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PHYSIOLOGIE - PHYSIOLOGY

Structural specializations of the ommatidia at the dorsal rim area of the compound eye in 2 hymenopterans

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The cornea at the dorsal rim of the honeybee's eye looks cloudy (grey area) due to pore canals penetrating the cornea from the inside. This affects the optics of this eye region and may be of significance for the detection of polarized skylight (Meyer and Labhart, Cell Tissue Res. *216;* Labhart, J. comp. Physiol. *141*). *Grey areas* were also found at the dorsal rim area of the eyes of *Ammophila* (Sphecidae) and *Andrena* (Andrenidae, Apoidea). Light and electron microscopy reveal that, compared to the honeybee, these corneae are more strongly perforated; they contain a system of interconnected caverns. In *Ammophila,* these cavities are partly filled with foamrubber-like material. In *Andrena,* the proximal portions of the cavities contain evaginations of the pigment cells with many microtubules. In both insects, the ommatidia in this eye region consist of 9 long receptors but in the adjacent part of the eye only 8 long receptors are present, as in the honeybee (Schinz, Cell Tissue Res. *162*).

Organization of stabilizing responses to backwards body pitch in the ankle muscles of normals and patients with unilateral labyrinthine lesions

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The interaction between muscle proprioceptive and vestibulospinal signals was investigated during an eyes-closed stance control task. Subjects stood on a platform which unexpectedly dorsiflexed the feet about the ankle joints, causing backwards body pitch. For normal subjects, segmental reflex EMG activity at 50 ms after rotation onset occurred symmetrically in the stretched soleus muscles of both legs. At 83 and 131 ms symmetrical EMG responses in the released tibialis anterior (TA) muscles returned the body to a stable stance. The first burst of TA activity occured some 62 ms after the onset of head angular acceleration induced by the platform movements. TA responses prior to 160 ms were assumed to be of vestibulospinal origin since they could not be elicited from sitting subjects.

Patients with recent vestibular lesions (spontaneous nystagmus present) had delayed, asymmetric TA responses. Correcting head angular accelerations were delayed with respect to normal, often started in the incorrect direction and were twice normal amplitudes.

Glutamate or a related substance may be the transmitter at the hair cell-primary afferent synapse in the vestibular labyrinth of the frog *(Rana temporaria)*

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We have chosen the isolated vestibular labyrinth of the frog to study the pharmacology of synaptic transmission between the hair cells and the primary canal afferents.

The effects of some neurotransmitter candidates on individual primary afferent activity was observed extracellularly and intracellularly. Bath application of glutamate ($\leqq 1$ mM), aspartate and some related agonists was found to consistently depolarize and excite these fibres in normal saline and when the presynaptic activity was depressed in high concentrations of Mg^{+2}. Unlike these acidic amino acids, other substances such as GABA, did not affect consistently the membrane potential or the spontaneous activity of the primary afferent. Antagonists (e.g. -- GDEE) of the acidic amino acid-induced excitation also decreased the amplitude of spontaneously occurring hair cell-evoked postsynaptic potentials without altering their freqency of occurrence. Taken together, these findings suggest that glutamate or a related compound is the transmitter between the hair cell and the primary afferent.

Does strict α-γ linkage occur during normal movement?

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From chronic recordings of spindle afferent discharge, length and e.m.g. of triceps surae during spontaneous and imposed movements in awake cats the time course of fusimotor activity was estimated by simulating the original responses (O) in anesthetized cats (A). Soleus muscle length (A) was controlled, via electromagnetic servo, by the original length record (O). The responses of soleus spindle afferents (A) were matched with the originals (O) whilst single static or dynamic γ-fibres were activated. γ-stimulation rate was modulated by the filtered e.m.g. (O), to simulate α-γ linkage. While selected primary afferents in A could adequately match responses to passively imposed movements (during brief anesthesia in O) simulations of strict α-γ linkage, i.e. of parallel activity in intra- and extrafusal muscle, always failed to reproduce the original discharge. In contrast, tonic fusimotor action could match original responses, e.g. during stepping or resisted stretch. Thus there is a measure of independence in the control of α- and γ-motoneurons.

Membrane currents in a developing parasympathetic ganglion

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The aim of this study was to determine the electrical properties expressed in cultured neurons dissociated from ciliary ganglia at a very early stage of the embryonic development (4 days). These neurons were 10 µm in diameter. Membrane currents were recorded 4 h after plating, using patch electrodes and the voltage clamp technique. We found that neurons that have just terminated their migration and are in the process of forming a ganglion, express several properties underlying membrane excitability. These cells possess voltage-dependent sodium, potassium and calcium currents as well as a calcium-activated potassium current. These currents resemble those previously described in the more mature ciliary ganglion (Bader et al., Devl Biol., 1982) but may differ in their density per unit membrane surface.

Control of the hypothalamo-neurohypophysial system (HNS) by portal vein chemoreceptors: spinal mechanisms

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Superfusion of the hepatic portal vein in anesthetized rats with 1 μM bradykinin (Stoppini and Baertschi, this congress) or hypertonic NaCl solutions activates the HNS. Spinal injection of xylocaine abolishes responses to both stimuli. Pretreatment of rats with capsaicin abolishes Substance P (SP)-immunoreactivity in portal vein and strongly reduces it at T8-T12; this pretreatment also abolishes HNS responses to bradykinin but not to NaCl. Spinal injection (8 μl) of capsaicin or SP at T8-T12 activates the HNS. Prior spinal injection of a SP-antagonist abolishes HNS responses to portal vein bradykinin but not to NaCl. These results suggest that SP fibres from portal vein to T8-T12 mediate HNS activation to a peripherally applied pain inducing peptide, and that portal vein osmoreceptors are not likely to be pain receptors. The neurotransmitter of peripheral osmoreceptor afferents remains to be identified.

The deoxyglucose method considered for studies of glucose metabolism in the chick embryo

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The uptake of 1-^{14}C-2-deoxy-D-glucose (DG) was measured (scintillation counting) in the young chick embryo (stages 4-7 HH) cultured in vitro. The total uptake of DG has been found to be proportional to the incubation time, and in addition, to the uptake of glucose as measured by the glucose oxidase method. A part of this uptake is independent of the cell metabolism (inhibitors) remains constant. The difference between the total and nonspecific uptakes should be due to the accumulation of the DG-6-phosphate, which is, according to Sokoloff (1977), proportional to the utilization of glucose by the tissue. The clearance of radioactivity from the embryo, as well as the relative proportions of the free and phosphorylated DG (chromatography and electrophoresis) have been measured, in order to define the optimal experimental conditions allowing the quantitative studies of glucose metabolism in the autoradiographs of the whole chick embryo.

The inward current Ih in vertebrate photoreceptors and If of cardiac cells: the same mechanism?

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Voltage clamp studies in solitary rod photoreceptors have shown the existence of an inward current, Ih, that is activated when the cell membrane is hyperpolarized (Bader et al., J. Physiol. *331* (1982) 253). Recently, we have recorded intracellularly in vertebrate photoreceptors with patch electrodes to further investigate the mechanisms underlying Ih. Comparing results in the presence and absence of Na on both sides of the membrane suggests that the charge carriers for Ih, namely Na and K, can permeate the same channel. Ih of photoreceptors resembles the inward current If described in cardiac pacemaker and Purkinje cells (Brown and DiFrancesco, J. Physiol. *308* (1980) 331; DiFrancesco, J. Physiol. *329* (1982) 485. The similarities are: a) the voltage range of activation, b) the block by caesium, c) the effect of extracellular potassium on the magnitude of the current, d) the time course, e) the charge carriers are Na and K and possibly in cardiac muscle, as in photoreceptors, permeate the same channel.

Role of c-AMP in the regulation of Na^+-dependent phosphate transport across renal brush border membranes

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The postulated role of c-AMP on protein phosphorylation and subsequently on sodium-dependent phosphate transport was investigated. Na^+-dependent phosphate uptake was determined with vesicles phosphorylated by 20 μmoles/l ATP either in the presence or in the absence of 0.1 mmoles/l c-AMP. Phosphorylation was performed under hypotonic conditions (dilution ratio 1:16). This procedure allows a transient permeabilization of the membranes and phosphorylation of the cytoplasmic surface of the membrane. Under hypotonic conditions, c-AMP stimulated phosphate incorporation into several proteins (e.g. 40,000 D, 46,000 D, 55,000 D). Although c-AMP affected protein phosphorylation, Na^+-dependent phosphate uptake was not significantly changed by c-AMP dependent phosphorylation when compared to vesicles phosphorylated in the absence of c-AMP. It is concluded that c-AMP dependent brush border membrane protein phosphorylation might not represent the final event in parathormone action.

Specificity of electrical coupling between individual *Aplysia* neurons in culture

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A cell when it exhibits electrical coupling (i.e. communication competence; Epstein and Gulila 1978) will couple with any cell that also shows this competence (with sufficient phylogenetic relationship). This has been demonstrated for many cell types with only few exceptions (Lowenstein 1981). Evidence for coupling specificity between *neurons* is presented using dissociated Buccal neurons and neurosecretory Bag cells from *Aplysia* in mixed primary cultures. Individual Buccal cells that electrically couple to homologous cells with a high frequency (> 80%) do not couple to neighboring Bag cells, which in turn exhibit an equally high frequency of coupling to other Bag cells. These cells are actually in contact with each other as shown by the presence of an inhibitory chemical Buccal → Bag cell-synapse. In conclusion, neurons in primary culture can exhibit specificity in electrical coupling.

Differentiation of the endplate membrane after denervation

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During development of a new endplate, the subsynaptic membrane becomes folded and the mean open time of the synaptic ion channels shortens from about 4 to 1 ms. To examine the role of the motor neuron in the differentiation of the subsynaptic membrane, we abolished the neural influences in developing ectopic endplates of rat soleus muscle by cutting the motor nerve before folding of the

junctional membrane and the change in channel gating had begun. The muscles were then either stimulated directly via implanted electrodes for 8–10 days or they were reinnervated by the soleus nerve at their original endplates. Electron microscopic and electrophysiological examination of the denervated ectopic endplates showed that junctional folds and the junctional type of channels appeared in the absence of the nerve terminal. This indicates that subsynaptic differentiation is programmed early during synaptogenesis and is then controlled solely by the muscle fibre independent of a neural influence.

The anatomy of some types of visual interneuron in the ant, *Cataglyphis bicolor*

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Visual interneurons in the brain of *Cataglyphis* are stained by applying the cobalt backfilling method for topographical investigation. By varying the period of diffusion, neurons of the 1st, 2nd or 3rd order (as counted from the site of injection) are filled. Depending on the site of injection, different types of visual interneuron are revealed. In the present investigation, cobalt is injected in the ocelli, the compound eyes and the ventral nerve cord. By this, a number of large interneurons is stained repeatedly and invariably. These interneurons can be categorized on the basis of the following criteria: the site and the geometry of the terminal arborization, the diameter of the axon, and the specific pathways along which the axon runs through the brain.

Proton-movements in rat jejunal brush border vesicles

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A continuous recording of the quenching of acridine orange fluorescence was used to monitor intravesicular acidification. In the presence of an outwardly directed K-gradient, valinomycin leads to intravesicular acidification; this observation documents a conductive pathway for protons. An outwardly directed Na-gradient provokes intravesicular acidification; this acidification was reduced to 35% by valinomycin in K-equilibrated conditions (membrane potential short circuit). A preset ΔpH was dissipated faster after pulse injections of sodium (maximal at 25 mmoles/l sodium); 100 µmoles/l amiloride reduced this sodium effect. Higher amiloride concentrations cannot be used in this technique because of unspecific effects. Chloride was unable to induce or to alter ΔpH. These observations document a sodium/proton exchange. The applied technique was unable to provide evidence for a Cl/OH exchange.

Milk-ejections induced by suckling and by cervicovaginal stimulation in lactating rats

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In the lactating rat, the secretion of oxytocin during nursing occurs at intervals ranging from 3 to 15 min. Each pulse of hormone causes a rather constant rise in intraductal pressure in all mammary glands and thereby induces the ejection of a spurt of milk. In post-partum rats anesthetized with a combination of pentobarbital and xylazine, weak cervicovaginal stimulation (CVS) caused the occurrence of an extra milk ejection that was superimposed on the rhythm evoked by suckling. The rise in intramammary pressure induced by CVS had a latency only slightly ($\sim$ 3 s) longer than that following the i.v. injection of oxytocin. Its amplitude was essentially all-or-none and similar in size to the suckling-induced milk ejections. Thereafter, the latter continued with the same frequency as observed before CVS. Following withdrawal of the pups, both CVS and suckling-induced milk ejections no longer occured. This data provides evidence for an interaction between suckling and CVS in eliciting the release of neurohypophyseal hormones.

When the photoreceptors in the retina of the honeybee drone are stimulated, K^+ activity in the glial cells rises more than Na^+ activity falls

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When drone photoreceptors are stimulated with light they gain Na^+ and lose K^+ into the extracellular space: most of this K^+ enters the glia (Coles and Tsacopoulos, J. exp. Biol. *95* (1981) 75). One possible mechanism for this K^+ entry is K^+/Na^+ exchange. To investigate this, we have made intracellular recordings with double-barreled microelectrodes that measured membrane potential and either Na^+ or K^+. When the photoreceptors in a superfused slice of retina were stimulated with a train of light flashes, 1 per s for 90 s, K^+ activity in the glia rose by 8 mM, SEM = 1 mM. With the same stimulation, Na^+ activity fell by only 1.5 ± 0.3 mM. Hence, unless there is some other factor, such as the transfer of Na^+ from a bound to a free state in the glia, the efflux of Na^+ appears to be much smaller than the entry of K^+ and only a small part of the K^+ entry is by K^+/Na^+ exchange.

Effect of parallel arms upon optimal foraging strategies of rats in various types of mazes

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Rats were trained to find food in transparent Plexiglass tunnels (section 12×12 cm; length 60 cm). 8 or 9 tunnels were either associated as an enclosed radial arm maze (situation a) or scattered on a table (situation b). Pathway length (situation b only), visit sequence and latency to visit all the tunnels was recorded during daily trials. Efficiency was estimated from the number of different tunnels visited in the first 8 choices. The presence of 2 parallel tunnels with adjacent accesses impaired choice efficiency in both situations. This was related to a constraint upon the sequence of visited tunnels and a difficulty to discriminate the parallel tunnels. The first factor was age-dependent.

Alpha-MSH: development of central control of pituitary peptide secretion

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We studied possible relationships between variations in serum MSH and the development of the central inhibitory control of this hormone by dopamine (DA). The innervation of rat intermediate lobe by DA fibers develops slowly and reaches a maximum 2 weeks after birth. Data on ^{3}H-spiperone binding and in-vitro release of MSH indicate that the intermediate lobe is responsive to DA at around birth. Serum MSH levels exhibit a peak at postnatal days (PN) 5 and 6. This peak appears to be linked with the onset of DA control as assessed by the DA antagonist flupenthixol, and with the development of feedback regulation stud-

ied by microfluorimetry of DA neurons. Recent observations with i.v. injections of anti-alpha-MSH on the 2 days of the MSH peak suggest that MSH secretion may be important for the maturation of the central loop.

Sodium transport in intestinal basolateral membrane vesicles

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Inside-out membrane vesicles were prepared from guinea-pig intestinal mucosa by differential centrifugation (BBA *688* (1982) 45). Na transport into the vesicles was measured by incubating them in a medium containing ^{22}Na and then separating them from excess medium by passage through a Dowex column. Nonspecific binding was estimated by incubating membrane sheets, obtained by treating vesicles with DOC/EDTA. Na uptake into vesicles attains an equilibrium after 30 min. Adding ATP at this time stimulates Na uptake which occurs against a concentration gradient. Addition of K + valinomycin to the medium reveals a 2nd component of Na uptake. The component stimulated by Na + K is inhibited by ouabain, and that stimulated by Na alone is sensitive to furosemide. 2 AT-Pases, a Na-ATPase and a Na-K-ATPase, have been detected in this preparation, and their sensitivities to inhibitors correspond to those of the transport mechanisms. We conclude that 2 ATP-driven Na pumps are present in the basolateral membrane of guinea-pig enterocyte.

Are calcium channels involved in the hydrosmotic effect of vasopressin (VP)?

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Voltage-dependent, Co-blockable Ca channels are involved in several types of stimulus-response coupling. To examine if such channels play a role in the hydrosmotic effect of VP, net water flow (Jw) was measured across toad bladders exposed to the following Ringer solutions on the serosal side: a) high K, b) 1 mM Co, c) Ca-free. Replacement of K for Na, a condition that depolarizes the basolateral membranes of the epithelial cells, did not change Jw, per se, but significantly enhanced the hydrosmotic effect of both VP (1 mU/ml) and cAMP (5 mM). This enhancement was even more marked in sulfate Ringer. In contrast, exposure to Co significantly reduced the hydrosmotic effects of VP and cAMP by 75% and 40%, respectively. Likewise exposure to nominally Ca-free Ringer diminished the same effects by 87% and 44%, respectively. These results suggest a participation of voltage-dependent Ca channels in the coupling of stimulus-hydrosmotic response of VP in toad bladder.

Comparative aspects of gaze stabilizing reflexes

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During locomotion, the position of the head passively oscillates around a center position. Vestibular and optokinetic reflexes compensate for these passive movements and reduce retinal image slip sufficiently to allow clear, unblurred vision within a certain working range. Stability of gaze results from compensatory head plus eye movements.

In unrestrained lower vertebrates (frog, turtle) slow phase as fast phase eye and head movements (measured with search coils) work in synchrony. Head movements compensate 80–90% of the imposed gaze shift. With respect to frog in the turtle the working ranges of vestibular and optokinetic reflexes are extended towards lower frequencies and higher velocities. This extension is due to the functional contributions of a brain stem network, that sotres neuronal activity related to slow phase velocity and that involves central vestibular neurons. Differences in response characteristics of central vestibular neurons (time constants, optokinetic modulation) parallel the behaviorally measured differences in the working ranges of both species. In conclusion: Compensatory head and ocular reflexes are organized according to the particular locomotory repertoire of a given species.

Recovery of the rat hippocampal choline acetyltransferase (CAT) levels following partial lesions of the fimbria

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The activity of the presynaptic marker enzyme CAT, in the whole left and right hippocampi, as well as the septum, was measured at 1-, 4- and 8-week intervals after partial lesions of the fimbrial bundle. Mean CAT activity in the ipsilateral hippocampus, taken as a percent of that in the hippocampi of unlesioned control animals, was reduced by 46% 1 week after the lesion. Spontaneous recovery of CAT activity occurred on the ipsilateral side as indicated by an increase in enzyme activity which was 66% and 77% of the unlesioned controls by 4 and 8 weeks respectively, following the lesion. In the contralateral hippocampi of lesioned animals, an enhancement of CAT activity over that of unoperated controls was observed at 8 weeks.

This study suggests that the hippocampal cholinergic system is capable of homotypic regeneration. The recovery following a partial lesion seems to involve partial replacement of degenerated cholinergic terminals as well as supplementation of undamaged ones on the intact side.

Recovery from motor asymmetry in rats with partial lesions of the substantia nigra

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Circling behavior after i.p. injection of d-amphetamine or apomorphine in rats with unilateral lesions induced by intranigral injection of 1, 2 or 8 µg of 6-OHDA, was quantified at various times after lesioning. The animals were then killed and tyrosine hydroxylase (TH), dopamine (DA) and metabolites were determined in the striatum. Rats with partial lesions responded only to amphetamine whereas those with complete lesions also responded to apomorphine. Amphetamine-induced turns as well as decreased TH and DA, and increased DA metabolism of ipsilateral striatum were proportional to the dose of 6-OHDA and thus to the degree of nigral degeneration. By about 35 days after lesioning, rats with partial lesions showed substantial to complete recovery from motor asymmetry. Animals with complete lesions, however, maintained the initial level of turning behavior. Normalization of motor asymmetry only in animals with partial lesions suggests involvement of the remaining DA system of the lesioned side in the recovery process.

Afferent fibers of the midbrain lateral tegmentum and their relation to the milk-ejection reflex in the rat

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The afferent pathways subserving the milk-ejection reflex in the lactating female rat were studied after electrolytic lesions placed in the midbrain lateral tegmentum. Bilateral lesions abolished the reflex on the day of the lesion, whereas the reflex persisted in rats with unilateral or sham lesions. In some animals, injections of horseradish peroxidase or True Blue were made immediately after the lesion using the same electrode. Retrogradely-labeled cells were found in various nuclei of the thalamus (e.g. reuniens, parafascicularis), hypothalamus (ventromedial, arcuate), medulla (gracilis, cuneatus, spinotrigeminal) and in the spinal cord (proprius, layer 10 of Rexed). The results suggest that the dorsal column-medial lemniscal system and the spinothalamic systems could both convey sensory information through or to the lateral tegmentum which is important for proper functioning of the milk-ejection reflex.

Presence of specific binding sites for prolactin in 2 toad urinary bladder-derived cell lines *(Bufo marinus)*

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Epithelial cell lines (TB-6c and TB-M) derived from toad urinary bladder, which maintained in culture a high degree of functional differentiation, expressed a significant amount of high affinity ($K_A = 10^{10}$ M^{-1}) binding sites for lactogenic hormones (up to 4000 sites/cell). Binding specificity was similar to that of PRL receptors present on rabbit mammary cells. When maintaining these cells in suspension at 28 °C, the concentration of PRL binding sites decreased significantly in absence of oPRL, but was maintained or increased in the presence of physiological amounts of oPRL (10^{-9} M), suggesting a positive influence of PRL on these toad bladder PRL receptors.
This finding of specific sites for PRL together with the observation that PRL exhibits antimineralocorticoid effects in these cells, confirms that PRL exerts specific biological action(s) for the control of electrolyte and water metabolism in the amphibians.

Morphological correlates of spinal pathways mediating the Mauthner cell-induced excitation and inhibition of spinal motoneurons

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One of the paired myelinated giant nerve fibers, the Mauthner axons, of the tench was injected with a fluorescent dye, Lucifer Yellow CH, in order to study a) the topography of its collaterals which are known to contact the ipsilateral spinal motoneurons directly (excitatory pathway), b) the occurrence and space frequency of dye coupling of these collaterals with other nerve cells (inhibitory pathway). Histological reconstruction of the Mauthner axons revealed a) that the number of its collaterals per spinal segment is fairly constant, b) that at least one Mauthner axon collateral per spinal segment exhibits the dye-coupling phenomenon with a midline crossing nerve fiber. This observation indicates the existence of electrically operating synapses (gap junctions) between the Mauthner axon collaterals and a certain type of commissural fibers which may represent the pathway mediating the Mauthner cell-induced short latency crossed inhibition of the spinal motoneurons.

