 152, gives hybridization signals both with a 65 bp fragment from the 5′ (noncoding) and a 70 bp fragment from the 3′ (coding) sequence of the cDNA clone 9d. Initial dideoxy sequence analysis of the 2.8 Kb fragment revealed colinearity of the cDNA with the genomic clone. We are now sequencing this and other genomic DNA clones in order to compare structural properties of the PV gene with the genes for calmodulin (Simmen, R.C.M., et al., JBC, in press) and other Ca^{2+}-binding proteins. This should allow a better understanding of molecular mechanisms involved in the evolution of Ca^{2+}-binding protein.

Cell free synthesis of human galactosyltransferase (lactose synthetase a protein)

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By metabolic labeling of HeLa cells, precursor forms of galactosyl-transferase (GT) were shown to migrate as 45 K and 47 K bands on SDS-PAGE. In presence of tunicamycin to prevent N-glycosylation, these forms moved as 42 and 44 K bands, resp. To investigate whether these two precursor polypeptides of GT are synthesized by different mRNAs, we used HeLa cells as source of total RNA for cell free synthesis using a wheat germ extract. GT was isolated from the translation mixture by immunoprecipitation and identified on SDS-PAGE/fluorography. Specific bands were quenched by addition of soluble antigen before immunoprecipitation. In vitro translated GT was shown to comigrate on SDS-PAGE with metabolically labeled GT from tunicamycin-treated HeLa cells as two bands with 42 and 44 K, resp. The relative amounts of these two forms were the same after both in vitro and in vivo synthesis, the 44 K form being in excess over the 42 K form. The two precursor peptides were strikingly similar by peptide mapping; thus, they originate from two closely related mRNAs. These results also suggest that translocation of GT through the membrane of the rER is not followed by signal peptide cleavage.

DNA sequence requirements for late gene expression in vaccinia virus

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Vaccinia is a large animal DNA virus that expresses its genetic information in a temporally well-regulated fashion. As a first step towards identifying the DNA sequences involved in gene regulation, we have sequenced a late gene encoding a major structural polypeptide. Various DNA fragments derived from the 5′-flanking region of the gene and including the mRNA start site, were introduced into the coding sequences of the tymidine kinase early gene by homologous in vivo recombination. The ability of these translocated putative promoter fragments to regulate transcription was monitored during the infection cycle of the recombinant viruses by nuclease S1 analysis. We conclude that 100 base pairs of 5′-flanking sequence of a late vaccinia gene are sufficient for correct temporal regulation of transcription.

Biosynthesis of β-glucuronidase in mouse liver

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Three differently processed forms of β-glucuronidase were identified. One was found in the microsomal fraction (M), the other 2 forms both in a light lysosomal (LL) and a heavy lyosomal (HL) fraction. The synthetic relationship of the 3 forms was studied by injecting ^{3}H-leucine into mice possessing microsomal β-glucuronidase (and the binding protein egasyn; C57Bl/6) and lacking it (C57Bl/6 YBR Eg°). Tritium incorporation into β-glucuronidase was determined in the 3 cell fractions. In C57Bl/6 mice, the incorporation in the M fraction was maximal at 12 h. The disappearance of labelled enzyme from this fraction coincided with a parallel increase in both lysosomal fractions, with a maximum at 48 h in the LL, and at 72 h in the HL fraction. In C57Bl/6 YBR Eg° mice, incorporation was maximal at 4 h in the LL fraction and only after 12 h in the HL fraction. Findings suggest that 1) the microsomal form is a precursor of the lysosomal forms and 2) the light lysosomal fraction contains primary lysosomes which are converted into secondary lysosomes (HL fraction).

Transcriptional regulation of mouse mammary tumor virus (MMTV): studies with homologous and heterologous promoters

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Internal deletions and substitutions using molecular linkers were introduced in the MMTV DNA region responsible for the glucocorticoid regulation of transcription. Their effect on the MMTV promoter, fused to the coding region of the thymidine kinase (tk) gene, was studied in cells stably transfected with the altered DNA. Sequences between −92 and −123 bp, containing most of the proximal binding site for hormone-receptor complexes were not required. The analysis of further constructs is in progress. In combination with the tk promoter, MMTV sequences from −108 to −204 bp conferred hormonal responsiveness to tk transcription when fused at −79 bp, but not at −197 bp. In fusions at −79 bp, high constitutive levels of aberrant tk transcripts were observed, for which MMTV sequences between −149 and −204 bp were required.

Structural studies on the light-harvesting chl a/b protein complex

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The chlorophyll a/b light-harvesting protein complex (LHC-II) of pea contains two major, N-terminally acetylated polypeptides of 27 kD and 25 kD. DNA sequencing data on the 27 kD polypeptide has recently been established (Cashmore, A.R., Proc. Natn. Acad. Sci. USA *81* (1984) 2960).

In order to study the nature of the difference between the two LHC-II polypeptides we fragmented the complex by chemical cleavage and sequenced more than 80% of the polypeptides. No difference in the primary structure of the two polypeptides was found. In the light of these experiments the chlorophyll a/b light harvesting polypeptides must have a common ancestral gene. The hydropathy profile of the amino acid sequence indicates three extended hydrophobic segments and a shorter one which presumably form three (maybe four) membrane-spanning helices.

Analysis of the nuclear migration signal of nucleoplasmin

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Nucleoplasmin is the most abundant protein in *X. laevis* oocyte nuclei. When injected into the oocyte cytoplasm it migrates to the nucleus (Mills et al., JMB *139* (1980) 561). Dingwall et al. Cell *30* (1982) 449) showed that the sequences necessary for migration were in the 'tail' of the protein, while the 'core' fragment did not accumulate in the nucleus.

A cDNA library of small oocytes of *X. laevis* has been constructed in λgt11. A 654 bp nucleoplasmin cDNA clone has been obtained by screening with antibodies. It contains the complete coding sequence for the carboxy-terminal tail and half of the core. The tail shows homologies to histones and other nuclear migrating proteins. It also contains a row of 12 glutamic acids and 2 aspartic acids, interrupted by a glycine.

Initial experiments, where purified bacterial β-galactosidase-nucleoplasmin fusion proteins were injected into the cytoplasm of oocytes, indicate that, while β-galactosidase is excluded from the nucleus, a fusion protein containing the last 42 amino acids of nucleoplasmin is targeted to the nucleus.

Measurement of DNA-breaks in single nuclei of myogenic cells before and after terminal differentiation

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As cells specialize and withdraw from the proliferative cycle, concomitant changes in the chromatine structure of cell nuclei i.e. DNA accessibility may influence the capacity of DNA-repair systems. Proliferating cells (myoblasts) from the embryonal pectoral muscle anlage were cultivated in vitro and irradiated before and after terminal differentiation. Since primary cultures always contain minor populations of other cell types and different developmental stages, a single cell assay based on the orange to green fluorescence ratio of cell nuclei stained with acridine orange was employed. After paratial alkaline unwinding, this ratio can be used as a semi-quantitative measure of DNA damage.

Induction of DNA breaks by X-rays did not show significant differences between myoblasts and differentiated myotubes in the dose range tested. The time dependence of repair for both developmental stages displays similar kinetics. On the other hand, the protective action of radical scavengers such as cysteine during irradiation was clearly seen in this system.

Meiosis promoting factor-like protein from mitotic yeast (*Saccharomyces cerevisiae*) induces cell division in mammalian somatic cells

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A MPF (meiosis promoting factor) was described in the cytoplasm of maturating oocytes of amphibians and echinoderms. A MPF-like activity has been demonstrated in the 105,000 g supernatant of Saccharomyces cerevisiae which induces oocyte maturation after intracytoplasmic microinjection (Weintraub et al., C. r. Acad. Sci. Paris *295* (1982) 787). This factor was only found in yeast cells which were synchronized in G2M (cdc16), but neither in G1 (cdc4), nor in late mitosis (cdc15). This yeast MPF is probably a protein, non dialysable, protease, heat- and Ca^{++} sensitive. In order to test its activity as a mitotic inducer in somatic cells, yeast MPF was encapsulated in liposomes (cholesterol, L-α-phosphatidylcholine, L-α-phosphatydil-L-serine) which were freshly prepared by reverse phase evaporation. The internalization of MPF into the cells was measured in using 14C-BSA as internal standard. The MPF-containing liposomes were introduced into L-929 mouse fibroblasts which were cultured in serum-free medium containing colcemid 1×10^{-6} M. Active MPF (cdc 16) increased the number of mitosis. This increase was observed after 7 h and increased until 48 h. It was also dose-dependent from 0,02 to 4 pg protein/cell, as observed after 24 h of culture. Inactive yeast MPF preparations from cdc4, cdc15, or from heat-inactivated cdc16 were without effect on mitosis in L-929 cells. Parallel microinjections into oocytes gave the same negative result. This work is the first direct demonstration that MPF is not only a meiosis but also a mitosis promoting factor.

Developmental expression of late-embryonic and sperm histone genes of the sea urchin

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In the sea urchin there are at least four developmentally regulated histone gene families, i.e. the early-embryonic, late-embryonic, sperm and cleavage stage histone genes. We have cloned all different late and sperm H2A and H2B mRNAs by priming reverse transcription with an oligonucleotide which is complementary to the highly conserved palindromic regulatory sequence present at the 3′ end of most eukaryotic histone mRNAs. Measurements of the mRNA steady-state levels suggest that the two different sperm H2B genes are only expressed during spermatogenesis in contrast to the sperm H2A gene which behaves like a late gene during sea urchin embryogenesis. The mRNAs transcribed from the late H2A and H2B genes start to accumulate at the hatching blastula stage, when the early histone genes are inactivated, and are most abundant during gastrulation. Sequence analysis of the cDNA clones revealed that the histone gene variants originated about 500 Mio years ago and have been maintained ever since in the various sea urchin species indicating the importance of the developmental programme of histone gene expression in the sea urchin.

Characterization of promoter sequences of the mouse albumin gene

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We have cloned and characterized the albumin gene of mouse strain A. This gene is very efficiently expressed in liver parenchymal cells but also produces low levels of mRNA in brain, pancreas and kidney. The transcriptional start site used by hepatocytes has been mapped by S1 mapping experiments and nuclear run-on experiments. Sequence analysis reveals several 8-nucleotide purine-pyrimidine repeats located in a region between 100 and 250 nucleotides upstream from the transcription initiation site. The same region is also hypersensitive to DNAase treatment of liver nuclei. Fusion-genes have been constructed that contain variable amounts of albumin gene 5′ flanking sequences ligated to the chloramphenicol-acetyl-transferase gene. These constructs are transfected into fibroblasts and albumin producing hepatoma cell lines to identifiy the sequences that control albumin expression in a tissue-specific fashion.

Chemiluminescence (CL) and leukotriene (LT) formation in horse eosinophils: no correlation

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Earlier work has shown that lipoxygenase can generate CL in vitro and that inhibitors of lipoxygenase reduce CL emitted by intact phagocytes. To assess whether lipoxygenase is a source of CL in intact cells, we have compared CL and LT formation (as assessed by ion pair HPLC) in horse eosinophils using the Ca^{2+} ionophore A23187 as the stimulus. CL and LT formation followed different kinetics. No CL was detected in the absence of exogenous glucose but LT's were formed. Increasing the concentration of glucose in the medium led to an increase in CL and a shift from spasmogenic to B-type LT's. Reduction of Mg^{2+} from 0.8 to 0.5 mM completely suppressed LT formation whilst CL was similar to the control even in the absence of Mg^{2+} from the medium. Our results suggest that Cl and LT formation are independent phenomena in eosinophils and that the contribution of lipoxygenase to light emission is insignificant.

Replication of the tetracycline resistance plasmid pSC101

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We have a series of mutations in the replication genes of the E. coli plasmid pSC101 caused either by insertion in vivo of the transposon Tn*1000*, or in vitro of a segment of DNA carrying an antibiotic resistance marker. These mutations have enabled us to map the origin of replication, a gene, *repA*, whose product is essential for replication and regions implicated in the regulation of replication. The *repA* protein represses transcription of its own gene. We are currently isolating and analyzing mutations which affect replication control and using gene fusion techniques to map transcriptional units within the replication origin. We want to elucidate the molecular mechanisms which determine plasmid copy number.

Cotransfection experiments of *ras* genes and human tumor DNA in rat embryo fibroblasts

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Transformation of primary rat embryo fibroblasts (REF) by transfection of cloned oncogene DNA is known to require two functions: a 'transforming' function (e.g. *ras*) and an 'immortalising' function such as *myc* (Land et al., Nature *304*, (1984) 596). By inoculation of REF transfectants into nude mice, we are searching for a *myc*-like function present in human tumors and able to complement cloned *ras* DNA. Progressively growing tumors were observed when *ras* and *myc* plasmids were cotransfected. Cotransfection of *ras* plasmid with various cell line and primary human tumor DNAs yielded tumor nodules in a fraction of the mice. Southern blot data will be presented, examining the presence of human oncogenes in the DNA of these induced rat tumors.

Interleukin-3-dependent expression of the *c-myc* and *c-fos* proto-oncogenes in mast cell lines

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Using established lines of mast cell precursors and Northern blot analysis, we studied the effects of IL-3 on cellular proto-oncogenes. Upon removal of IL-3 from the culture medium, factor-dependent cells stop dividing. During the first seven hours, however, normal amounts of total cellular mRNAs are maintained and this is reflected in unchanged levels of several transcripts, such as actin, c-Ha-*ras* and c-*fes*- In contrast, within 1½ h of IL-3 removal, the levels of c-*myc* and c-*fos* mRNAs decrease to very low levels, and addition of IL-3 at this stage quickly induces back the levels found in actively growing cultures. In factor-independent cells, which proliferate actively even in the absence of IL-3, high levels of c-*myc* and c-*fos* transcripts are maintained in the absence of the growth factor. The data suggest a model of hemopoiesis in which the c-*myc* and c-*fos* proto-oncogenes play an important role in mediating the proliferation- and differentiation-promoting effects of IL-3 on progenitor cells.

Changes of plasma membrane properties during lymphoid differentiation

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Enzymatic and ultrastructural properties of plasma membrane were studied on human lymphoblastoid cell lines at various stages of differentiation. Subcellular fractions obtained by isopycnic centrifugation from REH-6, NALM-1, RAJI, 6410 and KC 729-6 cell lines were assayed for plasma membrane marker enzymes. The ultrastructural topography was investigated on freeze-fracture preparations of intact cell by recording particle density of plasma and nuclear membranes. Enzymatic data correlated with the stage of differentiation, as supported by the enrichment of the enzymatic equipment of the plasma membrane and by homogeneous profiles of the marker enzymes. However, the ultrastructural properties did not correlate with the proposed differentiation stages being the particle density distributions similar in all investigated cell lines.

Isolation, characterization, genome structure of a Rous sarcoma virus derivative defective in replication and RNA packaging

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The extensive genetic diversity among the avian and murine leukemia and sarcoma viruses finds its origin in the ability of these viruses to mutate at a high frequency in the course of replication. Biochemical studies and RNA sequence data indicate that such a high incidence of mutation occurs in all viral genes.

The long term replication of RSV was studied by serial passaging of the virus in chick cells for a year. After 25 passages the original virus becomes a mixture of new ones; upon virus cloning one RSV derivative appeared of great interest with respect to cell fusion and lysis and was studied.

This virus is a 3'-Gag-pol α Src deletion mutant, codes for a 5'-Gag-Env. protein and needs an helper for propagation. Despite high levels of virus production and of viral RNA in the cell, the infectious titer and the RNA content of the virus are low. Expression of this virus and of its helper has been studied by protein and nucleic acid analyses and by transfection assays using proviral DNA cloned in λ-DNA. Results of the experiments will be presented and discussed.

Complexes between histone H1 and superhelical DNA

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We systematically explored the interaction of H1 with superhelical and relaxed DNA at increasing H1 to DNA input ratios

and different ionic conditions. Our experiments show that with increasing H1 to DNA ratios and at low ionic strength, H1 forms distinct soluble complexes with superhelical DNA prior to aggregation, which is not the case with relaxed DNA forms. Moreover, we examined the interaction of various H1-fragments with superhelical DNA and tested the protease cleavage of DNA-bound H1. Our data indicate that complexes of H1 with superhelical DNA might be a valuable model for the interactions of H1 in chromatin fibers.

Differential expression of mononuclear cell factor (MCF) mRNA that stimulates PGE_2 and collagenase production

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Translation products from *Xenopus laevis* oocytes of sucrose gradient fractionated poly(A)$^+$RNA extracted from 16 hour stimulated human peripheral monocyte-macrophage cultures induce the release of PGE_2 from human fibroblast and synovial cell cultures. This mRNA of ~ 16–17S encoded for a protein of ~ 13 Kd. Fractionated poly(A)$^+$RNA isolated from 48 hour phytohemagglutinin stimulated cultures exhibited a sedimentation value of ~ 10–12S and translated into a protein(s) possessing PGE_2 and collagenase inducing capabilities. Unlike the PGE_2 inducing material translated from mRNA of 16 hour stimulated cultures, found predominantly in the oocyte buffer, the collagenase and PGE_2 stimulating translation product(s) from 48 hour cultures was detected within the oocytes. Immunoprecipitation of a rabbit reticulocyte translation product that was encoded by biologically active sucrose fractionated poly(A)$^+$RNA (16 h cultures), migrated at M_r 36 Kd following gel electrophoresis. A large mRNA-protein precursor indicates that considerable processing occurs prior to the final destination of the mononuclear cell factor (MCF) molecule.

Regulation of human Class II gene expression

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The human Class II (HLA-D) genes are a multigene family whose products play a central role in the immune response. Expression of this set of genes is of interest because it is tissue restricted and developmentally controlled. We describe examples of coordinate regulation of the entire HLA-D region as well as expression limited to a single HLA-D subregion.

1) Exposure of macrophages to IFN results in the expression of mRNA for HLA-DP, DQ, and DR genes. The same observation was made with cells which normally do not express Class II genes; skin fibroblasts, melanoma, glioma, astrocytoma, osteosarcoma cell lines.

2) Patients with severe combined immunodeficiency (SCID) have a defect in Class II antigens. This defect results in a lack of expression of all HLA-D genes. The fact that the Class II genes are structurally intact and that the mutation maps outside of the MHC suggests the existence of Class II regulatory gene(s).

3) Another level(s) of regulation is likely because limited expression of genes from a single subregion was found in certain tumor cell lines and in some SCID patients.

A very strong enhancer is located upstream of an immediate early gene of mouse cytomegalovirus

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A strong transcription enhancer was identified in the 235 kb long genome of mouse cytomegalovirus (MCMV). An enhancer trap of linear simian virus 40 (SV40) DNA, lacking its own enhancer, was mixed with randomly fragmented MCMV DNA and cotransfected into CV-1 monkey cells. Selection for SV40 growth yielded SV40-MCMV recombinant viruses which had incorporated MCMV DNA to substitute for the SV40 enhancer. Two independent SV40-MCMV recombinants were found to contain overlapping MCMV DNA inserts of 343 bp and 313 bp. Within MCMV, these enhancer sequences are located upstream of the transcription initiation site of the major immediate early gene, between nucleotides −90 and −520. In transient expression assays, using cells from a variety of vertebrate species, transcription of a β-globin test gene was dramatically stimulated by the MCMV enhancer, several fold more than by the SV40 enhancer.

Partial characterization of the rabbit immunoglobulin λ light chain gene locus

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Rabbit λ chains are known to exist in at least two polymorphic forms: the c7 and c21 allotypes of the *c* locus. A fully characterized cDNA-containing clone encoding a constant (C) region of a rabbit λ chain has been described (Duvoisin et al., Eur. J. Immun. *14* (1984) 379). Southern blot hybridization analyses of rabbit liver DNA, using this cDNA as a probe, were carried out, suggesting the presence of a small number of Cλ-related genes; in addition the data show that *Eco* RI and *Bam* HI digested liver DNA of a c7$^+$/c21$^+$ rabbit contained a hybridizable fragment not found in the DNA of a c7$^+$/c21$^-$ rabbit. Cloning of the c21$^+$ specific DNA fragment is under way.

A rabbit liver genomic DNA library (Heidmann and Rougeon, EMBO J., *2* (1983) *437*) was screened with the Cλ-region cDNA probe. Restriction mapping and nucleotide sequencing analyses of the positive clones allowed us to identify four distinct genes. The predicted amino acid sequences suggest Cys-Pro C-terminal sequences as in horse λ chains. No termination codon was found in the coding region of these genes; the question of their expression has yet to be resolved.

Studies on the regulation of erythropoiesis in serum-free cultures

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Erythropoietin (epo) is the primary hormonal regulator of red cell mass in vivo. Evidence from studies in vitro indicates that other factors are required for differentiation of immature erythroid progenitors (BFU-E). Interleukin-3 (IL-3) induces colony formation by BFU-E while having similar effects on progenitors of other hemopoietic lineages. Colony growth by BFU-E, stimulated with epo alone, appears to require marow 'accessory' cells (AC), the nature and action of which is unknown. Investigations on AC activity were performed in serum-free cultures. Only pure erythroid colonies were formed in response to epo, suggesting that IL-3 is not involved. Addition of 200 uM hemin increased colony numbers but apparently via AC since the cloning efficiency decreased with decreasing cell numbers. Removal of Thy-1 positive cells by treatment with antibody plus complement had no effect on colony formation demonstrating that AC are not

T-lymphocytes. These results indicate an important role for AC in the regulation of erythropoiesis.

Bovine herpes virus 1 (BHV-1): DNA structure and restriction site maps of a new type

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Two types of BHV-1 trains can be differentiated by means of either endonuclease cleavage patterns, SDS-PAGE analysis or monoclonal antibodies. By using the same approaches, isolates originating from the brain of calves with meningoencephalitis, have recently been recognized as a third type of BHV-1, differing clearly from the hitherto known types. The genome structure of the new type as examined by electron microscopy of self-annealed single stranded molecules corresponded to that of known types. On the contrary, the restriction site maps constructed by conventional methods differed obviously. The significance of these results is discussed in view of the otherwise close relatedness between all the BHV-1 types.

Photosynthetic function and herbicide resistance: genetic and molecular analysis of photosystem II in *C. reinhardii*

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Analysis of photosynthetic mutants of the green unicellular alga *C. reinhardii* has allowed us 1) to study the expression and function of various proteins in the photosystem II (PSII) complex, 2) to determine the molecular basis for resistance and cross-resistance to *s*-triazine and urea-type herbicides, and 3) to develop a system that shows much promise for the transformation of chloroplasts. Point mutations have been identified at three different positions in the chloroplast gene *psb*A coding for the 32 kDa thylakoid membrane protein of PSII, each one of which causes an amino acid substitution that results in a different pattern of resistance to the herbicides atrazine and diuron. In addition, six different mutants which lack the 32 kDa protein were analysed, and all are deleted for both copies of *psb*A. These photosynthetic deficient strains may be ideal hosts for transformation with the chloroplast *psb*A gene isolated from wild-type cells or herbicide-resistant mutants, and the subsequent selection for restoration of PSII function.

Effects of insulin and IGF I on osteoblastic cells (OB) in a defined culture medium

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Calvaria cells of new born rats grown in a viscous (0.8% methylcellulose) α-MEME/F-12 medium up to 23 days form clones and have properties of OB: a high content in alkaline phosphatase and cAMP-responsiveness to PTH. Both features are expressed more markedly by OB cultured in this serum-free system than in standard tissue cultures. Proliferation of OB is favoured and starts 4–6 days after innoculation. Other cell types don't grow well (less than 5% fibroblasts after 7 days). 30% of cells form clones of more than 8 cells. The proliferation rate is increased by high initial cell density or addition of insulin (3–100 mU) or IGF I (20 ng). 30% of the cells (23 days) always remain as single cells. After 18 days of culture clones diverge and may show different behaviour: 1) The majority remains as clones or balls of cells containing up to 100 cells. 2) Cells spread out on the culture dish and remain in contact. 3) Some clones fall apart into single cells. In the present system OB obtained from a heterogeneous cell preparation by enzymatic digestion proliferate and appear biochemically highly differentiated.

Protein kinase C in human mammary tumor cells

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Protein kinase C (PKC) appears to be the major receptor for tumor promoters such as TPA (12-O-tetradecanoylphorbol-13-acetate). Since TPA has been shown to activate PKC as an agonist of the naturally occurring Diacylglycerol, the influence of TPA on the growth of a number of permanent breast cancer cell lines (MCF-7, T47-D, ZR75-1, MDA-MB231, HBL-100, BT-20) has been investigated. From our studies we assume that TPA and its analogues activate the PKC system which can be directly correlated with their tumor-promoting potencies.
To investigate the effects of tumor promoters on PKC we developed a rapid and sensitive method for the determination of PKC activity. The PKC cannot be accurately measured in crude cellular extracts, and requires a partial purification. This was achieved with an electrophoretic system under non-denaturating conditions, which allows the analysis of a large number of samples and requires only 10–250 µg of protein.

Characterization of acid phosphatase in *Schizosaccharomyces pombe*

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Acid phosphatase of *S. pombe* is coded for by two genes, *pho1* and *pho4*. The expression of the two genes is regulated differently. The gene products have been purified and characterized. They represent N-glycosylated cell surface glycoproteins and differ in their enzymatic properties. They can be deglycosylated with endo-H. The molecular weight of the protein mojety is 52,000 for the *phol*-product and 57,000 for the *pho4*-product. The carbohydrate moiety is not a requirement for enzymatic activity. A partially glycosylated (probably core glycosylated) a.pase has also been purified. In vivo studies show that unglycosylated and core glycosylated precursors of a pase are membrane-bound. Results will be presented that demonstrate that glycosylation of a.pase is a regulated process.

Isolation of the extra sex combs gene of *Drosophila* and its identification by *P*-element mediated transformation

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The extra sex combs (esc) gene is particularly interesting because it has been shown to be involved in the control of segment identity in all segments of *Drosophila*. We have cloned and identified this gene in several steps. First, a minilibrary was established from DNA microdissected from salivary gland chromosomes. With the microdissected clones a genomic library was screened, and the DNA comprising the entire deficiency Df(2L)esc^{10} (380 kb) was isolated. Then, the region containing *esc* was further narrowed down to 160 kb by a deficiency of the paired gene at the proximal limit and by a duplication carrying esc$^+$ at the distal border. After hybridization of this DNA with labeled cDNA prepared from polyA$^+$RNA of follicles or adult males, only two neighboring EcoRI fragments were labeled with follicle cDNA but not with cDNA from adult males. This region was subcloned into the Carnegie 4 vector and used for P-element

mediated transformation of embryos. In subsequent crosses the rescue of the esc$^-$ phenotype (female sterility) was verified and the transposon localized by in situ hybridization to salivary gland chromosomes.

Homeotic genes in *Xenopus laevis*

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X. laevis genes containing sequences highly conserved with the homeobox of *D. melanogaster* have been cloned and partially sequenced. Microinjection of genomic clones into oocytes has shown that at least one of them is efficiently transcribed. By Northern blot analyses it has been shown that one of the homeotic genes (XlH2) is transcribed in oocyte and again later on in early embryogenesis, while another (XlH1), first switched on at gastrula stage of embryogenesis, displays 3 differently controlled transcripts.

More recently we have obtained a series of new homeobox containing genomic and cDNA clones. Three of the cDNA clones were isolated from an oocyte cDNA library and thus are further candidates for maternally expressed genes involved in development. The cDNA clones will be used for Northern blot analyses and in situ hybridizations to sections of embryos. The genomic clones will be tested for transcriptional activity upon microinjection into oocytes and used for analysis of the structure and promoter sequences of these genes.

Amino-acid sequences of a colorless 8.9-kDa polypeptide and of a 16.2-kDa phycobiliprotein from phycobilisomes of *Mastigocladus laminosus*

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Two minor polypeptides with apparent molecular masses of 8,900 and 16,200 Da were isolated from phycobilisomes of the cyanobacterium *M. laminosus*. The complete amino-acid sequence of the smaller polypeptide was determined. This colourless polypeptide turned out to be composed of two polypeptides (each 67 residues long), which differed only in residue 9. A serine and a cysteine, respectively, were determined at this position. The longer polypeptide is a phycobiliprotein with an absorption maximum at 630 nm and a fluorescence emission maximum at 648 nm. Both its amino-acid composition and its N-terminal sequence were elucidated. Since both peptides were isolated from an allophycocyanin-I fraction, they probably belong to the allophycocyanin core of the phycobilisome.

Gene activity and developmental potential of unidifferentiated mouse teratocarcinomas

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The biochemical and biological analysis of unidifferentiated teratocarcinomas is the subject of this presentation. In collaboration with Dr. L. Stevens (Jackson Laboratory) we have started to investigate teratocarcinomas with homogeneous morphology showing only ectodermal, mesodermal or endodermal tissue derivatives, respectively. Such restricted teratocarcinoma lines are particularly suitable for the study of segregation of special cell lineages and germ layer diversification. Their morphological homogeneity also facilitates the biochemical comparison with the corresponding normal tissues in order to reveal their actual state of differentiation. Several isozymes that are tissue and stage specifically regulated during development have been chosen to investigate modulation of gene expression in the unidifferentiated teratocarcinomas. These results will be discussed in the context of reversible or stable alterations of gene activity during tumor progression. In the biological studies, cells from the teratocarcinoma lines have been microinjected into mouse blastocysts (preimplantation embryos at day 4 of gestation). The embryos containing tumor cells were then transferred into the uterus of pseudopregnant females to allow development to the fetal and adult stage. This in vivo approach will determine to what extent the unidifferentiated teratocarcinoma cells are able to participate in development and provide further information about the reversibility or stability of the neoplastic state.

Molecular approach of the mechanism of kidney tumor formation by MMTV

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A high incidence of spontaneous kidney adenocarcinomas has been reported in a substrain of a Balb/c mouse infected with MMTV. Two DNA probes representing unique cellular sequences adjacent to the two exogenous MMTV proviruses isolated from a Balb/c mammary tumor were used to screen by molecular hybridization a few primary kidney tumors and transplanted kidney tumors. One primary tumor containing one exogenous MMTV was presenting a DNA rearrangment and new mRNAs were transcribed in the domain specified by one of these probes called pBλ41.

A cell line, established in culture in vitro from one transplanted kidney tumor was shown to contain multiple exogenous MMTV proviruses whose expression is regulated by glucocorticoid hormones. Although, no DNA rearrangment was found in this cell line, a strong hormone stimulation of a 5.2 kb mRNA was observed for the pBλ41. This mRNA is a hybrid molecule with MMTV-LTR sequences linked to host cell DNA.

Thus the pBλ41 domain could represent a preintegration site implicated in tumorigenesis of epithelial cells from different organs.

Differential membrane targeting of 98 kD polypeptides related to α-subunit of Na,K-ATPase

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The biosynthesis and intracellular routing of α- and β-subunits (Sα, Sβ) of the Na,K-ATPase was studied in amphibian kidney cells (A_6 cells) in culture by using polyclonal antibodies prepared against the purified polypeptides. After short pulses (7 min) with ^{35}S-methionine, immunoprecipitates of Sα (98 kD) and coreglycosylated pre-Sβ were recovered from a pelleted membrane fraction (P) (100,000 × g, 30 min) of a sonicated cell homogenate. In addition, Sα antibodies detected a 98kd polypeptide in the supernatant (S) (5–10% of the pelleted Sα) that was apparently not associated with Sβ. After trypsin or proteinase K digestion (200 µg/ml, 60 min, 0 °C) of the cell homogenate, different protease-resistant fragments were observed in S and P. During a chase of 3 h, increasing amounts of non-digested Sα were recovered from P. The presence of a 98 kD polypeptide recognized by Sα antibodies in a light membrane fraction might indicate that Sα or a related gene product can follow a distinct biosynthesis pathway when processed in the absence of Sβ.

Actin in *Trypanosoma brucei*

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T. brucei is the causative agent of a number of servere diseases of man and cattle in Africa. Its cytoskeleton predominantly con-

sists of microtubules, which underlay the cellular membrane. No actin or actin-based structures have yet been detected. We have now demonstrated the presence of actin in this organism. Thin sections of Lowicryl-K4M-embedded trypanosomes were cut and stained for actin with the protein A gold technique (pAg). The cytoplasm of both, the cell body and the flagellum were labeled with gold particles, whereas the control sections, which have been treated with non-immune serum, followed by the pAg-complex, showed no labeling. Direct biochemical analysis showed that actin is present in trypanosomal cells in low concentrations. It differs from most other actins analyzed to date in that it binds to DNase I only very weakly. Actin genes of *T. brucei* have been isolated from a genomic bank by using human heart muscle actin DNA as hybridization probe.

Xenopus vitellogenin: protein structure derived from DNA sequences

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Yolk in vertebrate eggs serves as a source amino acids, lipids and inorganic phosphate in embryonic development. In *X. laevis* the yolk protein precursor, vitellogenin, is synthesized in the liver upon oestrogen induction. The heavily modified (lipidated, phosphorylated and glycosylated) vitellogenin is then secreted into the bloodstream from which it is taken up by oocytes and cleaved into the yolk proteins, lipovitellin I, II and phosvitin. To study the amino acid composition and structure of this storage polypeptide we have sequenced genomic and cDNA clones from the A2 gene, one of the four related vitellogenin genes. The cDNA and genomic sequences have been compared to establish, among other things, a map of the exons and introns. Based on these results we predict the amino acid sequence for the A2 vitellogenin protein. We present the arrangement of the different yolk proteins (lipovitellin I, II and phosvitin) on the vitellogenin precursor and discuss its structure and putative cleavage sites.

Cloning of the laccase gene from *Neurospora crassa*

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Polyadenylated RNA isolated from cycloheximide-treated *Neurospora* cultures was shown to contain laccase mRNA by in vitro translation and immunoprecipitation with anti-laccase antiserum. cDNA was synthesized using a deoxyundecanucleotide primer corresponding to the laccase-specific amino acid sequence Asn-Cys-Asp-Ala. The nucleotide sequence of the cDNA was found to be in complete agreement with the DNA sequence predicted from the partially known primary structure of *Neurospora* laccase. The cDNA sequence information allowed the synthesis of a unique 21-mer which was used for the screening of a genomic library from *N. crassa*. Four positive clones were identified by colony hybridization and their structure and function are currently analyzed.

Post-transcriptional mechanisms play a major role to increase immunoglobulin heavy chain mRNA levels during terminal differentiation of B-lymphocytes

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During the differentiation of B-lymphocytes to immunoglobulin-secreting plasma cells the synthesis of immunoglobulin heavy chain protein and the amount of corresponding mRNA increase by nearly two orders of magnitude. Surprisingly, however, the tissue-specific transcription enhancer element of the heavy chain gene is already fully active in pre-B-cells. This striking discrepancy between enhancer activity and mRNA level in immature lymphocytes was further investigated. We have performed nuclear run-on experiments to measure the transcription rate of the heavy chain gene in cell lines representing different stages of differentiation. Our results show that the heavy chain gene is transcribed in pre-B-cells to the same extent as in myeloma cells. Therefore posttranscriptional mechanisms are involved in controlling the increase of the heavy chain mRNA level upon maturation to a plasma cell.

Polymorphism and divergence of the HLA-DR β chain genes: a role for gene conversion

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The HLA-DR β chains carry the polymorphic determinants responsible for restriction of antigen presentation to T-cells. These polymorphic regions are thus implicated in interaction with antigen and T-cell receptor. Most of these polymorphic determinants are localized in the first domain of the β chain. We have been studying the molecular basis and extent of this polymorphism, first by analysis of the restriction enzyme polymorphism found in and around these genes and second by sequencing and comparing the first domains of eight cloned DR β chain genes. These eight genes include three pairs of loci from different haplotypes. Restriction analysis of genomic DNA by Southern blot hybridization shows that the DR haplotypes can be grouped on the basis of similar banding patterns. Polymorphic restriction site differences are found in both the intron and exon regions. Analysis of the eight sequences revealed: 1) Sequence microheterogeneity not evident by serology nor by Southern blot analysis; 2) Divergence between DR loci is of the same order if not greater than polymorphisms between alleles; 3) Intrachromosomal gene conversion between loci may play an active role in the generation of HLA-DR polymorphism.

The nucleotide sequences involved in bacteriophage T4 gene 32 mRNA stability

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To learn more about differential mRNA stability we have been studying the mRNA for the helix-destabilizing protein, gp32, of bacteriophage T4. The gene 32 mRNA is extremely stable. Moreover, by fusing the 5′ leader of gene 32 to sequences coding for an mRNA that is normally unstable, the resultant hybrid transcript is stabilized in phage-infected cells. We have shown this to be the case with the transcripts for β-galactosidase, galactokinase and HSV thymidine kinase. As the stabilization of the hybrid mRNA is seen only after phage infection, a phage-induced factor is presumably involved. We have begun a search for this factor, and have also attempted to identify the factor's target site on the 5′ leader of gene 32.

A dispersed tyrosine tRNA gene of *Xenopus laevis* with high transcriptional activity

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The tRNA genes of the frog *X. laevis* are more highly reiterated than in any other eukaryote so far examined, and they exhibit a

type of clustering that is so far unique. The best studied example is that of a 3.18-kb DNA fragment containing 8 tRNA genes which is tandemly-repeated 150-fold at a single chromosomal locus. Only one of these genes, coding for tyrosine tRNA, possesses an intervening sequence. A second kind of tyrosine tRNA gene has now been cloned and sequenced. It lies outside the major tRNA gene cluster, it is present in low copy number, and it contains a single base transition in the T loop, a shorter IVS and very different flanking regions. This dispersed gene is transcribed ~ 10-fold better in homologous S-100 extracts than the clustered tyrosine gene. Hybrid gene constructs show that the 5′ flanking sequences are responsible for the differential transcriptional activities of the two genes. In particular, the difference is maintained when each gene contains only 12 bp of natural 5′ flanking DNA. Oligonucleotides complementary to the IVS of each gene are being used as probes to test the proposition that the clustered gene is only expressed during oogenesis whereas the dispersed gene is transcribed in somatic cells.

Reactive oxygen generation in horse eosinophils and neutrophils

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The generation of reactive oxygen intermediates (ROI) is common to phagocytes of different cell lineages and represents an important mechanism of antimicrobial defense. To assess the pattern of ROI production in horse eosinophils and neutrophils, we have compared the luminol-dependent chemiluminescent (CL) response evoked by the Ca^{2+} ionophore A23187. The light emitted by eosinophils was strongly inhibited by superoxide dismutase (SOD) and catalase but was highly resistant to azide. Contrariwise, neutrophil CL was only slightly decreased by SOD and catalase but was highly azide-sensitive. Both isolated eosinophil peroxidase and myeloperoxidase were inhibited by catalase and azide but not by SOD. These observations document a distinct CL pattern in horse eosinophils and neutrophils which cannot be explained by a difference in the distinct peroxidatic enzymes of these two cell types. Additional experiments revealed different recognition specificity in ligand-induced ROI generation in eosinophils and neutrophils.

Microvillar membrane hydrolases migrate from the rough endoplasmic reticulum to the Golgi apparatus at individual rates in human colon carcinoma cells (Caco2)

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The kinetics of intracellular transport of newly synthesized intestinal hydrolases was studied by the pulse-chase technique and immunoprecipitation with monoclonal antibodies. Arrival at the trans-side of the Golgi apparatus was monitored by the acquisition of endoglycosidase H resistancy. The rate of transfer from rough endoplasmic reticulum to trans-Golgi (i.e. the half-maximal conversion of the core-glycosylated enzymes to their complex glycosylated form) was 20–25 min for dipeptidylpeptidase IV, 30–45 min for aminopeptidase N, 120–150 min for sucrase-isomaltase and > 240 min for lactase. Results obtained with a conformation-specific antibody suggest that both transfer to and migration through the Golgi apparatus is slow for sucrase-isomaltase. The data suggest that transport of surface membrane proteins to the Golgi apparatus is a selective possibly receptor-mediated process.

Chemically-induced apocrine secretion from yolk-sac epithelial cells

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In the process of developing an in vitro, short-term toxicity test model for ultrastructural observation, using ten-day old rat embryo yolk-sacs (Experientia *39* (1983) 665, and Tox. Lett. *18* (1983) 88) it was observed that certain lysosomotrophic drugs, such as cyproheptadine and gentamicin, caused an apical cytoplasmic secretion, which was seen also in vivo. Foreign material is taken up unselectively by pinocytosis during the pre-placental stage. Transcellular transport follows, mostly via the lysosomes. A portion of the lysosomal content is selectively transferred over the basal part of the cell into the vitelline capillary. The proteolytic waste apparently accumulates in the apical portion of the cell, and after reaching saturation, apical secretion occurs 24–48 h after drug application. Secretion results after cellular elongation into the apical lumen and de novo formation of membrane within the apical portion of the cell. Microvilli are immediately re-formed on both the cell and rejected part. The remaining cell and nucleus are morphologically unaltered. That such secretory processes occur in yolk-sac is not apparent in the literature.

Interleukin-2 receptor glycoprotein may be associated with Thy-1 in a detergent-resistant membrane domain

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A detergent-resistant plasma membrane domain has been isolated from P1798 T-lymphoma cells and contains a specific set of two surface glycoproteins of 25 kD (Thy-1) and 55 kD (Hoessli, D. and Rungger-Brändle, E., Exp. Cell Res. *156* (1985) in press). Using a monoclonal antibody (PC 61) directed against the interleukin-2 (IL-2) receptor on CTL-L cells, we have immunoprecipitated a soluble 55 kD glycoprotein from a P1798 detergent lysate, which 1) comigrates by 2-D electrophoresis and 2) yields similar V8 peptides, as the detergent-resistant 55 kD glycoprotein. These results may suggest that a subset of detergent-resistant IL-2 receptor and Thy-1 molecules are associated in the plasma membrane and are part of a detergent-resistant domain.

What are the factors which maintain the shape of metaphase chromosomes

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For a better understanding of the higher order structure of metaphase chromosomes it is important to know whether the integrity of the 30 nm chromatin fiber is necessary for the maintenance of the typical shape of mitotic chromosomes.

We have isolated metaphase chromosomes from Friend erythroleukemia cells of by a modification of the polyamine method earlier described by Laemmli's group (Lewis and Laemmli, Cell *29* (1982) 171). The presence of kinetochores and the shape of the chromatid axis were considered as criteria for morphologically intact chromosomes.

We examined both, chromosomes with all their histones present and chromosomes which were stripped of many of their non-histone proteins and in addition of histone H1, which is known to be essential for the formation of the 30 nm chromatin fiber. By looking at the protein composition and in parallel at the shape of such chromosomes, we found that also in the absence of histone H1 the chromosomal shape is maintained. This is in contrast to earlier observations in this laboratory in which a different chromosome isolation method was used (Labhart et al., 1983). We are investigating the cause of this discrepancy.

Molecular cloning of cDNA for chicken brain type creatine kinase (B-CK) by using antibody probes

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A cDNA library prepared from chicken gizzard RNA in the phage expression vector λgt11 was screened with affinity-purified anti-B-CK antibodies. cDNAs cloned into the Eco R1 site of the β-galactosidase gene in this vector in the correct frame and orientation give rise to immunologically detectable fusion proteins (a segment of β-galactosidase fused to protein sequences encoded by the cDNA insert). Cell lysates of the isolated clones were analysed by SDS-polyacrylamid gel electrophoresis followed by immunoblotting. Both, synthesis of the fusion protein (dependend on induction with IPTG) as well as presence of B-CK antigenic determinants within the product could be shown. A set of putative B-CK clones was further analysed by RNA blot analysis and DNA sequencing. The observed hybridization patterns with RNAs from different chicken tissues together with sequence data obtained so far confirmed the isolated cDNA clones as B-CK clones.

Altered gene expression in mouse cells induced by polyoma virus

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Polyoma virus induces S-phase in Go-arrested mouse kidney cells. Our goal is to characterize mouse genes responsible for the transition from Go to S-phase and to isolate the corresponding proteins. We prepared a cDNA library in λgt 10 of polya$^+$RNA extracted from cells 13 h after polyoma infection. At this time the viral T-antigens are expressed and cellular hnRNA synthesis is stimulated. However, onset of stimulated overall host RNA and protein synthesis and of cellular and viral DNA replication occurs about 3 h later. The cDNA library is being screened with cDNA probes made from polyA$^+$RNA extracted from infected and mock-infected cells. Differentially expressed cDNAs are subcloned and used to identify and quantify the corresponding mRNAs in mouse cells infected with SV40, polyoma wild type and polyoma mutants, in serum-stimulated 3T3 cells and in stimulated mouse thymocytes.

Transcriptional terminator sequences in the prokaryotic transposable element IS*1*

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The prokaryotic mobile genetic element IS*1* is known to exert a strong polar effect upon integration into an operon. Sequences responsible for this polar effect in one orientation of IS*1* have been mapped using a plasmid which has an IS*1* insertion between the ribosomal binding site and the ATG initiation codon of the gene *cat* for chloramphenicol acetyltransferase (CAT). The *cat* gene in this plasmid is transcribed from the promoter for the tetracycline resistance gene derived from pBR322. The degree of the *cat* gene expression in plasmid derivatives having deletions in IS*1* sequences was determined by the level of chloramphenicol resistance in the host cell or by measuring CAT enzyme activity. A strong *rho* dependent terminator was mapped between the two open reading frames of IS*1*, *InsA* and *InsB*, and a weak *rho* dependent terminator in the left end of IS*1*. Sequences for the former terminator were further characterized with the help of site directed mutagenesis.

The mammalian replicase: DNA polymerase α holoenzyme

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The DNA polymerase α responsible for mammalian chromosomal DNA replication exists as a multipolypeptide complex. Functional forms of DNA polymerase α can be isolated by using natural occurring DNA templates as replication probes and they are designated as DNA polymerase α holoenzymes (Ottiger, H.-P. and Hübscher, U., Proc. natn. Acad. Sci. USA *81* (1984) 3993; Hübscher, U., Trends biochem. Sci. *9* (1984) 390). The current status is: 1) The polypeptide composition of extensively purified holoenzymes is as complex as the bacterial DNA polymerase III holoenzyme; 2) Several different forms of DNA polymerase α holoenzymes exist that have possible functions at the leading and lagging strand of the replication fork; 3) Factors necessary for holoenzymes function can be separated from holoenzymes. In two cases successful reconstitution with factors was achieved; 4) The 'lagging type' holoenzyme is highly associated with the DNA primase. The latter appears to be part of the catalytic DNA polymerase polypeptide and 5) the 'leading type' holoenzyme is complexed with a DNA dependent ATPase. Evidence will be presented that this enzyme is a DNA helicase.

Tubulin genes in *Trypanosoma brucei*

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The genome of the causative agent of human sleeping sickness, *Trypanosoma brucei* contains a number of tubulin genes (10–15). These are mostly arranged in tight clusters of alternating alpha- and beta-genes. Though tubulin mRNAs are major mRNA species in these organisms, it is unclear which of the multiple genes are actually being transcribed. S1-mapping and primer extension experiments have now demonstrated a very homogeneous beta-tubulin mRNA population. The 5'-terminus of the mRNA contains a 35 bp mini-exon sequence, which is coded for by DNA sequences whoch are not present in the vicinity of tubulin genes. An analogous situation has been observed for genes coding for variable surface glycoproteins in trypanosomes. A site of DNase hypersensitivity has been discovered in proximity of one, but not of all other tubulin gene sets. This site, and its possible correlation to tubulin gene transcription, is currently being investigated.

Molecular cloning and characterization of a cDNA coding for mitochondrial aspartate aminotransferase (mAspAT)

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A 16S mRNA fraction 20-fold enriched with the specific message for mAspAT was prepared from chicken liver and used as a template for cDNA synthesis primed with oligodT (Gubler, U. and Hoffmann, B.J., Gene *25* (1983) 263). After dC-tailing the cDNA was inserted into the Pst I site of dG-tailed pBR322. A cDNA library consisting of 300,000 clones was obtained after high efficiency transformation. Colony screening of 10,000 clones using a synthetic oligonucleotide probe designed on the basis of the amino acid sequence gave 3 positive clones. One of these contained a plasmid carrying ⅔ of the coding sequence of mAspAT. The cDNA is ~ 1500 bp long and contains a noncoding region of ~ 700 bp at the 3' end. The cDNA corresponding to the coding region of the mRNA has been almost completely sequenced. With the exception of 2 positions, where glutamine

instead of glutamic acid codons are found the cDNA is in perfect accord with the known amino acid sequence.

Genome organization of the minute virus of mice (MVM) and its lymphotropic variant

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The following experiments were performed to probe the molecular basis for the tissue tropism of the fibroblast-specific (MVMp) and lymphocyte-specific (MVMi) variants of MVM: 1) We compared the nucleotide sequence of the two viruses. There are no major differences in the organization of their genomes or in the proteins they code for. The most striking differences are found in the hairpin structures thought to be essential for the replication of the genomic ends. 2) We tested the ability of hybrid viral genomes constructed from purified DNA fragments to replicate in the EL-4 lymphoblastoid cell line. Infectivity correlates with the presence of 5'-proximal information from MVMi, suggesting that interactions between the hairpins in viral DNA and tissue-specific host factors are essential for replication of the viral genome. 3) We constructed a cDNA library from cells infected with MVMi. Sequence data derived from it show that the coding assignments made previously for viral mRNAs have to be revised.

Molecular cloning of the gene of mitochondrial aspartate aminotransferase (mAspAT)

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A cDNA coding for mAspAT (see abstract by Jaussi et al.: Molecular cloning and characterization of a cDNA coding for mAspAT) was nick-translated and used as a probe for screening a chicken gene library which had been generously supplied by Dr. M. Ballivet. The library contains DNA fragments of ~ 15 kb, inserted in λ-phage L 47 (M. Ballivet et al., CSH Symp. Quant. Biol. 48, *1* (1983) 83). Approximately 10^6 phages corresponding to 10 genomes were examined. Eight strong signals were obtained, indicating that the gene is probably present as a single copy per genome. DNA from three positive phages was isolated and cleaved with Bam H1. Two fragments (3.5 kb and 1.7 kb) are common to all three clones and hybridize with the cDNA probe. The 3.5 kb fragment was partially sequenced and found to match a part of the cDNA coding for mAspAT.

Ca^{2+}-binding parvalbumin in rat testis

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Parvalbumin is known to be expressed in muscle and brain. In addition, parvalbumin cross-reacting material has also been found in a few non-muscle tissues e.g. the testis of rat. There, the Leydig cells, which elaborate androgenic steroid hormones, principally testosterone, are strongly immunostained. It is known that secretory activity of the Leydig cells is under control of the luteinizing hormone (LH). Maximum LH stimulation of testosterone production is only obtained in the presence of Ca^{2+}. Characterization of the immunological active component present in testis preparations has not been attempted so far because of the small quantities present e.g. estimated to be as low as 5 mg/kg in rat testis. Therefore, we isolated this immunoreactive protein and showed that its biochemical and immunological properties were indistinguishable from muscle parvalbumin. Further experiments will show if parvalbumin is involved in the regulation of testosterone production in the Leydig cells of rat testis.

Oligonucleotide-directed mutagenesis of gene 67 of phage T4: mutants in this gene produce form variants of the head

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Two amber mutations in gene 67 of phage T4 were constructed by oligonucleotide-directed mutagenesis and the resulting mutated genes were recombined back into the phage genome. The 67amK1 mutation is close to the amino terminus of the gene and phage carrying this mutation are unable to form mature phages on suppressor-negative hosts. A second mutation, 67amK2, which lies in the middle of the gene produces a small number of viable phage particles (5% of that seen in a wild type infection). The amber fragment produced by this mutation is still partly functional. In suppressor-negative hosts, both mutations produce polyheads and large numbers of proheads. The proheads are mostly (70%) icosahedral rather than having the usual prolate shape. This form variation is also observed in mature phages if a suppressor is used that inserts a wrong amino acid into the protein. About 50% of the phages that are produced are isometric and up to 10% have a biprolate structure with two tails attached.

Translational control of the heat shock response in *Drosophila melanogaster*

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One of the effects of temperature elevation above 35 °C is the selective translation of heat shock (hs) mRNA. We tested whether the transcript of a non hs gene is being translated at high temperature provided it is synthesized during the heat shock. A fusion construct between the alcohol dehydrogenase (adh) gene and the promoter of a hs gene was introduced into adh^- flies. Such transformed flies synthesize adh mRNA during hs in massive amounts but translation of this mRNA is restricted to non hs temperatures. Thus, the information that allows the translation machinery to discriminate between hs and non hs mRNA must reside in the primary sequence of the hs mRNA.
Internal deletions of different parts of the non translated leader region of a hs gene were performed in order to identify such a sequence element. Over 80% of the leader sequence are dispensable for efficient translation. Deletion to within 25 bases from the cap results in a drastic drop in expression both at high and low temperature.

Comparison of Sendai virus and influenza virus surface glycoproteins

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The nucleotide sequences of the Sendai virus (SV) F and HN surface glycoprotein genes were determined. The deduced primary structure of the proteins, when analyzed on a hydropathy plot, showed that the F protein was anchored in the membrane at its C-terminus but that the HN protein was unsually anchored in the opposite orientation. A similar situation exists for the two influenza virus (FLU) surface glycoproteins. Since the two SV and the two FLU glycoproteins share the same three activities (hemagglutinin, neuraminidase, fusion), their aa sequences were compared for homology. Limited but statistically significant homology was detected suggesting that SV and FLU shared

common ancestry. The data further suggests that intragenic rearrangement as well as gene concatenation has taken place during this evolution.

Histone gene expression during the cell cycle of *Physarum polycephalum*

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In the myxomycete *Ph. polycephalum* as in many other eukaryotes, synthesis of histone proteins occurs during S-phase of the cell cycle. Replication of the Physarum genome starts immediately after mitosis and lasts for 3 h. The following G2-phase takes 5 to 6 h. There is no G1-phase.
We studied the cellular abundance of H2B and H4 mRNAs during the cell cycle. The level of both mRNAs ran parallel and were very low in early G2. An increase was observed 4 h before mitosis. A plateau was reached 1 h later and was maintained through mitosis until mid-S. The mRNA levels then dropped to about $1/_{10}$ of the plateau. During G2 the level of both mRNAs were unaffected by a 1 h treatment with hydroxyurea. Treatment with this inhibitor during mitosis and early S caused a rapid disappearance of the mRNAs. The level of actin mRNA was unaffected by hydroxyurea. We suggest a tight coupling between the assembly of histones into chromatin and thereby regulation of the mRNAs in S-phase.

Fate of *Aedes albopictus* (C6/36) cells and Semliki Forest Virus (SFV) after prolonged cultivation at pH 6

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1) *Aedes* cells infected with SFV fuse, if the pH of the medium is lowered to 6. 2) Uninfected cells proliferate at pH 6. 1) and 2) provided the basis to study after fusion the coevolution of infected cells and virions under prolonged fusogenic conditions. Cultures A–U were infected and fused at pH 6 on the following day. At pH 6, after 10 days cell growth had resumed and a new monolayer was formed after 20 days. Fusion continued until the 5th weekly passage. From then on no fusion was observed if subcultures grown at pH 7 were exposed again to pH 6 even though the cells were persistently infected. Some cultures fused at pH 5. Only 2 virus strains were afusogenic at pH 6 (assayed on Vero and Aedes cells). Uninfected C6/36 cells could be grown at pH 6 for generations without losing their fusion capacity after infection.

Germ line and somatic DNA of the chromatin eliminating nematode *Ascaris lumbricoides* show short-period interspersion

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DNA from intestines and larvae of *A. lumbricoides* has been isolated and used as a source for somatic, DNA from spermatids as a source for germ line DNA. Following the protocol of Davidson et al. (J. molec. Biol. 77 (1973) 1), we have shown that both the somatic and germ line DNA of the chromatin eliminating nematode *A. lumbricoides* are arranged in a short-period interspersion or '*Xenopus* pattern'. By comparison of the amounts of clustered repetitive, single copy and interspersed sequences in the germ line and somatic genomes, we determined that the germ line limited genome consists of about 80% clustered repeats, mostly or exclusively of the satellite type, and roughly of 20% low abundant or single copy DNA sequences.

Characterization of sperm auto-antigens

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Combined application of immunohistochemistry and a nitrocellulose binding test for the detection of antisperm antibodies resulted in the recognition of cellular antigens relevant to infertility. Two of these were further studied. The first one has a MW of 10,000 and is sperm-, but not species-specific. Production of antibodies against this antigen results in infertility. It is a membranal antigen which might be used for suppression of sperm production through vaccination. The second antigen was detected on human sperms and lymphocytes. A single patient with a carcinoma of the testis in situ produces antibodies against this antigen. He has also antibody doposition in the malignant cells. Monoclonal antibodies against PAGE eluate of these two antigens are being prepared and analyzed.