The effect of captopril on brain angiotensin II (ANG II) receptors in spontaneously hypertensive rats (SHR-sp)

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ANG II-evoked firing of septal neurons occurred at a significantly lower threshold and showed an activity lasting far beyond the drug application in SHR-sp as compared to the normotensive Wistar Kyoto rat (WKY). In the present investigation we have studied brain ANG II receptors in SHR-sp which were treated with the converting enzyme inhibitor captopril (CAP) throughout their life span in order to keep the blood pressure at normotensive levels. The higher receptor sensitivity previously observed in SHR-sp was reduced by CAP. In contrast, no differences were found between WKY and CAP-treated WKY rats. Furthermore, the changes observed in SHR-sp were specifically related to ANG II. These data support the view that there exists a functional difference in the brain angiotensin system between SHR-sp and WKY rats. A possible link to central blood pressure control in SHR-sp should be considered.

Central effects of salmon calcitonin (CT) on gastric acid and pepsin secretion

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Acid secretion was studied in rats with a perfusion system of the stomach allowing repeated experiments with intracerebroventricular (i.c.v.) and s.c. administration of CT. i.c.v. CT shows a dose-dependant effect with a maximum reached with 10^{-10} moles. s.c. CT is as effective as i.c.v. CT, but at a 10 times higher dose. Both i.c.v. and s.c. CT effects persist for at least 90 min.
CT (up to 10^{-8} M) did not inhibit acid and pepsin secretion in the isolated mouse stomach, which was responsive to atropine (10^{-5} M). Moreover, less than 2% of [125-I]CT administered i.c.v. or s.c. was accumulated in the stomach.
In conclusion, i.c.v. administered CT inhibits gastric acid and pepsin secretion probably by a mechanism involving the central nervous system. CT has no direct effect on the isolated stomach.

Individually and group-housed female rats: a behavioral and reproductive comparison

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We have previously described (e.g. Behav. Brain Res. *4* (1982) 45) that individual housing of male rats induces behavioral alterations. Such studies concerned nonsocial behaviors as observed during openfield exposures. In the present experiments, female rats were compared after long-term individual or group housing in respect to openfield

behavior (nonsocial behavior), reproductive data and maternal behavior (social behavior). Results indicate that nonsocial behavioral differences as previously established for male rats are equally expressed in female animals. Reproductive data revealed some significant differences between individually and group-housed rats. Maternal behavior of differently housed mothers and the openfield behavior of the F_1-males will be described.

Effects of strophanthidin and extracellular K^+ on intracellular Na^+ activity in sheep heart

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Double-barreled Na^+-selective microelectrodes were used to study the effects of strophanthidin on intracellular Na^+ ion activity (a^i_{Na}) at different extracellular K^+ concentrations ($[K^+]_o$) in sheep heart Purkinje fibers. a^i_{Na} was 9.4 ± 2.3 mM (mean $\pm$ SD) in K^+_o 5.4 mM ($n = 10$) and decreased to 6.6 ± 2.3 mM when $[K^+]_o$ was doubled to 10.8 mM ($n = 10$). 5–10 min were required for a^i_{Na} to reach steady-state after the $[K^+]_o$ changes. The increase in a^i_{Na} after a 10-min exposure to a toxic concentration of strophanthidin (5×10^{-6} M) was dependent upon $[K^+]_o$. a^i_{Na} increased to 20.7 ± 2.8 mM in $[K^+]_o$ 5.4 mM ($n = 10$) and to 13.5 ± 2.4 mM in $[K^+]_o$ 10.8 mM ($n = 10$). The result that $[K^+]_o$ affects the strophanthidin-associated rise in a^i_{Na} may be one aspect of the benefits of increasing $[K^+]_o$ in digitalis intoxication.

Forskolin increases the water permeability of the skin and the bladder of toads *Bufo marinus*

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It has been recently shown that the diterpene forskolin (Fk) activates adenylate cyclase in intact cell systems. We investigated the effects of Fk on net water flow (Jw) across amphibian epithelia. In toad bladders, 0.5–10 µM Fk induced a reversible, dose-dependent stimulation of Jw. After 5 µM Fk, Jw was further increased by vasopressin, cAMP, theophylline or serosal hypertonicity. In toad skin, 10 µM Fk increased basal Jw from 0.66 ± 0.06 to 1.54 ± 0.11 µl/min cm^2 ($N = 16$, $p < 0.001$). Such an effect is similar to, but nonadditive to, that of vasopressin (100 mU/Ml). Preliminary results indicate that in both skin and bladder, the hydrosmotic action of Fk was not inhibited by the vasopressin blocker, methohexital, whereas KCl-Ringer and vanadate markedly reduced the Fk-induced stimulation of Jw. Forskolin can thus be used as a potent, nonhormonal activator of adenylate cyclase for studying the stimulus-response coupling of cAMP-mediated changes in water permeability.

Epidermal growth factor (EGF): stimulation of differentiating glial cells in culture

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Aggregating fetal rat brain cells grown in a chemically defined, hormone-supplemented medium undergo extensive maturation after an initial period of increased mitotic activity. The addition of EGF to the growth medium had only a negligible effect on the DNA synthesis, whereas the total protein content of the cultures was increased in a dose-dependent way. EGF-treated cultures showed also higher levels in glial enzyme activities; characteristic changes in cell surface properties; and an enhanced de novo synthesis of distinct proteins released into the medium. The stimulatory effects of EGF were irreversible, and the responsiveness of the cells was limited to a short period of development in vitro. These results show that EFG influences the development of cultured glial cells without interferring with their mitotic activity.

Modulation of synchronously bursting CA 1 pyramids by amine transmitters, an action on gK (Ca)?

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In hippocampal slices of the rat, in 0.2 mM Ca, 4 mM Mg, we have recently observed rhythmic depolarization shifts in CA 1 (Nature *300*, 448). Field bursts were recorded during bath perfusions with nM–µM of the following substances: an increase in burst frequency was caused by ACh, muscarine (blocked by atropine), histamine, impromidine (blocked by metiamide), noradrenaline, isoproterenol (blocked by propranolol), DBcyclic-AMP, dopamine (100 µM). Nicotine and phenylephrine had no effect while serotonin, dopamine (1 µM), adenosine and thiazolethylamine slowed the bursting. In normal solutions accelerating substances blocked, and slowing substances enhanced the slow afterhyperpolarization of CA 1 pyramids. High sensitivity and specificity make this response of the rhythmic bursts an excellent model for studying CNS pharmacology.

The action of proteases on the lung strip: an in vitro emphysema model

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The effect of 3 elastolytic proteases (pancreatic elastase, proteinase K and subtilisin) was compared with the effect of 3 nonelastolytic proteases (chymotrypsin, neutral protease and type I collagenase) on a) elastic properties (retractive force loss), b) contractility (response to histamine) and c) relaxation (response to adrenalin) of the guinea-pig lung strip. The elastolytic enzymes diminished the retractive force obviously to a greater extent than the nonelastolytic enzymes. None of the enzymes altered contractility with histamine and relaxation with adrenalin although the retractive force loss was irreversible under the conditions used. The evident but limited reduction of retractive force by the nonelastolytic enzymes was possibly based on the attack of microfibrillar proteins, or of other proteins contributing to the 'nylon-stocking elasticity' of the lung.

Regional distribution of calcitonin-binding sites in rat brain

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The regional distribution of calcitonin-binding sites in rat brain was examined by receptor autoradiography. Coronal and parasagittal sections (35 µm) prepared from perfused frozen brain were incubated with [125-I]salmon calcitonin ([125-I]sCT) at 4 °C for 24 h. Nonspecific binding determined in the presence of 1 µM sCT amounted to 5–20% of the total binding. The autoradiographs showed dense label-

ing over the hypothalamic region, the periventricular gray and the formatio reticularis. In the hypothalamus, the grain density was high in the anterior and dorsomedial parts, while it was lower in the ventrolateral and ventromedial areas. Dense labeling was also found over the amygdala, the zona incerta and the interpenduncular region. Binding was further seen over the nucleus arcuatus, nucleus supramamillaris and the lateral part of the pars compacta of the substantia nigra. The specific sCT binding seen by autoradiography corresponds to the regional distribution of the previously described high affinity binding sites in brain membranes (Fischer et al., PNAS *78* (1981) 7801). The well-defined topographical distribution strongly indicates a physiological role for CT in the mammalian brain.

Impact of oculomotor and vestibular research on clinical neurology

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Single neuron recordings and lesion studies in chronically prepared alert monkeys showed that the flocculus is an essential interaction site for visual and vestibular inputs to generate high velocity pursuit or optokinetic eye movements, and to adjust eye velocity to changing stimulus conditions, e.g. during combined visual-vestibular stimulation. This led to the development of easily applicable clinical tests defining a floccular lesion in patients. The loss of rapid eye movements in particular directions is a sensitive sign of brainstem pathology in patients. Because of parallel information processing the exact minimal lesion sites for vertical gaze palsies and the interaction of the horizontal and vertical eye movement generator for oblique movements we not known. Single unit recordings together with chemical lesions in the brainstem have defined such areas in the brainstem. Tests have been developed to indicate pathology of these structures in patients.

Spatiotemporal recoding of rapid eye movement signals in the monkey brainstem

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How is the image of a visual target, which is coded in retinotopic coordinates, transformed into a temporal discharge pattern of extraocular motoneurons to generate a rapid eye movement? Behavioral, anatomical and lesion studies, and the quantitative analysis of the discharge patterns of rapid eye movement related neurons in the brainstem and cerebellum of the awake Rhesus monkey have given many insights into the causal structure of this process of visuo-oculomotor physiology. Experimental findings will be discussed in relation to theoretical models.

The participation of basal ganglia in the control of isometric force

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The neural coding of force by neurons of the globus pallidus (GP) has been investigated in 2 monkeys trained to generate changes in isometric force on a transducer held between thumb and forefinger. The paradigm consists of a sequence of 2 step-and-hold increases in force, the first being lower (0.0–0.2 N) than the second (0.2–0.7 N).

The discharge patterns of 39 GPi and 16 GPe neurons have been classified into 2 main populations. The majority of the neurons (60%) have discharge patterns similar to those recorded in the finger region of area 4, and these patterns can be subdivided into phasic, phasic-tonic, tonic and tonic decreasing. About 30% of the tonic and phasic-tonic GP neurons showed a statistically significant correlation between firing rate and force, as do area 4 neurons. The other category of GP neurons (40%) displayed complex discharge patterns, seldom encountered in area 4, with sequences of tonic and phasic activations.

These observations support the idea that subcortical structures related to motor cortex may participate in the control of force in a similar way.

Eye movements evoked by stimulation of otolith organs

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Sensory inputs from the otolith organs elicited by changes in head posture relative to gravity help to stabilize gaze in space. We have studied in frogs the contribution of vertical eye movements to gaze stabilization during imposed static tilt or sinusoidal linear acceleration in the horizontal plane. In addition, single unit activity was recorded in trochlear motornucleus in order to evaluate the contribution of the eye periphery to the stimulus response dynamics.

The amplitudes of the evoked eye movements were in general quite small (of the order of 0.5°). When comparing our motoneuron data with the data obtained from otolith afferents (Blanks and Precht (1976)) little central processing of the peripheral signal in the maculoocular reflex in the frog is suggested. This is in contrast to findings from cat motoneurons, which indicate considerable central processing of the otolith afferent signal. It is concluded that maculo-ocular reflexes per se in the frog as in the rabit and in man perform relatively poorly in generating compensatory eye movements. However, otolith input in conjunction with canal afferent signals may contribute significantly to stabilization of gaze in space.

Intracellular electrophysiological recordings from the photoreceptors in the compound eye of the cricket *Gryllus campestris*

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As in the honeybee (Schinz, Cell Tissue Res. *162* (1975)) and in the ant *Cataglyphis* (Herrling, Cell Tissues Res. *169* (1976)), the ommatidia in the dorsal rim area (DRA) of the compound eye of crickets are characterized by anatomical specializations (Burghause, Zool. Jb. Physiol. *83* (1979)). 3 spectral cell types having maximal sensitivites at 340, 440 and 510 nm, respectively, were found. In the DRA, only blue receptors were recorded; they exhibit very high polarizational sensitivities (average: ca. 7) and extremely wide visual fields: $\Delta\rho$ is in the range 20–30°, but in a number of cells the angular sensitivity curve is to flat to allow determination of $\Delta\rho$. This property is due to the absence of both retinal screening pigment and corneal facetting in this part of the eye (Burghause, Zool. Jb. Physiol. *83* (1979)). $\Delta\rho$ in the unspecialized dorsal eye region is ca. 6.0°.

Noradrenergic fibers from brain stem to co-cultured spinal cord. Autoradiographic uptake and binding studies

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From biochemical, histochemical and electrophysiological studies there is strong evidence that axons from noradrenergic neurones located in the locus coeruleus project to the spinal cord. By means of autoradiographic uptake and binding studies we have mapped noradrenergic fibers projecting from brain stem cultures to co-cultured spinal cord of fetal rats. Uptake of ^{3}H-noradrenaline was observed in many neurones located in the brain stem explant whereas the co-cultured spinal neurones remained unlabeled. A great number of intensely labeled nerve fibers originating from brain stem neurones approached the unlabeled spinal neurones and appeared to form contacts with these cells. These findings are consistent with our previous observation of binding sites for ^{3}H-noradrenaline and labeled α- and β-antagonists on many large spinal neurones. They provide further evidence for the existence of α- and β-adrenoceptors in the spinal cord (Hösli and Hösli, Neuroscience (1982), in press).

Catecholamines and intracellular pH in slow-twitch muscle of the rat

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Intracellular pH and resting potential have been measured in vitro with recessed-tip and voltage glass microelectrodes on surface fibres of the isolated rat soleus (= slow-twitch muscle). The superfusate was buffered to pH 7.3–7.4 with 6% CO_2/21 mM HCO_3^- at 37 °C. Adrenaline (6×10^{-6} M) caused an intracellular acidification by 0.05 pH unit within 10 min (n = 4). This effect was not quickly reversible; it was dose-dependent and appeared at 6×10^{-9} M in the most susceptible preparations. It was also observed when adrenaline was applied during hypercapnic acidosis (14% CO_2, extracellular pH 7.0). Noradrenaline and isoproterenol (6×10^{-6} M) had a similar but smaller effect on intracellular pH: respectively 0.02 and 0.04 pH unit within 10 min (n = 4). The intracellular acidification took place simultaneously with the well-known hyperpolarization induced by these catecholamines. The mechanism of these observations will be discussed.

Coding of localization cues in the medial geniculate body of the cat

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400 units were recorded in the MGB of 5 cats under N_2O anesthesia. The effect of interaural intensity differences (IID) and interaural time differences (ITD) were studied for noise and tones. 89 units were categorized according to their sensitivity to IID into: a) 'Monaural like' (37%), b) 'lateralized' (23%) changing their response within one acoustic hemifield (for 90% the contralateral one), c) 'frontally oriented' (9%) responding with a given pattern over a limited IID range centered on the midline, d) 'nonoriented' (11%) and e) 'not classified' (20%). 1/4 of the units were influenced by ITD, but over large delay values. No units tuned to narrow regions of space were observed. In the MGB the acoustical space seems to be coded by only 1/3 of the units into 3 broad sectors: the midline, the contralateral and the ipsilateral acoustic hemifields.

Effect of long-chain triglyceride infusion on glucose uptake, storage and oxidation in man

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Glucose metabolism was studied in 25 healthy young men by using the euglycemic insulin clamp technique in combination with indirect calorimetry during long-chain triglyceride infusion (Intralipid 20% at 1 ml/min). In a control study, without Intralipid, the mean base line plasma free fatty acid (FFA) concentration was 385±8 μmoles/l, whereas with Intralipid FFA level was increased to 650±10 μmoles/l. At each insulin dose level, hyperlipidemia caused a significant reduction in total glucose uptake (5.9–3.5, 9.9–7.1 and 11.1–8.8 mg/kg min respectively, all $p < 0.001$), in glucose oxidation (2.4–1.6, 3.4–2.2 and 3.7–2.8 mg/kg min, all $p < 0.001$), and in glucose storage (3.6–1.9, 6.5–4.9 and 7.4–5.9 mg/kg min respectively, all $p < 0.001$). Thus, the inhibitory effect on glucose utilization due to elevated plasma FFA levels seems to involve the pathways regulating both glucose oxidation and glycogen synthesis.

A method for continuous measurement of β-emitting isotopes in mammalian preparations

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Continuous measurement of the influx and the cellular concentration of tracer isotopes is difficult for mammalian tissue when only weak β-emitting isotopes are available. The poor penetration of electrons in the preparation may be overcome by surrounding the tissue with tubing made of scintillating material, e.g. NE102A standard scintillation tubing (internal diameter 0.7 mm). The preparation is then continually superfused with physiological solution containing ~5 μCi/ml of the isotope. Counting efficiency using coincidence counting is 13% for ^{45}Ca and 7% for ^{14}C. Radioactivity in the external solution and extracellular space is estimated with radioactive extracellular markers. So far, we have studied ^{45}Ca uptake in frog sciatic nerve and guinea-pig taenia coli preparations, and both ^{45}Ca and ^{14}C-choline uptake in rabbit vagus nerve.

The rat's sleep during the estrus cycle

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As we have reported earlier, sleep and waking patterns of the female rat can vary, depending on the estrus cycle (Kleinlogel, 1975). In an 8-h recording session (8.00–16.00 h), during estrus, paradoxical sleep (PS) and non-PS are increased, whereas waking is decreased. These results raised the question of whether these sleep phenomena in estrus are rebound effects from the estrus night, when the rat may have been more active in searching for a sexual partner. We have therefore recorded the EEG continuously during the estrus cycle of the female rat. The results show that waking is increased during the estrus night, whereas PS and slow wave sleep are decreased. On the following day rebound effects are observed, substantiating our hypothesis from the previous daytime study.

Effect of 2,3-diphosphoglyceric acid (2,3-DPG) on the relative O_2-CO affinity of fully saturated human blood

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In order to investigate the effect of 2,3-DPG on O_2 and CO binding with human whole blood exposed to O_2 and CO partial pressures sufficient to saturate hemoglobin completely, Haldane's constand $M = HbCO/HbO_2 \cdot PO_2/PCO$ has been measured at pH 7.4 in 4 aliquots, A, B, C and D, of several individual bloods; in A, acid-citrate dextrose was added, in B, citrate-phosphate-dextrose-adenine (CPD-A), in C, CPD-A plus dihydroxy-acetone (60 mM). Aliquot D was treated as A but stored at 4 °C for 20 days. The average 2,3-DPG/Hb molar ratios of A, B, C and D were 0.6, 0.75, 1.1, 0.1 respectively. The blood samples had been tonometered with gas mixtures containing 6% CO_2 and various proportions of O_2 and CO in N_2. Aliquots A, B and D gave values of M ranging from 210 to 240. No significant correlation between M and concentration of 2.3-DPG was found. Our data suggest that at complete saturation 2,3-DPG has the same effect on O_2 and CO binding.

Gas transport and gas mixing during high frequency ventilation (HFV) in dogs

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Gradients of He and SF_6 built up along the airways by HFV were compared with those caused by conventional mechanical ventilation (CMV). The lungs were equilibrated with 1% He and 1% SF_6 and partially washed-out. Then the test gas concentrations were measured during deep expirations carried out by a special pump at constant low flow rate. From these expirograms 'Fowler dead space', slope of alveolar plateau, and concentration gradients were calculated. At HFV the dead space for He and SF_6 was identical and smaller than at CMV. Alveolar slopes were steeper for SF_6 than for He, but both were smaller than those obtained at CMV. At HFV the main concentration drop was found along the upper airways, whereas at CMV it was much deeper in the lungs. The data suggest that at HFV the gas transport along the conducting airways is not limited by diffusion. On the other hand, diffusion limits the alveolar gas mixing which is, however, more homogeneous at HFV than at CMV.

High frequency ventilation (HFV) and stretch receptor (SR) activity

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HFV inhibits spontaneous breathing more easily than does conventional mechanical ventilation (CMV). This apnea was shown to be a vagal reflex. To clarify the mechanism underlying the apnea during HFV, the timing of respiratory phases and the activity in SR single fibres were analyzed in anesthetized rabbits at standardized levels of mean airway pressure (Paw; 2–15 cm H_2O) and frequencies of HFV (f_{resp}; 5–30 s^{-1}). Expiratory duration (t_E) increased with Paw until apnea occurred. At a given Paw, however, T_E was prolonged by increasing f_{resp}. HFV modified the SR activity: the SR activity increased with each pump inspiration and decreased with expiration. This in turn interfered in the phasic SR discharges due to spontaneous breathing and resulted finally at a given Paw in higher instantaneous SR firing rate. It is concluded that SR, owing to their dynamic properties, are in addition stimulated by the high frequency oscillations at relatively low Paw and that the high SR activity may account for the apnea observed at HFV.

Propagation speed of light adaptation in photoreceptors of the honeybee drone

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In drone photoreceptors the receptor potential consists of an initial transient and a lower plateau. This decay is an expression of light adaptation, which has been found to spread through the entire cell and not to be restricted to the site of illumination (Bader et al., Vision Res. *22* (1982) 311). We have attempted to measure the spped of this propagation of light adaptation. The decay from the transient to the plateau is also observed when the retina is scanned slowly by a narrow slit of light. As the speed of the scanning is increased, however, the amplitude of the decay decreases markedly. This result would occur if the adaptation propagated as fast as the stimulus moved. The speed inferred from the results (> 1 mm/s) seems too fast to be explained by simple diffusion of even a small free ion.

Standing potential (SP) and c-wave during induced changes in flow and O_2 in perfused eyes

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We varied flow rate and oxygen supply and studied effects on ERG c-wave and SP in isolated, arterially perfused cat eyes. Increases and decreases in flow of a given perfusate induced parallel changes in O_2 supply. Because this variation in O_2 could cause changes in the parameters, we also changed the pO_2 while avoiding changes in flow, and subsequently, as a 3rd approach, varied the flow per se, keeping the supply of O_2 constant.The SP increased and decreased parallel with simple changes in flow. During hypoxia, in contrast, it tended to increase. Under hyperoxia, there was no systematic change in the SP. Under constant O_2 supply, flow increase per se induced a rise in the SP or left it unchanged, while flow decrease per se did not produce consistent results. The amplitude of the c-wave failed to vary as clearly as the SP during simple flow changes. However, it decreased in hyperoxia and increased in hypoxia. During variations in flow per se the c-wave did not change. These data deviate from the concept of 'covariation' between c-wave and SP under 3 experimental conditions: hyperoxia, increase in flow and increase in flow per se.

Functional organization of the tree shrew striate cortex

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Since no ocular dominance columns could be found in the tree shrew *(Tupaia belangeri)*, we have investigated the horizontal segregation of the LGN input into area 17. The correlation between neurophysiological and neuroanatomical data are as follows: 1. The majority of input is fed into layer IV. 2. Input into layers I, III, V, VI is predominantly from ON-OFF cells with transient components to light stimuli. 3. Input into layer IVa is predominantly from ON-center cells and into IVb predominantly from OFF-center cells. For both types, there is an additional or exclusive sustained component to light stimuli. 4. The region in layer IV where the response characteristics change shows a

structural correlate: The cell-sparse cleft. 5. The inputs into layers IVa, b are contra- and ipsilateral, the input into the cleft region is mostly dominated by the contralateral eye. 6. The receptive field sizes are especially small in layer IV. 7. The responses are generated by postsynaptic elements and can be blocked by cobalt ions.

The electrophysiology of visual cells in different eye regions of the ant *Cataglyphis*

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The following properties of visual cells are determined by intracellular recordings: 1. Assignement to the 3 anatomically different parts of the eye (Herrling, Cell Tissue Res. *169* (1976)) by measuring the optical axes (and comparing with optical data) or by intracellular staining. 2. Spectral sensitivity: UV ($\lambda_{max}=350$ nm) and green receptors ($\lambda_{max}=510$ nm) are found in all parts of the eye except in the dorsal rim area (DRA; type III in Herrling (1976)). There, only UV cells are recorded. 3. Polarizational sensitivity (PS) of green cells is small (average: ca. 2.0). In UV cells, PS is higher in the DRA (ca. 5.0) than in the other eye regions (ca. 3.5). 4. Angular sensitivity: In the frontal eye region (types I and II), $\Delta\rho$ is smaller (ca. 3.0°) than in the dorsal type I retina (ca. 4.0°). In the DRA, the visual fields are roughly elliptical ($\Delta\rho$ ca. 4.5° by 6.5°) and show also no marginal extension, which characterizes the DRA of the honeybee (Labhart, J. comp. Physiol. *141* (1980)).

Time-dependent influence of exogenous melatonin on sexual maturation of male rats

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Male rats were maintained under a light-dark cycle of 12/12 h and were injected s.c. once daily with 100 µg melatonin at different times of day from 20 to 40 days of age. Melatonin injections during the first 2 h of the photophase had no effect on pubertal development, while injections 7 and 9 h after the onset of light resulted in highly significant decreases of testes and seminal vesicles weights, plasma levels of testosterone, LH and FSH and pituitary GnRH receptor number. The strongest inhibition occurred just before the onset of darkness, whereas no effect was observed during the first 7 h of the dark phase. Melatonin injections 9 h after the onset of darkness had again an inhibitory influence, but injections 2 h later remained without effect. Our results indicate the existence of a biphasic circadian rhythm in sensitivity of the neuroendocrine-reproductive axis of male rats to melatonin during sexual maturation.

Calcium-dependent purine release produced by electrical activity in nerve

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In rabbit vagus nerve, labeled by incubation with ^{3}H-adenosine, electrical stimulation results in release of radioactivity which is found in inosine, hypoxanthine and adenosine (Maire et al., J. Phsiol. Lond. *323* (1982) 589). Futher experiments show that at constant extracellular Ca the first period of activity produces a large release; on subsequent stimulation a smaller release is found which is then not further decreased for several periods of activity. The release on stimulation is increased when the Ca concentration is raised, lowering the Ca lowers the release. The effect of Ca is partially inhibited by Mg. Exposure to the Ca ionophore A 23187 lowers the release on stimulation. A particularly large release is seen after the application of verapamil. The results suggest that purine release is related to an increase in intracellular Ca, and that the effect of the increase is larger when the Ca level is low.

Changes induced in superior cervical ganglion cell cultures by *Herpes suis* infection

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Herpes suis infection of superior cervical ganglia (SCG) in rats in vivo has been extensively studied by M. Dolivo et al. (TINS *3* (1980)). Thus we turned to SCG cell culture to further investigate the molecular mechanisms of this infection and its physiological consequences. 2-3 weeks old cultures, displaying a dense neurite network, were infected with 10^5 PFU/ml *Herpes suis*. 48 h after infection immunofluorescence labels predominantly the cell body. Acridine orange staining and electron microscopy show the chromatin condensed in patches. EM reveals the presence of nucleocapsids within the nuclei and fully enveloped viruses throughout the cell body and its extensions. Proteins released into the medium by the cells, as well as cell membranes, are altered by the infection, as their respective SDS-PAGE pattern show. Thus these cultures may provide a useful model for the study of the molecular basis of the *Herpes suis* infection.

Amplitude and time course of single fiber excitatory postsynaptic potentials (s.f.EPSPs)

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We have examined s.f.EPSPs of muscle spindle afferents in single motoneurons (MNs) in the anesthetized cat by means of multiunit spike triggered averaging (Neurosci. Suppl. *7* (1982) 135), in an attempt to separate pre- from postsynaptic factors responsible for the organization of input of primary afferents to MNs. The amplitude of s.f.EPSPs recorded within the same MN were directly related to the conduction velocity of the afferent fiber. However, the amplitude of s.f.EPSPs was *not* related to the size of the MNs. Shape indices of s.f.EPSPs were well predicted by the theoretical 'Rall-Model'. These results indicate that the number of terminals given off by an afferent fiber to a MN is directly related to the size of that fiber. They further suggest that the terminals are clustered at about the same electrotonic distance from the soma. The results indicate that the formation of connectivity is a random process within the constraints given by the size and architecture of the pre- as well as postsynaptic elements.

Biphasic effects of bradykinin on the rat portal vein

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The actions of bradykinin (BK), a potent hypotensive peptide in mammals, on the mechanical and electrical activities of the rat portal vein were investigated. Biphasic effects of BK on these activities were noted; an initial inhibition of spontaneous contractions was associated with cellular hyperpolarization, and a subsequent activation of contractions was paralleled by cellular depolarization and increased action potential frequency. The inhibitory-hyperpolarizing response was specific for B2-BK receptors, since

the analogue desArg^9-BK, which selectively activates the B1-receptor, induced only a contractile-depolarizing response. The stimulatory effect, but not the inhibitory effect, was markedly decreased by indomethacin, an inhibitor of prostaglandin synthesis. The initial response was abolished by apamin, a compound which probably blocks potassium channels. Activation of B2-receptors appears to modulate at least 2 processes: a membrane conductance for potassium and prostaglandin biosynthesis.

The identification of mass impregnated visual cells in the ant *Cataglyphis bicolor*

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HRP filled retinular cells are investigated electron microscopically. In the 2nd optic ganglion, the synaptic organization of UV receptors (R1-fibres) is studied by orthograde fillings. In the retina, specific types of R1-receptor cells are identified by impregnating these cells retrogradely via the corresponding terminals. The projection pattern of R1-cells are compared in different eye regions (for anatomical characterization see Herrling, Cell Tissue Res. *169* (1976)). In addition, lucifer yellow and cobalt ions are used in light microscopical investigations. It is suggested that the projection pattern of the specialized dorsal rim area differs from the pattern for the remainder of the eye.

Actin and myosin filaments in the early chick embryo

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The growth of the area opaca (AO) of the young chick bladtodisc seems to be modulated by a) the rate of the centrifugal migration of the cells located at the edge of the disc and b) the proliferation rate of the cells within the disc. The imbalance of these 2 processes results in tensions which flatten the epiblastic cells of the AO (Downie (1974)) thus probably modifying also the organization of cytoskeletal elements. Actin and myosin filaments were visualized using immunofluorescence methods (antibodies kindly supplied by Dr Gabbiani, Institute of Pathology, Geneva) and the location of fluorescent cells in the blastodisc (stages 3–7 HH) mapped. Isolated cells as well as cell groups have been observed and several morphologically different cell types have been recognized in the AO depending on the developmental stage and the region studied. Important variations in the fluorescence intensity of individual cells have been found corresponding probably to differences in the physiological state of the epiblastic cells.

Relation of cigarette puffing and inhalation to smoke yields of cigarettes

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Cigarette puffing (cigarette holder flow meter), respiratory inhalation (thorax impedance), and CO uptake (expired air) were compared accross 116 subjects smoking their habitual brand and within the subjects when switching to a lower smoke-yield cigarette. In both comparisons CO uptake remained unrelated to the type of cigarette smoked. Compensation for low yield cigarettes was achieved mainly through puff volume adaption. Quantitative analysis of the respiratory cycles following each puff suggests that some aspects of this cycle may change as a function of smoke yield measures. However, such correlations, although significant, explain only modest parts of the great interindividual variance in puffing and inhalation behavior.

Capsaicin application and FRAP histochemistry: technical improvements

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Local application of capsaicin on a peripheral nerve, or its transection, as well as i.p. injection in neonates lead to disappearance of fluoride resistant acid phosphatase (FRAP) from the central terminals of certain of its unmyelinated afferent fibres. Capsaicin being poorly soluble in water, and only with help of harmful chemicals, we use an innocuous vehicle, long known in medical practice: neutralized, sterilized olive oil. Capsaicin dissolves completely in the oil upon heating and stirring in a waterbath. Filtering is then necessary to eliminate impurities which provoke recristallization. Local application of this vehicle alone showed no effect on FRAP in mouse substantia gelatinosa, nor did i.p. injections in neonates (which, moreover, is harmless). FRAP activity in ganglion cells and terminals is best preserved as follows: perfuse with freshly prepared, phosphate buffered paraformaldehyde, then wash the tissue with 16% sucrose for up to a week at 4 °C. Cryostat sections are incubated for 40 min at 40 °C in a modified Gömöri medium.

Comparative study on paraquat and hyperoxia induced cytotoxic effects in cultured endothelium

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The toxic effects of Paraquat (PQ), a herbicide known to generate O_2 free radicals, were studied in confluent porcine aortic endothelial cells and compared to those resulting from exposure to 95% O_2. PQ related effects were dose- and time-dependent: no change was seen with 10^{-6} M, whereas decrease in the DNA and protein content of dishes by 50% and marked increase in LDH release were observed after a 5-day exposure to 10^{-4} M PQ. Reduced thymidine incorporation into DNA was observed already after 24 h. Exposure to 95% O_2 for 5 days resulted in a similar effect, equivalent to that of a PQ concentration between 10^{-5} and 10^{-4} M. The addition to culture medium of 2×10^{-7} M Se-methionine and the associated increase in glutathione peroxidase activity had no protective action on PQ induced toxic effect, but reduced LDH release by O_2 exposed cells. These differences suggest that lipid peroxidation could play a role in O_2 induced cytolysis whereas PQ related cytolysis could result from intracellular NADPH depletion.

Differential effects of lesions within the limbic system on locomotion and patrolling of rats in tunnel mazes

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In a tunnel maze with computer-assisted recording of locomotion patterns of rats, the effects of lesions within the dorsal hippocampus (hc) or the fornix (fo) on locomotion and patrolling were examined. The main purpose was to determine whether or not there always exists an interdependence between these parameters. Lesions were produced by the radiofrequency technique (RF) or the neurotoxin kainic acid (KA). As a consequence of lesions in the dorsal hc (RF), patrolling decreased to chance level whereas locomotion remained unchanged. RF-lesions affecting most of the fo, led to the patrolling deficit in addition to a persistent hyperactivity. KA-lesions were produced within the dorsal hc by injection of 2 different volumes. Microinjection of the smaller volume produced a

patrolling deficit only, the larger volume led to a hyperactivity in addition to the patrolling deficit. The behavioral variables locomotion and patrolling are therefore not always interdependent.

Hemodynamic effects of external airways obstruction

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To test the hypothesis whether external airways obstruction may limit cardiac performance, cardiac output (Q by N_2–CO_2 rebreathing), heart rate (h.r.), stroke volume (qst), systolic (APs) and diastolic (APd) arterial blood pressures, inspiratory (PIm) and expiratory (PEm) mouthpressures, endtidal CO_2 pressure ($PACO_2ET$), minute ventilation ($\dot{V}E$) and oxygen consumption ($\dot{V}O_2$) were measured in 8 young healthy subjects breathing through in- and expiratory resistances at rest (R, 32 $cmH_2O \cdot l^{-1} \cdot s$ at $0.5\,l \cdot s^{-1}$) and during steady state bicycling at 63 W (E, 45 $cmH_2O\ l^{-1} \cdot s$ at $2\,l \cdot s^{-1}$). The results were compared to control values obtained while breathing without resistance. For similar Pm deflections: a) $PACO_2ET$, h.r., APs increased by 5.7 mm Hg ($p < 0.001$) and 4.2 mm Hg ($p < 0.01$); 5.8% (NS) and 5.7% ($p < 0.05$); 8.2% ($p < 0.001$) and 9.4% ($p < 0.05$), at R and E, respectively. b) $\dot{V}E$ decreased by 30% ($p < 0.001$) and 18% ($p < 0.01$) at R and E, respectively. c) $\dot{V}O_2$, $\dot{Q}$, qst and APd were unchanged.
Since the product h.r. × APs, which is an index of myocardial $\dot{V}O_2$, increased by 14% ($p < 0.01$) and 17% ($p < 0.01$) at R and E, respectively, it is concluded that severe airways obstruction increases the load on the heart.

Use of vibration array to induce complex tactile sensation

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The present study was initiated to determine the best way to present information from other sensory modalities to the skin in view of a tactile aid for deaf or blind people.
An array of 16 vibrating points on an area of 0.8 cm^2 and a bracelet of 8 vibrators have been developed and tested. The psychophysical investigation revealed 3 modes of evoked tactile sensation depending on the way these arrays were actuated: vibration, apparent movement and texture. The sensation of vibration appeared when one or many points were vibrating synchronously for at least 100 ms at 10–600 Hz. The sensation of apparent movement was perceived when the points were stimulated one after the other for durations less than 100 ms. The time between each vibration had to be less than $^3/_{10}$ of the vibration time. On the wrist, speeds from 10 cm/s to 70 cm/s induced the best movement sensations. The passive texture sensation appeared for faster sequential activation of one point or for randomized activation of many points.

Stability constants of H^+ and Ca^{2+} for EGTA-buffered solutions

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A potentiometric technique was used to determine stoichiometric constants for the Ca-EGTA complex (K_{Ca}) and for dissociation of the 3rd (K_{a3}) and 4th (K_{a4}) protons from H_2EGTA at 25±0.2 °C. A glass electrode was calibrated for $[H^+]$ in a solution of an ionic strength (I) that approximated the EGTA-buffered solution. EGTA impurities were < 0.5%. Preliminary results (mean±SD) are shown below. The data imply an ~80% (I = 0.140 M) and ~500% (I = 0.404 M) greater free $[Ca^{2+}]$ than would be calculated from correcting tabulated constants. The binding of H^+ and Ca^{2+} to EGTA depends on the ionic strength and composition of the background solution.

Decreased thermic effect of infused glucose associated with insulin resistance

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By combining the euglycemic clamp with indirect calorimetry, the thermic effect of infused glucose (TEG) was measured in 10 lean subjects (Gr 1), 7 obese with normal glucose tolerance (Gr 2) and 12 obese with abnormal glucose tolerance and/or NID diabetes before (Gr 3a) and after weight loss (10.8 kg; Gr 3b). Hepatic glucose production (HGP) was measured. Insulin and glucose were infused to achieve steady state concentrations of 100 μU/ml and 5 mM respectively. Glucose uptake was 354, 441, 233, 232 mg/min and energy expenditure (EE) changed by +0.476, +0.293, −0.114 and +0.135 kJ/min in group 1–3b respectively. Increases in EE and glucose storage were well correlated. As glucose uptake was unchanged in groups 3a and 3b, the recovered response in EE in Gr 3b was partially explained by the lower absolute drop in HGP during the clamp after therapy. The smaller TEG observed with increased insulin resistance was mainly related to a lower peripheral glucose uptake rate and a higher absolute suppression of HGP.

Effect of light regimen on the action of melatonin at puberty in the female rat

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The action of melatonin on pubertal development of female rat was tested under 2 different light regimens, i.e. LD 12:12 (12 h of light/day) and LD 16:8 (16 h of light/day). Melatonin (100 μg, s.c.) was given daily, starting on day 15 of life, 3 h before darkness. Under a LD 12:12 regimen, melatonin given in the afternoon inhibits the pubertal-related growth of the pituitary, alters the content of pituitary gonadotropins during the following estrus cycles, probably by decreasing gonadotropins synthesis, and affects initiation or regularity of the first estrus cycles. However, in female rats housed since birth in LD 16:8, melatonin given 3 h before darkness does not have an inhibitory action. These melatonin-treated animals show normal initiation of cycling and regular 4- to 5-day cycles. The present experiment suggests that reduction of darkness has a modulatory role on the inhibitory action of melatonin during pubertal development in female rats.

Locomotion by rats through a tunnel maze: effects of caffeine on habituation modified by configuration

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The present experiments focused on first determining the dose-response relation between caffeine and locomotion

within a tunnel maze (see Psychopharmacology *78* (1982) 58, for maze description), and then evaluated the effect of maze complexity on the habituation of activity under the influence of a locomotion stimulating dose of caffeine. Each female Füllinsdorf rat received a single 30-min test given 15 min after injection of saline or 8, 16, or 32 mg/kg b.wt caffeine. In the simple configuration (outer alley bisected with a barrier), 8 and 16 mg/kg caffeine stimulated and 32 mg/kg caffeine reduced locomotion. In a 2nd experiment, using this simple configuration as well as 2 others of greater complexity, it was demonstrated that the slowing of habituation due to caffeine (16 mg/kg) was most pronounced for the simple configuration.

Binocular vision in the praying mantis

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If a praying mantis perceives an object within the binocular field, the position of the corresponding retinal images will differ for the left and right eye, respectively. This is due to the horizontal separation between the 2 eyes. The fixation of the object in the median plane of the head would thus require that the average of the 2 position angles is used to program ballistic saccadic head movements. My observations show that in mantis a binocular comparison of position information does in fact occur. However, since the saccades slightly undershoot the object, it is concluded that, of the 2, the smaller position angle is more heavily weighted than the larger one. In other experiments it has been shown that the binocular registration of target position is also used to estimate its distance (Rossel, in press).

Relation between premovement H-reflex facilitation and reaction time

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In a reaction time (RT) situation, the size of the H-reflex changes before movement initiation. The aim of the present investigation was to study how these reflex modifications depend on RT.

The subjects carried out plantar flexions of the right or left foot in a visual choice RT situation. Stimuli to evoke H-reflexes were applied bilaterally 20-500 ms after the onset of the visual stimulus. The following 3 effects were observed with increasing RT: a) both, the interval between onset of the visual stimulus and the facilitation (I1) and the interval between onset of facilitation and EMG response (I2) increased, b) the slope and c) the amplitude of the facilitation decreased. It can be concluded that the duration of the decision making process (within I1) and the preparation of the motor system for the movement (I2) increase with RT.

Heart-rate telemetry in rats: effects of cyclazocine

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24-h recording of heart rate (HR) of freely moving rats in the home cage by biotelemetry (Schlatter and Zbinden, Arch. Toxic. *5* (1982) 179) was used to evaluate the effects of various doses of cyclazocine (0.1, 0.75, 1.5, 3.0 mg/kg s.c.) on cardiac activity. This opioid with mixed agonistic-antagonistic narcotic properties altered the normal circadian rhythm of HR during 4 h following drug injection in a biphasic manner: During the first 30 min after the injection, when control HR was high due to the preceding manipulations (up to 550 bpm), cyclazocine treatment significantly attenuated this HR elevation. However, 1 h after the drug application, when control HR reached resting levels (300 bpm), HR of cyclazocine treated rats was increased. The duration of this HR-elevation was dose-dependent, while the magnitude was not. This between-group difference in the time course of HR was verified by an analysis of variance (repeated measures design), showing a significant treatment-HR interaction ($p < 0.0001$).

Neuronal activity in the substantia nigra of the waking monkey

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Single cell activity in the pars compacta (SNpc) and the pars reticulata (SNpr) of the substantia nigra was studied in 3 monkeys performing in a behavioral paradigm. SNpc neurons differed from SNpr neurons by histological localization and by electrophysiological properties. SNpc neurons changed, mostly increased, their activity moderately but consistently during, and sometimes preceding, large contralateral reaching movements, but not in relation to minor forearm movements nor following the sensory signals of the paradigm. In separate experiments on 2 anesthetized and awake monkeys, the activity of SNpc but not of SNpr neurons was suppressed by systemically given apomorphine, suggesting that the SNpc neurons were of dopaminergic nature. SNpr neurons were modulated in relation to sensory stimuli, different phases of contralateral and ipsilateral arm movements, mouth movements and eye saccades. The study shows an involvement of presumably dopaminergic SNpc neurons in motor related processes without detailed encoding of movement parameters. SNpr neurons, in contrast, represent in their activity a part of the complexity of basal ganglia output.

Localization of noradrenaline during retrograde axonal transport in sympathetic neurons in culture

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The uptake and retrograde transport of NA in axons of sympathetic neurons was investigated in vitro with dissociated rat sympathetic neurons grown in a culture dish divided into 3 chambers which allows a separate access to the axonal networks and their cell bodies. ^{3}H-NA (0.5×10^{-6} M) added to the axon chambers was taken up by the desmethylimipramine- and cocaine-sensitive uptake mechanism and a substantial part was rapidly transported retrogradely to the nerve cell bodies. In contrast to the storage of ^{3}H-NA in the varicosities, which was totally prevented by reserpine, the retrograde transport of ^{3}H-NA was only slightly diminished by reserpine. EM localization of the NA analogue 5-hydroxydopamine (5-OHDA) revealed large dense-core vesicles as the reserpine-resistant transport compartment. This retrograde transport of NA and 5-OHDA probably reflects the return of turned over synaptic vesicle membrane to the cell body.

Should directional movement discrimination necessarily be 'color-blind'?

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Neural mechanisms which compute direction of motion must compare or correlate input signals arising from neighboring regions of visual space. This study attempts to

predict the chromatic properties that the 2 input channels should possess in order to obtain good directional selectivity. Spatial intensity profiles of natural outdoor scenes were measured at various wavelengths by scanning color transparencies using a photodiode and color filters. The results indicate that directional selectivity is poor if the spectral sensitivity functions of the 2 input channels differ substantially from each other, and best if they are identical. Thus, colorblindness may be an unavoidable feature of neural circuitry geared for directional discrimination. Directionally-selective movement-detecting neurons in the bee, fly, ground squirrel and rhesus monkey are color-blind, although many of these animals possess color vision.

Bradykinin receptors in the hepatic portal vein area activate the hypothalamo-neurohypophyseal system (HNS)

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We showed previously that osmoreceptors in the hepatic portal vein area can activate the HNS (J. Physiol. Lond. *315* (1981) 217. To test for other sensory modalities we superfused various i.p. structures of anesthetized rats with 0.2 ml/1 µM bradykinin, a pain inducing peptide. Superfusion of the hepatic portal vein with bradykinin activated the HNS and increased vasopressin release, whereas superfusions of the vena cava or i.v. infusions had no effects. Responses to hypertonic NaCl solutions were abolished by prior superfusion with 0.5 ml/1 mM atropine sulphate, but responses to bradykinin were not affected, indicating the existence of separate receptor mechanisms for both stimuli. These results suggest that the hepatic portal vein may be a general chemosensitive area involved in blood volume homeostasis.

Etude de la résistance à l'insuline chez le rat génétiquement obèse *(fa/fa)*, utilisant une technique in vivo

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La technique du «clamp» hyperglycémique ou euglycémique a été adaptée à des rats Zucker normaux (FA/?) et obèses *(fa/fa)*. «Clamp» hyperglycémique: la réalité de l'existence d'une résistance périphérique in vivo est démontrée. «Clamp» euglycémique: la production hépatique du rat normal est de 3.5±0.9 mg/min, pour une insulinémie de 2.1±0.4 ng/ml, et diminue à 1.14±0.14 quand l'insulinémie est augmentée à 6.1±0.9 ng/ml. Chez le rat *fa/fa*, l'insulinémie de base (13.4±1.3 ng/ml) n'abaisse pas la production hépatique qui reste à 3.09±0.4 mg/min. L'augmentation de l'insulinémie du rat *fa/fa* à 22.7±3.1 ng/ml ne change pas la production hépatique. Conclusion: Une résistance à l'insuline des tissus périphériques et du foie est démontrée chez le rat *fa/fa*.