In vivo synthesis of nuclear lamin protein precursors: kinetics of processing and nuclear uptake

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The nuclear envelope-associated proteins lamin A and B are synthesized as larger precursors in vivo. Synthesis presumably occurs on free ribosomes. Subsequently, the two precursors are processed with different kinetics and in different cellular compartments suggesting that they may serve different functions: The lamin B precursor is processed within the cytoplasm with a half-time of 2–3 min; with similar kinetics both lamin proteins are transported to the nucleus. The lamin A precursor is then cleaved within the nucleus with a half-time of about 30 min; concomitantly both lamin proteins acquire a characteristic resistance to detergent extraction suggesting their insertion into a submembraneous protein network. The described biogenetic pathway involving precursor synthesis and processing is without precedent among nuclear proteins and may have considerable implications for current concepts of intracellular topogenesis.

A novel nuclear lamin protein identified by monoclonal antibodies

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The nuclear lamina, a structure closely apposed to the inner nuclear membrane, is believed to provide a framework important for nuclear envelope integrity and interphase chromatin organization. The quantitatively major constituents of the lamina in mammalian and avian species are known as lamins A, B and C. With the help of monoclonal antibodies we have identified a new lamin-like protein present in chicken tissues. This protein shares several characteristic properties, including location and extraction-resistance, with the known lamins and we therefore propose to call it lamin D. It has a molecular weight similar to that of lamin A (70,000) and an isoelectric point almost identical to that of lamin B. However, lamin D clearly differs from the previously known lamins as shown by both peptide mapping and immunological criteria. We conclude that lamin D most probably is the product of a separate lamin gene.

Calcium dependent and calcium independent phagocytosis in human neutrophils

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The phagocytic function of neutrophils is a crucial element in host defenses against invading microorganisms. Two main spe-

cific receptor-mediated mechanisms are operative on the phagocyte plasma membrane: one recognizing the C3b fragment of complement and the other the Fc domain of IgG. Anumber of evidence indicate that phagocytosis mediated by these two receptros differ in the number and nature of intracellular signals generated but their nature is unknown.
Here we present evidence by modulating and monitoring continuously cytosolic free calcium, Ca_i^{2+}, using the fluorescent calcium indicator quin2 (which behaves as an intracellular calcium buffer) during ingestion of C3b or IgG coated particules that in human neutrophils two mechanisms of phagocytosis coexist: a) A Ca_i^{2+} dependent and modulated phagocytosis, requiring localised ionic gradients, triggered by activation of the Fc receptor and b) a Ca_i^{2+} independent phagocytosis, still occuring in calcium depleted cells, where Ca_i^{2+} ten times bellow normal resting levels, triggered by the activation of the C3b receptor.

Biochemical heterogeneity of membrane vesicles shed from lymphoblastic leukemic cells

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The common acute lymphoblastic leukemia antigen (CALLA) is expressed on the surface of normal lymphocyte precursors and of lymphoblasts of patients with the common type of acute lymphoblastic leukemia. Circulating CALLA, detected in the serum of leukemia patients, was found associated with membrane vesicles isolated by ultracentrifugation. The membrane nature of the vesicles was assessed by conventional and freeze-fracture electron microscopy and determinations of plasma membrane associated enzymes. Vesicles were found to express CALLA antigen in association with the 5'-nucleotidase activity. The level of CALLA and 5'-nucleotidase recorded on membrane vesicles did not correlate with the level of both antigens mesured on the intact cells derived from the respective patients. These findings indicate that the shedding of membrane fragments from proliferating and pathological cells is a general phenomenon while the rate of shedding and the membrane composition of vesicles are peculiar to each type of lymphomalignancy.

Immunolocalization of glucosidase II in pig liver

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Glucosidase II removes the two inner α1–3 linked glucose residues from N-linked oligosaccharides. Rabbit antiserum against purified native pig kidney enzyme (Eur. J. Biochem. *141* (1984) 149) was applied to thin sections from frozen and Lowicryl K4M embedded pig liver followed by protein A-gold. In hepatocytes labelling localised to nuclear envelope, smooth and rough (RER) endoplasmic reticulum, transitional elements of RER, smooth surfaced profiles near to Golgi apparatus (GA) and autophagosomes (some CMP-ase positive). GA cisternae, nuclei, mitochondria, lucent lysosomes with ferritin-like content, and the lateral/sinusoidal membrane lacked specific label. Increasing antiserum concentrations gave low labelling at the biliary pole (frozen sections only). Lack of detectable glucosidase II in GA cisternae suggests transitional elements and/or smooth surfaced elements close to GA are sites of its segregation from non-ER proteins. Concentration of glucosidase II in ER is in accordance with biochemical studies.

Cell cycle regulation of histone gene expression

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Histone gene expression in most eukaryotic cells is tightly coupled to the S phase of the cell cycle. We are studying the molecular basis of this regulation in a *ts* cell cycle mutant of P815 mouse mastocytoma, K21Tb (Somat. Cell Genet. 7 (1981) 581). All endogenous H4 transcripts are drastically reduced in G1-arrested cells and increase gradually as the cells are shifted back to the permissive temperature. A cloned mouse H4 gene is regulated identically in stable K21Tb transformants. A detailed study of the regulatory information by surrogate genetics has been initiated. Transcripts of a fusion gene extending from the SV40 early promoter through the 3' part of the H4 gene up to the 3' processing site for H4 mRNA are also subject to regulation, whereas SV40-initiated transcripts which traverse the H4 processing signal and extend farther to a poly(A) signal are not. These results indicate that a genuine histone mRNA 3' structure is essential for changes in RNA metabolism during the cell cycle.

Morphological and immunofluorescence analysis of herpes virus suis infection in superior cervical ganglion cell culture

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The infection of the peripheral nervous system by a neurotropic virus, herpes virus suis (HVS), may be studied using cultures of dissociated superior cervical ganglion (SCG) cells as a model. Cultures displaying a rich neurite network were infected with 10^{-6} PFU/ml of HVS. At different times post infection the cultures were observed by phase contrast microscopy and the stages showing marked morphological changes further investigated by electron microscopy. Double labeling using antibodies against HVS and markers for neurons, Schwann cells and fibroblasts proved the neurons to be the preferential target of the virus.

Appearance of cytolytic granules upon induction of cytolytic activity in CTL-hybrids

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A CTL-line (B6.1) with specific cytolytic activity was found to produce poly C9-like, tubular complexes of two sizes (polyperforin 1 and 2). Isolated cytoplasmic granules from this cell line were hemolytic, contained arylsulfatase and assembled polyperforins on their surface. Antibodies raised against granules detected polyperforins and vice versa; they predominantly reacted with a 27 kD protein. Isolated granules contained two other main proteins with m.w. of 29 kD and 66 kD. By following hemolytic activity, a protein (perforin) with a m.w. of 66 kD, was isolated from disrupted granules on an HPLC-gel filtration column. Isolated perforin formed polyperforin 1 and 2 in the presence of Ca^{2+}.
We compared the B6.1 cell with a hybrid, PC60, derived from a cross between B6.1 and a non-cytolytic rat thymoma. This hybrid line exhibits inducible CTL activity. In induced PC60, polyperforins 1 and 2, cytolytic granules and the 27 kD protein were detected. Non-induced PC60 cells lacked cytolytic granules, the 27 kD protein and polyperforin 1, but contained polyperforin 2. These results suggest that cytolytic granules appear in parallel with the expression of cytotoxicity during T-lymphocyte differentiation.

Probing chromatin with psoralens: alterations during DNA excision repair

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We have developed a new procedure to probe for alterations in chromatin organization that may accompany the repair of carci-

nogen-induced DNA-damage. This approach includes: 1) Quantification of the accessibility of 8-MOP for 8-methoxypsoralen (8-MOP) as a function of repair time. 2) Analysis of the distribution of repair patches in this 8-MOP accessible DNA-fraction (MOPS-DNA). Results. 1) Within minutes after onset of repair, the accessibility of chromatin for 8-MOP alters dramatically and undergoes characteristic changes as repair proceeds. 2) The proportion of newly synthesized repair patches contained in MOPS-DNA changes with increasing repair time. Conclusion. 1) Our procedure monitors structural changes that occur within minutes after damage. 2) It is non destructive (as opposed to nuclease probing).

Delayed myelin basic protein synthesis impairs temporarily oligodendroglia and myelin metabolism

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The central nervous system (CNS) of young *mld* mutant mice contains few myelinated axons. In the absence of myelin basic protein (MBP), the myelin lamellae are poorly compacted. The myelin associated glycoprotein (MAG), is synthesized normally in the *mld* but its turnover rate is higher than in controls. Western blots revealed in *mld* optic nerves, the presence of MAG and its degradation product dMAG. Only MAG was present in controls. In the young mutants, by electron microscopy, approximately 70% of the oligodendrocytes showed abnormalities: dark cytoplasm completely filled with inclusion bodies positive for MAG; absence of recognizable Golgi apparatus; extended endoplasmic reticulum immunostained for MAG. After 60 days of age, when the MBP content increased, myelin lamellae were better compacted and the oligodendroglia fine structure was normal. At this developmental stage, dMAG was absent and the turnover rate of MAG was normal. These results indicate that MBP is important for the compaction and stability of CNS myelin.

Effect of tumor promoters and microtubules inhibitors on cell to cell adhesion

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In a study on a system of neoplastic (SGS) and normal syngeneic (FG) cells dedicated to the molecular basis of intercellular adhesion and contact inhibition, we investigated the role of the cytoskeleton and the related structures in cell-cell adhesion. The technique used consists of determining the percent of single cells labelled with ^{3}H-leucine adhering to a confluent monolayer. Preliminary results indicate that intercellular adhesion is increased in rat fibroblasts (FG) by exposure either to microtubules inhibitors like nocodazole and vinblastine or to an active tumor promoting agent like the TPA or to melittin, a polypeptide from bee venom. Our results indicate that 1) also the cytoskeleton partecipate to the determinism and to the regulation of cell-cell adhesion and 2) the higher intercellular adhesion of fibroblasts 'transformed' by TPA and melittin confirms what we have observed in our system.

Comparison of human-human and mouse-mouse fusions for hybridoma formation

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Four mouse myeloma cell-lines commonly used for cell fusions (NS 1, P3U1, SP 2/O, x63 Ag8653) and five human myeloma lines were compared for formation of heterokaryons after cell fusion with PEG. The cells were stained in vivo with fluorescent dyes (myeloma cells with Kodak rhodamine, lymphocytes with Hoechst bisbenzimide) which altered neither growth of cells nor secretion of monoclonal antibodies. Myeloma cells containing at least one nucleus from a mouse or human lymphocyte respectively (heterokaryons) were counted from fluorescence microphotographs and fusion-frequency was calculated as % heterokaryons among unfused myeloma cells.

Mouse myeloma lines fused in a range of 1 to 5%, whereas human myeloma lines showed a fusion-frequency of 5 to 25% (minimum three subsequent fusions). Although human cells fused more frequently no growth of hybridomas could be observed. In murine fusions almost every well contained growing clones.

Expression of a cluster of genes coding for the ribosomal proteins S12, S7 and for the elongation factor tu in *Euglena gracilis* chloroplast DNA

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We have shown that the genes for the ribosomal proteins S12 and S7 (*rps* L and *rps* G) and the gene for the elongation factor tu (*tuf* A) are clustered within *E. gracilis* chloroplast DNA (NAR *12* (1984) 2851). The gene arrangement is 5′ *rps* L *rps* G *tuf* A, somewhat similar to the *str* operon of *E. coli*, which contains in addition the gene for the elongation factor G *(fus)*. To study how these genes are expressed in the chloroplast, we have analysed by gel electrophoresis S1 nuclease resistant RNA:DNA hybrids, using chloroplast RNA and cloned DNA fragments, and sized chloroplast RNA by gel electrophoresis followed by blot transfer and hybridization with radioactive DNA fragments. The *tuf* A gene is transcribed as a spliced mRNA of 2 kb while *rps* L and *rps* G genes are cotranscribed as a dicistronic mRNA of 1.5 kb. The 1.5 kb transcript is much less abundant than the 2.0 kb transcript, suggesting that transcription of the two regions is under separate transcriptional and/or processing control unlike the situation found in *E. coli*.

Comparative analysis of the spacer regions of the two main rDNA size classes of *Ascaris lumbricoides*

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The rDNA of *A. lumbricoides* exists in two main classes which differ essentially in their spacers. The DNA sequences of the complete external transcribed spacer (= ETS), including the beginning of the 18S rDNA region, and the entire nontranscribed spacer (= NTS) have been determined on cloned representatives of the two organization forms. The main difference resides in an insert of 0.4 kb, present only in the NTS of the long form. This insert is flanked on both sides by a duplicated sequence of 100 bp length which exists in only one copy within the short repeat. Most other minor differences are localized within the NTS, whereas the transcribed regions show practically no differences. The high sequence conservation probably reflects the functional significance of this region. Moreover, there is a pronounced difference in GC content between NTS and ETS. The sequence of the putative promotor is discussed and compared with data from other organisms.

Molecular cloning of the *Neurospora* copper metallothionein gene

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Enriched *Neurospora* metallothionein mRNA was used as a template for cDNA synthesis primed by an undecanucleotide

which was synthesized according to the known amino acid sequence of the protein. The sequence of the cDNA obtained allowed the synthesis of a unique 21-mer corresponding to the amino terminal part of *Neurospora* metallothionein. Screening of a genomic DNA library from *Neurospora crassa* with this probe yielded several positive clones. DNA sequence analysis showed the *Neurospora* metallothionein gene to contain an open reading frame coding for a polypeptide of 26 amino acids in complete agreement with the published amino acid sequence. The coding region of the gene is interrupted by a short, 94 bp intron.

Hypersensitive sites, 'topoisomerase' activity and footprints on *Dictyostelium* ribosomal genes

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Using indirect end labelling, we have mapped the cutting sites of endogenous nucleases along the palindromic ribosomal genes of *Dictyostelium discoideum*. At the 3′ end of the transcribed region is the border of a sensitive zone which extends about 1000 bp into the 28S gene or in some cultures can extend right across the coding region. At the 5′ end of the transcribed region, between −350 and −1600 bp, is a hypersensitive region flanked by two sites which are cut only upon addition of SDS, a behaviour typical of topoisomerases. The two sites are situated at −200 and −2,230 bp. When cutting at these sites is inhibited by the prior addition of salt, a large footprint extending from 0 to −320 bp is visible. The footprint appears to be underrepresented in cells in which the rate of rRNA synthesis is reduced.
Control experiments with plasmid DNA showed none of the phenomena described above.

Isolation of a cDNA clone encoding PrP, a protein found in scrapie infected hamster brain

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PrP is a 27–30 kD protein accumulating in amyloid plaques in scrapie infected hamster brain. PrP purified from infected brain has a high level of scrapie infectivity. Its NH_2-terminus was sequenced and oligonucleotides encoding a 7 aminoacid segment were used to screen a cDNA library from infected brain mRNA. A positive cDNA clone with a 2 Kb insert was isolated and sequenced. The cDNA sequence encoded the known 20 amino-acids at the NH_2-terminus and 2 internal CNBr peptide sequences. Southern blots indicate a single endogenous gene in hamster, mouse and human DNA. Surprisingly, Northern blots revealed transcripts of 2 Kb length not only in infected but also in normal brain RNA. Therefore, expression of this gene is not specific for scrapie infection and the role of PrP in scrapie infectivity and pathology needs further investigation.

Changes in the chromatin structure following activation of a single copy gene in *Dictyostelium discoideum*

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We have studied changes in the chromatin structure of a single copy gene which is regulated during development of *D. discoideum*. Micrococcal nuclease digestion indicates the nucleosomal structure is lost when the gene is switched off but returns when the gene is inactivated. Specific regions of the neighbouring sequences become sensitive to endonuclease activity when the gene is active. The chromatin structure upstream and downstream from the gene has been examined using the indirect end-labelling method.

Repetitive sequences are found in mRNAs present in oocytes and stage 40 embryos of *X. laevis*

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We have used hybrid selection translation experiments to show that transcripts of two repetitive sequences, called R19 and R134, are present on sets of rare mRNAs in stage 40 *X. laevis* embryos. The R19 repeat selects mRNAs coding for three proteins, and the R134 repeat selects a different set of mRNAs coding for 16 proteins. Similar experiments performed with RNA from mature oocytes indicate that the R19 homologous mRNAs and 8 of the R134 homologous mRNAs are absent in maternal RNA, and must therefore be synthesized during embryogenesis. The other 8 R134 homologous mRNAs, on the other hand, are found in the oocyte as well as in the embryo. We also detect additional R134 containing mRNAs that appear to be restricted to the maternal RNA. Since the mRNAs that share the R134 sequence do not exhibit a common pattern of developmental regulation, it is unlikely that the repeat plays a role in coordinating the production of these mRNAs. Other roles are however not excluded.

Phospholipids in reconstituted porin membranes: conversion of one crystal habit into another by phospholipase

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Reconstitution of porin, a major outer membrane protein from *E. coli* with phosphatidylcholine yields three 2-dimensional crystal forms (Dorset et al., J. Mol. Biol. *165* (1983) 701). Two different trigonal lattices exhibit lattice constants of 93 and 79 Å. Image processing revealed that the channel orifices do not exhibit detectable differences, but that the areas between them vary in size. We have now confirmed their consisting of phospholipids by treating the large form with phospholipase. Electron microscopy shows lattice constants decreasing to 72 Å. Shrinkage of the lattices is either due to tighter packing, or to loss of the enzymatic degradation products. The results demonstrate that porin with its channels in an apparent transmembrane configuration (Engel, A. and Massalski, A., Ultramicroscopy *13* (1984) 71) is indeed cointegrated with phospholipids within a membrane.

T7-induced DNA-polymerase: cloning and expression of gene 5 of phage T7

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T7 DNA-polymerase which is essential for the replication of the phage T7 genome consists of two subunits, the phage-coded gene 5 protein (80 kD) and the host protein thioredoxin (12 kD). We have achieved a high overproduction of the phage subunit in transformed *E. coli* bacterias by cloning gene 5 of phage T7 into pBR322.
Expression of the cloned T7 DNA-fragment was found to be strongly influenced by thioredoxin, resulting in higher amounts of gene 5 protein in the absence of the host subunit. A higher copy number of the recombinant plasmid compared to pBR322,

as evident from the increased antibiotic resistance of the host cells, suggests an interaction of the T7 gene product with the replication control of the plasmid.
Studies dealing with the role of thioredoxin within the T7 DNA-polymerase were carried out after in vitro reconstitution of the holoenzyme by addition of the host subunit.

Effect of the tumor promoter TPA on human mammary tumor cells

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The mechanism of estrogen-dependent growth of breast cancer cells is poorly understood. Preliminary evidence suggests that estradiol acts via the induction of tumor-derived growth factors, which act via the EGF receptor. The tumor promoter TPA (12-O-tetradecanoylphorbol-13-acetate) is interfering with this system by down regulation of the EGF receptor via the activation of protein kinase C. Therefore we compared hormone-dependent (MCF-7, T47-D, ZR75-1) and hormone-independent (MDA-MB231, HBL-100, BT-20) human mammary carcinoma cell lines with respect to TPA effects. With the exception of the cell line T47-D, TPA inhibited the proliferation at concentrations of 10^{-8} molar.
Hormone-independent cell lines were shown to contain high levels of low affinity EGF receptor (app. $K_D = 8 \times 10^{-10}$ M), whereas the hormone-dependent cell lines exhibited low levels of receptor with high affinity (app. $K_D = 1 \times 10^{-10}$ M). In hormone-dependent cell lines TPA induces a transformation of the receptor to a low affinity state; affinity remained unchanged in the hormone-independent cell lines MDA-MB231 and BT-10.

Subcellular localization of $\alpha 2 \rightarrow 6$ sialyltransferase and sialic acid residues in rat intestinal cells

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Specific antibodies against rat liver $\alpha 2 \rightarrow 6$ sialyltransferase (J.B.C. *257* (1982) 13835) followed by protein A-gold, and *Limax flavus* lectin followed by fetuin-gold (J.H.C. *32* (1984) 1167) were applied to thin sections of Lowicryl K4M embedded rat intestine. In absorptive and goblet cells, sialyltransfrase was localized mainly in transmost Golgi apparatus (GA) cisterna (CPM-ase positive) with additional sparse labeling over an adjacent trans GA cisterna. Sialic acid distribution in the GA was similar. Sialyltransferase was also found outside the GA: in absorptive cells in cytoplasmic vesicles and the apical (brush border) and basolateral plasma membrane, and in goblet cells in the mucus droplet limiting membrane and the mucus. These structures were also positive for sialic acid. These findings suggest that sialylation in the GA is limited to trans cisternae. In addition evidence for ecto-sialyltransferase in intestinal cells is provided.

Maturation of antigenic sites of Sendai virus glycoproteins

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Antibodies raised against native forms of Sendai virus HN and Fo glycoproteins recognize antigenic sites whose formation follows different patterns. Antigenic sites of the Fo proteins are generated along with the protein synthesis, while those of HN are fully created only one hour after the completion of the protein synthesis. This delay reflects a need for native HN antigenic sites maturation. This maturation does not seem to depend on glycosylation per se, but rather reflects conformational changes associated with dimerization and tetramerization of the protein. These observations are verified in the use of monoclonal antibodies as well as of polyclonal rabbit antiserum. Implication of such observations for glycoproteins synthesis as well as for production of neutralizing antibodies will be discussed.

Are heat shock genes transcribed by modified polymerase?

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The expression of hybrid genes, in which human hsp 70, *Drosophila* hsp70 or 23 gene promotor segments were linked to an E. coli-β-galactosidase gene, was studied in *Xenopus oocytes*. *Drosophila* hsp70 genes compete with all hybrid genes. Thus, a common cellular component is involved in the regulation of the different hsp genes. The limiting factor appears not to be a specific promotor binding protein, since cloned promotor fragments without a protein coding region compete less efficiently. Thus, the amount of RNA polymerase engaged in heat shock gene transcription seems to be limiting. After short heat shock, hsp gene activity is low, but competition is still effective. Thus, the limiting factor is not normal polymerase II but, during heat shock, part of the polymerase (or a co-factor bound to polymerase during elongation) is modified and only then recognizes hsp genes.

Exocytosis from the retinal pigment epithelium is an actin-dependent mechanism and prevented by phalloidin

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The retinal pigment epithelium of the frog expells membrane material predominantly derived from the autophagic degradation into the extracellular space. In the lateral spaces, this material moves in a basal direction and accumulates within the basal infoldings. Then, it is locally released towards the Bruch's membrane and the choriocapillaris. We have investigated the nature of the coordinating moving force in exocytosis making use of phalloidin, a drug which stabilizes F-actin. Injection was made into the peribulbar eye space. The tissue was fixed in the presence of tannic acid. The cells reached by phalloidin were recognized by their permeability to tannic acid. Such cells showed severely impaired exocytosis. Membrane material was neither evacuated into the extracellular space, nor accumulated within the basel infoldings. No release towards the choriocapillaris was observed. It thus appears that exocytosis is mediated by a highly coordinated actin-dependent mechanism of the epithelial cells.

A T cell receptor cDNA clone from an allospecific cytotoxic T cell

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3F9 is a BALB/c allospecific cytotoxic T cell clone with specificity for H-2D^b. We have constructed a cDNA library in the bacteriophage lambda vector λgt11 and we have used a synthetic oligonucleotide complementary to the constant region of the β-chain of the T cell receptor to screen for crosshybridizing cDNA clones. Several isolated cDNA clones were characterized by restriction site mapping and DNA sequencing. We found a productive V_T-D-J_T-$C_\beta 1$ rearrangement. The V_T gene segment used in our cytotoxic T cell clone (anti-D^b) is identical to the V_T

gene segment expressed in a helper T cell clone specific of H-2^b and chicken red blood cells. In addition to this functionally rearranged cDNA clone we isolated a cDNA clone bearing the $C_\beta 2$ gene of the T cell receptor. However, the $C_\beta 2$ gene sequence is preceded by a DNA sequence that does not show any similarity to V_T or J_T sequences.

M-band formation in cultured differentiating chicken heart cells through introduction of MM-creatine kinase or specific MRNA by microinjection

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In contrast to skeletal muscle cells and mammalian heart muscle cells no transition from the embryonic and ubiquitous BB-CK to the muscle specific MM-CK takes place in chicken heart. Therefore no electron-dense material representing the so called m-bridges which are mainly formed by MM-CK, can be observed in myofibrils of chicken heart cells. In order to get more information on the control of expression and function of M-band proteins, chicken heart cells were microinjected with either MM-CK protein or with mRNA enriched for M-CK. In both cases appearance of MM-CK within the M-band of heart myofibrils could be observed by immunofluorescence, demonstrating translation of the injected message as well as specific binding of the translation product to the M-band of myofibrils. The M-band protein myomesin which is regularly found in heart myofibrils served as specific marker for assembled myofibrils in double immunofluorescence experiments.

The regulation of cAMP and $[Ca^{2+}]_i$ by hormones and neurotransmitters in GH_3 cells is correlated

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Measuring the cytosolic free $[Ca^{2+}]$, $[Ca^{2+}]_i$, with the fluorescent probe quin2 and cAMP by radioimmunoassay in GH_3 cells, evidence for a modulatory role of cAMP in the regulation of $[Ca^{2+}]_i$ has been obtained. Stimulators of adenylate cyclase, forskolin and VIP, raise $[Ca^{2+}]_i$ inhibitors of adenylate cyclase, somatostatin and carbochol, lower steady state $[Ca^{2+}]_i$. The effects of both stimulators and inhibitors of adenylate cyclase on $[Ca^{2+}]_i$ depend on extracellular $[Ca^{2+}]$. Exposure of GH_3 cells to pertussis toxin, known to abolish inhibitory regulation of adenylate cyclase, leads to a loss of the effects of somatostatin and carbachol on $[Ca^{2+}]_i$, while $[Ca^{2+}]_i$ can still be raised by forskolin and VIP. It is concluded that the regulation of $[Ca^{2+}]_i$ in GH_3 cells includes a cAMP dependent step. It appears that Ca^{2+} channels are the site of cAMP action, since it is known that the Ca^{2+} conductance of such channels is altered by cAMP dependent phosphorylation.

Quantitative assessment of the human neutrophil chemoattractant receptor system by flow cytometry analysis

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The chemoattractant receptor system of human neutrophils is a useful model to study stimulus response coupling mechanisms. Flow cytometry measurements allow the investigation of single living neutrophils under real time equilibrium conditions. On a laser based flow cytometer we determined the change in single cell fluorescence and transmission and perpendicular light scattering signals upon addition of chemotactic stimuli. Various techniques allow us to determine quantitatively the change of the following parameters as a consequence of neutrophil stimulation: receptor occupancy, internalization, F-actin formation, degranulation, oxidative burst, cytotoxicity, cell-cell interaction, phagocytosis, aggregation, and shape change. We made use of the fluoresceinated chemotactic peptide FNLPNTL to demonstrate changes in receptor occupancy elicited by other neutrophil directed stimuli. Simultaneously, metabolic events, e.g. increase in F-actin formation, degranulation, shape change, or oxidative burst were recorded and correlated with chemotactic peptide mediated stimulation.

DNA polymerase γ and cytochrome c oxidase activities in heat- and cold-sensitive mammalian cell-cycle mutants

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Activities of the two mitochondrial enzymes DNA polymerase γ and cytochrome c oxidase, and numbers of mitochondria per cell cross-section were determined after transfer of a heat-sensitive (hs, arrested at 39.5 °C) and a cold-sensitive (cs, arrested at 33 °C) cell-cycle mutant of the murine mastocytoma P-815 to the non-permissive temperature. In arrested hs and cs cells, numbers of mitochondria were nearly doubled, while a similar increase in cellular size and protein content was observed for hs, but not cs cells. In cs cells brought to 33 °C, both enzymes exhibited a parallel increase with a maximum after 3 days. In hs cells brought to 39.5 °C, cytochrome c oxidase activity increased to a maximum after 3 days, whereas DNA polymerase γ activity gradually decreased. In proliferating wild-type cells, temperature changes had only minor effects.