Afferent connections of the supraoptic nucleus (SON) of the hypothalamus in the rat

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To detect cell bodies which project to the hypothalamic SON, 10-50 nl of horseradish peroxidase or fast blue solutions were pressure-injected unilaterally in rats; both dorsal and ventral approaches to the nucleus were performed. In both cases, retrogradely labeled cells bodies were observed mainly ipsilateral to the injection in several nuclei of the brain stem (dorsal raphe nucleus, locus coeruleus, dorsal tegmental nucleus, dorsal parabrachial nucleus, nucleus of the solitary tract, lateral reticular nucleus), in the limbic system (septum, nucleus of the diagonal band of Broca, ventral subiculum) and in the subfornical organ. These data suggest that the SON is influenced by the same brain areas which project to its companion within the magnocellular system, the paraventricular nucleus.

O_2 uptake occurs faster than Na pumping in bee retina after a light flash

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After a flash of light the O_2 consumption (QO_2) by the photoreceptors of the drone bee increases to a peak of 60 µl g^{-1} min^{-1} and then declines to the base line with a time constant of 5.0±0.5 s (SD, n = 10). Superfusion of the retina with O-Na^+ Ringer reduced ΔQO_2 by 60%: this suggested that most of ΔQO_2 is necessary to produce ATP to drive the Na^+ pump (Tsacopoulos and Poitry, J. gen. Physiol. *80* (1982) 19). We have now measured intracellular activities of Na^+ and K^+ and find that the magnitudes of the light-induced changes support this hypothesis. However, the time course of the ion changes and other indices of Na^+ pumping is *slower* than the time course of ΔQO_2 (t). This raises the question of what stimulates the mitochondria: apparently it is not the ADP produced by the pump.

Effects of electrical gradients on properties of the passive pathway in tracheal epithelium

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The effects of applied transepithelial electrical gradients on ionic conductances and mannitol fluxes were studied in bovine tracheal epithelium in vitro. The tissue was clamped at a constant current (I = 1 mA, S-L or L-S) or at a constant voltage (0 or±20 mV) and the fluxes of ^{22}Na, ^{36}Cl and ^{3}H-mannitol were simultaneously measured. Active transport of Na^+ and Cl^- was abolished with ouabain. The total transepithelial conductance (G_t), Cl^- conductance (G_{Cl}), Na^+ conductance (G_{Na}) and mannitol permeability (P_{man}) were affected by the electrical gradients, in an asymmetrical fashion. The change in P_{man} was related to that of G_{Na}, but unrelated to the change of G_{Cl}. This suggests the existence of a common pathway for Na^+ and mannitol. The comparison of these data with previously reported measurements of current-induced volume flow indicates the existence of a selective channel for Na^+ and water through a paracellular pathway. The Na^+-water coupling cannot be a classical osmotic phenomenon.

Visual-vestibular interaction in the flocculus: eye movements, single neuron activity and effects of lesions

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Visual and vestibular pathways converge to generate compensatory eye movements, and to convey the subjective sensation of motion. Such convergence takes place in the vestibular nuclei. However, visual input to the vestibular nuclei is limited to low stimulus velocities. The flocculus

subserves high velocity visual-vestibular interaction to either enhance or reduce eye movements to exactly match stimulus velocities. After flocculectomy monkeys have difficulties in sustaining high velocity optokinetic nystagmus or to rapidly adjust the eye velocity to changing visual-vestibular stimulus conditions. These pathophysiological investigations on a single neuron level in chronically prepared, alert monkeys greatly facilitate interpretation of clinical data from patients with various cerebellar or vestibular disorders.

Effects of the juvenile hormone analogue ZR 515 on differentiation of the honeybee compound eye

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Differentiation of the bee's compound eye takes place during the pharate adult stage. To study the effect of ZR 515 on retina differentiation, pupae and pharate adults were removed from a brood comb, and ZR 515 (Dr G.B. Staal, Zoecon Corp.) was topically applied in concentrations of 30 µg, 10 µg or 1 µg dissolved in 4 µl aceton per bee. In control experiments, bees were treated with 4 µl aceton only. Then, each bee was placed in a size 0 gelatine capsule and raised at 35 °C and 75% rel. humidity until eclosion. Hatching bees were dissected and prepared for electron microscopy. In bees treated with high doses of ZR 515 shortly before or after apolysis, elongation of the twisted ommatidial cells did not occur, and the postretinal axon bundles did not shrink. Treatment after the onset of differentiation caused no malformation.

Polarized light navigation in bees: use of zenith and off-zenith e-vectors

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If a bee dancing on a horizontal comb is confronted with a single e-vector in the zenith, it exhibits bimodal orientation (α and $\alpha \pm 180°$; α, training direction). This is due to the fact that a zenith e-vector defines the direction of the solar and antisolar meridian, but does not allow for discriminating between the 2. If, in addition, a vertical e-vector is presented somewhere off-zenith, the situation becomes unambiguous, because in the daytime sky vertical e-vectors are always positioned nearer to the solar than the antisolar meridian. Bees, however, do not know this rule. They still exhibit ambiguous orientation ($\alpha+\varepsilon$ and $\alpha+\varepsilon \pm 180°$). From an analysis of the error signal ε it can be deduced that the bees invariably use a stereotyped celestial map (Rossel and Wehner, Proc. nat. Acad. Sci. USA *79* (1982) 4451 in which vertical e-vectors occur at right angles to the solar and antisolar meridian. What they apparently try to do is to match their internal map as closely as possible with whatever set of e-vectors they experience in the sky.

Functional role of light dependent synaptic plasticity in the fish retina

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Electronmicroscopic analysis of serial sections of the synaptic complex in teleost retinal cone pedicles and its computer-aided reconstruction revealed drastic, light dependent morphological changes. In light adapted animals, the processes of horizontal cells have numerous spinules which disappear in dark adapted animals. Intracellular recordings from horizontal cells were made at different adaptation levels, followed by HRP-injection and subsequent electronmicroscopic analysis. The disappearence of the spinules could be correlated with a loss of color opponency usually found in these cells. This suggests that the spinules, altrough they lack synaptic vesicles, are the site of sign inverting synapses in the feedback loop between cones and horizontal cells responsable for the color opponency.

Assessment of preretinal pH by ionselective microelectrodes

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HCl injection and raising pCO_2 depress components of the DC electroretinogram and decrease vascular resistance in the arterially perfused cat eye (Niemeyer and Steinberg, Vision Res., in press). These observations imply permeability of the blood-brain barrier to H^+ and possibly to CO_2. To test this hypothesis, we measured pH in the vitreous body within 100 µm of the retina using microelectrodes filled with a H^+ sensor (Ammann et al., Analyt. Chem. *53* (1981) 2267). Changes in pH from 7.4 to 7.0 of the perfusate were induced by raising pCO_2 or by adding 0.1 N HCl. Preretinally, these interventions resulted in delayed decreases in pH (approx. 0.25 units). Responses to changes in pCO_2 were faster than those to HCl. The data provide evidence for diffusion of acid moieties across the blood-retina barrier. Previously, this has been shown to be intact in perfused eyes.

Comparison of cortical projections to the pontine nuclei (PN) and to the basal ganglia (BG)

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The BG and the PN, considered to play an important role in movement initiation, belong to the chief recipients of descending cortical neurones. We have investigated the distribution and the amount of projection to these 2 structures by injecting various cortical fields with anterograde tracers in rats and marmoset monkeys. The relative contribution of cortical fields was assessed by measuring the projection volume as a percentage of the total volume of the target structure. In rats, the motor cortex, including the frontal pole, provides large and equal inputs to both BG and PN (63% in both structures). In monkeys, areas in front of the motor cortex project substantially more to the BG than to the PN. In rats and monkeys, the visual cortical projection is slightly more prominent in the PN than in the BG, whereas an inverse relation holds for the temporal cortex including the auditory cortex. The difference in the cortico-pontine and the cortico-striate projections may provide clues as to their respective functional roles.

Solubilization and characterization of the pituitary membrane protein carrying binding sites for gonadotropin releasing hormone

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CHAPS (3[(3-cholamido-propyl)dimethyl-ammonio]-1-propane sulfonate), a zwitterionic detergent was able to solubilize up to 70% of the available binding sites for gonadotropin releasing hormone (GnRH) that are present

in bovine pituitary glands, whereas triton X-100 and sodium dodecyl sulfate were ineffective. Characterization of these solubilized sites was achieved by using [(DTrp^6, (NEt)Pro^9, DesGly^{10}]-GnRH as radioiodinated tracer. Scatchard analyses indicated the presence of a single class of high affinity sites. The affinity constant K_A was $0.35\pm0.07\times10^{10}$ M^{-1} (n=5) for solubilized sites, whereas K_A value for membrane sites prior to solubilization was $0.53\pm0.13\times10^{10}$ M^{-1} (n=5). Binding specificity of the GnRH binding sites was not altered by solubilization. In summary, CHAPS appears to be quite suitable for solubilization of the GnRH receptor protein, an obligatory step for its purification.

Effects of smoking on vegetative reactivity to noise

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The calming effects of smoking under stress are paradoxical in that nicotine induces activational effects similar to stress. Various psychophysiological indices of vegetative activity (electrocardiogram, plethysmogram, pulse transit time and skin resistance) were continuously recorded in an experimental session consisting of pre- and posttreatment phases and a treatment phase involving real or sham smoking. Intermittent noise bursts, meaningful in nature, were presented during the pre- and posttreatment phase. Degree of subjective stress was reported after each noise burst. The experimental design involved 5 independent treatment groups (12 females/group): Smoking-deprived subjects who a) real or b) sham smoked, a c) nonsmoker sham-smoking group, and smoking-nondeprived subjects who d) real or e) sham smoked. The results suggest that the paradox may be understood with a cognitive model, involving the perception of interoceptive cues.

BIOCHEMIE - BIOCHIMIE - BIOCHEMISTRY

Control of factor XIIf in human plasma

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Factor XIIf (XIIf) is a plasma serine protease which activates prekallikrein (PK), plasminogen and Cl. To define the factors controlling XIIf in normal plasma (NP), we studied the inactivation kinetics of XIIf in various systems. The rate constant k′ for the inactivation of XIIf by a $^3/_5$ dilution of NP was 0.036 min^{-1}. In $C\bar{1}$-inhibitor ($C\bar{1}$-In)-deficient plasma, k′ was 0.012 min^{-1} indicating that $C\bar{1}$-In was the major inhibitor of XIIf in NP. Since k′ was 0.7 min^{-1} in PK-deficient plasma, the formation of a reversible XIIf-PK complex (Kd=25 nM) provided protection for XIIf against $C\bar{1}$-In. Further analysis of the dependence of k′ upon the dilution of inhibiting mixtures revealed that d^2k'/dI^2 was >0 with I=NP and <0 with I=purified $C\bar{1}$-In. This observation indicated that an enzymatic activity was formed by XIIf in NP which further contributed to XIIf inactivation. Since this activity was inhibited by EACA but was not adsorbed on lysine-agarose, it was probably dependent on $C\bar{1}$. These results contribute to a better understanding of the regulation of the plasma kinin-forming system.

Role of $(ADP\text{-ribose})_n$ in expression of hepatocyte functions

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The expression of 2 fetal enzymes by adult rat hepatocytes, γ-glutamyltranspeptidase (GGT) and pyruvate kinase K(III) isozyme (PK III), was preceded by a transient increase in nuclear $(ADP\text{-ribose})_n$-biosynthesis. When hepatocytes were maintained in the presence of 3-aminobenzamide or nicotinamide, the $(ADP\text{-ribose})_n$-modification of chromatin proteins and the expression of GGT and PK III was inhibited. The inhibition of both processes was reversible and the suppression of GGT- and PK III expression could not be achieved with the noninhibitory analogue, 3-aminobenzoic acid. The expression of α-fetoprotein and albumin was not affected by these same manipulations of $(ADP\text{-ribose})_n$-biosynthesis, which affect DNA repair, another chromatin function (Althaus et al., J. biol. Chem. *257* (1982) 5528). Conclusion: $(ADP\text{-ribose})_n$ plays a regulatory role in the fetal-genic expression of 2 liver functions in vitro.

Cold adaptation increases the number of glucose transporters in plasma membranes of brown adipose tissue

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Cold adaptation selectively increases lipogenesis in brown adipose tissue (BAT) of lean and obese (ob/ob) mice. Since in BAT, glucose transport may be rate-limiting for glucose metabolism, the effect of cold adaptation on the number of glucose transporters in plasma membranes of BAT was studied. [^{3}H] cytochalasin B binding and its inhibition by D glucose has been used to specifically quantitate the functional glucose transporters in plasma membranes prepared from BAT of lean, cold-adapted lean, obese and cold-adapted obese mice.

After cold adaptation, a 2.5-3-fold increase in the number of glucose transporters was found in plasma membranes of BAT of lean and obese mice. This increase could not be attributed to insulin since the level of the hormone was unchanged in plasma of cold-adapted lean mice and decreased in that of cold exposed obese mice. The data suggest the involvement of another mediator to account for the increase in the number of glucose transporters and lipogenesis in BAT from cold adapted lean or obese mice.

Exogenous Ca^{2+} mimics α-adrenergic responses of perfused livers

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Livers from fed rats, perfused with an isotonic bicarbonate buffer containing 0.01 mM Ca^{2+} and 1.2 mM Mg^{2+} responded to phenylephrine (0.5 μM) with an activation of glycogenolysis, an enhanced oxygen consumption and a transient uptake of K^+. Subsequent infusion of Ca^{2+} (1.27 mM) in the presence of phenylephrine caused an additional, identical response. In the absence of the α-agonist Ca^{2+} had practically no effect. Perfusion with Mg^{2+}-free medium and pretreatment with EGTA (2 mM) drastically enhanced the effects of Ca^{2+} in the absence of phenylephrine, whereas Mg^{2+} nearly suppressed the responses to Ca^{2+}. α-Adrenergic effects were not impaired by Mg^{2+}-free medium, but were markedly decreased in Ca^{2+}-depleted livers. Readdition of Ca^{2+} to Ca^{2+}-depleted livers in the presence of phenylephrine was maximally effective. In Mg^{2+}-containing medium plasma membranes are apparently impermeable to exogenous Ca^{2+}. Phenylephrine and Mg^{2+}-free medium may enhance the permeability. The results showing that under certain conditions exogenous Ca^{2+} has the same effects as phenylephrine suggest that the observed responses are mediated by Ca^{2+}.

The copper cluster in *Neurospora* metallothionein

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Neurospora metallothionein binds 6 Cu(I) ions per protein molecule, by coordinating the metal ions to 7 thiolate groups of cysteines. The protein can bind in vitro 2 mercurials per protein without loss of copper. This binding is responsible for typical modifications of the absorption, chiroptical and emissive properties of the native protein. In particular binding of mercury completely abolishes the luminescence emission of the protein. These results indicate that the 6 Cu(I) ions in *Neurospora* copper metallothionein behave as a single metal cluster. The metal-to-sulphur stoichiometry and the capability of Hg binding of *Neurospora* metallothionein strongly suggests that the copper-thiolate complex has the structure of a polymeric Cu(I)-μ-thiolate cluster. In this model, the 6 copper ions are bound to 5 cysteine residues bridging 2 Cu(I) ions each and 2 terminal cysteines coordinated to a single Cu(I) ion. The terminal cysteines provide the additional binding sites for mercury.

Evidence for 2 different forms of Hg(II)-metallothionein

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Mammalian metallothioneins contain 20 Cys in a total of 61 amino acid residues and bind 7 bivalent metal ions. With Zn(II), Cd(II) or Co(II) the protein forms unique adamantane-related metal-thiolate clusters in which each metal ion is bound tetrahedrally to 4 Cys ligands. With Hg(II) 2 spectroscopically different complexes are formed, depending on the metal-to-protein stoichiometry. Up to a ratio of 7 moles of metal per mole of protein, Hg(II) is bound tetrahedrally in tetrathiolate cluster structures. Above 7 moles of Hg(II), however, the metal changes to a lower coordination number, resulting at 10 moles of Hg(II) per mole of protein in a product containing dithiolate complexes exclusively. The 2 forms of Hg(II)-metallothionein also differ notably in their chemical properties. In the 7-to-1 complex 7 Cys are susceptible to oxidation by Ellman's reagent (DTNB) while no Cys is reactive in the 10-to-1 complex.

Are the 2 myosin alkali light chains generated by differential RNA splicing?

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Protein sequence data on the 2 alkali light chains of fast skeletal muscle myosin, LC1f and LC3f, indicate that they contain an identical stretch of 141 amino acids at their COOH ends, whereas the NH_2 ends of LC1f and LC3f contain 49 and 8 unique amino acids, respectively. Our data support the idea that such a 'building-block' pattern could be generated by differential RNA splicing. From a cDNA library of adult fast chicken muscle mRNA, 2 overlapping clones, $6E_4$ and $6A_8$, were isolated. Both clones hybridized to mRNA of LC1f and LC3f in hybridization-selection experiments. Sequence analysis of the clones, covering 244 bp, revealed no coding information. Clone $6E_4$ contained a putative polyadenylation site 21 bp from the vector junction, suggesting that the clones were derived from the 3'-noncoding end of the mRNA. Using clone $6E_4$, one genomic clone, containing a 15-kb insert, was isolated out of 250,000 plaques of a chicken genomic DNA library in Charon 4A. Restriction map data and sequence studies will be presented.

Rat hypothalamus contains factor(s) that is possibly involved in the control of insulin secretion

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Extracts from the ventrolateral hypothalamus (VLH) of rat were previously demonstrated to stimulate insulin secretion in vivo. After partial purification by gel chromatography, fractions from VLH extracts were tested in vitro by using isolated perfused rat pancreases. In presence of glucose 5 mM in the perfusion medium, the VLH fractions elicited a significant insulin release. Furthermore, these fractions potentiated the insulin secretion as stimulated by high levels of glucose (10 mM) or amino acids (6.6 mM) in the perfusion medium. A further purification of the active fractions from VLH extracts showed that the molecular weight of the factor(s) responsible for the insulin secretion promoting activity was about 800-1200 D. When incubating rat hypothalamic fragments, the incubates subsequently recovered had the same insulin secretion promoting activity than the active fractions obtained after partial purification of VLH extracts, supporting the notion that the hypothalamic factor(s) was releasable. Conclusion: The hypothalamus appears to contain releasable factor(s) that may play a role in the modulation, by the CNS, of insulin secretion.

Reduction of biogenic aldehydes in brain

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Biogenic amines, such as noradrenaline, dopamine and serotonin, are deaminated by monoamine oxidase to their aldehyde analogues. The aldehydes, especially those derived from β-hydroxylated amines (noradrenaline), are further reduced in the central nervous system to the alcohol products. 2 monomeric, NADPH-dependent oxidoreductases, classified as aldehyde and aldose reductase have been implicated in the reduction of these aldehydes. Both en-

zymes were isolated from human brain and other tissues, and their specificity for various biogenic aldehydes was tested. The K_m values for 4-hydroxyphenylglycolaldehyde, 4-hydroxyphenylacetaldehyde and indoleacetaldehyde were, respectively, 60, 1100 and 400 μM for aldehyde reductase and 3, 10 and 16 μM for aldose reductase. The lower K_m values of aldosereductase and the fact that in the liver, where aldose reductase activity is negligible, adrenaline is metabolized to the acid products, suggest that in brain aldose reductase catalyzes the reduction of the biogenic aldehydes.

Regulation of extractable adenosine 5′-phosphosulfate sulfotransferase activity by nitrogen nutrition in *Lemna minor* L.

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Adenosine 5′-phosphosulfate sulfotransferase (APSSTase) is an enzyme of the assimilatory sulfate reduction pathway which produces sulfur amino acids starting from sulfate. There was a 50–100% increase in extractable APSSTase activity of *Lemna minor* when NO_3^- was replaced by NH_4^+ in the nutrient solution with a parallel increase in protein content. In plants cultivated without a nitrogen source APSSTase activity rapidly decreased to a very low level. After addition of NO_3^- or NH_4^+ there was an increase in the extractable APSSTase activity. These reulations of APSSTase can contribute to the formation of adequate amounts of sulfur amino acids necessary for protein synthesis.

Specific killing of antigen-bearing cells by means of antibody-toxin conjugates

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Monoclonal antibodies (mAb) to lymphocyte surface antigens (Thy-1.2, HLA-DR) or rabbit anti-mouse immunoglobulins (RaMIg) were coupled to ricin A chain or gelonin in order to produce immunotoxins to be used in immune selection experiments. Purified conjugates were tested in vitro for their cytotoxicity. Results are as follows: anti-Thy-1.2-ricin is effective at 2.5×10^{-7} M toxin and can be used to select one Thy-1.2^- out of 10^4 Thy-1.2^+ cells; conjugate treatment of mouse bone marrow cells mixed with Thy-1.2^+ tumor cells leads to complete eradication of tumor cells without affecting stem cells colony formation; mAb-gelonin conjugates are as effective as mAb-ricin conjugates. Finally, RaMIg-gelonin conjugates can serve as universal killing reagents provided target cells are first coated with mouse Ab recognizing surface antigens or target cells.

Rat α_{2u}-globulin: an acute phase protein?

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Following turpentine induced inflammation, rat serum α_{2u}-globulin (α_{2u}G) increased from 20 to 34 μg/ml between 6 and 24 h and then returned to normal values at 48 h. The cell-free translation of hepatic poly(A)$^+$RNA, extracted after turpentine injection, showed that α_{2u}G mRNA levels were not altered for 20 h, but decreased from 24 to 48 h. These results can probably be explained by the observed injury-induced changes in renal function. Urine samples collected over 24 h after injury contained 50% less α_{2u}G than did urine collected from controls. Similar concentration changes occurred in urine amino acids, total protein and the creatinine coefficient. The reduction in urine α_{2u}G suggests that alterations in renal behavior are responsible for the increase in serum α_{2u}G. These results demonstrate that serum α_{2u}G is regulated early after injury at the physiological level and later by its hepatic synthesis. Unlike other acute phase proteins, it remains to be determined whether α_{2u}G can truly be considered a reactant of the acute phase response to injury.

Isolation of a glyceraldehyde-3-phosphate dehydrogenase inhibitor from human liver

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A peptide which inhibits the reassociation/reactivation of inactive monomeric glyceraldehyde-3-phosphate dehydrogenase (Döbeli, H., and Schoenenberger, G.A., FEBS Lett. *133* (1981) 123) was isolated to apparent homogeneity by trichloroacetic acid precipitation, ultrafiltration, Sephadex G-25, Dowex 1 and Dowex 50 ion exchange chromatography as well as HPLC (Lichrosorp RP-18). High voltage paper electrophoresis at pH 1.9 and 6.0 revealed one single ninhydrin negative spot which, however, exhibited a positive peptide reaction after chlorination and KI/starch treatment. Dansylation and hydrolysis followed by TLC revealed no amino acids. After redansylation the dansyl-aminoacids Glu, Asp, Arg, Ser, Thr, Gly, Ala and Pro were detected. The isolated peptide inhibitor was specific for GAPDH and did not affect the reassociation/reactivation of monomeric M-LDH nor that of H-LDH.

Isolation and biochemical characterization of D-malate dehydrogenase, a flavoprotein from rabbit kidney mitochondria

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A flavoprotein has been purified from rabbit kidney mitochondria which catalyses the oxidation of the D-stereoisomer of malic acid and, to a lesser extent, of a number of 2-hydroxyacids (e.g. D-lactate) to the corresponding keto-acids. The simple and rapid 3-step purification procedure yields a homogeneous protein as estimated from SDS-PAGE. The enzyme with a mol.wt of about 100,000 D contains a single FAD moiety per monomer and, contrary to the yeast D-lactate dehydrogenase (Morpeth, F.F., and Massey, V., Biochemistry *21* (1982) 1307) is devoided of Zn-metal, suggesting that a different mechanism of reaction is involved for the mammalian enzyme. Preliminary kinetic data using a variety of substrates and electron acceptors are discussed.

Choline acetyltransferase as a marker for cholinergic neurons in the cortex

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Only conflicting data exist on the possible presence of cholinergic neurons in the cortex. The acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT), represents a specific marker for the anatomical identification of cholinergic neurons. The enzyme has been localized immunohistochemically in rat cortex using a rat antiserum to pig ChAT. The specificity of the staining observed has been controlled by using antiserum preabsorbed with purified ChAT.

Scattered throughout the entire extent of the cortex, many round to oval, small diameter (5 µm) neurons containing ChAT-immunoreactivity were found. These neurons were present in all cortixal layers from II to VI. By differences in their fibre branching pattern, localization and staining intensity possibly 3 different types could be distinguished. The staining intensity observed in these neurons was only moderate, explaining earlier failures to observe cholinergic neurons in the cortex.

Function and structure of the transmembrane channel of *E. coli* outer membrane porin

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Porin facilitates diffusion of small solutes accross outer membranes of gram-negative bacteria, excluding molecules > 650 D. Incorporation of porin and phospholipids into planar membranes yields channels with discrete conductance steps which indicate that 3 channels are activated simultaneously. At high protein concentration, porin aggregates to ordered 2D arrays. EM and image processing reveal 3 distinct lattice types which have characteristic stain-penetrated triplets in common. A 3D structure analysis from EM tilt series of porin lattices identifies the stained triplets as 3 channels spanning the membrane and converging to a single outlet. This unique configuration allows a consistent interpretation of porin's electrical properties, although the structural difference between open and closed state could not be visualized as yet. Moreover, the 3D structure of porin extracted by EM was important to initiate the phasing of X-ray diffraction data at present in progress.

Changes of functional activities due to oxidative treatment of Clq

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Clq, a subcomponent of the first component of complement, was isolated from serum. The preparation was free from contaminating serum proteins, especially the other subcomponents of Cl, as shown by immunological methods. The influence of enzymatic labeling and chemical oxidation, followed by iodination, on the hemolytic activity and binding ability of Clq to complexed IgG and other molecules was studied. Oxidation was achieved by incubation of Clq with chloramine T or Jodo-Gen. Increasing the time of oxidation resulted in subsequent loss of the binding ability of Clq to complexed IgG and the loss of the hemolytic activity, whereas the binding toward heparin, fibronectin and fibrinogen remained unchanged. Therefore we conclude that the oxidation process leads to modification of the globular heads of Clq. Modification of the collagen like fragment of Clq cannot be excluded, but the results indicate that the binding sites in this part of the molecule are not decisively altered.

Regulation of transcobalamin II (TC2) secretion in cultured human fibroblasts

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It has been shown that cultured skin fibroblasts synthetize and secrete the vitamin B12 binding protein TC2 (Schweiz. med. Wschr. *112* (1982) 1435). Newly synthesized TC2 in the culture medium was quantified using a solid-phase radioimmunoassay and gel filtration. The rate of TC2 secretion which can be completely inhibited by the addition of cycloheximid, was influenced by several other agents as well: 1. Addition of cyanocobalamin (vitamin B12) to the medium, caused a significant decrease of TC2 secretion, probably as a consequence of increased uptake of the TC2-cobalamin complex. 2. Estrogen, when added to the medim, had no effect, but dexamethasone caused a significant inhibition of TC2 secretion. 3. Chloroquine and ammoniumchloride, both inhibitors of lysosomal proteolysis, each significantly increased TC2 secretion. Thus, intracellular lysosomal degradation of TC2 appears to downregulate the synthetic capacity of the fibroblast.

Quantitation of hemoglobin glycosylation by boronate affinity chromatography (BAC)

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Nonenzymatic glycosylation of hemoglobin (Hb) occurs at the N-terminus of the α- and β-chains and at some ε-amino groups. Ion exchange chromatograpy (IEC) resolves the β-N-terminally glycosylated hemoglobins, HbA1a-c, from the main HbA. Accurate quantitation of the glycosylation at all other sites (HbA-Glc) has therefore been difficult. We have utilized BAC and IEC to quantitate HbA1c and HbA-Glc. With hemolysates, the amount of glycohemoglobin retained on the boronate-affinity support Glycogel B was twice that of HbA1c and correlated with HbA1c ($r = 0.96$). The ratio HbA-Gluc/HbA1c determined by IEC was $1.05 +/- 0.2$ in this material. Heterogeneity revealed by BAC was small for HbA1b+c (92% of HbA1c; 84% of HbA1b retained) and significant for HbA1a (40% retained). These data show that nonezymatic glucosylation at the β-N-terminus of Hb is comparable to that at all other sites, and that total Hb glucosylation may be conveniently determined by boronate affinity chromatography.

Transcobalamin II secretion by human bone marrow cells in liquid culture

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Transcobalamin II (TC2) is an essential vitamin B12 binding plasma protein. TC2 genotype transformations after bone marrow transplantation have shown that part of circulating TC2 is marrow-derived (Blood *56* (1980) 560). Dextran sedimented bone marrow cells from healthy donors were cultivated in the presence of colony stimulating factor. Secretion of TC2 into the culture medium was investigated with a solid-phase radioimmunoassay for TC2 (RIA) and by TC2 phenotyping (PAGE). In spite of decreasing cell numbers in the course of cultivation (a plating efficiency for colony formation of 1:1000 was detected in a parallel agar culture), apo TC2 secretion (by RIA and PAGE) increased exponentially with time. Different TC2 phenotypes, corresponding to the serum types, were observed in 3 individual cultures. An attempt was made to separate the adherent cell population from the nonadherent cells, in order to identify the cell type responsible for TC2 formation in the mixed culture.

Structure of the rabbit IgA dimer receptor

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Secretory component (SC), synthesized as a transmembrane protein, acts at the basolateral surface of epithelial cells as the receptor that binds immunoglobulin A (IgA) dimer antibodies and mediates their transepithelial translocation (Kühn, L.C., and Kraehenbühl, J.-P., Trends Biochem. Sci. 7 (1982) 299). Proteolytic cleavage of the membrane form generates secreted SC, which remains tightly bound to the IgA dimer. The bound fragment is thought to protect the secreted Ig against proteolytic action in the mucosal environment. In the rabbit, both a high and low Mr family of SC are found; analysis of allotypic specificities and identical NH_2- and COOH-terminal amino-acid sequences suggest that the low Mr family results from a 25-kd deletion in the polypeptide chain of the high Mr SC family. The NH_2-terminal amino-acid sequence contains an interesting repeat of 6 amino acids with 2 serine and 2 hydrophobic residues, followed by glycine and proline. A model for the putative structure of the rabbit IgA dimer receptor is proposed.

Shape of factor VIII multimers in solution

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Factor VIII (FVIII), an extrinsic coagulation factor, is a macromolecular complex appearing in plasma as a mixture of multimers with mol.wts ranging from 0.5 to 20 millions. Conflicting evidence concerning the shape of FVIII multimers has been presented by electron microscopy, suggesting that FVIII molecules are either spherical aggregates of filamentous structures. To examine the shape of FVIII in solution we have labeled its surface by dye-sensitized photooxidation. Following illumination in the presence of a fluorescent dye (fluorescein or riboflavin) and a radioactive ligand (^{3}H-tryptophan), FVIII multimers were resolved by electrophoresis on SDS-2.5% polyacrylamide gels. Specific radioactivities of individual FVIII oligomers were plotted versus the number of protomeric subunits. The resulting curves indicate that the surface of subunits is very little affected by their polymerization, thus suggesting that large FVIII multimers are filaments rather than spheres.

Biological activity of disulfide intermediates of a snake venom toxin

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The conformational properies and stabilities of the disulfide intermediates formed during renaturation of the reduced toxin of *Naja naja philippinensis* (major toxin) are analyzed. The fully reduced toxin was oxidized by air at pH 7.1 and 37 °C. The products were reacted with iodoacetate or its amide and yielded 2 peaks by gel filtration, corresponding to the lower (native) and the higher (nonnative) hydrodynamic volume forms. HPLC (reverse phase, C-18) generated also 2 distinct peaks, a native-like and a denatured species. In both analyses, the native-like products proved to include molecules comprising 2, respectively 3 disulfide bridges, in addition to the natural toxin (which has 4 S-S). Furthermore, at least 3 different toxin analogs having 3 disulfides were detected by ion exchange chromatography and by isoelectric focusing. Binding experiments with acetylcholine receptor were carried out with the native and denatured toxins, and with modified analogs, in order to assert the occurence of an active lethal center. The aim was also to correlate activity with a stable and rigid molecular geometry exhibited by some disulfide intermediates.

A glycosylated membrane protein is released from the human T-lymphocyte surface in the induction of cell proliferation

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A 45-kD glycoprotein of marked charge heterogeneity can be labeled at the cell surface by ^{125}I, $NaIO_4/NaB^3H_4$ or reductive alkylation. It can be fractionated from cell homogenates in a vesicular membrane fraction. It is not solubilized by TX-100 under various conditions, but is eluted by 8 M urea or SDS from TX-100 insoluble fractions. The polyclonal cell activation by treatment with neuraminidase/galactoseoxydase, galactoseoxydase or $NaIo_4$ all result in the specific release of substantial amounts of a glycoprotein into the culture medium which appears derived from the 45-kD protein. In cell activation by phytohemagglutinin the 45-kD protein can no longer be surface-labeled for about 16 h after mitogen exposure; blanking off by bound mitogen, receptor rearrangement and release from the cell surface all may play a role. It is postulated that the release of this glycoprotein is functionally significant in cell activation; the released material might accrue the role of a growth-regulating factor.

Effects of citrate, in vitro, on the calcium-retention capacity of isolated rat liver mitochondria

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The uptake of Ca^{2+} by mitochondria was supported by succinate, and rotenone was used to prevent metabolization of added citrate. Net movements of Ca^{2+} into and from mitochondria were monitored with a pCa electrode. The intramitochondrial load of calcium required to induce uncoupling of the organelle, with resulting back flow of Ca^{2+} into the medium, was dose-dependently increased by citrate (in the range 0.1-3.0 mM) as well as by ATP (±oligomycin). Citrate was effective at concentrations similar to those present in the cytosol of the liver cell. When added together, citrate and ATP exerted supra-additive effects. The data suggest that citrate may help tissue to survive a transient overflow of Ca^{2+} into cells, as may occur in various pathological conditions. The protective effect of citrate appears to rely, not only on preservation (related to proton cotransport with the anion) of the electrical potential of the inner membrane, but also on intramitochondrial effects of citrate.

Interaction of neuropeptides with artificial lipid membranes compared to peptid structure-activity relationships

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The interaction of ACTH agoniosts and antagonists with phosphatidylcholine and that of dynorphin and enkephalin-derivatives with phospatidylcholine, phosphatidylserine and cerebroside sulfate vesicles was investigated by hydrophobic labeling with a phenylcarben-precursor. This model system characterizes the behavior of peptides at a hydrophilic/hydrophobic interface. Analysis of the exact

location of labeling revealed that the N-terminal part of ACTH and dynorphin penetrates into the hydrophobic core of membranes, whereas the C-terminal part remains on the surface. Labeling intensity paralleled the corticotropic activity of ACTH-derivatives. Labeling of opiate peptide derivatives paralleled K-, δ- and μ-receptor specificity. The results show that lipids reflect the organization of pharmacological information of the investigated peptides. They also correlate with the observed cross-reactivity of opiate peptides and pro-opiomelanocortin products.

Differentiation of desensitized and down-regulated states of the beta-adrenergic receptor

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When rat glioma C6 cells were desensitized by the β-agonist (−)isoproterenol, their high affinity binding sites for the hydrophilic antagonist CGP-12177 decreased more rapidly than those for dihydroalprenolol (DHA). This differential effect in β-antagonist binding was attributed to a shift to lower affinity for CGP-12177 by the desensitized receptor . Exposure of C6 cells to other agents that elevated cAMP levels caused a parallel loss in both DHA and CGP-12177 binding sites. Downregulation of β-receptors by these effectors was not accompanied by a shift in affinity for CGP-12177. Agonist-mediated desensitization was not blocked but the subsequent down-regulation of receptors was inhibited by cycloheximide.

Demonstration of 2 forms of IGF II in human and monkey brain

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Using indirect immunofluorescence in preliminary studies with monkey brains, we could demonstrate the presence of an IGF II-like protein. The neuronal cell bodies of cortex showed granular fluorescence. IGF II could be extracted form a synaptosomal, but not from the nuclear fraction. 2 forms of IGF II-like immunoreactivity were found (M_r 36-38,000 and 7500, resp.) upon extraction and gel filtration of IGF from different areas of human brain. The highest concentration of the 7.5 K-form occurs in the anterior pituitary (23 pmoles/g wet weight). The relative amounts of the 2 forms of IGF vary in the 24 regions of brain examined. We suspect a precursor-product relationship of the 2 forms. We feel, however, that it is premature to speculate on the function of IGF in brain.

Interaction of ω-substituted analogs of dicarboxylic amino acids with aspartate aminotransferase (AspAT)

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The action of substrate analogs containing a nitro, phosphonyl or 3-hydroxyisoxazolyl group instead of the distal carboxylate group on mitochondrial AspAT was investigated. 3-Nitroalanine, an analog of aspartate, was efficiently converted to nitropyruvate. This product could be conveniently assayed photometrically ($\varepsilon_{336} = 17{,}200\ M^{-1}\ cm^{-1}$). For the nitroalanine anion, the actual substrate, a K_m' of 0.7 mM, comparable to that of aspartate, and a k_{cat} of 6% of that for normal substrates was measured. Strong product inhibition ($K_i \sim 80\ \mu M$) was observed. On prolonged incubation AspAT was inactivated by a modification of pyridoxal phosphate with nitropyruvate. 3-Phosphonoalanine was accepted by AspAT too, although with a much lower affinity than aspartate, and underwent rapid transamination. In contrast, 2-amino-4-phosphonobutyrate and ibotenate, analogs of glutamate, reacted very slowly at about the rate of alanine. These compounds did not inactivate the enzyme.

Plasma kallikrein activates blood neutrophil respiratory burst

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Human plasma kallikrein (KAL) is a serine protease which aggregates human blood neutrophils (PMN) and increases their oxygen consumption. To assess whether this increase in PMN oxidative metabolism resulted in the production of active oxygen species, we measured the generation of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) by cytochalasin B-treated PMN following cell exposure to KAL. Substantial amounts of O_2^- and H_2O_2 were formed when PMN were challenged with KAL concentrations ranging between 0.2 and 1 U/ml (0.09-0.45 μM). For example, when KAL was 0.45 μM, the production rate of O_2^- and H_2O_2 was respectively 0.95±0.18 and 0.096±0.018 nmoles/min per 10^7 PMN. For comparison, similar amounts of O_2^- and H_2O_2 were produced when PMN were activated by the synthetic tripeptide f-Met-Leu-Phe (0.01-0.1 μM). In contrast, PMN exposed to 0.45 μM prekallikrein, the zymogen form of KAL, or to 0.45 μM KAL which had been inactivated by protease inhibitors failed to generate detectable amounts of O_2^- or H_2O_2. These observations support the conclusion that catalytically active KAL stimulate the NAD(P) H-oxidase system of human PMN.

Purification of bovine colostrum sialyltransferase to homogeneity by affinity chromatography

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Sialyltransferase (ST) was purified 1.6 million-fold from bovine colostrum (yield: 26%; 0.3 mg from 22 l) by modifications to the affinity chromatography procedure of Paulson, J.C., et al., J. biol. Chem. *252* (1977) 2356. Modifications included casein precipitation by chymosin and synthesis of a CDP-hexanolamine analogue affinity ligand by a novel and time-saving procedure. Pure ST moved as a single band, $M_r = 42$ kD on SDS-PAGE. The enzymatic product (trisaccharide) using lactose as acceptor substrate was isolated by gel chromatography and its structure determined. Rabbit antibodies to ST are being characterized.

Thiamin diphosphate binding to and stimulation of pyruvate decarboxylase activity

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Yeast pyruvate decarboxylase (EC4.1.1.1, M_r 230,000) is a 4-subunit enzyme with a $(\alpha\beta)_2$-structure. Cofactors are thiamin diphosphate (TDP) and Mg^{++}. The enzyme as it is isolated has 2 coenzyme molecules/4 subunits. Incubation of the enzyme with various amounts of TDP, Mg^{++} being in excess, increases the activity about 1.8-fold ($n_H = 1.9$, $K_{app} = 0.7$ mM). The analysis in the time regime yields a sigmoid coenzyme concentration/time constant profile explained on the basis of a fast preequilibrium of coenzyme

binding followed by a slow conformational change ($1/k_{conf} = 200$ s). These results are underlined by fluorescence titrations yielding a biphasic, sigmoid binding curve, which can be decomposed numerically into a high and low affinity binding phase of 2 coenzymes each. The preliminary binding constants are: 15 ± 2 µM ($n_H = 0.97 \pm 0.06$) and 110 ± 5 µM ($n_H = 2.00 \pm 0.05$), respectively. Thiamin does not increase the enzymic activity and binds weaker by a factor of 2.

Mammalian DNA polymerase α holoenzyme functioning on in vivo like templates

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In analogy to the *E. coli* replicative DNA polymerase III, we functionally define 2 forms of DNA polymerase *α*: the core enzyme and the holoenzyme (Hübscher, U., Gerschwiler, P., and McMaster, G.K., EMBO J. (1982) in press). The core enzyme is not able to elongate primed single-stranded DNA templates, in contrast to the holoenzyme which functions well on in vivo like templates. Using these criteria we have identified and partially purified DNA polymerase *α* holoenzyme from calf thymus and have compared it to the corresponding homogeneous DNA polymerase *α* (defined as the core enzyme) from the same tissue. The holoenzyme is able to use single-stranded parvoviral DNA and M13 DNA with a single RNA primer as template. The core enzyme, on the other hand, although active on DNAs treated with DNase to create random gaps, in unable to act on these 2 long single-stranded DNAs. Evidence will be presented that the high mol. wt DNA polymerase *α* polypeptide contains the primase activity necessary to initiate DNA synthesis on single-stranded DNA.

Early segregation of temporary efferent projections in the visual cortex of kittens

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In visual cortices of adult cats few neurons send bifurcating axons either to ipsilateral and (via the callosum) contralateral areas or to different contralateral areas (Segraves and Innocenti, Neurosci. Lett. Supp. *10* (1982)): distinct sets of neurons give rise to the various corticocortical projections. At birth, many neurons, strikingly in area 17, project temporary axons through the callosum; early after birth, these axons are eliminated depriving most of area 17 of its callosal output: apparently, the transitorily-callosal neurons form permanent connections within the ipsilateral hemisphere (Innocenti, Science (1981)). Using retrogradely transported fluorescent fast-blue and diamidino-yellow, we now found that distinct sets of efferent neurons have already formed at birth. In particular, distinct sets of transitorily-callosal neurons project to different contralateral temporary target areas (still other neurons project ipsilaterally). Why this differentiation of temporary projections should go that far is unclear; it may achieve economy of morphogenetic information: a common mechanism could guide the differentiation of cortical efferents throughout the cortex independent of their later fate.

Changes of peptide hydrolase activities in wheat leaves during senescence

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A shift of the pH optima from the acid to the neutral range during senescence was observed for the azocaseolytic activity in crude extract. Different elution profiles for young and senescing leaves were obtained by chromatography on DEAE cellulose columns eluted with a linear NaCl gradient (pH 7.5). 3 major peaks were observed. Peak I was not bound by DEAE cellulose under the conditions used, was more active at pH 7.5 than at pH 5.4 and reached maximal activity during senescence. The other peaks (II and III) were found in the NaCl gradient, showed higher activities at pH 5.4 than at pH 7.5 and were the predominant forms in young and in mature, green leaves. These data suggest, that the shift of the pH optimum in crude extracts during senescence was due to changing proportions of the different endopeptidase activities. It remains open, whether the increasing endopeptidase activity in peak I plays a major role in leaf senescence.

Effect of cations and guanyl nucleotides on agonist and antagonist binding to angiotensin II receptors

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The effect of cations and guanyl nucleotides was examined on the binding ^{125}I-labeled angiotensin II (AII), the octapeptide agonist, and (Sar^1, Ala^8)-AII, an antagonist analogue, to rat adrenal and uterine membranes. Sodium ion increased ^{125}I-AII binding to adrenal membranes by 50% at 140 mM, but had no effect on binding to uterine membranes. Calcium and magnesium ions (2 mM) enhanced agonist binding to both tissues. These effects were due to an increased affinity of the receptors for the agonist. In contrast, the cations reduced the binding of ^{125}I-(Sar^1, Ala^8)-AII to both adrenal and uterine receptors, a decrease due to receptor loss without change in affinity. Gpp(NH)p inhibited agonist, but not antagonist binding to adrenal and uterine receptors. Thus, agonist binding does not display the same dependence of Na^+ in all target tissues, and AII agonist and antagonist show striking differences in their sensitivity to known modulators of binding.

Nucleoprotein structure of *Physarum* ribosomal DNA

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Nucleoli of *Physarum polycephalum* contain about 100 molecules of free rDNA, not incorporated in the large chromosomal DNA. Each rDNA molecule is an inverted repeat of 60 kbp size. Most of the nontranscribed sequences are satellite-like, with numerous repeats within repeats. The central nontranscribed spacer has few nonrepetitive sequences.

We have investigated the chromatin structure of the rDNA in 2 respects. We wish to compare a) mitotic nuclei to G2 nuclei and b) G2 nuclei to naked DNA. Our approach is based on cutting DNA in isolated nuclei with several restriction enzymes and analyzing the purified DNA by blot hybridization.

At the present stage of analysis we cannot find significant differences in nucleoprotein structure between G2 and mitotic nuclei. However, comparing digests of nuclear chromatin with those of naked DNA we find several

protected restriction sites in the chromatin. These sites are probably localized in the central spacer region. Further work will be done on the exact identification of these protected regions.