Developmental and molecular analysis of mutants in the *Antennapedia (Antp)* locus in *Drosophila melanogaster*

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The *Antp* locus is a prominent member of the *Antp* complex, a homeotic gene cluster on the third chromosome. The locus is associated with a dominant transformation of antennae into legs and a recessive lethal transformation of the mesothorax into prothorax and head structures. Developmental analysis of a newly discovered allele ($Antp^{PW}$) revealed several new aspects of the homeotic transformation. Transplanted imaginal discs of homozygous $Antp^{PW}$ larvae show a transformation of part of the mesothorax into head structures. A strong correlation between the localization of $Antp^+$ transcripts as determined previously by in situ hybridization in the wild type and the sites of mutant defects caused by the recessive loss of *Antp* function is observed. Molecular analysis of these mutants suggest that the dominant effect might be due to gene fusion which leads to an altered expression pattern of the *Antp* gene.

The Vi element: a repeated DNA sequence interspersed in the vitellogenin locus of *Xenopus laevis*

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The Vi element was originally identified through its presence in the 5′ flanking region of the *X. laevis* vitellogenin B2 gene where it interrupts the extensive homology with the corresponding region of the closely related B1 gene. Although it is present only about 540 bp upstream of the transcription initiation site of the B2 gene, it does not appear to interfere with the oestrogen-controlled expression of this gene. Three other copies of the Vi element have now been found in a total of 150 kb of DNA isolated from the vitellogenin locus [viz. 1) upstream of A1 gene, 2) in the spacer region between the A1 and B1 genes and 3) in a 3′

proximal intron of the A2 gene]. The Vi element is bounded by an imperfect 12 bp inverted repeat sequence and is flanked by 4 bp direct repeats which differ between elements. These properties indicate that the Vi element may be an example of a vertebrate transposable element or the evolutionary relic of such a sequence.

Subtypes of glial cells in optic nerve cultures and their interaction with cocultured neurons

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Dissociated optic nerve cultures from 10 day old or adult rats contained, besides some fibroblasts ($LETS^+$), flat 'reactive' protoplasmic astrocytes ($GFAP^+$) and fibrous astrocytes ($GFAP^+$) with long, dichotomously branched processes. In the oligodendrocyte family 3 subtypes could be distinguished: polygonal cells with few processes and immature membrane characteristics (tetanus toxin $(TT)^+$, O_4^+, mostly galactocerebroside $(GC)^-$, myelin basic protein $(MBP)^-$); cells with 'classical' morphology (small, round cell body; few, fine, long processes; TT^-, O_4^+, GC^+, MBP^+); and highly branched cells with many radial processes (mostly TT^-, O_4^+, Gal C^+). Sympathetic or sensory ganglion neurons or embryonic retina cells attached to and grew well on both types of astrocytes. The 'classical' oligodendrocytes also associated closely with the neurons and neurites. In contrast, the highly branched oligodendrocytes did not allow neuronal attachment and fiber growth on their territory, thus representing a non-permissive substrate for neurite regeneration.

A monoclonal antipeptide antibody recognizing a human N-*ras* p21 gene product

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We generated monoclonal antibodies directed against a synthetic peptide representing positions 172–180 of the human N-*ras* protein p21 which has 189 amino acids. A hybridoma (DRA 5221) was isolated and cloned. Its antibody precipitated a protein of 21 kD present in normal 3T3 cells and found at higher levels in N-*ras* transfectants. Immunoprecipitation analysis of N-, H-, and K-*ras* transfectants will be presented. A second band of 23 kD was also observed. The relationship between p21 and p23 was examined using different cell lines.

An inducible enhancer-like element in the upstream region of a mouse metallothionein gene

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Co-transfection of enhancer-less SV40 DNA (the so-called enhancer-trap; Weber et al., Cell *36* (1984) 983) along with sonicated DNA from the mouse metallothionein I gene resulted in a recombinant virus, SVMTI. This virus produces sufficient quantities of T antigen only in the presence of cadmium and zinc ions and is, therefore, heavy metal-dependent for its propagation. In place of the SV40 enhancer, SVMTI contains the upstream sequences of the metallothionein gene from nucleotide −63 to −188 in an inverted orientation with respect to its natural configuration, and also a 60 bp segment of unknown origin. Preliminary data suggest that the 60 bp extra DNA can be deleted without affecting the properties of the virus. In transient expression assays, the metallothionein DNA, in particular if present in several tandem copies, enhances the expression of a linked β-globin gene over a large distance from a 3′ position.

The primary structure of *Bacillus cereus* neutral proteinase

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Bacillus cereus neutral proteinase is an extracellular calcium-binding zinc metalloendopeptidase. It's molecular weight is 35,000 d and it is thermostable up to 60 °C. The complete amino acid sequence was established. The molecule was splitt into two main parts, DP I and DP II, by an acid cleavage. DP I yielded four fragments by cleavage at trp, and DP II three fragments by cleavage at asn-gly with hydroxylamine. Cleavage by trypsin and chymotrypsin yielded a C-terminal peptide and some overlaps. The peptides were sequenced with a Beckmann 890 C sequencer. The amino acid sequence is 76% homologous to thermolysin, a neutral proteinase thermostable up to 80 °C. The two sequences are compared with respect to the characteristics which may affect their thermostabilities: metal binding sites, salt bridges, hydrophobicity and other important, but more subtle amino acid residue exchanges.

The use of transducing SV40 viruses in DNA transfer experiments

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We have constructed transducing SV40 vectors harboring the origin of replication as well as sequences encoding SV40 late-functions, but lacking the genes specifying early functions. These sequences were replaced by an equivalent amount of DNA consisting of the chloramphenicol acetyl-transferase (CAT) gene fused to two different eucaryotic promoter sequences (α-amylase Amy-1^a, SV40 early) upon transfection into cos cells, which complement the early functions in trans, such constructs are replicated and packaged into infectious particles. Cos cells infected with such particles contain very high levels of CAT activity. We hope that these transducing viruses will be useful in a wide variety of gene transfer experiments in which the currently used transfection techniques are not feasible.

A 34 kD cellular protein phosphorylated by RSV transforming product is associated with small cytoplasmic ribonucleoprotein particles (RNPs)

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In RSV transformed chicken embryo fibroblasts (CEFs), the tyrosine phosphorylation of a 34,000 daltons cellular protein (34 kD) is greatly enhanced. This was shown to be a consequence of the phosphotransferase activity of RSV transforming protein: $pp60^{src}$.

The subcellular localization of 34 kD protein was investigated by biochemical fractionation of uninfected CEFs: 34 kD is mainly found in a crude membrane fraction, half behaves like a peripheral membrane protein, the remainder being more tightly bound. We have purified to homogeneity a 34 kD containing RNP from normal cells: it sediments at 9S suggesting a M_r of about 150–200 kD in good agreement with gel filtration data and has a buoyant density of 1.35 g/cm^3 in CsCl after formaldehyde fixation. This RNP is composed of 4 different polypeptides and contains an RNA moitie with tRNA like mobility.

Ca^{2+}, calmodulin-dependent and independent activities in spinach leaves and pea seedlings

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Purified chloroplasts were prepared from spinach leaves and pea seedlings and tested for NAD kinase activity. In both plants, a Ca^{2+}, calmodulin-independent activity could be detected in the stroma, which contained only trace amounts of calmodulin. An additional activity, sensitive to Ca^{2+}-calmodulin, was found associated to the pea chloroplast envelope, but the enzyme was oriented towards the cytoplasm. Most of the Ca^{2+}, calmodulin-dependent activity was recovered with a sedimentable (pea) or soluble (spinach) chloroplast-depleted cellular fraction. In this context, the occurrence and presumed role of calmodulin in the chloroplast is questioned.

Psoralen-crosslinking of DNA as a probe for the structure of SV-40 replicating minichromosomes

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Psoralen was used to crosslink replicating SV-40 chromosomes. The DNA was extracted and examined by spreading under fully denaturing conditions. The distribution of single-stranded bubbles corresponding to nucleosomal DNA was analysed (mapping of nucleosomes). The first nucleosome on the leading strand is formed at about 230 nucleotides from the 3′ end of the nascent DNA, whereas the first nucleosome on the lagging strand is located at about 330 nucleotides from the growing point of the replication forks. Viral chromatin synthesized in the presence of cycloheximide appears deficient in nucleosomes. The distribution of the 'old' nucleosomes on the two daughter strands suggest that the nucleosomes are randomly segregated in clusters.

Processing of the polymeric Ig receptor in the rat liver

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We have examined the processing of the polymeric immunoglobulin receptor in the rat liver by a combination of subcellular fractionation techniques, biosynthetic labelling and Western blotting analysis. The receptor, known as membrane secretory component (SCm), is synthesized as a core glycosylated transmembrane glycoprotein on the rER, which is subsequently terminally glycosylated in the Golgi with a half-time of about 30 min. Analysis of purified bile canalicular and sinusoidal plasma membranes was performed by Western blotting using a monoclonal antibody directed against the cytoplasmic tail of the receptor. The results indicate the intact receptor to be present in the sinusoidal plasma membrane, whereas the canalicular plasma membrane contains the membrane anchoring domain of the receptor which is generated upon cleavage of SCm. Studies are under way to determine the site and mechanism of receptor cleavage and the fate of the anchorage domain.

Clusters of axonal proteins with similar environmental modulation profiles: cues for proteins that cooperate in particular axonal functions?

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A heuristic approach to the assignment of functions to axonal proteins was undertaken by screening for axonal proteins that were environmentally modulated. Dissociated dorsal root ganglia (DRG) cells were plated in the center chamber of a compartmental cell culture system that allows selective analysis of axonal proteins. The outgrowing axons crossed the barrier between center and side chamber through a thin film of medium, whereas the cell somas were retained in the center chamber. Peripheral or central glial cells and spinal cord cells (neurons and glia) were co-cultured with the DRG axons in the side chamber. Metabolic labeling, 2D-SDS-PAGE and computerized quantitation of the individual axonal proteins revealed that the co-cultured cells modulate the synthesis of a few axonal proteins of DRG neurons differentially. These proteins subdivide into groups with a common distinct modulation profile in the different co-culture conditions. The proteins exhibiting comodulation might be involved in the same, yet to date unknown, axonal functions and stick out as intriguing proteins for further studies.

Interferon-induced protein with homology to protein Mx in cells from influenza virus resistant wild mice and human donors

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Interferons(IFNs)-α/β (but not IFN-γ) activate the allele Mx^+ in cells of the resistant inbred mouse strain A2G and thereby induce the synthesis of a nuclear 75,000 dalton protein and an antiviral state which is particularly effective against influenza viruses. Similarly, many wild mice from Europe and California were found to resist experimental infection and proved to be genetically Mx^+. Protein Mx was induced in these cells by IFN-α/β but not by IFN-γ. Moreover, their chromosomal DNA was identical to A2G DNA when Southern blots were probed with Mx^+ specific cDNA. A similar protein crossreacting with polyclonal and monoclonal antibodies was found in peripheral blood lymphocytes from human donors and in various human cell lines treated with either natural leukocyte IFN or recombinant hu IFN-α_2. Again, this human protein was not induced by hu IFN-γ. We conclude that IFN-induced proteins with structural and functional similarities may be conserved during evolution.

A new molecular model of DNA recombination based on micrographs of joint molecules involved in strand exchange

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Direct electron microscopic visualization of recA-DNA complexes involved in consecutive stages of strand exchange reaction (Stasiak et al., 1984, Cold spring Harbor Symp. Quant. Biol. 49, in press) allows the following conclusions: 1. Initial contact of recombining molecules occurs between recA covered single-stranded DNA and naked double-stranded DNA. 2. During the step of homologous recognition double and single-stranded DNA segments are placed together in the same recA-DNA filament. 3. Displacement of single stranded DNA is late in the recombination process and occurs only after achievement of homologous alignment.

The tumor promotor TPA acts on cells transformed by polyomavirus middle T antigen to produce a highly transformed phenotype

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According to R.A. Weinberg and his colleagues, the cancerous state of a cell is triggered by transforming as well as immortaliz-

ing genes (Nature *304* (1983) 596). v-src, the transforming protein of Rous sarcoma virus is thought to stimulate diglyceride synthesis (Nature *308* (1984) 770). Interestingly, diglyceride is known to activate protein kinase C, as does the tumor promotor TPA (Nature *302* (1983) 750). From these findings one might have expected that TPA and transforming proteins, such as v-src and polyoma virus middle T antigen can mutually replace each other in the process of malignant cell transformation. However, such a model is likely to be too simple. We have found that TPA acts on middle T antigen-transformed, immortalized rat 3T3 cells to produce in agar suspension fast growing variants. Several of these variants are no longer spindle shaped but are of a highly transformed phenotype.

3′ processing of histone pre-mRNA: the novel U7 small nuclear RNA

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3′ processing of sea urchin H3 histone pre-mRNA depends on a small nuclear RNP which contains an RNA of nominally 60 nucleotides length, referred to as U7 RNA. The U7 RNA can be enriched by precipitation of sea urchin U-snRNPs with lupus antibodies of the Sm-serotype. We have prepared cDNA clones of U7 RNA from two different sea urchin species. The RNA sequences derived from the analysis of eight U7 cDNA clones show neither homologies nor complementarities to any other known U-RNA. Their most striking feature is their base complementarity to the 3′ conserved sequences of histone pre-mRNA. Six out of nine bases of the conserved CAAGAAAGA sequence of the histone mRNA precursor and 13 out of 16 nucleotides from the conserved palindrome can be basepaired with the presumptive U7 RNA sequence suggesting a unique hybrid structure for a processing intermediate formed from histone precursor and U7 RNA. U7 RNA is being further characterized.

Cloning and characterization of *Xenopus laevis* actin genes

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A cDNA library and a genomic library from *X. laevis* have been screened for actin coding sequences. Two cDNAs were identified which represent an α actin of the type expressed in adult heart muscle and an α actin of the type expressed in adult striated muscle. Sequencing of genomic clones led to the identification of 3 different types of α actin genes. Two of them code for the isolated cDNAs and the third could represent a second type of striated muscle actin gene, however, absence of intron sequences suggests it to be a pseudogene. Hybridization of restricted genomic DNA with actin cDNA generates a complex pattern of bands suggesting the presence of 10–20 genes per genome. More specific probes containing the exon coding for amino acids 1–41 of either the cardiac or the striated actin gene light up only 2–3 EcoR1 fragments each. Two additional genes closely related to either the cardiac or the striated muscle actin gene seem thus to be present in the *X. laevis* genome.

Time sequence of meiotic stages in bovine oocytes matured in vitro

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Bovine oocytes were aspirated from small (≤ 6 mm) follicles of ovaries collected immediately after slaughter. Each pair of ovaries yielded an average of 10 to 12 compact cumulus oocyte complexes (40 to 60% of the total number of collected oocytes) which were cultured in order to study the time sequence of meiosis. The oocyte culture was terminated after various time periods and the chromosome configurations were determined after staining with Giemsa. For the first 10 h after oocyte collection the predominant chromosome configurations were fine threads and contracted threads. For the next 6 more than 60% of the evaluated oocytes showed the bivalent chromosomes of diakinesis. This stage was followed by the first meiotic division resulting in the chromosome configuration of metaphase II. After 20 h of culture 70% of the evaluated oocytes had already reached metaphase II.

Adeno-associated virus (AAV): a vector for stable integration and expression of a foreign gene

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The parvovirus AAV was studied as a vehicle to stably integrate and express a foreign gene in human cells. We constructed an AAV vector containing the prokaryotic gene NEO which provides a dominant selectible marker for mammalian cells, namely resistance to the antibiotic G418. When this vector was transfected into human 293 or HeLa cells, G418 resistant cells were obtained at frequencies of up to 2×10^{-4}. When the vector was first packaged into AAV particles and then introduced into cells by particle infection, G418 resistant cells were obtained at frequencies close to 2×10^{-2}. Cell clones derived from either transfections or infections were isolated and the number and arrangement of the integrated NEO gene copies were determined. The AAV NEO vector was rescued from NEO transformed cells by infection with wild-type AAV and helper adenovirus. Subsequently, the vector could be recovered as amplified episomal DNA from a Hirt lysate or from the yield of particles in the cell lysates.

Protein phosphorylation is implicated in the meiotic arrest of mouse oocytes: effect of forskolin and phorbol esters

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In mammals, meiosis reinitiation is induced by the preovulatory surge of gonadotrophins in vivo, but occurs spontaneously in vitro when the oocytes are isolated (denuded) from their follicle. We report that forskolin, an activator of adenylate cyclase, maintains the meiotic arrest of denuded oocytes and increases intracellular levels of cAMP. Active tumor-promoting phorbol esters (PDBu and PMA), known to activate the calcium and phospholipid-dependent protein kinase, are also able to prevent the spontaneous meiosis reinitiation, in contrast to the inactive one (phorbol monoacetate). Retinoids appear to antagonize the phorbol esters inhibitory action on meiosis reinitiation, whereas the ionophore A23187 neither potentiates nor antagonizes this inhibition. These data suggest a role for protein phosphorylation (cAMP-dependent and calcium and phospholipid-dependent) in the control of the meiotic arrest of denuded mouse oocytes.

Mapping of structural determinants in human alpha-interferons responsible for high antiviral activity in mouse cells

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Human IFN-α1 but not -α2 has significant antiviral activity on mouse cells (relative murine to human activity: 3500). To iden-

tify the sequence(s) responsible, we prepared hybrid genes, with different lengths of 5′ moiety of the IFN-α2 and 3′ moiety of the IFN-α1 gene (Weber and Weissmann, NAR *11* (1983) 5661). The cognate IFNs were generated in E. coli and purified. A hybrid protein with 45 C-terminal IFN-α1 residues (of which 6 differed from those in IFN-α2) retained activity on mouse cells. Single and multiple amino acid replacements were generated in the critical region. Replacing Gln^{125} in IFN-α2 with Arg (as in IFN-α1) or Arg^{121} by Lys increased the relative activity on mouse cells 250- and 30-fold, respectively, suggesting that this region may be essential for productive interaction with the murine receptor.

Relationship between chloroplast ARC, ARS sequences and origins of replication in *Chlamydomonas reinhardii*

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We have previously isolated several chloroplast DNA segments that promote autonomous replication in yeast (ARS, Vallet et al., EMBO J. *3* (1984) 415) and in *C. reinhardii* (ARC, Rochaix et al., Cell *36* (1984) 925). Waddel et al., Nucleic Acids Res. *12* (1984) 3843, have localized two authentic chloroplast origins of DNA replication one of which is located on a 5.3 kb EcoRI restriction fragment. The latter also contains an ARC and an ARS element. A fine structure analysis has revealed that the ARC element and the origin of replication are located 1.5 kb from each other. The ARS sequence has been localized in a region which overlaps neither the ARC site nor the origin of replication. Similarly restriction fragments containing the origin of replication from the chloroplast DNA of *Euglena gracilis* (Schlunegger et al., Biochim. biophys. Acta *739* (1983) 114) are unable to promote autonomous replication in yeast.

Lack of induction of citrate synthase (CS) in amphibian epithelia responding to aldosterone

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Aldosterone-dependent sodium transport is thought to be linked to the induction of CS. We tested this hypothesis in the urinary bladder of the toad *Bufo Marinus* and in cultured cells (TBM). In both models, stimulation by aldosterone elicited a significant increase in Na^+ transport. The citrate synthase activity, however, did not increase significantly.Immunoprecipitation of biosynthetically labeled CS from induction could be a characteristic of H^+ secreting cells (mitochondria rich cells in toad bladder). In a fraction enriched in mitochondria rich cells, we observed a minimal change in CS activity, despite a significant increase in H^+ transport measured by reverse short circuit current in presence of amiloride. Our negative results suggest that the induction of CS is not a prerequisite for the stimulation of Na^+ and H^+ transport by aldosterone.

Ethylene-induced increase in translatable mRNA for chitinase in bean leaves

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In many plants, the hormone ethylene causes a strong increase in the activity of chitinase, an enzyme with a potential defensive function against pathogens (Boller et al., Planta *157* (1983) 22). Using a rabbit antiserum raised against purified bean chitinase, we investigated the mechanism of chitinase induction by ethylene. Excised bean leaves were pulse-labelled in vivo with 35S-methionine. The newly formed polypeptides were precipitated with the chitinase antiserum. Much more radioactivity was present in the immunoprecipitates from ethylene-treated than from control bean leaves. Poly-adenylated RNA was extracted from bean leaves and translated in vitro in a rabbit reticulocyte lysate. Immunoprecipitation of the translation products and SDS gel electrophoresis yielded a single band with a MW of 36,000 (2000 dalton larger than native chitinase). Ethylene treatment caused a rapid and strong increase in this product, indicating that ethylene increased the level of translatable mRNA for chitinase.

Structure and sequence of the chloroplast genes coding for the ribosomal proteins S12 and S7 from soybean

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The *rps* G and the *rps* L genes coding, respectively, for the ribosomal proteins S7 and S12 are part of the str operon in *E. coli*. S7 is an assembly protein interacting with the 16S rRNA and S12 functions in the read-out process and is somehow involved in streptomycine resistance. Both genes were mapped and sequenced on the chloroplast genome of *Euglena gracilis* (Montandon and Stutz, NAR *12* (1984) 2851). We have now sequenced the chloroplast *rps* G and *rps* L genes of soybean in order to compare identical chloroplast genes of different evolutionary level. The soybean chloroplast *rps* G gene codes for a protein of 155 aminoacids which is to about 35% homologous with the E. gracilis chloroplast gene. The *rps* L gene contains two large introns and a major ORF (143 codons) in the second intron. Sequence homology between the two chloroplast S12 proteins is in the range of 65%. The domains around lys 42 and lys 87 (crucial in streptomycine resistance?) are well conserved. The 5′ intron bounderies follow a recently suggested consensus i.e. 5′-GPyGPyG- (Koller et al. Cell *36* (1984) 545) as seen in the multiply split *psb* A and *rbc* L genes of the Euglena chloroplast genome.

Expression of gene constructs transfected into chicken hepatocytes in primary cultures

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In *Xenopus laevis*, vitellogenin is encoded by a family of 4 related genes named A1, A2, B1 and B2, all of which are under oestrogen control in the liver. The 5′ ends of these 4 genes have been sequenced. Comparison between the *X. laevis* genes and the single gene which has been isolated in the chicken has revealed, besides other homology blocks, a short dyad-symmetrical sequence GGTCANNNTGACC at similar positions upstream of the five genes. We have now developed an expression system, that consists of primary cultured chicken hepatocytes which can be transfected. Genes under the control of the SV40 early promotor are faithfully expressed in these cells. We are currently analysing the hormonally-controlled expression of vitellogenin gene constructions in this system.

Identification and localization of creatine kinase (CK) isoenzymes in retina

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Isolated chicken retinas were shown by cellulose polyacetate electrophoresis and immunoblots to contain brain-type BB-CK and mitochondrial-type MiMi-CK. BB-CK was shown by immunofluorescence to be highly concentrated within both rod and cone cells of the photoreceptor cell layer. Most of the fluorescence staining with anti-B-CK antibodies was found within

the myoid and ellipsoid portion of the inner segments of the photoreceptor with some additional staining of the peripheral regions of the outer segments. Localization of MiMi-CK corresponded to the ellipsoid portion of the inner segments, a region known to be rich in mitochondria. The finding of high local concentrations within photoreceptor cells of compartmentalized CK isoenzymes as well as the high concentration of total creatine (10–15 mM) indicate an important function of CK for vision and suggest that visual perception, like muscle contraction and sperm motility, may depend on a phosphoryl-creatine (CP) metabolism whereby efficient distribution of energy from mitochondria to sites of utilization is mediated through a CP-shuttle.

Enhancer activity correlates with the transforming potential of avian retroviruses

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Avian retroviruses without an oncogene, such as Rous-associated virus 1 (RAV-1), RAV-2, and td-mutants of Rous sarcoma virus (RSV), can nevertheless cause tumors by integrating near a cellular proto-oncogene like c-myc, thereby deregulating its expression. The virus RAV-0, on the other hand, is known to be non-oncogenic even in long-term in vivo infections. The major difference between transforming and non-transforming viruses is found in the U3 region of the long terminal repeat which is known to harbor the promoter/enhancer elements. We therefore wanted to see whether viral oncogenicity was correlated with enhancer activity. Using a variety of techniques (including the SV40 'enhancer trap' from which we obtained RSV-SV40 recombinant viruses), we demonstrate a strong enhancer within the long terminal repeats of both RSV and RAV-1. In contrast, no enhancer is present in RAV-0 (although RAV-0 has functional promoter elements). Our data therefore strongly support a concept of oncogenesis by enhancer insertion.

The complete amino acid sequence of the Bchlc-associated polypeptide of chlorosomes from the green thermophilic bacterium *Chloroflexus aurantiacus*

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Chloroflexus aurantiacus is a gliding filamentous bacterium which inhabits alkaline hot spring effluents at temperatures from 50–70 °C. The photosynthetic apparatus of *Chloroflexus* consists of reaction center units and a light-harvesting Bchla-protein complex (absorbing at 805 and 805–865 nm) which are located in the cytoplasmic membrane. A second Bchlc-containing light-harvesting complex is situated in extramembrane vesicles (chlorosomes). This Bchlc-associated polypeptide (absorbing at 740 nm) was seqenced by manual Edmann degradation and with a Beckmann 890 c sequencer. It contains 51 amino acid residues to give a molecular weight of 5592. One His and the high amount of 9 Asn and Gln were found. The latter are candidates for Bchl-polypeptide interaction sites. We consider a pigment-protein-complex consisting of 6–8 Bchlc-molecules per monomer to be possible.

Nucleosome phasing or sequence specific cleavage of micrococcal nuclease in BR2-chromatin of *Chironomus?*

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Nuclei of cultured *Chironomus* cells are digested with micrococcal nuclease. The DNA is electrophoresed, blotted and hybridized with probes of BR2-sequences. The BR2-gene contains 10 to 20 kb long blocks of tandem repeats of either 215 bp (α-repeat) or of 195 bp (β-repeat) (for ref. s. Höög & Wieslander, PNAS *81* (1984) 5165). Limited restriction digestion of BR-DNA produces ladders of multimers of 215 bp or 195 bp, respectively. The micrococcal nuclease digest of BR2-chromatin shows 1) Up to 30 and even more multimeric bands and 2) small oligomers (mono- to decamers for the α-repeat, and mono- to tetramers for the β-repeat) with a repeat length of about 180 bp. The repeat length of the fragments longer than 11- or 5-mers, respectively, is the same as that of the underlying DNA, namely 215 bp or 195 bp, respectively. The region of the transition from the smaller to the longer repeat (11- or 5-mers, respectively) is smeared. Possible models are discussed.

Pathway of infecting virus and newly synthesized viral proteins in PR8 influenza virus infected cells: immunoperoxidase labeling in the electron microscope

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Various compartments are involved in the productive infection of cells with influenza viruses. Infection occurs via the endocytic pathway through fusion of the viral envelope with the endosomal membrane. This event is mediated by the hemagglutinin (HA) molecule, the fusion activity of which is activated by the acid pH of the environment. We have used a monoclonal anti-HA antibody specific for the acid-induced conformational change of the molecule to localize with peroxidase labeling the passage of the virus through an acid compartment (endosome). Biosynthesis of virus-coded proteins was studied by following the pathway of the HA and nucleocapsid protein (NP). Newly synthesized NP could first be detected evenly dispersed in the nucleus. Later, a membrane-bound form was found in the cytoplasm which migrated to the plasma membrane for viral assembly.

Mechanism of action of IFN-α and of IFN-γ in human cells infected with influenza a virus

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Interferon(IFN)-α and IFN-γ bind to separate receptors on the cell surface, and they induce sets of proteins which are quantitatively and/or qualitatively different. Taking influenza A virus as a model we asked 1) whether both types of IFN elicit the same antiviral response and 2) whether the antiviral pathways are similar. We have found that both IFN-α and IFN-γ induced a potent anti-influenza virus state in human embryonic cells. Polyadenylated viral mRNAs were quantitated by hybridization to radioactive genomic RNA, followed by separation of the hybrids by affinity chromatography on oligo(dT). Viral protein synthesis was quantitated by labeling cells with ^{35}S-methionine and separation by two-dimensional gel electrophoresis. Primary transcription was not significantly inhibited by either type of IFN, but a strong inhibition of viral protein synthesis was observed. It seems therefore that both types of IFN inhibited influenza virus replication at the same level. This does not necessarily imply that the antiviral pathways are identical at the molecular level.

Chick superior cervical ganglion neurons grown in long-term culture: factors influencing their adrenergic and cholinergic properties

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Neurons dissociated from the embryonic chick superior cervical ganglion were separated from ganglionic non-neuronal cells us-

ing a continuous density gradient formed with Percoll. The sympathetic neurons were grown for 3–4 weeks in serum-containing medium on a polyornithine substrate pre-coated with heart-conditioned medium. Both catecholamines (CA) and acetylcholine (ACh) are synthesized and accumulated by these neurons, but the amount of CA is higher and increases much more over time in culture than the amount of ACh. The cultures become therefore much more adrenergic with time. 50% of the neurons from 8-day embryos take up (^{3}H)-norepinephrine whereas up to 90% of the neurons from 12-day embryos do. The cholinergic properties of the neurons increase when chick eye extract of heart-conditioned medium are added to the culture medium. In the presence of ganglionic non-neuronal cells, liver conditioned medium, increasing nerve growth factor concentrations or increasing neuron densities, however, only the adrenergic properties of these neurons increase.

GENETICS

A homeo box containing gene isolated from the *engrailed* region of *Drosophila* and the spatial distribution of its transcripts

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The *engrailed (en)* locus of *D. melanogaster* which is known to be involved in subdividing segments into anterior and posterior compartments has the characteristics of both a homeotic and a segmentation gene. Using homeo box cross-homology we have isolated both genomic and cDNA clones from the *en* region (48A). The homeo box is different from other homeo boxes with respect to homology (only ~ 55%; cf. 75–80%) and because it contains an intron. A major *en* RNA of 2.6 kb and an other species of 1.3 kb are detected in embryonic RNA. Accumulation of transcripts peak at the time when germ band extension is terminated (~ 6 h). By in situ hybridization to tissue sections the transcripts are first detected shortly after the onset of gastrulation as narrow bands (1–2 cells in width) in the region posterior to the cephalic furrow. At 6 h of development 14 hybridization bands are seen reiterated along the germ band. The transcripts appear to be located in cells which belong to the posterior compartments of segments as expected from previous genetic analyses.

Genotoxicity of the carcinogens azaserine (ASER) and streptozotocin (STR) in the *Drosophila* somatic mutation and recombination test

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ASER is a typical pancreatic carcinogen (IARC *10* (1976) 73). STR has affinity for the β-cells of pancreatic islets and, in inducing necrosis there, provokes hyperglycaemia (IARC *17* (1978) 337); it is carcinogenic in liver, kidney and pancreas. Both substances (originally from *Streptomyces* spp.) have been used as antineoplastic drugs. Both are mutagenic in bacterial assays and in the sex-linked recessive lethal assay of *Drosophila*. We studied their genetic effects in *Drosophila* wing cells to contribute to the validation of the somatic mutation and recombination assay for the rapid detection of mutagens and carcinogens (Graf et al., Environ. Mut. *6* (1984) 153). Feeding of 3rd instar larvae for 48 h revealed both compounds to be strongly genotoxic in this in vivo test. With ASER, clone induction due to mitotic crossover or mutation was about 1.5 times as high as with STR, as borne out by the respective exposure/effect relationships.

Use of wide host range R-plasmid R388 for in vivo and in vitro genetic engineering in *Pseudomonas putida*

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There is an increasing interest in applying in vivo and in vitro genetic procedures established in *Escherichia coli* to other gram negative bacteria. We used derivatives of wide host range *inc*W plasmid R388 to extend genetic methods to *Pseudomonas putida* to study catabolic genes of TOL plasmid pWW0. Two applications will be presented. 1) an R388-pACYC177 hybrid was used to clone mutant genes of catabolic enzymes and complementation tests using such clones were carried out in *P. putida;* 2) an R388::Tn*10* derivative was used to make stable cointegrates between R388::Tn*10* and pBR388 clones in vivo. This is a simple method to transfer from *E. coli* to *P. putida* genes cloned in pBR322 (and other *E. coli* specific vectors) and to maintain them in the latter strain.

A genetic analysis of size control in the *Chlamydomonas reinhardii* flagella

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The flagella of *Chlamydomonas reinhardii* are organelles whose size is closely regulated. A genetic analysis of mutant strains defective in their control of flagellar length has been pursued in order to better understand the means by which cellular dimensions are specified. Both long- and short-flagella mutants have been isolated, along with mutants with abnormal numbers of flagella. Six mutant strains with abnormally short flagella were chosen for comparative analysis. The six short flagella *(shf)* mutants fall into three recombinationally distinct complementation groups. All possible *shf, shf* double mutants were constructed in order to study the interactions of the various mutations. Depending on the alleles present, double mutant cells either had short flagella or were flagellaless. Revertant strains with wild-type length flagella were isolated from several mutant backgrounds. Analysis of these revertants revealed in some cases the presence of new extragenic suppressor mutations. The biochemical composition of mutant and revertant flagella was also studied. In many cases, consistent differences between mutant and wild-type flagellar polypeptides can be detected.

Overlapping promoters of *Pseudomonas putida* TOL plasmid

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The *Pseudomonas* are a group of bacteria that collectively exhibit an extremely wide range of metabolic activities, and are therefore of considerable interest for both basic and applied studies. We are currently analysing expression of the TOL plasmid catabolic operon of *Pseudomonas putida*, which encodes enzymes of the *meta*-cleavage pathway for the degradation of benzoate and toluates. Expression of this operon is positively regulated by the *xylS* gene product. We have sequenced the promoter region of the operon and localized its transcription initiation sites. Two overlapping promoters are responsible for the regulated expression of the operon in both *Escherichia coli*

and *P. putida*. Isolation, characterization and comparison of mutations which created new promoters allowed the derivation of consensus sequence for *P. putida* promoters which is significantly different from that of *E. coli*. A broad host range expression vector was constructed, containing these promoters and the *xylS* gene. Features of this vector will be discussed.

Characterization of IS*903*.B, a variant of IS*903* and IS*102*

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An internal, 1057 bp long segment of the transposon Tn*2680*, which is homologous to Tn*6*, is itself an active transposable element. It can mediate transposition of unrelated genes by flanking them. It is a close structural and functional relative to IS*903* and IS*102* and is given the designation IS*903*.B. Nucleotide sequence analysis revealed that 34 nucleotides differ between IS*903*.B and IS*903*, while 61 nucleotide differences are found between IS*903*.B and IS*102*. All three elements have the same length, the same 18 bp long perfect inverted repeats and generate 9 bp target duplications upon insertion. The nucleotide substitutions in IS*903*.B do not affect the size of the large open reading frame, but they affect its amino acid sequence. The C-terminal end of the expected product (about one third of the entire size), however, is identical in all 3 IS elements. In contrast, the short open reading frame of IS*903*.B terminates at an amber stop codon earlier than that of IS*903* and IS*102*. Since transposition of IS*903*.B functions with equal efficiency in either permissive or nonpermissive bacteria, the small open reading frame may not be of functional relevance, at least not in its full length.

Glucocorticoid resistance is a dominant trait in hybrids between cytolytic T-lymphocyte lines and AKR-thymomas

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We have screened several cytolytic T-lymphocyte lines and AKR-thymomas as well as CTL × thymoma hybrids from two crosses for their sensitivity to the glucocorticoid (GC) analogue dexamethasone (dex), and have found that CTL-lines and cytolytically active, IL-2-dependent (CTL-like) hybrids are resistant to the cytostatic or cytolytic effects of dex; thymomas and thymoma-like hybrids (cytolytically inactive, IL-2-independent), however, are sensitive to these effects of the drug. The CTL-phenotype behaves like a dominantly inherited trait in these crosses, and the same is true for the resistance to GC. The GC resistance of the CTL-lines and the CTL-like hybrids is not due to the absence of functional hormone receptors, as these cells contain several thousand hormone binding sites/cell and translocate the GC-receptor complex efficiently into the nucleus. The macrophage-activating factor release by the CTL-lines and CTL-like hybrids is inhibited by dex.

Detectability of mutagens in somatic and germ cell assays with *Drosophila melanogaster*

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Drosophila melanogaster provides assay systems which allow the detection of induced mutations in either germ cells or somatic cells. Mutagenicity testing of chemicals using somatic cell assays is faster and more flexible than testing with germ cells. In the other hand, the germ cell tests in particular the sex-linked recessive lethal assay have been validated extensively already. At present data are collected in order to validate somatic assays, especially the wing spot test. Somatic assays detect induced mitotic recombination and possibly nondisjunction in addition to the induction of gene mutations, deletions and other types of chromosome aberrations. The data available at the time of the meeting will be used to compare the detectability of different classes of mutagens and promutagens in several *Drosophila* somatic and germ cell assays.

PHARMACOLOGY

Fast cytotoxicity assay using dye exclusion and retention in two-parameter flow cytometry

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A reliable and fast cytotoxicity assay to detect chemicals which affect membrane permeability is described. The fluorochrome ethidium bromide (EB) is known to be excluded from the intact cell, staining only nucleic acids of membrane-damaged cells. On the other hand, fluorescein diacetate freely penetrates intact cells and is hydrolyzed to its fluorochrome fluorescein (F) which is retained in the cell due to its polarity. The different emission spectra of EB and F allowed us to filter selectively and to measure both dyes independently in single cells of a double-stained cell population.

Freshly isolated rat thymocytes were selected because of 1) ease of isolation without enzyme treatment, 2) large numbers available, and 3) homogeneous size distribution. Two-parameter analysis was done in a Partec flow cytometer. Proper triggering and data analysis was performed by an online computer (Ahrens). Concentration-dependent cytotoxicity of various detergents was demonstrated.

Choline acetyltransferase in cholinergic nerve endings: partition into hydrophilic and hydrophobic activities

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Choline acetyltransferase (ChAT) and acetyltransferase (AChE) activities present in the purely cholinergic synaptosomes isolated from the electric organ of the fish *Torpedo* could be partitioned into hydrophilic and hydrophobic activities by treating the synaptosomes with the non-ionic detergent Triton X-114. Reproducibly 85% of ChAT was found as hydrophilic and 10% as hydrophobic activity. AChE was present mainly as hydrophobic activity, this enzyme being an integral protein of the synaptosomal plasma membrane (SPM) of *Torpedo*. Triton X-114 treatment of the isolated SPM confirmed the existence of the hydrophobic form of ChAT activity. No other membrane fraction isolated from the electric organ contained significant levels of membrane-bound ChAT. The velocity of choline acetylation by the hydrophilic and hydrophobic ChAT had app K_m for choline of 0.47 ± 0.17 and 1.04 ± 0.37 mM, respectively. The interaction of hydrophobic ChAT with SPM has now to be investigated.

Comparative metabolic disposition of caffeine in rats, mice and Chinese hamsters

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The comparative metabolic disposition of [1-Me^{14}C] caffeine has been studied in three rodent species: the rat, the mouse and the Chinese hamster. No inter-specie difference appeared in urinary and fecal excretion of radioactivity while 1-methyl demethylation was significantly more important in the rat with 20.6 ± 0.8% of the dose recovered as $^{14}CO_2$ compared with the Chinese hamster: 16.1 ± 2% and the mouse: 13.9 ± 0.9%. HPLC and t.l.c. analysis of 1-methyl labeled metabolites excreted in the urine showed that the rat is characterized by a significantly higher excretion of trimethyluric acid, trimethylallantoin, theophylline and the uracil derivative of caffeine. The Chinese hamster excreted more paraxanthine, 1-methylxanthine and the uracil derivative of paraxanthine. In the case of the mouse higher amounts of 1,3 and 1,7-dimethyluric acid were found. The mouse was also characterized by the presence of an unknown polar metabolite amounting to 22 ± 3% of urine radioactivity.

Effect of captopril on food intake in rat

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Large doses of captopril exert effects possibly due to interference with peptidase other than converting enzyme. Thus, large doses of intracerebroventricular (i.c.v. 10, 30, 100 or 300 mcg) captopril in satiated rats dose-dependently increased food intake, but depressed water intake. Naltrexone (1 or 3 mg/kg b.wt s.c.) 15 min before 300 mcg captopril i.c.v. depressed the increase of food intake. The increase of food intake induced by 3 mg 2-deoxy-glucose i.c.v. was significantly increased by 300 mcg captopril i.c.v. *Subcutaneous* captopril (3–100 mg/kg b.wt) had no effect on food intake in satiated or in hungry rats, but significantly increased water intake. The observations suggest a possible influence of captopril with the metabolism of endorphins.

Clinical effectiveness of amitriptyline (AT): a clinical, pharmacokinetical and pharmacogenetical study

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In 30, mostly endogenous, depressive patients, the relationship between the clinical effectiveness of a treatment with 150 mg AT daily during 3 weeks and free and total plasma levels of AT and nortriptyline (NT) has been investigated. 22 patients improved by more than 50% (Hamilton). On day 22, mean ± s.d. (ng/ml) levels were: total plasma AT: 102 ± 59.1; free plasma AT: 6.35 ± 3.44; total plasma NT: 85 ± 60.0; free plasma NT: 7.59 ± 5.15. No simple relationship between clinical and biochemical parameters was observed. Half of the patients were submitted to the debrisoquine- and some to the mephenytoin-test. In poor metabolizers of either of these drugs, there was a tendency to higher levels of AT and NT. The PM patients differed from the extensive metabolizers (EM) by a lower ratio of (AT − OH + NT − OH)/AT in plasma. From a theoretical point of view, the monitoring of all of the above-mentioned parameters (free and total plasma levels of the parent drug and metabolites) seems justified. However, the example shows that the higher is the number of relevant parameters, the more difficult is the interpretation of the data with regard to their clinical importance.

Effect of caffeine on enzymatic induction in the rat as evaluated by using the model of demethylation of ^{14}C-aminopyrine to $^{14}CO_2$

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The purpose of the present study was to determine, in rats, the effects of caffeine (CAF) pretreatment on the demethylation of ^{14}C-aminopyrine (^{14}C-AMPY) by measuring the resulting $^{14}CO_2$ in expired air. The kinetics of $^{14}CO_2$ were measured for each animal before and after pretreatment over a 4-h period after administration of 4 mg/kg ^{14}C-AMPY (~ 4 μCi). CAF pretreatment consisted of giving 25 mg/L in the drinking water for 3 days to rats. A comparison of area under curve values for $^{14}CO_2$ elimination using each animal as its own reference indicated that CAF induced the enzymatic system and thus caused a 20% increase (6 animals). Comparative experiments with phenobarbital (PB) pretreated rats showed that PB caused a AUC increase of 25%. These results show that, in rats, short CAF pretreatment results in a significant induction of the liver enzymes involved in the demethylation mechanism, i.e., almost as much as that obtained with PB pretreatment.

Pharmacokinetics of Oxetorone, an antimigraine drug, in man

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Pharmacokinetic studies of ^{14}C-labeled Oxetorone (Nocertone, Labaz Lab.) were performed in 6 male volunteers after oral adm. of 60 mg of the drug. The max. plasma concentration was 533 ng eq/ml at 1 h 30′ and mother product value was 78 ng/ml at 30 min. The absorption was fairly rapid ($t_{1/2}$ = 0.49 h). For a two compartment open model, all parameters were determined as for example, $t_{1/2}$ α = 1.69, $t_{1/2}$ β = 26.65, V_c/F = 88.6, AUC = 10.293 ng h/ml. In vitro protein binding was 97%, while in vivo it was 93% at 1 h and 85% at 3 h. Over a 72-h period, 35% adm. dose was excreted in urine and 38% in the feces. Unchanged drug and metabolites were quantified in plasma. In the urine, unchanged drug (3–7% at 13 h and 3 h, respectively) and 8 metabolites were quantified. In view of certain known properties of the drug, the prolactin level was also quantified in all plasma samples; no modification of basal levels were observed.

Single nephron cadmium-metallothionein or cadmium uptake: an in vivo study by micropuncture

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Renal tubular micropunctures and microinjections were carried out in rats to investigate cadmium (Cd) uptake by single nephron in vivo. Free-flow glomerular and proximal tubule micropunctures indicate that, in rats pretrated and acutely infused with cadmium, which had measurable levels of Cd-metallothioneins (CdMt) in plasma (measured by radioimmunoassay), uptake of Cd by proximal tubules could not be accounted for by CdMt reabsorption only: tubular uptake of Cd not bound to Mt was predominant. Similarly, microinjections of either inorganic Cd (Cd_i) or CdMt into proximal tubules indicate that a larger tubular uptake occurred after Cd_i than after CdMt microinjections. Thus, results from both technical approaches suggest that, at least under the present experimental conditions, uptake of Cd_i from the lumen of proximal tubules is more extensive than that of CdMt.

The selection of more or less salted food pellets by normal and adrenalectomized rats

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Choosing between water and moderately hypertonic NaCl solutions, adrenalectomized rats drink more saline than controls and secure a salt intake permitting survival (Chr. Meuli and G. Peters, Nato Research Workshop, Camerino 1984). We investigated the choice between more or less salted food pellets in animals drinking water. Choosing between pellets containing 6 g NaCl/kg and similar pellets containing either 12 or 18 g NaCl/kg, adrenalectomized rats ate a greater proportion of the more salty food than controls eating less food but secured a smaller total salt intake ($\sim 30\%$) than controls. Choosing between 6 and 24 g NaCl/kg containing food, adrenalectomized rats reached a daily NaCl intake of 62% of controls. The salt intake secured in many adrenalectomized rats was too small to permit survival: 9/23 adrenalectomized rats died after 6 to 11 days of the choice situation: Survivors given a choice between pellets containing no NaCl and pellets containing 12 or 18 g NaCl/kg ate enough high salt pellets to survive for another 10 days. The motivation for salt intake, in many adrenalectomized rats, thus, was too weak to overcome the decreased motivation for eating salt-enriched food pellets in amounts sufficient to insure survival.

Enzymatic basis of the debrisoquine/sparteine type polymorphism of drug oxidation

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The mechanism of the common metabolic defect known as debrisoquine polymorphism was investigated in liver microsomes from patients phenotyped in vivo as extensive (EM, n = 10) or poor (PM, n = 5) metabolizers. 1'-Hydroxylation of the model substrate bufuralol (Bu) revealed: 1) in EM oxidation is selective for (+)-Bu, 2) PM microsomes are characterized by an increased K_m (EM: 18 ± SD 6 μM, range 10–28 μM versus PM: 118 ± 85 μM, range 38–218 μM), 3) PM present a loss of substrate stereoselectivity and a reduced V_{max}. The increased apparent K_m in PM microsomes may reflect an enzyme conformational change or activity of other enzyme(s) with overlapping substrate selectivity. The recent isolation from human liver of a second cytochrome P-450 species able to oxidize Bu (P-450 Buf II) with low affinity/stereoselectivity favors the second hypothesis.

Calcium flux studies in vascular smooth muscle cells

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The effect of various vasoconstrictors on calcium fluxes in smooth muscle has been examined using cells originating from the rat aorta. A 3–4-fold increase in $^{45}Ca^{2+}$ influx was observed when cells were depolarized by high concentrations of KCl. A variety of calcium channel blockers inhibited depolarization induced influx with potencies similar to those observed in relaxing K^+ induced vasoconstriction in aortic rings. The following IC_{50} values were observed: La^{3+} 1.7×10^{-5} M; nitrendipine 3×10^{-9} M; PY 108-068 3×10^{-9} M; (+)PN 200-110 0.8×10^{-9} M. Calcium channel agonists induced an increase in calcium influx which again reflected pharmacological activity observed in aortic rings. This influx was also blocked by calcium antagonists. Calcium efflux was measured in $^{45}Ca^{2+}$ preloaded cells. Addition of vasoconstrictor hormones or neurotransmitters increased the rate of calcium efflux. Vasopressin, angiotensin II and platelet activating factor were among the most potent in stimulating efflux.

Improvement in shuttlebox performance by genetically-selected low-avoidance rats due to amphetamine injections is reduced after drug discontinuation

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Roman low-avoidance (RLA/Verh) rats are selected and bred for poor two-way avoidance performance in a shuttlebox, showing excessive freezing behavior when confronted with a shock stimulus. The present study sought to determine if this pattern could be reversed by amphetamine (Amph) injections. 4 groups of 7 RLA/Verh rats each *(A–D)*, were given 7 sessions of 50 shuttlebox trials, every 3 to 5 days, 20 min after i.p. injections of *A:* 7 × 0 mg/kg (control), *B:* 3 × 0 and 4 × 2.0 mg/kg, *C:* 3 × 2.0 and 4 × 0 mg/kg and *D:* 3 × 2.0, 1 × 1.5, 1 × 1.0, 1 × 0.5 and 1 × 0 mg/kg Amph. It was found that, whereas Amph considerably improved performance, this was accompanied by a proportional increase in spontaneous activity (inter-trial responses). That learning was not enhanced by Amph was further indicated by the fact that the avoidance improvement gradually disappeared when the drug was discontinued. The return to control levels was accompanied by a similar reduction in activity.

Botulinum toxin blocks acetylcholine release in the *Torpedo* electric organ

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The effects of type A Botulinum toxin (BoTX) were tested on synaptic transmission in the *Torpedo* electric organ. BoTX stops transmission by blocking the neurally evoked release of acetylcholine (ACh). During the same time, spontaneous ACh release transiently increases whereas both the frequency and amplitude of miniature potentials decrease. BoTX does not reduce the ACh content of the tissue but the level of phosphocreatine is unexpectedly depressed. This preparation is being used to investigate the mode of action of BoTX on cholinergic nerve terminals.

Type II collagen (CII) induced delayed type hypersensitivity (DTH) in DBA-1 mice

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Autoimmunity to CII is considered one mechanism involved in the pathogenesis of human rheumatoid arthritis. While working with CII induced arthritis in DBA-1 mice we became interested in their susceptibility to CII induced DTH. DBA-1 mice were primed with 0.1–1000 μg of bovine cartilage CII 4 days prior to challenge; 10 μg i.p. was found to be optimal. Mice were challenged with 40 μg CII in the ear. Swelling was highest (37 ± 16%, n = 24) after 48 h as compared to challenge only controls (17 ± 8%, n = 27). DTH responsiveness was transferred to naive animals by 100×10^6 spleen cells or 50×10^6 splenic T cells from primed animals. The results show that CII can induce a DTH response in DBA-1 mice. The swelling of 20% is not as marked as with e.g. SRBC as antigen but is reproducible. Further investigations should indicate whether there is a link between CII induced DTH and arthritis as has been suggested for human rheumatoid arthritis.

Effect of muscarinic agonists and antagonists on the isolated rat sympathetic ganglia – a comparison with effects on the guinea pig ileum

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Two putative muscarinic (m) receptor subtypes have been defined on the basis of the differential inhibition by the non-classical m antagonist pirenzepine (PZ). The superior cervical ganglion is enriched in the M_1 class which is highly sensitive to PZ while the guinea pig ileum is rich in M_2 receptors where PZ has a 30-fold lower activity. I have now investigated a number of classical m agonists and antagonists in these two models and found that the rank of order of activity of these compounds in the two models was different. With the exception of McN-A343 which was active only in the ganglion it was the following for the agonists: ileum: oxotremorine > muscarine > arecoline > RS86 > aceclidine > pilocarpine > AF-30; ganglion: oxotremorine > muscarine > RS 86 > arecoline > pilocarpine > AF-30 > aceclidine. The activity of the antagonists showed the following rank of order: ileum: scopolamine(S) > 4-DAMP > hexahydrosiladifenidol(H) > PZ; ganglion: S > 4-DAMP > PZ > H. In conclusion muscarinic agents differ in their M_1 and M_2 activity.

DSIP effects involve different pathways

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The natural occurrence and sleep-inducing effects of DSIP have been demonstrated in several species. The molecular mechanisms of action, however, remain unclear. DSIP administrations affected the levels of serotonin in rat brain depending on the time of day, and the peptide injected into mice also influenced amphetamine-induced locomotor activity and hyperthermia that are mediated through catecholaminergic pathways. Furthermore, DSIP significantly reduced nocturnal increase of N-acetyltransferase activity in the rat pineal in a dose-dependent manner. This enzyme is known to be regulated through adrenergic receptors. Finally, DSIP in doses from 5 to 30 µg/kg i.v. attenuated CRF-induced release of corticosterone in rats apparently at the level of the pituitary. In conclusion, DSIP seems to interfere with serotonergic and catecholaminergic pathways as well as to influence hormone levels and activities probably by modulating the respective receptor-sites/regions.

Excitation of hippocampal pyramidal cells by caffeine

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In the rat hippocampal slice preparation, bath application of caffeine caused a dose-dependent increase of the extracellularly recorded EPSP and population spike in CA 1 with a threshold dose of 10 µM. Intracellular recordings revealed a caffeine evoked decrease in resting membrane potential, an increase in input resistance, a reduction of the long afterhyperpolarization and a decrease in accommodation. All these effects are probably mediated by a decrease in potassium conductance. The maximal response to caffeine was increased in the presence of adenosine and the adenosine dose-response curve was shifted, in parallel, to the right in the presence of caffeine. These results are consistent with a competitive antagonism of adenosine by caffeine. We conclude that stimulation of the central nervous system by caffeine can result from disinhibition of central neurones and suggest that this is a consequence of the antagonism between caffeine and the adenosine normally active in the extracellular fluid.

Different responses of vascular muscle to α_1- and α_2-agonists

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By the use of selective agonists and antagonists it was not possible to differentiate convincingly between α_1- and α_2-adrenoceptors in the rabbit main pulmonary artery (RMPA) under in vitro conditions. Furthermore, α_1- and α_2-agonists produced similar changes of the membrane potential of the smooth muscle cells of the RMPA. Yet, contractions to α_1- and α_2-agonists differed in several aspects including different susceptibility to inhibition by calcium withdrawal and calcium antagonists, to temperature reduction, to manoeuvres that moved the membrane potential into either the depolarizing or hyperpolarizing direction and to inactivation of guanine-nucleotide binding regulatory protein by pertussis toxin. It is concluded that in the RMPA stimulation of a common α-adrenoceptor recognition site by α_1- and α_2-agonists initiates different responses in the subsequent effector systems.

The pharmacological specificity of cysteine sulfinic acid, an endogenous excitatory amino acid, in the cat caudate

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Neuronal membrane potentials were recorded in halothane anesthetized cats during iontophoretic application of drugs and simultaneous stimulation of the cortico-caudate pathway. Cysteine sulfinic acid (L-CSA) elicited a type I regular firing pattern (Herrling et al., J. Physiol. *339* (1983) 207) while on the same cells, N-methyl-D-aspartic acid (NMDA) caused the cells to fire on 100 ms and longer depolarization shifts, the type II pattern. The selective NMDA-antagonist 2-amino-7-phosphonoheptanoic acid (AP-7) selectively inhibited the effects of NMDA, often achieving total abolition, while L-CSA induced excitations were moderately inhibited only at much higher AP-7 application currents. With kynurenic acid, it was relatively easy to completely antagonize L-CSA, NMDA and quisqualate excitations. From this we conclude that L-CSA interacts predominantly with non-NMDA excitatory amino acid receptors in the cat caudate.

^{3}H-Glycogen hydrolysis induced by K^+ and adenosine in mouse cerebral cortical slices: comparison with VIP and monoamines

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Vasoactive Intestinal Peptide (VIP) and the monoamines norepinephrine (NE), serotonin (5-HT) and histamine (HIS) promote, when applied to mouse cerebral cortical slices, a concentration-dependent hydrolysis of ^{3}H-glycogen newly synthesized from ^{3}H-glucose. We have now examined the effect on ^{3}H-glycogen levels of factors such as K^+ and adenosine, whose concentration is known to increase in the extracellular space upon increases in neuronal activity. We have observed that K^+ induces a concentration-dependent glycogenolysis. A nearly 50% decrease in the polysaccharide content is observed already at 10 mM K^+, a concentration that may be reached in the extracellular space upon electrically-induced depolarization. Increases in K^+ concentration up to 30 mM result in the hydrolysis of virtually all the newly synthesized ^{3}H-glycogen. In comparison the maximal decrease in glycogen content induced by VIP, NE, 5-HT and HIS is only 70%. Adenosine 10 µM hydrolyzes 50% of the newly synthesized ^{3}H-glycogen. This effect is mimicked by the adenosine analog L-PIA 1 µM.

Potential-dependent modulation of cardiac Ca channels by dihydropyridines (DHP)

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Isolated ventricular cells of guinea pigs contain high-affinity DHP binding sites. ^{3}H-Nitrendipine bound to a single population of sites (K_D = 1.2 nM and B_{max} = 3.4 × 10^5 sites/cell) in a rapid and reversible manner. The DHP Ca channel blocker nifedipine (IC_{50} 5.1 nM) and agonist Bay K 8644 (IC_{50} 89 nM) inhibited radioligand binding. Nifedipine suppressed the peak Ca current (I_{Ca}) in voltage-clamped ventricular cells, the extent of which was greater at holding potentials (V_h) of −40 mV (IC_{50} ~ 20 nM) than −50 mV (IC_{50} ~ 700 nM). Bay K 8644 caused an enhancement of peak I_{Ca} which was greater at V_h of −40 mV than −60 mV. In guinea-pig papillary muscles, the negative inotropic concentration-response curve of nifedipine was shifted progressively to the left when extracellular K was elevated stepwise from 5.9 mM (IC_{50} 480 nM) to 37 mM (IC_{50} 0.63 nM). The results suggest that the high-affinity DHP site in cardiac cells mediates an inhibition or enhancement of peak I_{Ca} in a potential-dependent manner.