Partial characterization of monoclonal antibodies against insulin-like growth factor I and II (IGF I and II)

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Monoclonal antibodies of the IgG type against IGF I and II have been tested with regard to their affinities for different IGF variants and to the epitopes directed against. Using dual binding of antibody to IGF I, each of 8 antibodies could be assigned to 1 of 3 groups (groups A-C). Testing different tryptic and synthetic peptides of IGF I for competition with IGF I, the epitope recognized by group C antibodies was found to be within the sequence 22-36. The 2 antibodies against IGF II recognize the sequence 27-45. All antibodies of group A and one of group C differed in their affinity towards human IGF's and IGF's of other species.

Structure and topology of membrane proteins specific for functional T-cell subsets

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Lyt-2/3 antigens have recently been shown to play a role in the binding of target cells by cytolytic T-lymphocytes. The structure and the mode of association with the membrane of these molecules has been studied by surface and hydrophobic labeling in conjunction with immunochemical methods. Among the 3 polypeptides of mol. wts of 28 kD 32 kD and 37 kD, the 2 latter are surface expressed and structurally related as indicated by peptide maps. In addition both run as multiple spots in 2-dimensional electrophoresis suggesting a microheterogeneity in their carbohydrate moiety. Finally their susceptibility to labeling with hydrophobic nitrene and carbene precursors indicates that both have domains embedded in the lipid matrix. In contrast, the 28 kD polypeptide is homogenous in charge and poorly accessible to surface as well as hydrophobic labeling. The membrane insertion and oligomeric assembly of these 3 polypeptides is currently under investigation.

Naturally occurring autoantibodies with tissue-homeostatic functions in sera of healthy humans

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We detected naturally occurring IgG autoantibodies (AA) to red blood cell membrane proteins in sera of healthy humans (J. Immun. *128* (1982) 1695). One of these AA was directed to an integral membrane protein, band 3. It was purified on immobilized band-3 protein. Purified AA was specific to band 3, but cross-reacted with a protein in the band-4.2. region which revealed peptides common to band 3. It bound to exoplasmic portions of band 3 and to the 65-K chymotryptic fragment on immune replicas. AA were obtained from IgG of 4 individuals as well as pooled IgG (Schweiz. Rotes Kreuz) and represented a fraction of 5×10^{-5} of the total IgG. AA were not donor-specific and had a 10-fold higher functional affinity to dimers and oligomers of band 3 than to monomers on solid phase assays. They preferentially bind to band-3 oligomers on senescent red cells in vivo, as shown by immunoprecipitation of cell-bound IgG and analysis of its coprecipitated target.

A novel mechanism for organ-specific metastasis

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Earlier this laboratory (Tao et al., Int. J. Cancer *23* (1979) 854) reported the isolation of a liver colonizing line of the B16-F1 melanoma designated L8-F1. Subsequently it was found that the growth of macroscopic tumors was related to the organ distribution of injected F1 but not L8. One day post injection, the number of viable F1 cells is 10 times higher in the lung than in the liver, kidney and spleen. Primarily lung tumors are observed. L8 cells, however, are found in equal numbers in these same organs after 24 h. Injection of these cells, results primarily in liver tumors and a few lung tumors. These results suggest that organ preference is due to a growth affect rather than to specific recognition. Studies in vitro support this conclusion. The rate of adhesion of F1 and L8 to liver cell monolayers is identical. However, a significant difference in the ability of the 2 melanomas to grow in co-culture with hepatocytes is seen. The growth of L8 is stimulated by liver cells, while F1 is inhibited.

Mode of action of the insecticide DDT on the activation of phosphodiesterase by calmodulin

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Dichlorodiphenyltrichloroethane (DDT) inhibits calmodulin (CaM)-mediated stimulation of phosphodiesterase (PDE) (Hagmann, J., FEBS Lett. *143* (1982) 52). Our purpose was to investigate whether this inhibition is due to binding of DDT to CaM and/or to the CaM-binding domain of the enzyme. Polyacrylamide gel electrophoresis of a mixture of CaM and ^{14}C-labeled DDT shows complex formation provided Ca^{2+} is present. DDT does not bind to troponin C or to parvalbumin. The K_d of the complex, estimated by the degree of inhibition of CaM effect on bovine brain PDE, is ca. 10 µM. Binding of DDT to PDE (whose effect is independent of the enzyme catalytic site) is likely, since DDT decreases the pronounced positive cooperative response of PDE to CaM activation but not V_{max}. In conclusion, the inhibitory effect of DDT on CaM-activated PDE results both from binding of the insecticide to CaM and from a suppressive action on the CaM-induced allosteric transition in the enzyme.

Structural changes in calmodulin and melittin upon complex formation

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In the presence of Ca^{2+}, calmodulin (CaM) forms a high-affinity, 1:1, complex with melittin, the amphiphatic peptide of bee venom (Comte et al., Biochem. J., in press). Our purpose was to identify the concomitant conformational changes in both polypeptides. Circular dichroism (CD) suggests that upon complex formation the α-helical content of melittin increases from 14 to 86%. The fluorescence of the unique Trp residue of melittin undergoes a marked blue shift in the presence of CaM and Ca^{2+}. Both changes are identical with those induced in melittin by neutral or acidic detergents. Near-UV CD shows that melittin markedly enhances the ellipticity of the Tyr belonging to CaM.

The interaction apparently implies Tyr-99 of CaM, which is also involved in enzyme activation. In conclusion, whereas localized structural changes occur in CaM upon complex formation with melittin, the latter undergoes drastic structural rearrangements; these lead to a configuration close to the elucidated tridimensional structure.

An induced mitochondrial protein correlating with steroid 18-methyl oxidase activity

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The enzymatic oxidation of the corticosterone 18-methyl group to an aldehyde group results in the production of the mineralocorticoid aldosterone in zona glomerulosa of the adrenal cortex. It has been shown that this enzymatic step is carried out by a mitochondrial cytochrome P-450 mixed function oxidase. Potassium depletion of rats for 2 weeks nearly abolished 18-methyl oxidase activity. Replenishment of those rats with potassium chloride for 48 h reinduced 18-methyl oxidase activity severalfold. We could show that this induction of 18-methyl oxidase activity was accompanied by the induction of a specific protein of M_r 49,000 in zona glomerulosa mitochondria. This protein increased in parallel with 18-methyl oxidase activity after 11, 15, 24, 39 and 48 h of potassium chloride replenishment. The good correlation between the increase in 18-methyl oxidase activity and the increase in the mitochondrial protein supports the hypothesis that this protein represents the cytochrome P-450 mixed function oxidase responsible for steroid 18-methyl oxidation.

Repetitive character of cell binding site in sponge aggregation factor

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3 different approaches were used to show that the cell binding site in *Microciona prolifera* sponge aggregation factor (MAF) has a repetitive character:
1. Each of the isolated MAF subunits and tryptic fragments contained the intact cell binding site, with binding affinity to the cells decreasing proportionately with their size.
2. Peptide mapping of the MAF subunits and fragments revealed that each consists of the repeated sequences of the smallest tryptic fragment (M_r 5000).
3. Intermolecular cross-linking of the smallest fragment into polymers of the dimension of the intact MAF showed a binding affinity equal to that of intact MAF.
This evidence supports the concept that multiple weak interactions add up to the high affinity interaction of MAF with the cell surface which is necessary for species-specific reaggregation of sponge cells.

Partial purification and in vitro translation of the mRNA for copper metallothionein from *Neurospora crassa*

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Total cellular RNA was extracted from cultures of *Neurospora crassa* grown on a minimal medium supplemented with 500 µM copper. Polyadenylated RNA was selected by oligo-dT cellulose chromatography and size fractionated by isokinetic sucrose density gradient centrifugation. Aliquots of each fraction were translated in a rabbit reticulocyte lysate system. The carboxymethylated, ^{35}S-cysteine labeled products were chromatographed on Sephadex G-50. Fractions coeluting with an authentic carboxymethylated *Neurospora* metallothionein sample were pooled and analyzed by means of SDS-polyacrylamide gel electrophoresis followed by autofluorography. RNA prepared from cultures grown on copper-free medium showed no detectable levels of metallothionein mRNA activity.

Nerve activity increases the phosphorylation of myelin basic protein (MBP)

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Our studies using 'back phosphorylation' have shown that incubation of rat optic nerves with depolarizing agents reduces the amount of dephosphorylated MBP relative to control. We now show that if samples are treated with a phosphatase before 'back phosphorylation' then the amount of dephosphorylated MBP is similar. This indicates that the reduction in dephosphorylated MBP reflects an increase in endogenous phosphorylation induced by depolarization. Experiments with optic nerves mounted between glass pipettes and bathed in physiological solution, show that it is possible to activate nerves by electrical stimulation of one end and to record the action potentials elicited at the other. Our results indicate that in activated nerves MBP phosphorylation is increased and the magnitude of this change is related to the number of potentials conducted. These results suggest that there exists a coupling mechanism between the axon and its myelin sheath and may provide clues to the function of MBP phosphorylation.

Isolation of a Mg^{2+}-ATPase from spinach chloroplast envelopes using a calmodulin affinity column

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Envelopes from spinach chloroplasts contain at least 21 proteins and 37 polypeptides (Siegenthaler, P.A., and Nguyen, T.D., Biochim. biophys. Acta (1982), in press). On the other hand, the presence of calmodulin has been reported in spinach leaves (Simon, P., et al., Pl. Cell Rep. *1* (1982) 119). The aim of this investigation is to determine if some of these proteins have a specific affinity for calmodulin and what are their function.
Chloroplast envelope membranes were solubilized in triton X-100 and applied to a sepharose 4B calmodulin column in the presence of Ca^{2+} and phosphatidylcholine. The EGTA-eluted fraction contains 2 major proteins as revealed by isoelectric focusing and electrophoresis in native gels. These 2 proteins represent 3–4% of the total proteins. One of them shows an ATPase activity which is Mg^{2+}-dependent and activated by the addition of calmodulin and a mixture of triton X-100 and phosphatidylcholine.

Partial sequence and alignment of the CNBr-fragments of the catalytic chain of the human complement subcomponent $C1\bar{s}$

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C1s was isolated by a rapid, 2-stage method involving affinity chromatography of serum on IgG-sepharose, followed by the separation of the resulting mixture of subcomponents C1r and C1s on DEAE-sephacel. The purified single-chain C1s proenzyme form was converted to the active 2-chain $C1\bar{s}$ by incubation at physiological pH for

18 h at 37 °C. After reduction and alkylation the light and heavy chains were separated on DEAE-sephacel. Cleavage of the C1s light chain (M_r 27,000) with CNBr followed by gel filtration on sephadex G-50 yielded at least 7 fragments (with an M_r ranging between approx. 1500 and 9000) whose N-terminal sequences were determined by automated Edman degradation. The fragments show varying extents of sequence homology with the known primary structures of serine proteases. By comparison with these proteases the alignment of the CNBr-fragments in the catalytic chain of C1s was established. The combined sequence data obtained correspond to approx. 40% of the primary structure of this chain.

cAMP-dependent protein phosphorylation in the *Aplysia* nervous system in vitro and in *Aplysia* neuron R15 in vivo

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In *Aplysia* neuron R15 physiological experiments have suggested a role for cAMP-dependent protein phosphorylation in the regulation of an identified K^+ conductance (Levitan et al. 1983). When proteins of neuron R15 are labeled in vivo by intracellular injection of $\gamma[^{32}P]$ATP and separated by 2-dimensional gel electrophoresis a great number of phosphoproteins can be resolved. Under these conditions cAMP stimulates the phosphorylation of specific proteins. A detailed study of cAMP-dependent protein phosphorylation in homogenates and subcellular fractions of the *Aplysia* nervous system in vitro was performed in order to investigate if some of these in vivo substrates could also be found in vitro. 18 phosphoproteins, observed both in vivo and in vitro, were analyzed by 2-dimensional gel electrophoresis. Of the 11 phosphoproteins that were affected by cAMP in vitro, 6 were also found to be substrates for cAMP-dependent protein kinase in neuron R15.

Stimulation of ketogenesis and inhibition of fatty acid esterification by norepinephrine in isolated rat hepatocytes

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Previously, we observed a potent ketogenic effect of norepinephrine (NE) in man. To investigate the effect of NE on hepatic ketogenesis, hepatocytes of fed rats were incubated with various concentrations of 1-14C-palmitate and NE. During 60 min incubation with 0.5 mM 14C-palmitate and 50 µM NE, fatty acid oxidation doubled during NE: Conversion of 14C-palmitate into 14CO2 increased from 7±1% to 13±2% of total fatty acid uptake, and into 14C-ketone bodies from 7±1% to 13±2%, respectively (x±SEM, n=6). Total ketone body production increased from 34±2 to 45±3 nmoles/mg dry weight. 14C-palmitate uptake increased by 14±3%. Fatty acid esterification diminished during NE as shown by a decrease of 14C incorporation into liver lipids from 77±4% to 58±3% of total palmitate uptake. Dose-response studies showed a half maximal effect of NE on palmitate oxidation at 1 µM NE. Thus, NE exerts a stimulatory effect on hepatic ketogenesis mainly by diverting fatty acids into the pathways of oxidation away from esterification.

Structural model of intima (type VI) collagen

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Intima collagen was studied by electron microscopy (rotary shadowing and negative staining) and by ultracentrifugation. The monomer (M_r=170,000) consists of a 105 nm long triple helix terminated by a small globular domain (M_r=30,000) at one end and a large globular domain (M_r=50,000) at the other end. The monomer was produced by selective reduction of interchain disulfide bridges. Prior to reduction dimers, tetramers and larger filamentous structures were found. Dimers are lateral staggered aggregates of 2 monomers aligned in an antiparallel fashion. This gives rise to an inner 75 nm long region of 2 slightly intertwisted triple helices flanked by the large globular domains. The outer triple helical segments (length 30 nm) with the small globular domains at their ends emerge at both sides of this structure.

Composition of vesicles released from erythrocyte membranes

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Release of membrane vesicles was induced either by incubation of human erythrocytes with dimyristoylphosphatidylcholine (DMPC) liposomes, by ATP depletion or by a combination of both procedures. Immunoelectrophersis techniques showed that DMPC induced vesicles contained the major integral membrane proteins but no cytoskeletal components. In vesicles obtained after extensive ATP depletion spectrin and ankyrin were also present. When red blood cell ATP levels were decreased before incubation with DMPC, vesiculation started earlier and the resulting vesicles contained some spectrin and ankyrin. The amount of cytoskeletal proteins in these vesicles increased with decreasing ATP content of the incubated erythrocytes. ATP depletion (and a concomitant change in membrane protein conformation) apparently facilitates the DMPC-induced vesiculation but it is not an obligatory prerequisite for the release of erythrocyte membrane vesicles.

Purification and some properties of a α_2-β_1 glycoprotein from horse plasma

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During the fractionation of horse protease inhibitors, a byproduct of horse antithrombin III was isolated in a highly purified form. The protein migrated in agarose gel electrophoresis just in front of the β_1 region. The glycoprotein seemed composed of a single peptide chain. The mol.wts determined by gel filtration on sephadex G-100 and by pore limit PAGE were 65 and 83 kD respectively. Isoelectric focusing showed a highly heterogenic banding pattern, with several bands focused between 5.1 and 6.5. Neither serological nor biochemical relation was found between the horse α_2-β_1 glycoprotein and human and bovine plasma proteins.

Purification of a neutral insulinotropic peptide from porcine duodenum

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Physiological studies suggest that the insulinotropic activity of the gut cannot be entirely attributed to the presently known gut factors; we thus decided to identify other biologically active peptides in a crude extract of porcine duodenal mucosa.

This extract, which exhibits a strong insulinotropic action on a in situ rat pancreas, was divided into fractions by preparative isoelectric focusing. Biological activity was observed not only in the pH region corresponding to GIP and other known peptides (8.1 and above), but also in the neutral region (pH 7.0). The latter fraction was isolated from the crude extract by cation exchange chromatography. The absence of GIP in this fraction was verified by RIA and high pressure liquid chromatography. Gel filtration indicated an apparent mol.wt over 6000 for the active peptide.

The biological activity of the neutral fraction was confirmed using an isolated rat pancreas preparation and was shown to be glucose-dependent.

Phosphatidylinositides are substrates for the phospholipase C reaction stimulated by TRH in clonal pituitary cells (GH_3 cells)

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Turnover of phosphatidylinositol (PI) is accelerated by TRH in GH_3 cells (Schlegel, W., Roduit, C., and Zahnd, G.R., FEBS Lett. *134* (1981) 47), leading to a rapid increase in incorporation of ^{32}P-phosphate into PI after TRH stimulation. Concomitantly phosphatidylinositol-4-phosphate (DPI) and phosphatidylinositol-4,5-diphosphate (TPI) show a transient loss of ^{32}P labeling, which could be due either to dephosphorylation or to a phospholipase C reaction. Prelabeling of GH_3 cells with ^{3}H-inositol allows to monitor changes in PI, DPI and TPI but also in the water soluble products of the phospholipase C reaction, the inositolphosphates. The rapid rise in labeled inositol-1,4-diphosphate and inositol-1,4,5-triphosphate after hormonal stimulation indicates that DPI and TPI are substrates in a phospholipase C reaction accelerated by TRH. The breakdown of phosphatidylinositides (DPI, TPI) could lead to a rapid liberation of membrane bound Ca^{2+}, and thus initiate the changes in Ca^{2+} distribution observed after TRH stimulation.

Hyperinsulinemia of preobese and obese *fa/fa* rats is a vagus-nerve mediated abnormality

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Glucose-induced insulin secretion was studied in vivo in preweaned 17-day-old normal and preobese Zucker *(fa/fa)* rats. Basal insulinemia was normal, but following a glucose challenge, insulinemia increased to levels markedly higher in preobese than in normal rats. This hypersecretion of insulin was reversed to normal by acute pretreatment with atropine.

Isolated perfused pancreases from adult obese rats oversecreted insulin, following arginine infusion, a defect brought to normal by atropine. In vivo, a 30-sec electrical vagus nerve stimulation potentiated glucose-induced insulin secretion to a much greater extent in adult obese than in controls. Acute bilateral vagotomy in adult obese rats produced a decreased insulin output, an effect that was not observed in control rats.

It is concluded that a) the increased insulin secretion of preobese Zucker *(fa/fa)* rats is an early abnormality that is mediated by the vagus nerve; b) increased secretion of insulin in adult obese *fa/fa* rats continues to be partly vagus-nerve mediated.

Insulin binding to human skin fibroblasts is regulated by glucose

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Glucose regulates the responsiveness of human fibroblasts to insulin; this finding lead us to study insulin binding at various glucose concentrations. Human fibroblasts derived from skin biopsies were cultured under standard conditions with 10% fetal calf serum. For binding experiments the cells were washed and the medium was replaced with DMEM containing variable glucose concentrations. Binding of mono-^{125}I-(Tyr A14)-insulin to the attached fibroblasts was determined after 3 h of incubation at 14 °C. High glucose concentrations (3g/l) during the binding experiment reduces binding of insulin at low concentrations (10^{-11} to 10^{-11} M) and changes the apparent affinities and binding capacities for insulin. The effect can be inhibited by blockers of glucose transport (cytochalasin B, phloretin). The demonstration of a direct regulation of insulin receptors by glucose may have important implications for the mechanisms controlling insulin responses in vitro and possibly in vivo.

Conformational changes in mitochondrial aspartate aminotransferase detected by a covalently attached fluorescent probe

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A conformation-sensitive fluorescent probe was attached to Cys-166 by reacting the enzyme with monobromotrimethylammoniobimane. Though Cys-166 is situated 25 Å away from the active site, its microenvironment changes during transamination as indicated by a 10-fold increase in reactivity toward SH-reagents (JBC *253* (1978) 3158). Labeling with the fluorescent probe decreases k_{cat} to 20% of its initial value. The fluorescence properties of the pyridoxal and pyridoxamine form are identical (λ_{em}^{max} 473 nm, λ_{exc} 380 nm). Addition of aspartate plus oxalacetate, or of substrate analogs decreases the fluorescence intensity by 40–60% with a blue shift of ~ 4 nm. Similar changes are observed with the apoenzyme. The data indicate that conformational transitions may occur on binding of specific ligands independently of coenzyme substrate interactions. This finding is consistent with X-ray crystallographic observations on interdomain movements (Ford et al. PNAS *77* (1980) 2559).

Determination of cholecystokinins in rat brain by high pressure liquid chromatography with electrochemical detection

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A simple method for the determination of CCK-4 and CCK-8 sulfate, based on high pressure liquid chromatogra-

phy with direct electrochemical detection (HPLC-ECD), has been developed. The 2 peptides are separated on a RP-18 column (2.1 × 150 mm) in less than 5 min and detected electrochemically at a potential of +1.0 V. The detection limit (= 3 × baseline noise) determined with CCK-4 is < 0.1 pmoles. Using this method the levels of CCK-4 and CCK-8 sulfate in rat brain cortex, hippocampus, striatum and brain stem were measured and found to be comparable to those reported in the literature using RIA methods. In cortex eg the levels are: CCK-4 33±5, CCK-8 sulfate 286±70 pmoles/g wet tissue (mean±SD, N = 6). Our results indicate that HPLC-ECD is sensitive enough to measure accurately cholecystokinins in rat brain tissue and is therefore an attractive alternative to presently used standard methods based on RIA. Due to its simplicity it might become the method of choice also for the determination of other neuropeptides.

Ca^{2+}-binding proteins in normal and rachitic rat brains

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Parvalbumin (PV), a high affinity Ca^{2+} receptor, is present in the brain predominately in a distinct subpopulation of neurons (Nature *293* (1981) 300). A similar distribution has been reported for the vitamin D-dependent Ca^{2+}-binding protein (Vit D-CaBP) (Nature *294* (1981) 765). This fact prompted us to investigate if the expression of PV and Vit D-CaBP is coupled. After the separation of prepurified Vit D-CaBP on 18% SDS PAGE we cut the dominant band (M_r 9000–10,000) and injected the dried and powderized gels directly into rabbits for the induction of monospecific antibodies. The antisera will be used for protein isolation with an immunoabsorbent affinity column and for immunhistochemical mapping of different tissues (brain, muscle, skin, testes, prostate, kidney) from normal, Vit D-deficient and repleted rats. Heat soluble protein fractions of these tissues were analyzed on HPLC for the presence of Ca^{2+}-binding proteins. Preliminary results indicate variations of the PV content depending on the Vit D-status.

The chloroplast fructose 1,6-bisphosphatase studied in a model system

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The fructose 1,6-bisphosphatase is a chloroplast enzyme whose activity is controlled by light. The system transmitting the light signal to the enzyme and thereby activating it by reduction is the ferredoxin/thioredoxin system. We have used its isolated and purified components to study activation and deactivation of fructosebisphosphatase in vitro with the aim to better understand its behavior observed in vivo. Our results indicate that oxygen deactivates the light activated enzyme via the ferredoxin/thioredoxin system. In the presence of physiological substrate concentrations the activated enzyme forms an enzyme-substrate complex which is stable at pH 8 (a pH reported for the chloroplast stroma during illumination) even under aerobic conditions. Deactivation of the active complex in the dark is achieved by oxygen after a shift of the ambient pH from 8 to 7 (as observed in the chloroplast stroma during a transition from light to dark). The presence of Mg^{++} is not necessary for the activation of the enzyme.