Do drug-induced changes of the phospholipid-composition in cultured cells reflect a part of the pharmacological drug action in vivo?

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Chronic exposure of cultured human fibroblasts to certain amphiphilic drugs produce an increase of cellular phospholipids (PL), microscopically visible as granular inclusions. According to subcellular fractionation studies PL and drugs accumulate within the lysosomes as nondegradable complexes. Exposure of cultures to 0.1–5.0 µM of the tricyclic antidepressant desipramine not only raises the total PL-content but also changes the relative amount of the individual PL. This change of the PL-pattern is manifested in lysosomes and in other membrane fractions. Experiments with 15 amphiphilic drugs have shown that these substances induce variable changes of the PL-composition which are, however, very similar for drugs of the same therapeutic group (antidepressants, neuroleptics, antimalaria drugs, etc.). Specific PL-matrix changes might induce functional changes of membrane proteins, that supposedly cause the desired effects of certain drugs in vivo.

Suramin inhibits binding and mitogenic activity of platelet derived growth factor in vitro

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The polyanion suramin was recently found to inhibit binding of ^{125}I-PDGF (platelet derived growth factor) to Balb/c 3T3 cell membranes (Williams et al., J. biol. Chem. *259* (1984) 5287). We used cultured Swiss 3T3 cells to investigate the mode of action of this compound. In a coincubation assay suramin inhibited ^{125}I-PDGF binding with an $IC_{50} \approx 50$ µM. However, it was inactive in another assay, in which only agents interacting with the receptor, but not agents interacting with PDGF can inhibit. Since the competitive and with suramin equipotent inhibitor protamine sulfate did inhibit, we may conclude that suramin inhibits by binding to PDGF. In line with this model is the ability of suramin to readily dissociate bound ^{125}I-PDGF. Suramin also inhibited PDGF-induced DNA synthesis and cell growth, with an IC_{50} comparable to that of PDGF binding. Due to its cytotoxicity at higher doses, however, suramin may not be of use as an inhibitor of the action of PDGF in vivo, but as a research tool only.

Characterization of human spinal cord neurons in monolayer cultures

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Dissociated spinal cord cells from 8- to 9-week-old human embryonic tissue can survive for up to 7 weeks in monolayer culture. The neurons have been identified by their ability to bind antibodies to tetanus toxin and neurofilament protein and by their electrophysiological properties. The cultures exhibit high levels of choline acetyltransferase (CAT) activity and they synthesize and accumulate ^{3}H-acetylcholine using ^{3}H-choline as a precursor. There are more cholinergic neurons in the anterior part of the spinal cord as compared to the posterior part. Therefore it is possible that a large part of the cholinergic neurons derive from the mononeuron pool. Approximately 60% of the neurons are labeled with ^{3}H-GABA as shown by autoradiography. In addition, the neurons can synthesize ^{3}H-GABA using ^{3}H-glutamate as a precursor but there is no synthesis or uptake of norepinephrine. When the cells were grown for 3 weeks in the presence of serum from patients with amyotrophic lateral sclerosis, there was no difference in the levels of CAT, glutamic acid decarboxylase and LDH as compared to control serum.

Cholinergic neurons in septal slice cultures: histochemistry and acetylcholine metabolism

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Cholinergic neurons can be demonstrated in slice cultures prepared from the medial septum-diagonal band complex of the embryonic rat brain, by choline acetyltransferase immunocytochemistry and acetylcholinesterase pharmacohistochemistry. When incubated with [^{3}H]choline, the cultures synthesize [^{3}H]acetylcholine and release it both spontaneously and upon depolarization with 50 mM K^+. The spontaneous, as well as the depolarization-induced release of [^{3}H]acetylcholine are calcium-dependent. These results show that slice cultures might provide a new, complementary tool for the investigation of the mechanisms regulating the release of acetylcholine in central cholinergic neurons.

Corticosteroid-induced stimulation of collagen synthesis in human skin fibroblasts

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Collagen synthesis was determined in normal human skin fibroblasts during log growth phase (5% O_2 atmosphere) with and without triamcinolone acetonide (TA). After labeling for 5 h, ^{14}C-proline incorporation into collagen (CP) and noncollagen protein (NCP) was measured according to Peterkofsky and Diegelmann (Biochemistry *10* (1971) 988). The solubilized TCA/tannic acid-precipitate of medium and cell layer was digested with purified bacterial collagenase. Enzymatically non-degradable protein (= NCP) was reprecipitated with TCA/tannic acid, the peptides derived from CP remaining in the supernatant. Radioactivities were expressed as cpm per µg of total protein. Our results indicate that TA markedly stimulated CP-synthesis over the whole concentration range (0.001–1 µg/ml) with a peak of more than 300% at 0.01 µg/ml. Furthermore, the amount of relative CP vs total protein synthesis increased significantly, indicating a preferential stimulation of CP-synthesis.

Circadian food intake rhythm disturbance after chronic methamphetamine treatment depends on route of application

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Chronic treatment of rats with methamphetamine (M) induces tolerance to its anorexic effect (daily food intake), as well as disturbing circadian feeding rhythmicity (increased food intake in the early light phase). To test whether these effects are dependent on route of drug application, we designed a cross-tolerance experiment. Constant delivery of M to naive rats via an s.c. implanted osmotic minipump (~ 8 mg/kg per day) caused a marked anorexia followed by rapid and total tolerance within 4 days, together with a slight increase of feeding in the early light phase. Periodic delivery of M via the drinking water (~ 8 mg/kg per day) caused mild anorexia followed by slow and partial tolerance within 7 days, together with a large increase of food intake in the early light phase. Crossed application of M resulted in a similar profile to that found in naive rats with the disappearance of the previous phenomena. Thus there appear to be two separate mechanisms of M tolerance depending on the route of application of the drug: The circadian rhythm of feeding is markedly disturbed only after periodic administration of M in the drinking water, that does not bypass the liver.

Debrisoquine(D)/sparteine(SP)-type polymorphism in drug oxidation: a model of the active site of the involved cytochrome P450 isozyme

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Common structural and reaction characteristics of substrates (S) with inherited polymorphic metabolism (D/SP-type) are: 1) lipophilicity, 2) a basic nitrogen atom (N), 3) reactions on this N are not under genetic control. Hypothesis: 1) The positively charged N represents the S binding site, 2) the S have to be in a similar configuration as the rigid dextrometorphan. Results: 1) The distances from N to the reaction site are 6.7 to 7.2 Å for: D-7-hydroxylation (OH), dextrometorphan-O-demethylation (dem.), bufuralol-1'- and 4-OH, 4-methoxy amphetamine-O-dem., nortriptyline-10-OH, perhexiline trans-4'-OH (M3), metoprolol α-OH. 2) Aromatic or aliphatic rings adjacent to the reaction site are nearly coplanar in this model. 3) The protein binding site is not enantioselective. 4) Regioselectivity in bufuralol OH is induced by the asymmetric center in the aminoethanol side chain. 5) S binding site, lipophilic plane and reaction site are not in one plane. D-4-OH (N – reaction site 3.8 Å) seems to be catalyzed via different binding or another but genetically linked isozyme.

Ethane and pentane formation measured by capillary gas chromatography

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Oxygen centered free radicals induced by various toxic chemicals can cause a peroxidative destruction of lipid membranes of cells. Ethane and pentane are produced during the following fatty acid hydroperoxide decomposition. A highly sensitive method has been developed to measure these hydrocarbons in cell cultures or in vivo in a subcutaneous air pouch of rats. We used a capillary gas chromatograph, equipped with a flame ionization detector, achieving a limit of detection of 1 vpm. Lipid peroxidation was induced in freshly isolated cells with 100 μM CCl_4. Hepatocytes (10^6 cells) formed 240 pmol pentane/h and 2×10^6 cells derived from a granulation tissue produced 140 pmol pentane/h. This demonstrates that even small amounts of hydrocarbons produced during chemically induced lipid peroxidation in intact tissues in vitro can be detected.

Genetic polymorphism of debrisoquine/sparteine oxidation: in vivo 'transformation' of extensive metabolizers (EM) into poor metabolizers (PM) by quinidine (Qd)

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Qd is a potent in vitro inhibitor of some hepatic microsomial oxidations (Otton, Life Sci. *34* (1984) 73). Using metoprolol (Me) and bufuralol (Bu) as probes we investigated the influence of Qd on the polymorphic oxidation of Me in vivo and of Bu in human liver microsomes. EM received (±)-Me p.o. before, 2 and 48 h after 50 mg Qd sulfate p.o. Me, α-OH-Me and O-demethyl-Me were measured in plasma and urine with an enantioselective HPLC assay. Qd increased total Me plasma concentration 3-fold. Moreover, Qd abolished the known stereoselectivity of Me metabolism. Conclusion. Qd causes a metabolic deficiency undistinguishable from the PM phenotype. It may serve as a specific probe for the cytochrome P-450 responsible for this polymorphism.

Arthritis induced in Wistar rats after immunization with type II, 1a2a3a and M collagens

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Collagen-induced arthritis (CIA) in rats is used as an animal model for human rheumatoid arthritis. In addition to type II collagen used for the induction of arthritis we investigated the arthritogenicity of 1a2a3a collagen. Another purpose of this study was to examine whether Wistar rats from different breeding colonies were equally susceptible to CIA. Bovine collagens were injected in ICFA i.d. in the tail at days 0 and 7. Amounts of 100 μg/injection were sufficient to induce CIA. Higher amounts of collagen did not influence the onset and course of arthritis. The average day of onset was day 14. The incidence of CIA was 80–90% with type II and 1a2a3a collagen and 20% with M collagen. Female Wistar rats obtained from Mollegaard (DK) were the most susceptible for CIA. We conclude from our experiments that Wistar rats from various breeding colonies respond differently with respect to CIA. This may explain variations in incidence seen in the literature.

Effects of extracellular choline on acetylcholine release from striatal slices

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Cholinergic neurons require choline as the immediate precursor for the synthesis of the transmitter, acetylcholine (ACh). Slices from rat striatum were superfused with physiological solution. ACh and choline levels were measured both in the effluent and in tissue samples removed at the beginning and at the end of the collection period. Addition of choline (20 μM) to the medium enhanced ACh release both at rest (by 300%) and during electrical stimulation at 15 Hz (by 200%). Atropine (1 μM) increased the evoked release of ACh and hemicholinium lowered both ACh release and tissue content. In choline-free medium, the total amount of ACh + choline released during an experiment was 5–15 times higher than the decrease in tissue levels of these two compounds during the same experimental period. It is

concluded that ACh release is dependent upon the availability of extracellular choline and that a pool of bound choline provides a source of free choline.

Study of caffeine in in vivo mutagenicity tests

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The in vitro mechanism of action of caffeine on the DNA is very complex. It was shown in the past that caffeine can either directly affect the DNA or act as inducer or inhibitor of mutagens/carcinogens depending on the test procedure.
In the present investigation therefore the property of caffeine in in vivo mutagenicity tests was studied. In outbred mice doses over 100 mg of caffeine/kg b.wt were necessary to cause chromosomal aberrations. At similar doses (100 mg) chromosomal aberrations were induced in a mutagen sensitive inbred strain. Higher doses (300 mg) of caffeine were required for induction of sister chromatid exchanges (SCE) in hamsters. These findings suggest that under in vivo conditions caffeine only interacts with the DNA at dose levels which are exceedingly higher than the human exposure.

Genetic polymorphism of mephenytoin oxidation: evidence for a defect in cytochrome P450 function in human liver microsomes

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Genetically deficient metabolism of the anticonvulsant mephenytoin (M) involves the stereospecific 4-hydroxylation of (S)-M. M-demethylation to nirvanol (N) is not affected. The molecular basis of this polymorphism, was investigated in liver biopsies of 12 phenotyped (urinary 4-OH-M) patients. Eleven subjects were extensive metabolizers (EM, 99.7 ± SD 44.9 µmol 4-OH-M/8 h) while 1 subject was a poor metabolizer (PM, 1.8 µmol 4-OH-M/8 h). In vitro oxidation of (R)- and (S)-M in liver microsomes was determined by a HPLC assay and showed cytochrome P450 dependence. A striking decrease in the (S)-M-4-hydroxylation in the PM liver microsomes was observed (0.49 nmol (S)-4-OH-M mg prot/h vs 3.98 ± 2.0 in the 11 EM's). The hydroxylation of (R)-M was not affected (R/S-ratio: PM = 0.76, EM's = 0.10 ± 0.03). These data provide convincing evidence for a microsomal hydroxylation defect and suggest a quantitative or qualitative deficiency of a cytochrome P450 isozyme.

Validation of computerized fingerpulse-photometry (CFP) by lidocaine (L) bioavailability (BA) to assess portosystemic shunt (PSS)

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CFP using glyceryltrinitrate (GTN) has been proposed to quantitate PSS in liver cirrhosis (Gastroenterology *82* 629). We compared CFP to LBA in 16 patients with varying degrees of impaired liver function. The inverse ratio of height (H) vs distance from peak to dicrotic incisure (D) of a fingerpulse wave was assessed by CFP. D/H changes after 600 µg of po GTN were compared to the response to i.v. doses to estimate BA as a measure of PSS. To estimate LBA, 125 mg L and 30 mg tri-H2-L (DL) were simultaneously administered po and i.v., respectively, to the same patients. L and DL in serum were measured by capillary GC/chemical ionization mass spectrometry. BA of GTN and L ranged from 2 to 40 and from 23 to 92%, respectively. They correlated significantly ($r = 0.83$, $p < 0.001$) being related by LBA = −15.7 + 0.57 CFP. Thus, CFP underestimates PSS by 43% on average which is probably due to intestinal metabolism of GTN. PSS can accurately rapidly and noninvasively be determined by CFP.

Nitroprusside (NP) inhibits platelet function probably by elevating cAMP

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We investigated whether cAMP has any role in mediating the inhibition by NP of human platelet function, since effects of NP on adenylate cyclase (AC) systems are disputed. NP and Ro 15-2041, a phosphodiesterase inhibitor, prevented aggregation and serotonin release with IC50 of 0.7–3.5 µM as well as thrombin-induced elevation of intracellular free calcium (Quin-2 method). 3 µM NP elevates platelet cGMP (measured by RIA) from 0.04 to 2.3 fmol/10^6 platelets and cAMP from 9.6 to 13.8 fmol/10^6 platelets. Ro 15-2041 up to 1000 µM had no effect on cGMP but cAMP rose from 12.1 to 16.8 fmol/10^6 platelets. Since small increases of cAMP in platelets are difficult to measure, we tried to exclude a possible effect of NP via stimulation of AC by blocking AC with 3',5'-dideoxyadenosine (DDA). 300 µM DDA prevented the NP-induced increase of cAMP but not of cGMP. 100 µM DDA partially prevented the inhibition of aggregation by NP or PGI_2. Conclusion. NP might inhibit platelet function by elevating intracellular cAMP, provided a 50% increase is sufficient.

Competition of organic anions with ^{14}C-pyrazinoate (PZA) uptake in renal cells from rabbit proximal tubule

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The uptake of the organic anion PZA (10^{-4} M) was measured at equilibrium after 30 min incubation at 37 °C in isolated nonperfused proximal tubular S_2 segments, in the absence (control) or in the presence of an inhibitor of organic anion uptake. The uptake in controls was 4020 ± 240 fmoles·nl^{-1} tissue water ($n = 24$). The passive uptake component, measured at 15 °C in the presence of 10^{-2} M cyanide was 457 ± 17 fmoles·nl^{-1}. Thus, the active component of uptake was 3563 fmoles·nl^{-1}. The effects of competitive inhibitors at 10^{-4} and 10^{-3} M were tested on PZA uptake: Probenecid decreased uptake by 66 and 80%, p-aminohippurate by 52 and 83%, urate by 51 and 86%, and salicylate by 45 and 94%, respectively. These data show that probenecid the classical and usually most potent inhibitor of organic anion transport did not inhibit totally PZA uptake. In contrast, salicylate induced a more pronounced inhibition. PZA might be transported partially by a mechanism different from that of probenecid.

Effect of gamma linolenic acid on platelet aggregation

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Gamma linolenic acid (GLA), an essential fatty acid (EFA) is found in rather high levels in different plants. EFA are known to lower thrombogenic effects of platelets (Vergroesen et al., 1978). For therapeutic reasons GLA is of great interest. The purpose of the presented work was to see if there is an effect of GLA on rat platelets.
Rats were fed an EFA free diet until their body weight did not increase any longer. At this time one group of EFA deficient (EFAD) rats got i.v. GLA, another group i.v. arachidonic acid and a third group of EFAD rats had no treatment. 30 min later blood samples were taken on all rats to perform platelet aggregation (PA) with platelet rich plasma induced by collagen/$CaCl_2$

(CC) or ADP. Aggregated and non aggregated platelets were fixed for electronmicroscopic investigations. First results showed differences between the groups in PA induced by CC or ADP.

Subminiature electroplaque potentials are present in *Torpedo* electric organ

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The spontaneous release of acetylcholine at *Torpedo* nerve-electroplaque junction has been studied by means of extracellular microelectrodes. To improve the signal to noise ratio, the resistance to ground was increased by applying a slight negative pressure to the inside of the pipette. In these conditions, the major MEPP's population exhibited a classical bell-shaped distribution and in most cases another population of smaller amplitude and skewed distribution could also be recorded. The time to peak vs amplitude relation of these skewed MEPPS corresponded to that of the bell-shaped population and their relative number when compared to the bell MEPPS was identical during bursts of low or high frequency. As at the neuromuscular junction, botulinum toxin caused a reduction of the overall MEPP's frequency and a selective blockage of the bell-shaped population, leaving only the skewed MEPPS.

Dopamine synthesis and release in rabbit retina in vitro: effects of high K^+, adenylate cyclase activators, reserpine, (±)-amphetamine and (±)-3-PPP

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As previously described (Ofori et al., Experientia *40* (1984) 648), rabbit retinae were used to study dopamine (DA) biosynthesis and release in vitro. K^+ (50 mM) and the adenylate cyclase activator forskolin (50 µM) both stimulated tyrosine hydroxylase activity (THA) but two other adenylate cyclase activators 2-chloroadenosine (100 µM) and VIP (650 nM) did not. K^+-stimulated THA but not forskolin-stimulated THA was inhibited by (±)-3-PPP (100 µM). K^+-stimulated THA was greater in Ca^{2+} free medium. K^+ also induced a release of endogenous DA. This effect was strongly reduced but not totally abolished in Ca^{2+} free medium. Thus DA synthesis and release could be controlled by different mechanisms and/or receptors (autoreceptors?). DA release was 53% by reserpine (100 µM) and 34% by (±)-amphetamine (100 µM). Both agents together at 100 µM each released 86% of endogenous DA. Rabbit retina is thus useful for studying in vitro, the regulation of DA biosynthesis and release, using a reliable analytical method.

Voltage-dependent binding of dihydropyridine Ca-channel ligands to intact cultured cardiac cells

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We have used monolayer cultures of newborn rat heart cells to study specific binding and competitive interactions of dihydropyridine derivatives in vivo. Under control conditions in Ca-free Hank's solution, where cells contracted spontaneously, the K_D for 3H-nimodipine binding was 3.5 ± 1.6 nM. This value decreased significantly to 0.7 ± 0.14 nM under depolarizing conditions when most of the NaCl in the medium was replaced by KCl. Maximal binding capacity was 325 and 211 fmoles/mg protein, respectively, but could be determined reliably only in depolarized cells. Increasing the Ca concentration of the medium to 1.8 mM had no effect on the binding constants. Competitive displacement of 3H-nimodipine by the antagonist nisoldipine or the agonist BAY k 8644 was biphasic. High affinity displacement by both ligands was voltage dependently enhanced, but the effect was more pronounced with the antagonist. The voltage sensitivity of binding affinity suggests that dihydropyridines bind preferentially to inactivated Ca channels.

Properties and modulation of Ca^{2+} channels in cardiac cells

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Voltage dependent Ca^{2+} channels are widely distributed in excitable membranes and are involved in the regulation of many cellular functions. An important property of these channels is their modulation by neurotransmitters and drugs. Recently it has become possible to measure directly properties of single Ca^{2+} channels in cardiac cells. We have analyzed single Ca^{2+} channel currents (i) in myocytes from rat hearts in the absence and presence of isoproterenol or 8-bromo-cAMP. We have found that both compound have similar effects on Ca^{2+} channel properties. They increase the opening probability (p_o) of individual Ca^{2+} channels while i remains unaffected. Analysis of the gating kinetics of Ca^{2+} channels showed a) an increase in the mean open times of Ca channels, b) a reduction in time intervals between bursts of channel openings, c) a reduction in failures of Ca^{2+} channels to open upon depolarization, and d) an increase in burst length. These kinetic changes by isoproterenol and 8-bromo-cAMP can account for the increase in p_o. Since the macroscopic Ca current, I_{Ca}, can be described by $I_{Ca} = N \cdot p_o \cdot i$, the increase in p_o accounts for the well-known increase in I_{Ca} by β-adrenergic catecholamines, cAMP-dependent phosphorylation of Ca^{2+} channels is a likely metabolic step involved in their modulation. Another class of drugs that modulates Ca channel gating are the 1,4-dihydropyridines which can either enhance or reduce I_{Ca} by either prolonging the open state of the channels or by facilitating the inactivated state. These drug effects are independent of phosphorylation reactions.

Mapping a $GABA_A$/benzodiazepine receptor complex in rat and human brain using monoclonal antibodies

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Monoclonal antibodies, raised against a purified $GABA_A$/benzodiazepine receptor from bovine cerebral cortex, have been used to visualize receptor immunoreactivity (RI) in rat and post-mortem human brain. The distribution and density of RI in rat CNS was similar to that of receptor radiolabelling (RR) using ^{3}H-Ro 15-1788, e.g. RI (on neuronal cell bodies and processes) was high in cerebral cortex, olfactory bulb, ventral pallidum, hippocampus, dentate gyrus, substantia nigra, cerebellum, spinal cord and retina but absent in white matter, pineal and pituitary. The appearance of RI in rat brain during ontogeny coincided with that of RR. Moreover, in post-mortem human brain, e.g. in hippocampus and dentate gyrus, the distribution and density of RI and RR were virtually identical. In conclusion, RI in rat and human CNS reveals the distribution and density of a $GABA_A$/benzodiazepine receptor complex with a resolution superior to receptor autoradiography. The monoclonal antibodies could be used to diagnose, in post-mortem human brain, receptor dysfunction possibly associated with CNS disorders such as epilepsy.

Verapamil stimulates potassium efflux from cardiac Purkinje fibers

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Potassium efflux was measured in unstimulated and electrically stimulated calf Purkinje fibers pretreated with ouabain $6 \cdot 10^{-7}$ M. [K^+] was measured with a potassium-sensitive electrode (Metrohm) in a closed circuit filled with 5 ml Tyrode solution containing 4 mM potassium. Under control conditions, there was no significant difference in potassium efflux within a defined observation period between unstimulated (3.8 ± 1.3 µg $K^+ \cdot min^{-1} \cdot g$ wet weight^{-1}; $\bar{x} \pm SD$) and stimulated fibers (3.5 ± 2.1 µg $K^+ \cdot min^{-1} \cdot g$ wet weight^{-1}). After addition of verapamil $4 \cdot 10^{-6}$ M, potassium efflux increased by $19 \pm 7.5\%$ in unstimulated and by $23 \pm 7.8\%$ in stimulated fibers. Under control conditions potassium efflux per minute was estimated to be 0.28% of the total potassium content of Purkinje cells in unstimulated preparations and 0.26% in stimulated preparations (22% of the fiber volume are Purkinje cells).

Muscarinic receptors in slice cultures of rat brain

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Muscarinic acetylcholine receptors (mAChR) in homogenates from organotypic slice cultures of rat *hippocampus* have been examined using the radioligand ^{3}H-QNB as a marker. Maximum specific ^{3}H-QNB binding amounted to 300 fmol/mg protein and a K_D of 0.17 nM was determined. Atropine and scopolamine inhibited total ^{3}H-QNB binding by 90–95%. With the antagonist pirenzepin, only a 40% inhibition was obtained. Furthermore, the cholinergic drugs carbachol, decamethonium, tubocurarine and neostigmine had no influence at all on total ^{3}H-QNB binding. The mAChR content varied between cultures with explants from different brain areas: hippocampus > striatum > septum > spinal cord > cerebellum. These in vitro results are in good agreement with results obtained in situ by other investigators and suggest that the ^{3}H-QNB binding observed in our cultures is indeed correlated to specific mAChR sites. With the aid of selective neurotoxins and autoradiography it should be possible to establish the presence of mAChR on specific neurons.

Metabolic activation of cultured human lymphocytes

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The in vitro human lymphocyte test is valid for use in mutagenicity/carcinogenicity screening. However, the lymphocytes are not efficient in converting pro mutagens/carcinogens into the ultimately reactive species. Attemps have recently been made to solve this problem by directly adding liver microsomal fractions (S-9) to the cell culture. Unfortunately, it was shown that this approach leads to artefact. The present techniques by immersing an S-9 containing dialyse bag into the culture or using a preincubation step were shown to be suitable to induce metabolic activation. Results obtained with complex and reactive compounds as well as with pro mutagens which are representive for a large class of substances showed that these techniques were efficient.

Experimental cyclosporine nephropathy

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Renal dysfunction is commonly found in cyclosporine (CSA) treated patients. Preliminary experiments in rats indicated that similar nephropathy occurs in this species reflecting closely the situation in man. Renal functional changes in CSA administered rats included reduction of the glomerular filtration rate and defective tubular transport. Alterations of the proximal tubule (S3) consisted of vacuolization, inclusion bodies and microcalcification. No tubular necrosis was observed. Arteriolar changes – only found in spontaneously hypertensive (SH) rats – consisted of media necrosis and myofibroblast proliferation. Furthermore, CSA caused tachycardia, hypertension and stimulation of the renin-angiotensin system. It is postulated that CSA nephropathy may be caused by direct renal effects as well as by indirect mechanisms. In summary, rats, especially of the SH strain, may be used as en experimental model to investigate CSA nephropathy.

Caffeine consumption (CC), anxiolytic drugs and anxiety in psychiatric outpatients

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Recent reports indicate that excessive CC is associated with anxiety (A) in psychiatric patients. The quantitative extent of this relation and the influence of concomittant anxiolytic medication remains, however, to be clarified. In 50 consecutive newly admitted psychiatric outpatients CC and A was assessed by a questionnaire; in addition a screen for anxiolytic medication with EMIT in plasma and urine was performed. Neither in the whole sample, nor in patients with considerable CC (> 200 mg/daily) and an A score > average in the normal population, a significant relation could be established. If only subjects with negative drug screening results were considered, a correlation $rs = 0.70$ ($n = 14$, $p < 0.005$) was found. Our results confirm that there is a strong relation between CC and A, which is restricted to patients not treated with anxiolytics. The question remains to be clarified whether systematic reduction of CC in psychiatric patients allows to diminish medication.

Do leukotrienes contribute to the analgesic activity of NSAIDs?

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Leukotrienes (LT) possess antinociceptive activity in inflammatory pain by antagonizing effects of painful stimuli such as bradykinin (Schweizer et al., Eur. J. Pharmac. *105* (1984) 105). Cellular LT production is increased by analgesic non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin or naproxen whilst prostaglandin (PG) synthesis is effectively inhibited (Brune et al., Agents Actions *14*, No. 5/6 (1984) 729). We have tested several NSAIDs for their LT- and PG-inhibiting activities in vitro and tried to correlate the results with their analgesic activity in mice, rabbits and man. We found that the most selective cyclooxygenase-inhibitors were also the most active analgesic NSAIDs, whereas drugs inhibiting both cyclooxygenase and lipoxygenase in equimolar concentrations usually had a weaker analgesic potency. These results suggest that NSAIDs with the capacity to enhance LT-formation by only blocking the cyclooxygenase pathway may have a higher analgesic activity (against inflammatory pain) than compounds which inhibit PG- and LT-production to the same extend.