D-b-hydroxybutyrate mediates recovery from glucose deprivation in neonatal mouse brain cell cultures

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Neonatal mouse brain cell cultures have been used to study the effect of glucose deprivation. Brain cells were cultured for 8 or 14 days. Subsequently, the cultures were either continued in 13 mM glucose (controls) or exposed for 24 h to low glucose (2.1 mM) with or without D-b-hydroxybutyrate (BOB). Cerebroside sulfotransferase (CST) activity which closely parallels sulfatide synthesis was assayed immediately and after 5 days of recovery in medium with 13 mM glucose. At day 9, CST specific activity had dropped to 34.6±SE 1.6% in cultures kept in 2.1 mM glucose. Addition of 1.0 or 6.5 mM BOB raised CST activity to 53.2±3.5% and 65.5±2.2%, respectively. After 5 days of recovery, the beneficial effect of BOB was even more expressed. A similar pattern was observed if cultures were glucose-deprived at day 14, but CST activity loss was less and recovery was more complete than in the younger cells.

Transmembrane control of the accessibility of band 3 carbohydrate of human erythrocytes to galactose oxidase

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The accessibility of galactose residues to galactose oxidase and subsequent modification of aldehydes with [^{14}C]-arylamines (Biochemistry, in press) was studied. Modification of intact 0 Rh^+ cells resulted in 0.45±0.14 moles of label per mole of band 3 protein, while identical treatments of isolated membranes and spectrin-free vesicles yielded 1.38±0.15 and 1.2±0.14 respectively at saturating concentrations of galactose oxidase. This suggested that cell lysis or detachment of the bilayer from the cytoskeleton alters the conformation of an integral membrane protein, as visualized by exposure of additional galactose residues. Their location within band 3 protein was studied by 1-dimensional peptide maps and gel filtration of pronase digests of purified band 3 obtained from either ^{14}C-modified cells or from ^{14}C-modified ghosts, originating from ^{12}C-modified cells. Oligosaccharides with approx. mol. wts of 10–20 kD were more strongly labeled in ^{14}C-modified ghosts than in ^{14}C-modified cells.

Tumor metastases and cell deformability: 1. Differences in deformability of melanoma tumors as measured by a pressure filtration technique

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The ability of a tumor cell to deform may help it to squeeze through vessel walls and thus invade neighboring host tissue. In order to determine if any correlation existed between metastatic potential and tumor cell deformability we tested various B16 melanoma lines for their ability to pass through small pores of Nucleopore filters under low pressure. We obtained the following results: 1. The most highly metastatic line (NR4, mets = 79%) passed through the filters with the greatest speed and was thus the most deformable. 2. F1A of lowest metastatic potential (28%) was the slowest. 3. 2 other melanoma lines F1 and NR10 which were intermediate in their metastatic potential (55%) moved through the filters at an intermediate speed. Thus, the in vitro results correlated with metastatic potential in

vivo. Studies are presently in progress to characterize the deformability of other types of tumor cells and to elucidate the role of the cytoskeleton in this process.

Isolation of a GABA/benzodiazepine receptor complex of bovine cerebral cortex

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The GABA/benzodiazepine receptor from bovine cerebral cortex was purified by affinity chromatography on a new benzodiazepine-agarose. The benzodiazepine binding protein was enriched 1800-fold. SDS-polyacrylamide gel electrophoresis showed the presence of 2 major bands of M_r 53,000 and M_r 57,000. [^{3}H]flunitrazepam, after UV irradiation, was incorporated irreversibly into both bands of the isolated protein. A high affinity binding site for GABA was copurified with the benzodiazepine binding site and the 2 sites were shown to reside on the same physical structure. The dissociation constants were 10 ± 3 nM for [^{3}H]flunitrazepam and 12 ± 3 nM for the GABA-agonist [^{3}H]muscimol. The maximum specific binding for [^{3}H]muscimol was 4.2 nmoles/mg protein, the ratio of muscimol to flunitrazepam binding was between 3 and 4. The purified complex had a pharmacological profile that corresponds to the receptor specificity found in membranes. A preliminary physical characterization has been carried out.

Membranolysis by the 9th component of complement (C9)

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Human C9 has potential membranolytic activity. C9 acquires this activity by heat-induced polymerization on membranes. Incubation of purified C9 with carboxyfluorescein containing single bilayer vesicles for 1 at 46 °C resulted in marker release, which correlated with hydrophobic binding of C9 to the vesicle membrane. Lipid entry of C9 was accompanied by an increase of β-pleated sheet structure within C9. Vesicle lysis was caused by polymerization of C9 to tubules containing 12–18 C9 protomers (Poly C9). Electronmicroscopical studies showed that the poly C9 tubule forms a 10 nm wide transmembrane channel. The data indicate that isolated C9 can be transformed from a water soluble monomeric protein to a membrane bound amphiphilic protein.

Gene transfer into T-cell lines

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We have cloned different oncogenes into the pSV2 vector of Mulligan and Berg (Science *209* (1980) 1422) and tested their biological activity by 3T3 transformation and growth in selectionmedia. We want to use these composite vectors as a tool to introduce different oncogenes into T-cell lines and study the effect of oncogene expression on the growth properties of the T-cells and their responsivness to antigenic stimulation. As a first approach the plasmid containing the *E.coli* xanthine-guanine-phosphoribosyltransferase gene was transfected into the thymoma cell line 136.5 by the calcium phosphate method. A clone 136.5gpt was obtained which was dependent on xanthine in the presence of selectionmedia containing mycophenolic acid (MPA). An activity converting 14C-xanthine into 14C-XMP was found in cellysates of 136.5gpt but not in 136.5. The clone was stabile when passaged in selectionmedia, but lost the capability to grow in selectionmedia after prolonged passage in DMEM 10% FCS. We conclude, that T-cells can be used as transfection targets.

The onset of liver glycogen synthesis in starved and refed rats

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Rats were inured to a daily 4-h feeding period. Hepatic glycogen was synthesized at a rate of about 10 mg/g liver/h. The onset of glycogen deposition was accompanied by an inactivation of phosphorylase and phosphorylase kinase but without activation of glycogen synthase which was already partially active in the starved state. Cyclin AMP and protein kinase were unchanged after 1 h of feeding. The concentration of fructose 2,6-bisphosphate, which may be related to the glycogen stores, increased during the refeeding period. All these parameters were measured in liver biopsies sampled on anesthetized animals. In unanesthetized rats, however, the values were not the same and the changes depended on the nutritional condition of the animals.

Effects of endogenous calcium-activated thiol-protease on human platelet membrane glycoproteins

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Human blood platelets, either simply washed or surface-labelled by the periodic acid/NaB^3H_4 technique were incubated with purified platelet Ca^{2+}- activated protease for various times. The aggregation response of these platelets to bovine von Willebrand factor (bvWf) and to thrombin was measured and compared to control platelets. The supernatant and the platelets from the experiments with surface-labeled platelets were analyzed by 1 and 2-D gel electrophoresis, followed by fluorography and densitometry. After only 3 min incubation with protease platelets no longer aggregated to bvWf. The response to low amounts of thrombin (0.5 µ/ml) was also reduced after 3 min but became even weaker after longer incubations. Comparison with the densitometry data indicated that the aggregation response to bvWf is roughly a sigmoidal relationship with the amount of GPIb present and is lost when more than 80–90% of the GPIb has been removed. The loss of response to thrombin is more complex and appears to be related to both GPIb and GPV.

Synthetic hexapeptide models of metallothionein

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Metallothioneins are unique metalloproteins in which 7 bivalent metal ions (Zn, Cd, etc.) and all 20 Cys of the polypeptide chain are arranged in 2 discrete adamantane-related metalthiolate clusters with 3 and 4 metal ions, respectively (Otvos and Armitage, PNAS *77*, (1980) 7094). The formation of the clusters may be attributed to the abundance of chelating -Cys-X-Cys- sequences in the protein. To explore the metal-binding features of such structures, we have now chemically synthesized the hexapeptides SCVCAA, ACKCAA and ACSCAA. Titration of these peptides with Cd (II) documents the formation of 2 mononuclear complexes of 1:1 and 1:2 metal-to-peptide stoichiometry and of a binuclear 2:3 complex. The latter is

characterized by a red shift of the first Cd(II)-thiolate absorption band and by changes in circular dischroism indicative of metal-bridging thiolate ligands. Similar features are associated with the CD(II)-thiolate clusters in Cd(II)-metallothionein.

2 forms of phospholipase B in plasma membranes of *Saccharomyces cerevisiae*

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Plasma membranes of *S. cerevisiae* contain a phospholipase B. After solubilization of the membranes by the zwitterionic detergent SB 12 and purification by gel filtration phospholipase B was identified as a single glycoprotein band with an apparent mol.wt of 145,000 by SDS PAGE (Witt et al., Biochim. biophys. Acta *711* (1982) 403). In this investigation we present evidence, that the plasma membrane contains phospholipase activity in at least one other glycoprotein form with an apparent mol.wt of 210,000. The carbohydrate moities of both forms of the enzyme are susceptable to an endoglycosidase H from *Streptomyces griseus*. By SDS PAGE a stepwise removal of carbohydrates could be demonstrated. Degradation products of the same apparent mol.wts were obtained from both forms of the phospholipase B. From this results it can be concluded, that this enzyme exists in at least 2 forms different in the carbohydrate chains which are of the 'high mannose' type.

Fragments of bovine von Willebrand factor which influence platelet aggregation by the intact factor

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Bovine von Willebrand factor (bvWf) was isolated from bovine plasma by cryoprecipitation and gel filtration and its biological activity determined by the aggregation response which it produced on formaldehyde-fixed human platelets. The purified bvWf was reduced and alkylated and the monomer (mol.wt ~ 200,000) was isolated by gel filtration on Ultrogel AcA44. The fractions containing the monomer were pooled and concentrated. This concentrated monomer solution did not cause aggregation of fixed platelets but inhibited their aggregation by intact bvWf (complete inhibition with 15 times more monomer than intact). Intact bvWf was also treated with trypsin (250 U/mg bvWf; 15 min, 37 °C) and the fragments separated by gel filtration on a Ultrogel AcA44 column. A large fragment (mol.wt ~ 120,000) could inhibit aggregation of fixed platelets by the intact factor. On reduction this fragment was split into 2 smaller peptides (mol.wts 45,000 and 25,000).

The possible role of calmodulin in leukotriene metabolism of eosinophils

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Eosinophils (25×10^6 cells/ml) incubated with the ionophore A23187 (10 µg/ml) release leukotrienes (LT) into the medium, which can be separated by ion-pair RP-HPLC on a 3-µm Spherisorb ODS II column (250×4.6 mm) into at least 14 compounds. 4 of them were identified as LTB_4 and its stereoisomers and 4 showed pronounced slow reacting substance activity and were identified as LTC_4, LTD_4 and probably as its stereoisomers. Chlorpromazine (100 µM), phenothiazine (25 µM) and trifluoperazine (100 µM, TFP), known calmodulin antagonists, suppressed the LT formation almost completely. In order to know if inhibition takes place not only on phospholipase - but also on lipoxygenase level, eosinophils were incubated with arachidonic acid (50 µg/ml) in the presence or absence of TFP. While in the absence of TFP the LT formation was normal, TFP inhibited the transformation of external arachidonic acid into leukotrienes, an indication that calmodulin could also be involved in the lipoxygenase reaction.

ZELL- UND MOLEKULARBIOLOGIE
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Isolation and characterization of a chicken M-creatine kinase gene

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With the available cloned M-CK cDNA (Rosenberg et al. (1982) a phage (Charon 4A) library of genomic chicken DNA was screened. The clone λgMCK1 was further investigated by restriction analysis and southern blotting. A Eco R1 fragment was shown to hybridize with the available M-CK cDNA sequences and with cDNA from muscle poly A+ RNA. Further characterization is in progress and includes the analysis of the subclones of the genomic M-CK fragments, S1 nuclease mapping and the sequencing of the cDNA clones and part of the genomic M-CK DNA. Further genomic clones containing sequences adjacent to the M-CK gene will be isolated using fragment subcloned into the πVX-miniplasmid.

From periphery to cortex: consequences of radical peripheral manipulations during embryonic life on the barrelfield of the mouse

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The whiskerpad-to-barrelfield system of the mouse has been the subject of this study. Whiskers begin to develop at ca. 12 days of gestation; the barrels (their topological representations in layer IV of the parietal cerebral cortex), at ca. 4 days after birth. Experiments were made to test whether modifications of the 'whiskermap' can be produced by manipulating the whiskerpad during gestation (at 16, 17 and 18 days) or at birth. Operations were: a) removal and reimplantation of the whiskerpad in the same position, b) removal, c) 90° and 180° rotations, d) amputation of the hand, e) replacement of whiskerpad by dorsal skin, f) transplants of supernumerary whiskerpads, g) transplants of cornea. The operations done during gestation produce

more outspoken changes than those done after birth. Results show different modifications of the barrelfield; the most conspicuous being an enlargement of the barrelfield and an increase of the number of barrels consequent to an enlarged periphery.

A cellular protein phosphorylated by RSV transforming gene product is associated with ribonucleoprotein particles

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In RSV transformed chick embryo fibroblasts, the tyrosine phosphorylation of a cellular protein of 34,000 daltons (34 kd) is greatly enhanced. This was shown to be a consequence of the phosphotransferase activity of RSV transforming protein $pp60^{src}$. We report, that in both normal and transformed cells, the majority of 34 kd is associated with large structures in presence of Mg^{++} and that a fraction of this protein behaves as a ribonucleoprotein.

Organization of the rDNA repeating units of *Ascaris lumbricoides*

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The ribosomal DNA of *Ascaris lumbricoides* exists in at least 3 different molecular forms, an 8.8 kb ($\approx 88\%$), an 8.4 kb ($\approx 10\%$) and a 13.5 kb ($\approx 2\%$) long repeat. The length difference between the 8.8 kb and the 8.4 kb rDNA is due to the lack of a 450 bp long spacer fragment located 870 bp upstream of the 18S 5′ gene end. In contrast, the 13.5 kb rDNA contains a 4.7 kb long 'insertion element' in the 26 S coding region. In situ hybridization experiments demonstrated that the rDNA is localized at a single site on an autosomal chromosome. The in vivo initiation sites in the 2 main length classes were determined by S_1 mapping. Furthermore, we established the entire sequences of the external transcribed spacers both in the 8.8 kb and 8.4 kb repeating units. Currently we are working out a homologous in vitro transcription system for RNA polymerase I.

Modulation of climbing fiber activity causes changes in the postsynaptic membrane of Purkinje cells

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The number and freeze-fracture plasma membrane organization of Purkinje cell spines were studied in rats: a) after destruction of climbing fibers (cf) by 3-acetyl-pyridine (3-AP); b) after injection of colchicine into the inferior olivary nucleus to inhibit transport in cf and c) during the tremor period induced by harmaline. 3-AP and colchicine caused a significant increase in the number of dendritic spines in the molecular layer but no change in the number of spine synaptic profiles. The plasma membrane (P-face) of increased spines appeared similar (> 1000 intramembrane particles -IMP-/μm^2) to that of the postsynaptic targets of cf, i.e. the spines of large dendrites. The latter were affected by harmaline which decreased the number of IMPs ($1495 \pm 50/\mu m^2$ to $767 \pm 39/\mu m^2$). These changes in a population of Purkinje cell spines when cf activity is altered suggest a role of presynaptic afferents in the maintenance of the postsynaptic membrane.

Loss of microsomal β-glucuronidase and structural change of its lysosomal form in cultured mouse hepatocytes

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In some organs, lysosomal β-glucuronidase is also located in the endoplasmic reticulum (microsomes) and bound to the anchor protein egasyn. In freshly prepared hepatocytes of C57 Bl/6, approx. 40% of total activity was present in the microsomal form. During culture, this form disappeared within 6 days and concomitantly, a new form appeared in the lysosomes. This suggested a correlation between the absence of β-glucuronidase in microsomes and a lysosomal enzyme of particular structure. The correlation was confirmed in spleen which normally lacks the microsomal enzyme and was found to contain a lysosomal form electrophoretically identical with that of 6 days cultured hepatocytes. From this observation and from the fact that the microsomal enzyme, fully active at acid pH, was less than 5% active at neutral pH we conclude that the accumulation of β-glucuronidase in the endoplasmic reticulum may be important not for catalysis but for the control of directing and packing the enzyme into the lysosomes.

Immunocytochemical detection of ecto-galactosyltransferase

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A monospecific rabbit antihuman milk galactosyltransferase (GT) antibody was used to detect cell surface (= ecto) GT in 2 systems: 1. Formaldehyde fixed primary cultures form fetal calf kidney explants were grown to subconfluency and investigated for cell surface immunofluorescent staining for GT and, by permeabilizing cells with triton X-100, for intracellular (Golgi) staining. Whereas intracellular staining was juxtanuclear, cell surface staining was punctate and appeared randomly dispersed in these nonpolarized cells. 2. In human enterocytes, GT was detected by immunoelectron microscopy using the protein A-gold technique on thin sections of Lowicryl K4M embedded biopsy specimens. In these polarized cells, ecto-GT was concentrated over brush borders, apparently membrane-associated with external orientation. Less intense labeling was found on basolateral membranes. Goblet cell mucus was also heavily labeled for GT. Upon secretion, mucus-associated label spreads over the brush border glycocalix and forms a layer of label distinct from the membrane-associated label.

Use of primary cultures of adult rat hepatocytes to study induction of DNA synthesis in liver

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Primary cultures of adult rat hepatocytes do not undergo cell division, although a small proportion of the cells ($< 5\%$) synthesize DNA (incorporation of ^3H thymidine). This synthesis is stimulated by compounds known to induce liver hyperplasia and by suspect promoters of hepatocarcinogenesis. Using this convenient system we have compared the effects of nafenopin, a hypolipodaemic compound, and phenobarbital, a model promoter of hepatocarcinogenesis. Nafenopin induced peroxisome proliferation and increased the extent of DNA synthesis in the cultured hepatocytes.

Phenobarbital had no effect upon DNA synthesis. The 2 compounds also differed in their effects upon the rate of dedifferentiation of the cells, as assessed by morphology or the appearance of the 'fetal' enzyme, γ-glutamyl transpeptidase. Phenobarbital appeared to stimulate dedifferentiation, whilst nafenopin stabilized the differentiated state. Such intriguing differences may give some insight into the multifaceted nature of liver tumor promotion.

Analysis of a H2A histone terminator by surrogate genetics

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As determined recently, the generation of authentic 3′-termini of a *Psammechinus miliaris* H2A mRNA is dependent on a highly conserved inverted DNA repeat and, in addition, on spacer sequences. Bisulfite mutagenesis of DNA cloned into the single-stranded phage M13 generated a number of point mutations which disrupt the dyad symmetry of the conserved sequence motif. Analysis of the transcripts produced in the *Xenopus laevis* oocyte show that the production of authentic 3′-termini is diminished or abolished by dyad specific mutations. To delimit the necessary sequences downstream of the dyad symmetry more closely, we constructed a series of mutants, deleting different extents of spacer sequences. Removal of the sequences up to nucleotide position +125 had no significant effects on the generation of 3′-ends. However, only 50% of the wild-type levels of 3′-ends were produced, when all the spacer sequences up to nucleotide +26 were deleted.

Mouse teratocarcinoma and embryonic development: comparison of protein patterns in nerve and muscle differentiation

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Teratocarcinoma lines were established which differentiate in vivo exclusively to either immature neural or muscle tissue as shown by histological sections. These unidifferentiated tumor lines were compared with each other and with the corresponding normal tissues at different stages of embryonic development by 2-dimensional gel analysis. Over 1000 different newly synthesized polypeptides were resolved on the autoradiogramms. About 30 proteins were found to appear in the teratocarcinoma-derived neuroblastoma TDN 2283 but not in the teratocarcinoma-derived rhabdomyosarcoma TDR 114, whereas another set of proteins was specific for the latter. Muscle and brain tissues from day 8 embryos to day 15 fetuses were scored for these sets of tumor line-specific proteins. The identification of cell lineage-specific proteins in the unidifferentiated teratocarcinomas and in the corresponding normal tissues corroborates their significance in development. Antibodies raised against such cell lineage-specific proteins will offer a means to follow their temporal and spatial distribution during mouse embryonic development.

Enzyme activity profiles in mouse teratocarcinomas: a quantitative ultramicroscale analysis

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9 tumor lines with different developmental capacities were derived from spontaneous as well as from one induced teratocarcinoma: 3 teratocarcinoma-derived rhabdomyosarcomas TDR 602, TDR 694 and TDR 114; 2 teratocarcinoma-derived neuroblastomas TDN 2151 and TDN 2283; 2 teratocarcinoma-derived endodermal tumors TDE 274 and TDE 113; 1 multipotential teratocarcinoma OTT 2289 and 1 undifferentiated teratocarcinoma OTT 2158. Quantitative analysis of 10 catabolic enzymes, i.e. alkaline and acid phosphatase, α- and β-galactosidase, α- and β-glucosidase, α-mannosidase, α-fucosidase, β-glucuronidase and hexosaminidase was carried out at the 20 cell level, and specific enzyme activity profiles were established for each of the tumor lines studied. These profiles may be used for the biochemical identification of a tumor type at the single cell level in addition to morphological and biological criteria. (Blüthmann, H., Vogt, E., Hösli, P., Stevens, L.C., and Illmensee, K., Differentiation (1983) in press.)

Isolation and characterization of multiple alleles for murine pancreatic alpha-amylase

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The aim of our studies is to elucidate the role of the genetic background in the regulation of α-amylase gene Amy-2 expression in the mouse pancreas. For this purpose we have isolated the genes and their mRNA products form mouse strain CE which, unlike mice from inbred strain A/J, expresses not only one but at least 4 different alleles in the pancreas. The 4 mRNA species of CE mice differ from the mRNA species of strain A/J by about 1% in sequence. Gene counting experiments using a probe specific for the first exon indicate the presence of about 10, i.e. about twice as many Amy-2 sequences, in the CE genome. At least 4 different Amy-2 genes plus flanking regions have been isolated from a genomic CE library by using cosmid vectors. The genes range in size from 9 to 15 kb and are interrupted by 9 introns each. Unlike the Amy-2 gene from strain A/J, they all contain duplicated 5′ termini. The functional significance of this unusual sequence arrangement remains to be established.

Cloning and characterization of ARS sequences in the chloroplast genome of *Chlamydomonas reinhardii*

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It has been shown recently that a small mitochondrial restriction fragment of *Xenopus laevis* which contains the mitochondrial origin of replication acts as autonomously replicating sequence (ARS) in yeast (Zakian, V., Proc. nat. Acad. Sci. USA *79* (1981) 2264). In order to screen for ARS sequences in the chloroplast genome of *C. reinhardii* we have constructed a pBR322 derivative which contains the arg4 locus of yeast. Total chloroplast DNA was cut either with HindIII or MboI and the fragments were inserted into the arg4 plasmid. Pools of the hybrid plasmids were prepared and used for transforming a yeast arg4 strain. Until now we have cloned and identified 3 small chloroplast DNA regions which act as ARS in yeast and which may contain chloroplast replication origins. One of these regions has been sequenced.