Catalysis of neuropeptide-receptor interactions by the lipid phase of target cell membranes and its implications for the interpretation of binding and effect coupling studies

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We have recently discovered specific, biologically significant neuropeptide-membrane interactions that provide new insights into structural requirements of receptors and molecular mechanisms of neuropeptide-receptor interactions and may have predictive value for drug design. Regio-, conformation- and orientationselective reactions of ACTH (1–24), dynorphin (1–13), and enkephalin with planar and vesicular lipid bilayer membranes have been reported (e.g. Biochemistry *22* (1983) 4257, *23* (1984) 1808, 1811). Examination of the structural data leads to the concept that the lipid phase of the target cell membrane catalyzes agonist- and antagonist-receptor interactions. Such catalysis has profound effects on association and dissociation rates and thermodynamics, as well as the interpretation of macroscopic ligand binding and dose-response characteristics. Alternative explanations of 'high affinity–low capacity, low affinity–high capacity' binding, and apparent negative cooperativity phenomena are provided by our new concept.

Mutagenic compounds in heated food

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Recently numerous studies revealed that several heated food products contained mutagenic substances. Maillard model systems employed in our laboratory provided evidence that several parameters such as pH, temperature, amino acids, dry matter and length of heating, which may vary between food products and mode of heat processing greatly influenced the mutagenic outcome. We further showed that mutagen-free 'heat reaction' food products can be produced when bearing in mind the above parameters. To enable the testing of final food products it was necessary to establish meaningful extraction procedure to extract and purify possible mutagens. We showed that a simple solvent extraction method was efficient for routine use if solvents were employed which did not interact with the test substance. However, for risk assessment it is essential to identify and quantify such mutagens as well as inhibitors which may be present in heated food products.

Stimulation of lipid peroxidation by asbestos

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This investigation is part of a study designed to elucidate the mechanism of the carcinogenic effect of asbestos. The influence of asbestos fibres on lipid peroxidation was measured by the formation of malondialdehyde (MDA) or ethane/pentane in an in vitro model. Arachidonic acid (1.33 mM) or rat liver homogenate (2 g/100 ml PBS) were used as substrates. Lipid peroxidation was initiated with 1 mM cumene hydroperoxide (CHP). A statistically significant, dose-dependent increase in MDA and ethane/pentane formation was found both with Fe^{2+} and crocidolite. Since metal ions are known to catalyze peroxidation and Fe^{2+} is a component of crocidolite $[Na_2Fe_3^{2+}Si_8O_{22}(OH_2)]$ it is suggested that asbestos fibres catalyse lipid peroxidation. The subsequently released membrane lipids are then further converted to reactive species.

PHYSIOLOGY

Visual and vestibular effects on postural adaptation

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Dorsiflexing platform rotations under eyes open conditions produced a correlated pattern of EMG activity in tibialis anterior and soleus in 10 subjects. Stabilizing forward angular accelerations of the body followed the onset of medium latency (ML) TA muscle activity at 124 ms. A pulse of backward head angular acceleration followed the 114 ms onset of trapezius activity.

The area under EMG responses reduced as acceleration of body and head decreased in frequency and amplitude with subsequent rotations. A second group of normals tested with eyes closed, only demonstrated adaptation in ML trapezius responses. The correlation between angular acceleration of the head and trapezius activity was reduced.

Head angular accelerations coinciding with ML neck muscle activity were reduced and TA stabilizing responses were always less than normal in patients with bilateral peripheral deficits. We conclude that normal sway stabilizing responses depend on an intact vestibulo-spinal system, and adaptation on a visual-vestibular system to stabilize the head relative to body motion.

A sodium-activated potassium current in cultured vertebrate neurones

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Cultured neurones from dissociated avian parasympathetic and sensory ganglia were studied in voltage clamp. An outward current carried by potassium ions and supressible by extracellular 4AP and TEA was found to be initiated whenever a sodium current entered the cell. This potassium current had the following characteristics: 1) it was suppressed by TTX at a concentration which blocked the fast inward sodium current, 2) it was suppressed by removal of extracellular sodium, 3) it could not be detected at voltages more depolarized than the sodium reversal potential, 4) inhibition of inactivation of the sodium current increased the duration of this potassium current. These observations were made in the absence of extracellular calcium and in the presence of 5 mM EGTA in the intracellular fluid. We conclude that this potassium current is activated by an increase in the intracellular sodium concentration. Our results suggest that the quantity of sodium entering a cell during a single action potential is sufficient to activate this potassium current.

Effect of mechanical stimulation, substance P and VIP on the tension and membrane potential of the pig coronary artery smooth muscles. Role of endothelium

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The involvement of the endothelium on the responses (tension and membrane potential of smooth muscle cells) induced by three vasodilators has been studied on the pig coronary artery. Mechanical stimulation, substance P ($ED_{50} = 0.38 \pm 0.09$ nM, mean ± SEM, n = 7) and VIP relax the open ring of anterior descending branch of pig coronary artery precontracted by Ach 10^{-5} M. Although substance P induces an hyperpolarization,

which precedes the relaxation (15 ± 0.7 mV, mean ± SEM, n = 10 for submaximal stimulation), the decontraction induced by VIP is not accompanied by a change in transmembrane potential. Effects of mechanical stimulation and substance P require the presence of an intact endothelium. VIP relaxes the coronary artery in the presence and in the absence of an intact endothelium.

Functional characteristics of the newborn (NB) rabbit collecting tubule (CT); role of ADH and PGE_2

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The NB animal has a weak urinary concentrating ability. To investigate the role of the CT in that defect, the passive diffusion of Na and water was measured in isolated CT of NB (3–5 days) and adult (AD) rabbits perfused with a fluid hypotonic (200 mOsm/kg, [Na] = 100 mEq/l) to the bathing medium (300 mOsm/kg, [Na] = 160 mEq/l). In the absence of ADH, the water absorption (J_v) was not different from zero in both cases ($J_v = 0.19 \pm 0.21$ nl/mm·min, n = 19, and 0.10 ± 0.09 nl/mm·min in NB and AD respectively, p:NS). In the same conditions, no net backflux (J_{Na}) was seen in AD ($J_{Na} = 2.1 \pm 24.4$ pEq/mm·min) in opposition to NB ($J_{Na} = -86.3 \pm 22.9$ pEq/mm·min, $p < 0.05$). Transepithelial resistance (R) was significantly lower in NB ($R = 83.2 \pm 11.6\,\Omega \cdot cm^2$, n = 10) when compared to AD ($R = 220.6 \pm 55.0\,\Omega \cdot cm^2$, n = 6, $p < 0.01$). In addition, ADH or PGE_2 had opposite effects on J_v and J_{Na} in the NB and the AD CT. Both structural and functional differences of the NB may contribute to the NB concentrating defect.

The effects of peptides from thrombic digestion of bovine myelin basic protein (BMBP) on the bioelectric activity of frog spinal cord

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BMBP is known for its depolarizing effects on the bioelectric activity of frog spinal cord. We cleaved the BMBP Peak 2 specifically with thrombin at a single site (Arg97–Thr98). The two resulting peptides were isolated by cation exchange chromatography. They both caused a dose-dependent (0.01–0.001 mM) depolarization of the ventral and dorsal roots of the hemisected frog spinal cord. BMBP and peptide A (MG: 10 kD) both had similar effects on the DC recorded activity. Peptide B (MG: 8 kD), with a higher charge than peptide A, was less active. This indicates that the depolarization probably depends on configuration rather than on charge. Both peptides, in contrast to BMBP, were ineffective in increasing the AC recorded spontaneous activity. These results show that MBP peptides, which could occur in demyelinating foci (e.g. in multiple sclerosis, where enzymatic activity is very high), still have strong depolarizing actions which might contribute to neuronal disturbance.

A new, ultrasonic respiratory flowmeter

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The flowmeter presented calculates the mean velocity of the respiratory gas flow by measuring the differential velocity of bi-directional ultrasonic pulses along a respiratory tube. The latter includes a fast temperature sensor and a symmetrical transmission channel with two ultrasonic transceivers. The respiratory tube has a length of 90 mm and a diameter of 20 mm, hence exhibiting minimal flow resistance and a small dead space. An electronic system generates an ultrasonic burst every 2 ms and calculates the actual mean flow by detecting the difference between the received signals. The experimental flowmeter covers a wide flow range (linear response 0 to 10 l/s, corresponding to Fleisch PT1–PT3) with a resolution of 10 ml/s. The volumetric measurement is practically not affected by moisture (condensed vapor, salivation) and by changes in temperature. It is planned, by means of a microprocessor, to compute simultaneously with the flow the mean mol. wt of the respired gas mixture.

Mechanism of phosphate (Pi) transport adaptation in rat renal (R) and intestinal (I) brush border membrane vesicles (BBMV)

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We have examined whether adaptation of Pi transport to low Pi diet could be mediated by changes in the Na dependency of the Pi carrier. This was studied by measuring Pi uptake by BBMV ([Pi] = 0.1 mM, pH 7.4) at various external Na concentrations ([Na]) in R and I BBMV prepared from animals adapted to either low (LPD) or high (HPD) Pi diet. The [Na] at half maximal Pi transport, ($K_{0.5}^{Na}$) was not modified by dietary Pi, in both I and R BBMV. LPD increased maximal Pi transport from 26 ± 5 to 88 ± 6 in I BBMV and from 1800 ± 205 to 3025 ± 380 pmoles/mg·10 s in R BBMV. Under both LPD and HPD R BBMV, lowering pH to 6, dramatically increased $K_{0.5}^{Na}$. At 500 mM Na, Pi transport adaptation to LPD was equally enhanced at pH 6 and 7.4. Thus, Pi transport adaptation is expressed in I as well as R BBMV. In both tissues, LPD does not appear to change the $K_{0.5}^{Na}$ of the Pi transporter. In R BBMV, low pH increases $K_{0.5}^{Na}$ without altering the expression of Pi transport adaptation to LPD.

Quantal analysis at the Ia-motoneurone junction

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The amplitude of 1024 excitatory postsynaptic potentials (e.p.s.p.s) elicited in cat spinal motoneurones by impulses in single group Ia fibres were measured, and the probability density of the fluctuations in peak amplitude of each e.p.s.p. was calculated together with the probability density of the recording noise amplitude. The maximum likelihood estimators for the means and membership probabilities of individual components of a finite mixture of normal distributions were used to remove the noise from the distribution of the fluctuating e.p.s.p. amplitudes. Most e.p.s.p.s fluctuated between different components with peak amplitudes which were integer multiples of the increment between successive components. The amplitude of this incremental e.p.s.p. ranged from 75 μV to 125 μV. The results suggest that the incremental e.p.s.p. (unit-e.p.s.p.) reflects the all-or-nothing action of an individual bouton, and that the probability of transmission failure varies at the different boutons making up a single Ia-motoneurone contact system.

Chloride enters receptors and glia in response to light or raised external potassium in the retina of the bee drone, *Apis mellifera*

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We have made measurements of Cl^- activity (a_{Cl}) and membrane potential (V_m) with double-barrelled, ion-selective microelectrodes in photoreceptors and glial cells in superfused slices of drone retina. In the dark, the Cl^- equilibrium potential, E_{Cl}, was

indistinguishable from the membrane potential in both types of cell. When the membranes were depolarized, either by illumination of the photoreceptors or an increase in $[K^+]$ in the bath from 7.5 to 18 mM, E_{Cl} followed V_m with a lag that was less than 10 s in the glia but more than 100 s in the photoreceptors. Stimulation with a train of light flashes, at one per sec for 90 s, caused a_{Cl} to increase by 4.9 mM, SEM 0.3 mM, N = 5 in the photoreceptors, and by 3.8 ± 0.3 mM, N = 11 in the glia. The entry of KCl into the glia contributes to extracellular ion homeostasis in this nervous tissue.

Buphenine changes the electroretinogram but not the flow in perfused cat eyes

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Buphenine (B), a presumably β_2 related vasodilatator, is used to treat degenerative retinal diseases. We studied effects of B on electroretinogram (ERG), optic nerve response, intraocular pressure and flow rate (reflecting vascular resistance) in perfused cat eyes by continuous injections for 10 to 35 min, reaching concentrations from 5×10^{-6} to 10^{-4} M. An unexpected finding was the absence of consistent effects on flow at concentrations that caused large increases in the ERG b-wave and decreases in the c-wave. The latter effects were dose-dependent, reversible and were accompanied, at medium and high doses, by changes in the configuration of the optic nerve response. Intraocular pressure was unaffected. The data suggest neural and/or glial effects of B rather than vasodilatation. However, redistribution of microcirculation within the retina cannot be ruled out.

Early propagation of virulent and avirulent rabies strains from the eye to the brain

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Penetration in the brain of the rabies challenge virus strain (CVS) and of its two avirulent mutants (Av01, Av02) was studied in adult rats after inoculation of the viruses in the anterior chamber of the eye. The primary sites of penetration of the CVS are: 1) intraocular parasympathetic oculomotor fibers, 2) fibers of pretectal origin, 3) intraocular fibers of the ophthalmic nerve. The Av0 strains have lost the capacity to invade the two former groups of fibers but their penetration into the trigeminal system was not impaired. Neither the CVS nor the mutants infect primarily the intraocular adrenergic terminals and the optic nerve. The mutants, but not the CVS, were able to infect the lens. These results indicate that 1) the cholinergic receptor may not be the only receptor for the virus, and, 2) the rabies virus propagates within the nervous system by means of the retrograde axoplasmic flow.

The CVS spreads throughout the brain within 1 week. The mutants invade the brain as well but the infection is slow, involves different cerebral structures and clears up completely in 3 weeks.

The effects of lesions in the ventro-posterior thalamic complex, dorsal columns and lateral cervical nucleus on the milk ejection reflex

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Bilateral electrolytic lesions of some somatosensory structures in the thalamus and spinal cord were made in order to assess their participation in the afferent limb of the milk ejection reflex in the rat. Lesions involving the lateral cervical nucleus blocked the reflex whereas animals with lesions in the dorsal column of the spinal cord or in the ventro-posterior complex of the thalamus displayed milk ejection frequencies similar to those of control animals. Unilateral injections of horseradish peroxidase were made after lesioning the lateral cervical nucleus. Anterograde labelling was seen contralaterally in the external nucleus of the inferior colliculus, the intercollicular zone, and in the thalamic ventro-posterior complex. Sparse projections ipsilateral to the injection site were also observed. The results suggest that the projection of the lateral cervical nucleus on the mesencephalon is part of the pathway which conveys the sensory information from the suckling stimulus.

Junctional resistance of isolated adult rat ventricular cell pairs

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An enzymatic procedure was used to isolate ventricular cells from adult hearts. Pairs of cells were chosen to study the electrical properties of the nexal membrane linking the cells. Both cells of the pair were attached to suction pipettes (tip size 2–3 µm) in order to voltage clamp each cell. With this method transjunctional voltage gradients of variable amplitude and either polarity were applied to investigate the voltage and time dependence of the nexal membrane. Transnexal current-voltage relationship was found to be linear and independent of the direction of current flow over the voltage range tested (±40 mV). It was not affected by the transsarcolemmal membrane potential at which the measurements were made (range: −90 to +30 mV). There was no sign of a time-dependence of the nexal membrane resistance for pulse durations from 0.2 to 10 s. The measurements revealed values for the nexal membrane resistance on the order of 2 to 4 MΩ in the presence of 5 mM $[Ca^{2+}]_0$.

Rapid change of presynaptic intramembrane particles during transmission of a single nerve impulse

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By using fast-freezing techniques we have cryofixed small pieces of *Torpedo* electric organ at 1-ms intervals before, during and after the passage of a single nerve impulse. Freeze-fracture replicas were examined in the absence of any chemical fixation. Synaptic transmission is accompanied by a marked increase in the number of particles in the presynaptic membrane. The change is very brief, appears at the same time as the electrical response, and returns to the resting value within a few ms. The density of large pits denoting endo- or exocytosis does not increase during the passage of the nerve impulse. We propose that this transient ultrastructural change is a manifestation of the process of transmitter release.

Inhibition of blood platelet aggregation by synthetic peptides corresponding to the carboxy terminal of the γ-chain of fibrinogen

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The binding of fibrinogen to the glycoprotein IIb/IIIa complex at the surface of activated blood platelets is a prerequisite for platelet aggregation. C-terminal peptides of the fibrinogen γ-chain – which was reported to be involved in the binding of fibrinogen to the platelet receptor – were synthesized and tested. The highest antagonistic activity was obtained with the C-terminal pentadeca- and dodecapeptides. Shortening of the peptide

from either end resulted in considerably lower activity. The relative activities compared to the dodecapeptide were for the C-terminal deca-, nona-, hepta- and tetrapeptides 58%, 35%, 40% and 13%, respectively, and for the undecapeptide lacking the C-terminal Val only 10%. Platelet aggregation in vitro was inhibited by these peptides independently of the agonist used. The degree of inhibition was however dependent on the fibrinogen concentration and points to a competition for the same binding site at the receptor.

Horizontal optokinetic ocular nystagmus (OKN) in the pigmented rat measured with the magnetic search coil technique

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OKN was induced by rotation of a pattern of bright dots projected onto a cylindrical screen. Stimuli consisted either of velocity steps of pattern rotation (2–60°/s) or sinusoidal oscillations (0.05–2.0 Hz, 2–20°/s). Gain of step responses in binocular vision ranged between 0.8 and 1.0 for pattern velocities up to 20–40°/s. Step response profiles showed a gradual increase in slow phase velocity (SPV) reaching steady state after a time period roughly proportional to stimulus velocity. Initial SPV measured within 500 ms after stimulus onset reached between 2 and 4°/s and was largely independent of stimulus amplitudes above 10°/s. Occasionally rats showed fast rises in SPV at the onset of the step response profile. Gain of step responses in monocular vision was, for temporo-nasal stimuli similar to that measured in binocular condition, while for naso-temporal stimulation gain was much smaller even at low stimulus velocities. Sinusoidal modulation of SPV was linearly dependent on stimulus velocity. Gain was relatively constant (0.8), while phase lag increased strongly with stimulus frequency.

Aggregating brain cell cultures as a model to study gliosis

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Proliferation of astrocytes due to CNS lesions is a well-known phenomenon. However, the factors inducing this cellular response are still obscure, and their elucidation may require the development of suitable in vitro systems. Serum-free aggregating cell cultures of fetal rat telencephalon mimic normal brain development. In these cultures, the mitotic activity is limited to the first two weeks and is followed by a prolonged period of cellular maturation. During the phase of cellular proliferation, astrocytes are responsive to epidermal growth factor (EGF). Treatment of the cultures with cytosine arabinoside (Ara-C, 0.4 μM) irreversibly abolishes both the mitotic activity and the response to EGF, without affecting cellular maturation. If cultures previously treated with Ara-C are partially dissociated by mechanical means, astrocytes are induced to proliferate, and at the same time, become again responsive to EGF. These findings suggest that aggregating brain cell cultures offer a promising model to study astrocytosis.

Evidence for hydroxyl: sulfate exchange in basolateral rat liver plasma membrane vesicles

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Sulfate is actively transported into intact hepatocytes. In order to delineate the driving forces for hepatic uptake of $SO_4^=$ we investigated the effects of transmembrane pH gradients on $SO_4^=$ transport in highly purified basolateral (sinusoidal) rat liver plasma membrane vesicle preparations (J. Cell Biol. *98* (1984) 991). It was found that an outwardly directed OH^- gradient (pH 8.0 in/6.0 out) stimulated $^{35}SO_4^=$ (0.1 mM) uptake 8-fold compared to pH equilibration (pH 8.0 in = out)[4] and 2- to 4-fold above equilibration $^{35}SO_4^=$ uptake (overshoot). Initial rates of this pH gradient stimulated $SO_4^=$ uptake exhibited saturability with increasing concentrations of $SO_4^=$ (K_m 13.4 ± 4.6 mM; V_{max} 11.7 ± 1.8 nmoles · mg^{-1} · min^{-1}) and were inhibited by 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene (DIDS) and the protonophor carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP). These findings demonstrate the presence of a carrier mediated OH^-: $SO_4^=$ exchange system in basolateral rat liver plasma membranes.

Functional restriction of the paradoxical sensitivity reducing action of dynamic γ-motoneurons

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The distinguishing feature of dynamic γ-action is that it enhances the sensitivity of spindle Ia afferents to muscle stretch. Yet dynamic γ-efferents also act paradoxically, by reducing Ia sensitivity to small disturbances at fixed mean length, especially when these are slow (below 1 Hz; Hulliger et al., J. Physiol. *253* 811). Yet the present findings show that normally this paradoxon is likely to be restricted to fast disturbances superimposed on extremely slow background movements. Stimulation of dynamic γ-axons enhanced Ia sensitivity to 4 Hz sinusoids superimposed on slow triangular movements, unless the latter were very slow (below 3 mm/s) and the former very small. Even then paradoxical action was seen only when passive Ia sensitivity was pronounced (at long mean length) but not when sine frequency was low (in marked contrast to its presence at fixed mean length). Thus dynamic γ-efferents may be viewed as providing nearly uniform enhancement of spindle Ia sensitivity.

Cephalic phase insulin secretion in Zucker rats

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Cephalic phase insulin secretion, elicited either by saccharin or glucose ingestion, was studied in adult lean and genetically obese Zucker rats. In both groups of rats, it occurred in the first 2 min post-ingestion and taken in absolute values, it was exaggerated in the obese rats. The existence of cephalic phase insulin secretion confirmed that the neural efferent limb of the reflex functions in the obese rats as well. Expressed in percent increment of basal values, cephalic phase of insulin was blunted in the obese when compared to their lean controls. The cephalic phase insulin secretion is probably partly responsible for the high abnormal oral glucose tolerance in the obese rats, being ineffective on the liver metabolism.

Calcium fluxes and intracellular calcium buffering in rabbit vagus nerve

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The incorporation of ^{45}Ca into the rabbit vagus nerve was continuously monitored during superfusion of the preparation with ^{45}Ca containing solutions. La^{3+}, which displaces the membrane-bound Ca was used to identify the intracellular Ca compartment. It was found that the rates of labeling and the amounts of both the membrane-bound and the intracellular

compartments increase with increasing the external Ca^{2+}. When Na_{out} was withdrawn, an important incorporation of ^{45}Ca was observed as well in the membrane-bound as in the intracellular compartment. Further, electrical stimulation did not produce a measurable increase of the total ^{45}Ca, although results with quin-2 show that during electrical activity the intracellular free Ca increases as much as during exposure to Na-free solution. These observations suggest an involvement of Na_{in} in the Ca buffering capacity of axoplasm in this tissue.

Effects of various O_2 concentrations on protein synthesis by cultured endothelial cells

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Studies on primary cultures of porcine aortic endothelial cells at confluence have shown that a 2-day exposure to 95% O_2 resulted in a 75% inhibition of the ^{3}H-thymidine (TdR) incorporation into DNA and a 82% reduction in TdR kinase activity. To see whether this inhibition of DNA synthesis could be secondary to inhibition of protein synthesis, we decided to study the effect of a 1–5-day exposure to various O_2 concentrations on the rate of ^{3}H-phenylalanine (PA) incorporation into protein. The size of the free PA pool was also measured. The cells exposed to 95% O_2 showed a decrease in ^{3}H-PA incorporation into protein which was linearly correlated with the duration of exposure (by −14%/day). The intracellular pool of PA remained unchanged. The different time course of these O_2-related effects suggests that the O_2-related reduction in DNA synthesis is more likely to result from a direct toxic action of hyperoxia on TdR kinase than from a primary effect on protein synthesis.

Effects of neurohypophyseal peptides on ganglionic transmission

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The actions of arginine-vasopressin and oxytocin on rat superior cervical ganglion were investigated with intracellular recording techniques. The peptides, perfused at micromolar concentrations, blocked initiation of APs and decreased the amplitude of fast-EPSPs in 45% of the cells tested. This synaptic depression was accompanied in the majority of the cells by a slight depolarization of the post-synaptic membrane. The peptides decreased the frequency of the miniature-EPSPs and the quantal-content of fast-EPSPs without affecting the acetylcholine sensitivity of the cells. The depressant action of the peptides was not antagonized by atropine, phentolamine and naloxone but was totally and reversibly abolished by a highly potent vasopressor response antagonist. It was concluded that the peptides depress ganglionic transmission by acting at specific receptors located in the presynaptic terminals.

Myocardial ischemia: dependence of resting membrane potential and extracellular K^+ on pH

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Extracellular pH(pH_e), intracellular pH(pH_i), resting potential (V_M) and extracellular K^+ were measured in perfused guinea-pig hearts with microelectrodes. Interruption of coronary perfusion was followed by a small initial decrease of pH_i to a steady-state level (pH_i 6.85 after 10 min at pCO_2 50 mm Hg), by a large decrease of pH_e, by a depolarization of V_M and by extracellular K^+ accumulation. During ischemia pH_e and pH_i were varied by changing the pCO_2 in the atmosphere of the recording chamber. Intra- and extracellular acidification (increase of pCO_2 from 5 to 400 mm Hg) produced a decrease of V_M of 10 mV and additional accumulation of extracellular K^+. Alkalinization (decrease of pCO_2 from 400 to 5 mm Hg) was followed by a hyperpolarization and extracellular K^+ depletion. Our results suggest a direct relationship between changes of pH_i, V_M and K^+ movements between the intra- and extracellular space.

Effects of pulmonary stretch receptor blockade on vagal reflexes during high frequency ventilation

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High frequency ventilation (HFV) is known to prolong expiration up to an apnoea. This effect is probably not solely due to the Hering-Breuer inflation reflex (HBIR), as it occurs during HFV at lower lung volume than during lung inflation, and as the HFV-induced apnoea is, contrary to inflation-induced apnoea, associated with increased diaphragmatic activity. To assess the role of the pulmonary stretch receptors (SR) in lung reflexes during HFV, the SR were blocked by SO_2 in anesthetized rabbits. After SR-blockade the HBIR was abolished and the HFV-induced prolongation of expiration was diminished. The HFV-induced apnoea, however, persisted and the accompanying diaphragmatic activity was even higher than before blockade. It is concluded that at least a part of HFV effects on respiration is due to stimulation of other receptors than pulmonary stretch receptors.

A voltage-sensitive K^+ channel in the apical membrane of a cultured renal epithelium (JTC-12)

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We have identified a voltage-sensitive K^+ channel in the apical membrane of a cultured renal epithelial cell line: JTC-12, using the patch clamp technique. Under physiological conditions (cell-attached patch configuration with Krebs-Ringer in both the pipette and the bath) spontaneous channel activity was observed in all patches. The channel had a mean ohmic conductance of about 96 pS and displayed complex burst kinetics. The burst kinetics displayed a strong voltage dependence: The mean burst life time increased and the mean closed time between bursts decreased as the apical membrane potential was depolarized. At large depolarizing potentials up to six channels could be observed simultaneously in a single patch. It is proposed that this channel may play an important role in the maintenance of the apical membrane potential and hence driving forces for a number of electrogenic sodium/solute cotransport systems.

On-line image analysis of cell and tissue cultures: recording of mechanical tensions and movements generated in the chick embryo

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Development of cell cultures has been often studied using time-lapse microcinematography. This technique is however not suitable for physiological experiments where informations are needed in real time.

A processing of pictures taken by a TV camera allows an on-line recording of events taking place in the preparation. This is achieved by subtracting a memorized reference image from the images taken onward. The result, an image of the change, facilitates detection and interpretation of growth, mechanical tension, migration and shaping.
This approach has been used to study the early development of the chick embryo cultured in vitro. Analysis of images recorded under temperature variations, normoxia-anoxia cycle, electrical stimulation, and inhibitors of cytoskeletal elements helps to understand the origin and distribution of mechanical forces within the embryonic disc.
The early embryonic development could be modulated by a subtle mechanical equilibrium between the forces generated by the embryonic and extraembryonic regions.

Changes of rat brain neurotransmitter systems in chronic relapsing experimental allergic encephalomyelitis (CR-EAE)

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Neurochemical studies of serotoninergic (5-HT) and noradrenergic (NA) neurotransmitter systems by HPLC in five CNS regions, and immunological studies in various peripheral organs, were performed in rats with CR-EAE (two attacks and one recovery phase) and in controls. Immunologically, the lymph node cellularity could be clearly correlated with clinical signs. The neurochemical results showed that both the neurotransmitters 5-HT and NA were reduced in lumbosacral spinal cord (LS) during the whole course of the disease, whereas in the craniothoracal (CT) part only NA was reduced in the two attacks, returning to normal in the recovery phase. In LS and CT the precursors Tyr and Trp were greatly elevated during the attacks. 5-HIAA (a 5-HT turnover marker) was increased in the first attack and then decreased in the later stages indicating destruction of nerve fibers. A correlation of these results with clinical signs is only partially possible.

Seasonal variations in thermoregulatory and psychometric parameters in healthy subjects

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In a cross-sectional study, covering the period of June 1981 to June 1982, thermoregulatory and psychometric data from 285 healthy subjects (147 ♂, 135 ♀; 20–71 y) were analyzed for seasonal rhythms. One-way ANOVA with respect to month revealed significant variations in oral temperature (minimum in November, $p < 0.05$), in acral rewarming time after local cold stress (peak in October, $p < 0.003$) and in cold sensitivity (peak in November, $p < 0.03$), whereas basal acral temperature did not vary significantly with time of year. Psychometric data revealed seasonal patterns in subjective 'tiredness' (peaks in winter and late spring, $p < 0.03$), and 'depressed mood' (peak in December, $p < 0.05$). When grouped according to the four seasons, changes in 'nervousness', 'masculinity', and the subjective classification into 'morning- or evening type' were also found. Furthermore, sex differences in mean, amplitude and time course of seasonal variations were observed in most parameters. These control data are important in comparing mesurements from pathophysiological states with cyclic mood swings such as affective illness.

An automatized Y-maze for multimodal discriminatination training of mice

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Mice are trained in a computer-controlled Y-maze to discriminate between a pair of visual or vibrisso-tactile stimuli. Movements of the animal are recorded by photocells and monitored graphically. Mice run to a decision point, choose between two tubes that contain the discriminanda (those for touch are parts of the walls), and return to a start point. Inappropriate behavior is punished by blows of compressed air. Movable gates are used to direct the animal, and ensure a forward passage.
The program analyzes starts (one-way avoidance), run time, side preferences (inspections and choices), and choice strategies based on discrimination, alternation, and recollection of the last trial. This permits determining whether discrimination errors are based on nonsensory factors. Besides neurosensory studies, the apparatus may be used for analyzing brain lateralization, and drug or gene effects on cognitive mechanisms.

The recovery of parameters of finite mixtures of normal distributions from a noisy record and its application to the quantal analysis at central synapses

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Analysis of amplitude fluctuations of Ia single-fiber e.p.s.p.s is handicapped by high noise levels due to spontaneous synaptic potentials. It is possible to remove the masking effects of the noise from the underlying distribution of the fluctuating e.p.s.p. if the following assumptions are made: 1) The mechanisms generating the noise are statistically independent of those causing the fluctuations in the e.p.s.p. 2) Noise and signal add linearly. Since the variance of the gaussian distributed noise amplitude can be determined, the problem is reduced to finding the means and membership probabilities of the components of a normal mixture distribution. A Monte-Carlo study was carried out to evaluate empirically the performance of the maximum likelihood estimators for the means and membership probabilities of mixtures of normal distributions. Problems of resolution which arise from sample size and high noise levels are discussed.

Electrophysiological studies of the effect of bradykinin on the rat portal vein

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The rat portal vein shows a biphasic response to bradykinin (BK) in vitro: a suppression of the spontaneous contractions (40–60 s) followed by an increase in myogenic activity. The ionic mechanism responsible for the inhibitory phase has been studied by means of intracellular recordings of the electrical activity. The inhibition was the result of hyperpolarization (max. 15 ± 2 mV, $n = 20$) which blocked the spontaneous discharge of action potentials. Among the K^+-channel inhibitors tested, TEA (10^{-3} M) and 4-AP (10^{-4} M) induced depolarization without preventing the inhibitory phase, whereas apamin (10^{-9} M) had no effect on the resting potential while abolishing the BK-induced hyperpolarization. When BK was applied to tissues depolarized by high $(K^+)_0$ (50 mM), no hyperpolarization occurred, whereas the BK-inhibitory effect was present in tissues depolarized by NA (10^{-6} M). The same results were obtained in the presence of the Ca^{2+} inhibitor nifedipine (10^{-6} M). Thus, the transient inhibitory effect of BK on the electromechanical activity of the rat portal

vein appeared to be connected to hyperpolarization resulting from the activation of slow K^+ channels.

Effects of smoking on visual evoked potentials in rapid information processing

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In 11 inhaling and in 10 noninhaling smokers visual evoked potentials (VEP) and continuous performance were measured for 30 min before and 30 min after smoking. The subjects were required to watch single digits on a TV screen presented in a fixed pseudorandom order and to detect triples of either odd or even consecutive digits by pressing a button. Digit presentation rate was continuously adapted to the subject's actual performance. Evoked brain potentials were analyzed for the digits of all correctly answered triads.

No late components were seen for the first digit, a late negative shift (CNV) with the second digit and a late positive component (P300) with the third digit. The magnitude of the CNV (voltage × time) correlated partially with performance level, while magnitude of P300 correlated in part negatively with performance. Postsmoking improvements of performance generally correlated with a shortening of the N90-latency while for CNV differential effects of smoking were seen in the two groups.

Transport of inorganic phosphate (P_i) by an established intestinal epithelial cell line ($CaCo_2$)

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$CaCo_2$ are derived from a human colon carcinoma. The uptake on P_i was analyzed in subconfluent monolayers or in isolated vesicles of the apical membrane. A divalent cation precipitation method was used for membrane isolation; alkaline phosphatase was enriched 10fold whereas marker enzymes for the basal-lateral membrane and for different intracellular organelles were not enriched. The same properties were found for P_i uptake into monolayers as into vesicles. Transport of P_i was specifically stimulated by a sodium (Na) gradient with an apparent K_m for Na of 66 ± 27 mM and for P_i of 214 ± 17 µM. Increasing Na reduced the apparent K_m for P_i. A Hill analysis suggested the involvement of more than one Na ion (n = 1.6). The apparent V_{max} of the transport system was reduced with increasing confluence of the monolayer. It is concluded that $CaCo_2$ cells contain a Na-P_i cotransport mechanism in the apical membrane.

Vasopressinoic acid (VA) increases water permeability in amphibian epithelia

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It has been reported that VA inhibits the antidiuretic effect of vasopressin (VP) in rat kidney but, paradoxically, stimulates net water flow (Jw) in toad bladder. We reexamined the effects of VA in amphibian epithelia and measured Jw by means of automatic techniques. In toad bladder, VA in the range of 1 to 100 nM did increase Jw in a dose-dependent manner; at 100 nM the effect of VA was not significantly different from that elicited by 1 mU/ml of VP (N = 9). As previously reported for VP, the action of VA was enhanced by quercetin (N = 11, $p < 0.01$) and inhibited by methohexital (N = 8, $p < 0.001$), trifluoperazine (N = 7, $p < 0.01$) and vanadate (N = 9, $p < 0.001$). In toad skin, VA increased Jw by 47% (N = 10) and by 286% (N = 11) in low and high responders, respectively ($p < 0.001$). In conclusion VA induces a hydrosmotic response in amphibian epithelia, which is sensitive to the same array of agents that modify the stimulation of water flow induced by VP.

A device with increased light collecting efficiency for measurement of optical parameters in small sample volumes

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The use of optical probes as indicators for biological processes has increased over the last years (potentiometric dyes, fluorescent labelled proteins, fluorescent Ca^{2+}-indicators, etc.). Due to limited apperture conventional fluorometers only detect a small fraction of the light emitted in all directions. Placing a sample at the focal point of a surrounding elliptical mirror allows detection of nearly 100% of emitted light in the second focal point (F2). The sample can be illuminated with parallel light at 90° to the long axis, with fluorescent or scattered light detected in F2, while light transmittance (on-axis) is detected outside the ellipse, yielding information about absorbance or transmittance of the sample (allowing simultaneous monitoring of two parameters). Without electronic signal conditioning 4 pmoles of Na^+ fluoresceinate in 20 µl is detectable with a signal/noise ratio of 10:1.

Performance of senescent rats in spontaneous exploration and shock-motivated avoidance tasks

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Age-related changes in maze exploration and active avoidance learning were studied in senescent (30 months or older), middle-aged (17 months), and young adult (5 months) EMD:Wi-AF/Han rats. With increased age, open field avoidance increased, and patrolling efficiency (a measure of spatial concept development) was reduced in maze tests. Following mixed housing of the rats of the two younger groups for 13 months, retesting in the same maze paradigm yielded weaker differences. Senescent rats were also found to exhibit poorer one-way avoidance learning than younger controls in a test consisting of up to a maximum of 30 trials given in a single session. Further experiments with these rats provided evidence that this deficit was probably not due to either sensory or motor impairments in the senescent group.

The rat iris – a peripheral target for studying interactions between innervating neurons

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Apart from the well-known sympathetic and parasympathetic innervation originating from cell bodies in the superior cervical ganglion (SCG) and ciliary ganglia respectively, the rat iris is innervated by nerves containing substance P (SP)-, somatostatin-, vasoactive intestinal polypeptide- and enkephalin-like immunoreactivity.

SP, mainly localized in trigeminal sensory nerves, noradrenaline present in sympathetic, and choline acetyltransferase (ChAT) in parasympathetic nerves were used to monitor innervation of the iris after sympathetic and sensory nerve lesioning.

Unilateral surgical removal of the SCG of adult rats led to the destruction of noradrenergic fibers and to a gradual increase in SP but did not affect cholinergic nerves in the iris. However,

ChAT activity in the iris significantly increased after combined sympathetic and sensory denervation.
These results indicate that important regulatory interactions occur between innervating nerves. Measurement of endogenous nerve growth factor (NGF) in the iris by a specific immunoassay indicated that the iris produces NGF-like activity upon denervation suggesting that innervating neurons compete for this protein within this target.

Speech characteristics and psychophysiological reactivity during two speech tests: SI and 'self-disclosure'

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The standardized Type A interview (SI) representing a social interaction and a 'self-disclosure' test (SD) consisting in a 4.5 min tape recorded self-report with a 5 min preparation time were presented to the same 26 students within a single psychophysiological recording session. Blood pressure was significantly elevated immediately after each test and recovered during the following rest periods to baseline level. Cardiovascular parameters (ECG, ear pulse transit time and finger pulse amplitude) showed strong but similar stress reactivity during the two tests. Skin conductance responses were more frequent during the SI than during the following SD. Recovery during the rest periods was in all these measures nearly complete too. In contrast, the EMG tended to increase consistently.
Some speech characteristics, such as speech rate, expiratory sighs and self-involvement were correlated between the SI and SD, and this was also the case for the magnitude of the individual physiological responses.

The influence of temperature, humidity and delay changes on the reliability of automatic respiration-analyzing systems

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Open, computerized respiration-analyzing systems are not only used in medical routine examination, but also for scientific studies on cardio-respiratory physiology. High standards are therefore demanded for the reliability of such systems. Usually, however, raw data, acquired by gas analyzers and flowmeter, are calculated with expected or standard values of temperature and humidity in order to determine the breathing parameters. In addition, a fixed delay (the time difference between gas concentration changes and the corresponding gas concentration signal, which is due to the gas sampling technic) for the synchronization of gas and flow data is accepted. By developing a new respiration-analyzing system which includes fast, continuous temperature measurement and breath-by-breath delay determination, it has been rendered possible to analyze the influence of changes in the above-mentioned parameters.

Changes in free calcium in mammalian nonmyelinated nerve fibers followed by quin-2

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The fluorescent dye quin-2 can be used to measure the intracellular free Ca concentration in mammalian nerve fibers (Pralong et al., Neurosci. Lett. *18* (1984) S 338) where methods developed for giant axons are not applicable. In fibers of rabbit vagus the mean Ca concentration at rest is 50 nM. A reversible increase in Ca is seen when external Ca is raised or Na lowered, or when acetylcholine is added. Electrical activity (10 Hz, 30 s) causes an increase in free Ca, which is inhibited by omission of external Ca or application of Cd (100 μM), suggesting that the increase in free Ca is due to Ca influx rather than to liberation from intracellular Ca stores.

Validation of the measurement of glucose turnover during non steady state in the rat

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The most widely used method to measure glucose turnover in the non steady state uses a one compartment model. However the model gives the best results when it is assumed that only a part of the total glucose pool takes part in a rapid change. The previously suggested pool fractions vary from 0.2 to 0.75, these values having been derived from experiments in dogs. As yet, no validation has been performed in rats, animals which are increasingly being used for the study of glucose kinetics in vivo. In this study the one compartment model was validated for the rat and the best pool fraction was experimentally determined. It is concluded that the model gives reliable results but only if it is assumed that 20% of the glucose pool ($p = 0.2$) is in the rapidly mixing part of the compartment.

Effects of substance P and related peptides on hippocampal neurones

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Using unitary extracellular recordings from hippocampal slices, we found that substance P (SP), when added to the perifusion medium in the micromolar range, caused an increase in the rate of firing of a proportion of neurones. Other, nonmammalian tachykinins, i.e. physalaemin and eledoisin, were also excitatory and usually more potent than SP. The excitatory action of tachykinins was direct, rather than synaptically mediated, because the neurones retained their responsiveness in high magnesium media causing synaptic uncoupling. Tachykinin-sensitive neurones were usually spontaneously active and since they responded to an orthodromic input by a train of spikes, they were deemed to be nonpyramidal cells. Tachykinins did not interact with muscarinic acetylcholine receptors, for the response to these peptides was unaffected by atropine. Structural analogues of SP which antagonize its peripheral effects did not block the action of tachykinins in the hippocampus, suggesting either that the potency of the analogues tested is too low or that the hippocampal action of tachykinins is mediated by a receptor distinct from the peripheral SP receptors.

Is there a thermogenic defect in human obesity and diabetes?

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The metabolic rate (MR) of six lean, nine obese and three diabetic subjects was measured before and during the infusion of glucose at exactly the same rate (609 mg/min) and euglycemia was maintained by adjusting the rate of insulin infused. At 2 h, propranolol was infused (bolus 100 μg/kg, 1 μg/kg·min) for a further 75 min. During glucose/insulin infusion alone MR increased significantly by 0.13 ± 0.02 in the lean and the obese and by 0.14 ± 0.03 kcal/min in the diabetics, with similar rates (~ 380 mg/min) and costs of glucose storage ~ 0.40 kcal/g. With propranolol MR fell significantly in the three groups and the energy cost of glucose storage fell to 0.23 ± 0.04, 0.17 ± 0.05

and 0.20 ± 0.04 kcal/g in the lean, obese and diabetics respectively. The thermogenic defect observed in human obesity and diabetes is related to the rate of glucose storage rather than a decreased sympathetically mediated thermogenesis.

Comparison of Pi-uptake in rat, rabbit and calf small intestinal BBMV

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Duodenal and jejunal brush border membrane vesicles (BBMV) were isolated by the Ca^{2+}-precipitation method. Considering the differences in the D-glucose transport properties, we decided to investigate the six vesicles populations for the Pi transport systems. Looking at the time course of Pi uptake, no significant variation could be observed between the different intestinal segments and animal species but the kinetical parameters present the following variations: 1) In between each species, K_m values (affinity constants obtained by Michaelis double reciprocal plot calculation) are practically identical for the duodenum and jejunum, but the V_{max} is much higher in the duodenum. 2) K_m in rabbit and calf exhibit similar values (0.24 ± 0.05 mM), whereas those in rat are much lower (0.12 ± 0.02 mM). 3) The Na^+-independent Pi uptake is much higher in the jejunum. On the other hand, the specific activities of BBM marker enzymes are different in the six vesicles populations despite of the fact that the enrichment remains more or less constant.

Projections of the reticular complex of the thalamus (RE) onto functionally characterized regions of the cat medial geniculate body (MGB)

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The single unit response properties to sounds were studied by extracellular recordings in the subnuclei of the MGB. In order to determine the origin of the afferents to the MGB, horseradish peroxidase (HRP) was then injected in such physiologically defined regions. As a result of HRP retrograde axonal transport, labelled neurons were principally seen in the inferior colliculus and auditory cortex, representing the two main sources of inputs to the MGB: In addition, this study demonstrates that RE projects in a diffuse way onto the ventral and dorsal divisions of the MGB. HRP stained cells in RE were located in its ventral posterior third, which coincides with a sector of RE physiologically defined as auditory in the rat. RE has connecting properties compatible with a role of modulator in the processing of the acoustic information by thalamo-cortical auditory centers.

Superposition of ballistic movements on steady contractions

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At the onset of an acoustic warning signal, human subjects carried out steady isometric flexions of different intensity with both feet. After a fixed interval, a light was turned on at which they superimposed, as fast as possible, a ballistic movement on the steady contraction. The intensity of the *intended* ballistic contraction was kept *constant* by instructing the subjects to carry out movements of constant amplitude without giving them any feedback about their performance. Dorsal and ventral steady as well as dorsal and ventral ballistic contractions were tested.
The relation between torque amplitudes of steady and ballistic movements depended on whether the two movements were likewise or oppositely directed. If the two movements were in the same direction the ballistic movement did not depend on the steady movement. If they were in opposite direction the superimposed ballistic movement increased in size with increasing steady movement. An analysis of e.m.g. records revealed that this direction-specific relation was based on an inhibition of the antagonist. Functional implications of these results on the synaptic organization of motoneurons ared discussed.

The splenic nerve, a major pathway to the endocrine pancreas

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Parasympathetic pathways to the rat endocrine pancreas are known to follow the vagi along their abdominal trunks, their coeliac branches, their gastric branches and the hepatic branch. Little is known of them beyond the coeliac branch, the most important one functionally but leading to other organs such as the small intestine and the spleen. We now show that the splenic branch, a mixed (sympathetic and vagal) ramification of the coeliac branch following the splenic artery, elicits insulin secretion when electrically stimulated. Rectangular stimuli were delivered at 15 Hz during 4 min and blood samples withdrawn through a jugular catheter, before and during the stimulation. Insulin secretion, in terms of relative incremental area, was roughly 50% of the secretion obtained by stimulating the right cervical vagus, indicating that this splenic branch is a major pathway of the insulinotropic fibers.

α_2-Adrenergic stimulation of rat brown adipose tissue respiration

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Brown adipose tissue (BAT) respiration is activated by norepinephrine (NE) via β_1-adrenoceptors and to a lesser extent via α_1-adrenoceptors. Measurements of respiratory rates were performed on BAT fragments with clonidine (10^{-7} to 10^{-6} M), and α_2-agonist added alone or in combination with NE or isoproterenol (ISO). Results show that clonidine potentiated the effects of both NE and ISO on the respiratory rate and that there was no potentiation when the α_2-antagonist, yohimbine (10^{-6} M) was added. In purified BAT plasma membranes, both basal and ISO-stimulated adenylate cyclase activities were unaffected by clonidine. These results indicate that, in contrast to isolated brown adipocytes in which clonidine inhibited respiration, in tissue fragments, it had a potentiating effect.

Phosphate transport in the gut of the ruminant

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Sheep must absorb daily 3–8 times as much phosphorus (P) as man due to the recirculation of P via saliva. P transport across the luminal border of intact abomasum and small intestine was thus measured in vitro according to Danisi and Straub (Pflügers Arch. *385* (1980) 117). Segments of gut from three young sheep (5–8 months) and two older sheep (9–12 months) were incubated with either sodium (Na) or choline (four assays/sheep). Na-dependent P transport appeared to be only in the ileum and was significantly higher in young sheep (224 ± 29 nmoles/h·cm^2) than older sheep (26 ± 3). Na-independent P transport in the ileum was lower in younger (3.0 ± 0.4) than older sheep (9.8 ± 0.7). In abomasum duodenum and jejunum, P fluxes in the presence and absence of Na were not different. In the sheep

excess P is excreted via the feces. The presence of Na-dependent P transport in the ileum, where P solubility is low, may provide an important control over P balance.

Alpha$_1$-adrenergic mediation of membrane depolarization in rat brown fat

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Lipolysis and heat production in brown fat are mainly mediated by binding of noradrenaline to β-adrenergic receptors and activation of the adenylate cyclase pathway, although an initial event in response to stimulation is an α-adrenergic, stimulation frequency dependent, rapid, membrane depolarization. Since α receptors have been divided into two classes: α_1 receptors involved in the elevation of Ca_i^{2+} and α_2 receptors in the inhibition of adenylate cyclase, this type of receptor was characterized. A pulse of phenylephrine 10^{-5} M (mainly α_1 agonist) applied on superfused brown fat was followed by a transient depolarization from -57 ± 2 mV to -32 ± 4 mV within 10 s. In the presence of prazosin 10^{-6} M (α_1 antagonist) the amplitude of the rapid depolarization (4 Hz, 100 stimuli: 22 ± 2 mV, n = 6) was decreased by 85% ($p < 10^{-3}$) but in the presence of yohimbine 10^{-6} M (α_2 antagonist) it was not (23 ± 2 mV and 21 ± 2 mV, n = 6). Thus the first, rapid, membrane depolarization in brown fat results mainly from α_1 receptor activation.

Active ion transport and O_2 consumption in bovine tracheal epithelium

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The relationship between ion transport Ji and O_2 consumption Jr was studied in short-circuited bovine tracheal epithelium. Ji is the sum of Cl^- secretion J_{Cl} and Na^+ absorption J_{Na}. Jr is made of two parts: Jr basal, unrelated to Ji, and Jr suprabasal, which is a function of Ji. The measurements were made at 37 °C and at PO_2 near 600 Torr. Special care was taken concerning temperature stability, microbial contamination and O_2 leaks. Jr and Ji were stable for at least 5 h. J_{Na}, J_{Cl} or Ji were selectively inhibited with amiloride, furosemide, ouabain or K^+ suppression. Jr basal was found to be invariant and unaffected by the inhibitors. The relative contribution of either J_{Na} or J_{Cl} to Jr suprabasal and to Ji was not different: a plot of % change in Jr suprabasal vs % change in Ji gave a straight line ($r^2 = 0.96$, 51 observations). A unique stoechiometric ratio of about 13 ions/O_2 was found for Cl^- as well as for Na^+.

Whole body protein synthesis and resting energy expenditure (REE) in very low birth weight infants

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10 very low birth weight infants (< 1500 g) were kept under standard thermoneutrality conditions and received a milk formula providing 99 kcal/kg·d metabolizable energy and 3 g protein/kg·d. These infants grew at an average rate of 15 g/kg·d. Their REE was 58 kcal/kg·d indicating that 41 kcal/kg·d was retained. Estimates of body composition from the energy balance coupled with N balance method showed that 25% of the gain was fat and 75% was lean tissue. Whole body protein synthesis and breakdown were determined using repeated oral administration of ^{15}N glycine for 60–72 h, and ^{15}N enrichment in urinary urea was measured. Protein synthesis averaged 11.2 g/kg·d and protein breakdown 9.4 g/kg·d indicating that 1.8 g prot/kg·d was retained. Muscular protein breakdown, as estimated by 3-methylhistidine excretion, contributed to 12% of the total protein breakdown. The elevated REE observed in these infants is related to their rapid rate of weight gain which is accompanied by high rate of protein synthesis.

Behavioral effects of MPTP-induced dopamine cell lesions in monkey

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MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a newly described neurotoxin for dopamine cells. Two monkeys were lesioned by injecting MPTP (0.33–0.36 mg/kg) i.v. on five consecutive days. One or two days after the last dose, animals showed a flexed posture and strong hypokinesia. Lateron, occasional tremor and rigidity developed in one animal. All symptoms reverted with several daily doses of L-Dopa with benserazide, a regimen that was maintained during the period of severe hypokinesia. A remarkable recovery occurred several weeks after the lesion. In a simple reaction time paradigm in one animal, strong increases in reaction time, EMG-reaction time and movement time were observed. Dopamine content in the caudate and putamen of this animal was decreased by 88–95%. In cresyviolet-stained histological sections, a comparably severe cell loss in the pars compacta of substantia nigra was seen.

Recovery of action potential, birefringence signal and twitch tension from a preceding activation in single frog skeletal muscle fibers

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During activitation of skeletal muscle a three component birefringence signal (BS_1–3) can be observed. BS_2 is considered to signal changes in free myoplasmic calcium concentration. (Oetliker, 1982, J. Muscle Res. and Cell Mot. *3* (1982) 247). To further test this hypothesis the recovery from a conditioning stimulus of BS_2, the AP and twitch tension were compared. At 22° the action potential was refractory for 2.9 to 5 ms and recovered to 90% of its original amplitude in 2 to 3 ms. At 2.9 to 5 ms the BS_2 had an amplitude of about 20% and reached 90% (steadily increasing) only after 80–90 ms, while the mechanical trace showed potentiation of extratension at about six times threshold interval. These findings strongly suggest that BS_2 cannot reflect the activity of the contractile proteins and they are in agreement with the hypothesis that BS_2 reflects changes in myoplasmic Ca^{2+} concentration.

Cortical influence on interactions between neurons in the acoustic thalamus of the cat

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Spike trains of 364 pairs of single units were recorded in the Medial Geniculate, Suprageniculate and Reticular Nuclei of the thalamus of three nitrous oxide anesthetized cats with an array of six microelectrodes. Corticofugal influences were suppressed by intermittant cooling of ipsilateral Auditory Cortex. The cross-correlograms between these pairs revealed a class with flat curve ('no interaction', 76%), a class with a peak in the centre and duration up to 25 ms ('sharp common input', 12%), a class similar to the previous one but with longer duration ('broad common input', 4%), a class showing excitation of one cell onto the other (1%) and finally a class with correlograms hard to

interpret (7%). Out of 87 pairs showing an interaction, 50% were affected by cortical cooling, the strength of the interaction being increased (40%) or weakened (10%). Moreover the observed interactions were modulated by the acoustic stimulation for 34 pairs.

Kinetics of O_2 consumption induced by a single flash of light in the photoreceptors of the barnacle

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In the drone retina, the measurement of the kinetics of O_2 consumption following a flash of light provided surprising results: the increase of O_2 consumption (ΔQO_2) precedes the activation of the Na^+ pump (Tsacopoulos et al., Nature *301* (1983) 604). We are measuring the O_2 consumption in the rudimentary lateral eye of the barnacle *(B. eburneus)* which contains only three large photoreceptors (100 µm ∅). Using O_2-sensitive microelectrodes, we record a transient drop of PO_2 (ΔPO_2) at the surface of the eye after a brief flash of light. ΔQO_2 is calculated from ΔPO_2 considering the preparation as a sphere consuming O_2 uniformly. We find that: 1) the time course of ΔQO_2 is similar to that of the post-illumination hyperpolarization caused by the electrogenic action of the Na^+ pump; 2) substitution of Na with choline in the bathing solution reduces the amplitude of ΔQO_2 and this reduction occurs faster when the preparation is stimulated with flashes than when it remains in the dark.

Effects of smoking on vegetative reactivity to noise in light versus deep inhalers

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A smoker's depth of smoke inhalation largely determines the dose of nicotine delivered. Two groups of smokers were therefore selected (12 per group) whereby light inhalers generally showed a pre/postsmoking CO boost in respiratory tidal air of 1.0 ppm or less while deep inhalers typically exhibited boosts of 3.0 ppm or greater. This study sought to compare these two groups in an experiment involving psychophysiological stress reactivity to intermittent environmental noise bursts (95 dBA peak intensity) before and after smoking following 15 h of deprivation. Degree of subjective stress was reported after each noise burst while electrocardiogram, photoplethysmogram, pulse transit time, electromyogram, respiration, and skin conductance were continuously recorded. The results indicate light inhalers to derive a greater stress dampening effect from less nicotine than do deep inhalers from more.

Interactions of neurohypophysial and opioid peptides in hippocampal slices

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Opioid peptides depress the spontaneous firing of oxytocin-responsive interneurones in hippocampal slices (Mühlethaler et al., Brain Res. *308* (1984) 97). We now show that these effects are mediated by a *mu*-subtype opiate receptor. Indeed, DAGO, a *mu*-opiate agonist, decreased or even suppressed the firing of these neurones, an effect reversed by naloxone. U-50488, a *kappa*-opiate agonist, had no effect. When the slices were synaptically uncoupled by high external magnesium, oxytocin excited nonpyramidal neurones and DAGO still inhibited them. Thus, opioids and oxytocin exert direct, opposite effects on the same population of interneurones. An action of opioids on the excitability of pyramidal cells was found to be indirect and is probably mediated by the same interneurones bearing *mu*-type receptors, since in the presence of DAGO, the amplitude of the intracellular synaptic potential (EPSP) elicited by stimulation of Schaffer's collaterals increased.