Bacteria-fed *Tetrahymena* express a large surface protein absent in axenically grown cells

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Tetrahymena pyriformis grown in axenic conditions lack a large - ca. 250 kd - external protein (i-ag) present in several other ciliates. We have found that extracts of bacteria-fed

cells contain a molecule similar to the i-ag's. An antiserum produced against these extracts agglutinates both axenic and nonaxenic cells. Fluorescence in immunolabeled cells is principally associated with buccal structures. Labeling of surface extracts blotted on nitrocellulose or DBM paper reveals 2 main antigens. One of them is present on both types of cells, while the other, i-ag like, is only found in nonaxenic cells. Neither of the 2 components is detected in exponentially growing cells. Results suggest that i-ag of ciliates, whose function is at present unknown, could play a role in feeding processes.

Reduction of the number of oligodendrocytes enhances their proliferation rate

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We have previously shown that the proliferation rate of oligodendrocytes (O) in culture is age-dependent. We have now investigated whether the genetically determined proliferation rate of O can be influenced by extrinsic factors. In neonatal mouse brain cell cultures, O carrying the antigenic marker galactocerebroside (GC) were eliminated by complement dependent anti-GC antibody mediated cytotoxicity at day 14 in vitro. 4 days after the withdrawal of the cytotoxic medium, 20% of the normal number of GC^+O reappeared. Their proliferation rate at that time was estimated by 3H-thymidine autoradiography combined with GC immunostaining. The proliferation rate of O in 18-day-old untreated cultures was $13\pm1.9\%$. The proliferation rate of O in cultures where their number was reduced by cytotoxicity was enhanced to $26.6\pm5.1\%$. These results indicate that reduction of the number of O in culture increased the proliferation rate of O.

Independent lateral mobility of the (Na^+, K^+)-ATPase subunits in the plasma membrane of toad leukocytes

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The surface expression and lateral mobility of the glycoprotein and catalytic subunit of the (Na^+, K^+)-ATPase was studied on toad leukocytes by immunofluorescence and cytofluorometry using the biotin-streptavidin bridge technique. On fixed cells the distribution was homogeneous with complete overlapping of the 2 labeling patterns. On viable cells labeled at 4 °C, patches were observed with little overlapping between the 2 subunits. After warming, each of the 2 subunits capped in a different area of the cell surface. When 1 subunit was capped and the cells fixed, the distribution of the other subunit was homogeneous. The kinetics of capping and reexpression of subunits at the cell surface were analyzed by cytofluorometry. Our results indicate that the 2 (Na^+, K^+)-ATPase subunits are loosely linked in the plasma membrane.

Expression and processing of human preproparathyroid hormone in *Escherichia coli*

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In order to study processing and secretion of human preproparathyroid hormone (hpreproPTH) in *E. coli*, we have constructed vectors designed to efficiently express the previously cloned complementary DNA (cDNA) for hpreproPTH in transformed bacteria using a previously described approach. A *lac* promoter DNA fragment including a ribosomal-binding site was placed at varying distances in front of the coding sequence for a hybrid protein fusing the 'prepro' coding sequence of hpreproPTH and the enzymatically active carboxyterminal fragment of β-galactosidase. Clones expressing hybrid protein were recognized in indicator agar plates. Using plasmids from clones efficiently expressing the hybrid protein, the entire coding sequence for hpreproPTH was reconstructed. Plasmid encoded proteins were radiolabeled using the maxicell technique. On gel electrophoresis 2 immunprecipitable peptides have been identified. The larger has the size of hpreproPTH, and the smaller comigrates with hPTH, suggesting that the hpreproPTH is correctly processed. After cell fractionation β-lactamase, but not hPTH, is found soluble in the periplasmic space.

The affinities of myomal and myometrial progestin receptors to different steroids

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Benign tumors of the smooth muscle are rare in human beings except in the uterus. Here they are frequent but grow only in presence of active ovaries indicating that tumorous and nontumorous cells respond differently to identical ovarian stimuli. In the present work we determined the affinities of various steroids to the progestin receptors present in the $100{,}000\times g$ supernatant of the 2 tissues. This was accomplished with a competitive binding assay using promegestone as labeled ligand. The 2 receptors had the same affinities to all steroids as long as they had the same conformation as progesterone in either the A or D ring. However, if the conformation of both was altered as in 17α-hydroxypregnenolone, 20α-hydroxypregnenolone and dehydroepiandrosterone then the receptors of the tumor exhibited a higher affinity. Thus, subtle differences exist between the steroid binding sites of the 2 receptors. This could in part be responsible for the different responses of the 2 tissues to identical ovarian stimuli.

Glucocorticoid regulation by mouse mammary tumor virus (MMTV) DNA

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To characterize the regulatory region of viral DNA, we have joined a cloned MMTV DNA fragment containing the long terminal repeat (LTR) to a DNA fragment containing the coding sequence of the herpes simplex thymidine kinase gene lacking its own promoter. Introduction of this hybrid molecule into mouse Ltk^- cells by transfection yielded tk-positive cell clones. Deletion mutants lacking portions of the LTR upstream of the viral promoter were constructed and introduced into Ltk^- cells. Tk^+ clones were obtained, and their pattern of transcription will be reported.

Effect of DNA methylation on globin gene expression

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Using a novel DNA methylation technique, we set out to identify DNA sequences which are responsible for the effect of DNA methylation on gene expression. The method used allows to in vitro-methylate specific segments of cloned eukaryotic DNA, which is then brought back into

an eukaryotic cell to investigate in vivo the biological significance of the introduced segmental methylation pattern. The human γ-globin gene and its 5′ flanking region, cloned into M13, was studied by this approach in mouse L-cells. Globin gene expression is inhibited by total methylation of the γ-globin and M13 DNA sequences, whereas the γ-globin gene is clearly transcribed in control experiments with unmethylated DNA. Methylation of either the M13 vector sequences or the globin structural gene alone has no effect on globin transcription. By contrast, globin gene expression is blocked, once the 5′ flanking region is methylated. Consequently, DNA methylation in the promoter and upstream sequences is sufficient to prevent γ-globin transcription, while DNA methylation in the structural gene is not interfering with transcription.

Immunological investigations of muscle creatine kinase (M-CK) in various tissues and species

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Polyclonal and monoclonal antibodies were produced against chicken and hamster muscle creatine kinases (M-CK). The distribution of M-CK in various tissues of chicken and hamster was reinvestigated using sensitive enzyme linked immunosorbent assays (ELISA). The cross-reactivity between M-CKs from different species was tested by immunoblotting. Rabbit antichicken M-CK (RaChM-CK) bound neither to rat, rabbit and hamster M-CK nor to chicken brain type CK (B-CK). Competition ELISA showed the same results. These methods were also used to investigate the reactivity of different M-CKs with rabbit antihamster M-CK antibodies. The degree of immunological cross-reactivity between the different mammalian and avian M-CKs, in spite of their identical catalytic activity, may be an indicator of evolutionary changes.

Monoclonal antibody directed against a yolk sac carcinoma: reactivity during mouse embryonic development

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Monoclonal antibodies were raised by immunization of rats with the LT mouse teratocarcinoma-derived yolk sac carcinoma 113, and fusion of their spleens with mouse myeloma P3X63Ag8.653. Among the monoclonal antibodies reacting with 113 cells in indirect immunofluorescence, one was selected for furher characterization. This antibody reacting with several endodermal mouse tumors was used to follow the differentiation patterns of embryoid bodies from different mouse teratocarcinomas, of mouse embryos at various pre- and postimplantation stages as well as to establish the possible endodermal origin of some structures in adult mouse tissues. It was shown that this antibody brightly stained the endodermal layer of the 6.5-day embryo and kept a restricted location to the parietal yolk sac up to 9.5 days. Furthermore this antibody was unreactive with the unfertilized and fertilized egg, 2- and 4-cell embryo, morula, blastocyst, trophectoderm and zona pellucida. From all adult tissues tested, only the testicle exhibited a bright fluorescence lining the tubules, and the ovary showed a positive reaction on some follicles. This monoclonal antibody detecting an antigen of limited distribution should be useful in following a gene product characteristic of endodermal differentiation.

Intravascularly injected peroxidase labels all and only the cells which contain acid phosphatases resistant to glutaraldehyde in the brains of chick embryos

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If 5–10 µl of 10% horseradish peroxidase (HRP) is injected intravascularly in 13–16-day-old chick embryos, much of it rapidly enters the intercellular space of the brain, whence it is taken up by phagocytes and by some types of dying neuron (Clarke, Neurosci. Lett. *30* (1982) 223). The phagocytes probably include microglia and 'gitter cells', judging from the morphology revealed by their ingested HRP. If the HRP-labeling reflects phagocytosis and autophagy, one might expect the labeled cells to be rich in acid phosphatases, but tests of this idea were inconclusive in formaldehyde-fixed material, since even healthy neurons generally contained substantial acid phosphatase activity. But fixation with 3% glutaraldehyde selectively suppressed the activity in HRP-unlabeled cells, leaving it virtually unaffected in phagocytes and in HRP-labeled dying neurons. The title claim was established in sections double-reacted for HRP and acid phosphatase.

Functional dissection of the reduplicated promoter of a *Xenopus laevis* histone H4 gene and its use in an expression vector

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DNA sequencing of a *X. laevis* histone H4 gene and its flanking regions has revealed a reduplicated promoter. Three 5 bp direct repeats at the termini of the 106 bp long reduplicated motifs are indicative for an excision-translocation event. Each of these motifs contains a 5′TATAA3′ box and a 5′CAAT3′ box. To analyze the sequence requirements for the transcription initiation of this gene, promoter mutants were injected into frog oocytes. Transcription efficiency dropped drastically when sequences between -170 and -90 bp from the CAP site of the mRNA were deleted. This 'surrogate genetics' approach may help to define the physiological regulation of transcription of the H4 gene in our homologous expression assay. We have also linked this H4 gene promoter to a rabbit β-globin cDNA in order to produce bona fide globin protein in injected oocytes.

Biological experiments on Spacelab

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We are presently preparing 3 experiments for flight on Spacelab. The objective will be the study of the behavior of human lymphocytes in culture at $0 \times g$. Experiment 1 ES 031 will be performed in Spacelab-1 in 1983, AO8/32/CH will be integrated in the Biorack in 1985 by ESA and 781240 is selected by NASA for a Life Sciences dedicated mission in 1986. Although the effect of $0 \times g$ on cells cannot yet be predicted, simulated low-g has shown a depressing effect on lymphocyte reactivity in vitro. Previous observations with lymphocytes of crew members of US and Soviet spaceships have shown, that reactivity towards mitogens is weakened after flight. We will use cells from conventional donors and from flight crews after few days in space. Several biochemical parameters will be determined and cell ultrastructure will be analyzed by electron microscopy. The projects outlined here shall contribute to the study of

adaptation of living systems to the space environment and to the development of biotechnology in space as well.

Spontaneous, in vitro, malignant transformation of a basophil/mast cell line

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Permanent cell lines displaying several basophil/mast cell differentiated traits (presence of histamine and IgE receptors, specific stains) can be relatively easily isolated in vitro from murine hemopoietic organs. these lines are strictly dependent for their continued proliferation on specific growth factor(s) and they fail to give rise to tumors in vivo. We have now observed and documented a case of spontaneous, in vitro transformation of such cells. The transformed cells, in contrast to their untransformed counterparts, no longer require factor(s) for continuous growth but they still respond to its addition by an increase in growth rate and in cloning efficiency. When injected in vivo, they are highly tumorigenic. Contrasting with these drastic changes in growth properties, the expression of basophil/mast cell traits has remained unaffected. Furthermore, the transformed cells are still diploid and no retrovirus appears to have been involved in the transformation process.

Formation and elimination of synapses in developing human visual cortex

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Developmental changes in synaptic density in human visual cortical area 17 (striate cortex) ranging from 28 weeks of gestation to 71 years of age were determined in material prepared for electron microscopy with the phosphotungstic acid method. The results were correlated with measurements of the volume of striate cortex in celloidin sections. 2 periods were defined: one in which synapse formation is predominant, ending at about postnatal age 8 months, and a subsequent longer period characterized by synapse elimination, lasting until about age 10 years. When synaptic density is at its peak (at 8 months) the total number of synapses in area 17 equals about 3.5×10^{12}, a number that is diminished by about 1.4×10^{12} by age 11. Exuberant synaptic connections early in development may impart to the immature cerebral cortex plasticity which is lost in the adult.

RNA associated with Simian virus 40 large tumor antigen: sequence of T_1-oligonucleotides

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We have shown previously that SV40 large T-antigen, a virus-coded protein which accumulates in the nuclei of infected cells, is an RNA binding protein (PNAS *79* (1982) 1139). The sequence of more than 40 T_1-oligonucleotides derived from T-antigen protected RNA fragments has been established. In T-antigen-associated RNA extracted from monkey cells, extensive sequence homologies were found between 6 T_1-oligonucleotides and specific regions of SV40 transcripts; these regions were located a) in the intron and at the 3'-end of the early transcript, b) at the 3'-end of the attenuator RNA, and c) at the common 3'-end of the late VP2 and VP3 transcripts. In T-antigen-associated RNA extracted from mouse cells, only one T_1-oligonucleotide was homologous to a SV40 transcript. The majority of the T_1-oligonucleotides seems therefore to originate from cellular transcripts. The determined RNA sequences will be discussed in terms of T-antigen recognition signals.

Interactions of viral proteins with Rous sarcoma virus (RSV) RNA in virions and in RSV-infected fibroblasts

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Rous sarcoma virus is an attractive model to study essential biological processes since viral RNA can direct reverse transcription, translation, splicing and virion assembly. Which features in this RNA could account for these functions, their control and modulation? Viral *gag* protein P19 is known to bind tightly to RSV RNA (Sen and Todaro, Cell *10*, 91) and recently we have determined the binding sites of P19 onto the viral RNA, and shown that P19 could inhibit both translation and reverse transcription in vitro (Darlix and Spahr, J. molec. Biol. *160*, 147). Using antibodies against viral *gag* proteins, we report that P12 can also bind tightly to RSV RNA in the virus. In RSV-infected fibroblasts preliminary experiments indicate that both P12 and P19 are binding to RNAs. Work is now in progress to determine the nature of the RNAs interacting with viral proteins P12 and P19 and what could be the controls exerted by P12 and P19 on the functions of RSV RNA in vivo.

Regeneration of alfalfa plants from somatic cells cultivated in vitro after long-term subculture at 4 °C

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The modern technology of genetic engineering can be applyed to crop improvement. One of the difficult steps remains the regeneration of entire plants from in vitro cultivated somatic cells. Many researchers have observed a loss of morphogenetic potential after repeated subculture of in vitro cultivated somatic cells. In the search of factors stabilizing the morphogenetic potential we have produced about 2000 clones of an alfalfa cell line and selected one capable of regeneration into entire plants after subculture of about $1\frac{1}{2}$ years at 4 °C.

Human T-cell lymphokines: induction, kinetics and molecular cloning

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Lymphocytes, stimulated with mitogens or strong antigens secrete mediators with profound effects on other leucocytes (lymphokines). Induction and kinetics of appearance of the following 3 lymphokines produced by T-lymphocytes were studied: γ-interferon, measured by its antiviral effect, TCGF and BCGF, by their proliferation-inducing activity for T-cells and B-cells, respectively. Poly-A RNA from induced large scale human T-cell cultures was fractionated on sucrose gradients and their mRNA size estimated by in-vivo translation in *Xenopus* oocytes. cDNA libraries established from the sized mRNA are being screened for the lymphokine gene sequences in question.

Physical mapping of BHV-1 strains

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Bovid herpesvirus type 1 (BHV-1) causes 2 different clinical pictures; infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV). The 2 virus types are not distinguishable by their biological behavior in vitro. But since a characteristic difference in their virulence and organ affinity is manifested in vivo, these properties should be coded for on their genomes. Restriction enzyme analysis of various IBR and IPV virus strains showed, that the restriction patterns differ in fact between the IBR and IPV virus group. To localize these differences we mapped the genomes of an IBR and an IPV reference strain in view of cloning distinct regions of the genomes and for further characterization. For the same purpose we mapped an IPV wildtype strain DNA and the one of its attenuated daughter strain used for vaccination. Restriction enzyme analysis showed the latter to possess a short deleted region.

The induction of cytolytic activity in hybrids between murine cytolytic T-cell lines and a rat T-lymphoma requires 2 factors

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Somatic cell hybrids between a murine cytolytic T-cell line and a rat T-lymphoma, C58NT.D, acquire cytolytic activity during culture, for 3 days, in medium supplemented with supernatant from Con A stimulated spleen cells (CS). These hybrids do not depend on T-cell growth factor (TCGF) for proliferation. Fractionation of CS on reverse phase HPLC has shown that this induction requires 2 different factors, one of which coelutes with TCGF. The 2nd 'cytotoxicity inducing activity' (CIA) is distinct from γ-interferon and macrophage activating factor.

Cyclic AMP-dependent protein kinases in normal and abnormal human spermatozoa

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The possible involvement of cAMP-dependent protein kinase in asthenozoo- (A), teratozoo- (T) and asthenoteratozoospermia (AT) was investigated.

Photoaffinity labeling by 8-N_3-/^{32}P/cAMP revealed the presence of 2 specific cAMP-binding activities of mol.wt 52,000 (R-II) and mol.wt 47,000 (R-I) which were quantitatively equally distributed in normal and abnormal spermatozoa. In contrast, the seminal plasma of the respective groups incorporated the 8-azido cAMP photolabel in similar quantities into 2 additional functional cAMP-binding proteins (mol.wts 42,000 and 37,000).

In addition, the total cAMP-dependent protein kinase and total cAMP-binding activity (sperm homogenate and seminal plasma) were quantitatively comparable within N, A, T and AT.

A mouse mammary tumor virus (MMTV) DNA containing an aberrant LTR retains hormone-dependent expression

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Long terminal repeats (LTR) of MMTV DNA contain sequences involved in glucocorticoid stimulation of transcription, as assayed by DNA-transfer experiments. An aberrant cloned DNA derived from circular unintegrated MMTV DNA retained the hormone effect in transfected cells, despite a rearrangement that deleted the region of the LTR containing transcriptional regulatory signals. This result might be explained by the presence of other binding sites for the hormone receptor complex (HRC) on the MMTV genome. We are now trying to determine precisely where transcription starts in this aberrant clone. By computer analysis of sequence data of other glucocorticoid regulated genes and other regions of MMTV DNA which have been shown to bind the HRC in vitro we are defining a consensus sequence involved in the binding of the HRC.

Cloning of the genes involved in the *myo*-inositol active transport in a *Pseudomonas* species

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Pseudomonas sp. J.D. 34, isolated from soil, was shown to be able to utilize *myo*-inositol as sole carbon source. An active transport system for *myo*-inositol uptake allows the cell to grow on low concentrations of this cyclitol. Mutants deficient in the active transport system were isolated after nitroso-guanidine mutagenesis by screening for loss of the ability to grow on low *myo*-inositol concentrations. These mutants show linear uptake kinetics. A gene bank of the w.t. *Pseudomonas* sp. was generated by cloning 35 kb DNA fragments of its chromosome in the broad host range cosmid pMMB34 into an *Escherichia coli* K12 strain. The cloned genes were then introduced by mobilization from *E. coli* into the transport negative pseudomonas mutants. Transconjugants were screened for restored growth on low *myo*-inositol. 3 clones were found which were able to restore the active *myo*-inositol uptake in 2 different mutant strains.

Aristolochic acid: an old drug is a mutagen

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Extracts from the leaves and roots of *Aristolochia clematitis* L. have been used as medicines since the ancient egyptian and greek times. The most important active ingredient is aristolochic acid, a nitrogen containing, water insoluble compound which is neither an alkaloid nor a glycoside. This compound was fed to 72-h-old *Drosophila* larvae which were trans-heterozygous for the 2 genetic wing hair markers multiple wing hairs and flare (mwh +/+ flr). The wings of surviving adults showed genetically marked, induced cell clones proving a mutagenic activity of the aristolochic acid. This result, together with a positive Ames test (Robisch et al., Mutation Res. *105* (1982) 201), induction of chromosome aberrations and SCEs in human lymphocytes (Abel, unpubl.) and the observation of multiple carcinomas in treated rats (BGA, Pharm. Z. *126* (1981) 1373) indicates that aristolochic acid is a genotoxic carcinogen.

Construction of a cosmid cloning system for gene cloning into a broad host range of gram-negative bacteria

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A cosmid cloning system has been developed which is useful for the construction of gene banks of a broad range of bacterial species. The cosmid vector pMMB34 allows selective cloning of 36-kb fragments of partially *Sau* 3A digested DNA. Rearrangements of the insert DNA and formation of polycosmids is avoided allowing highly efficient cloning. The genes cloned with this cosmid vector in *E. coli* can then be introduced by mobilization into many gram-negative species to permit study of gene expression and complementation. Because mobilization is efficient, the vector has the advantage that it reduces the restriction barrier and enables relatively efficient transfer between bacterial species which specify different restriction systems. It thus reduces the necessity of obtaining suitable restriction deficient derivatives. We have used the vector to generate a gene bank from the chromosome of *Pseudomonas* sp. J.D. 34 and have isolated genes which complement a strain mutant in *myo*-inositol transport.

Structure and transcription of the *Antennapedia* locus of *Drosophila*

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Mutations at the homeotic locus *Antennapedia* result in an altered developmental program of particular cells in *Drosophila*. In heterozygous adults the antenna is transformed into a 2nd leg, whereas the homozygous embryos show a transformation of the 2nd and 3rd thoracic segment towards the first segment. We have cloned this gene in order to study its function at the molecular level. Chromosome rearrangements associated with *Antennapedia* phenotype were mapped at the DNA level. By northern blot analysis of mRNAs and the isolation of cDNA clones the composition and number of transcripts from the *Antennapedia* gene have been studied and compared over the course of development.

Cyclosporin A, an immunosuppressor, prevents activation of the ribosomal genes in mouse T-lymphocytes

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Addition to mouse thymocytes in vitro of concanavalin A (Con A) plus interleukine 2 (IL 2) led by 3-4 h to stimulated hnRNA synthesis, followed (at 8-9 h) by stimulated overall cellular RNA and protein synthesis. Cyclosporin A (CS-A; 0.3 µg/ml), added together with Con A plus IL 2 completely inhibited blastic differentiation (Wiesinger, D., and Borel, J.F., Immunobiology *156* (1979) 454); however, stimulation of hnRNA synthesis occurred whereas CS-A prevented activation of the ribosomal genes. Addition of CS-A after the activation of the ribosomal genes remained virtually without effect on 45S pre-RNA synthesis. We presently attempt to define the genes which are activated (±CS-A) during the first 7 h after addition of Con A plus IL 2.

Stimulation of Na^+ transport and Na^+,K^+-ATPase synthesis by aldosterone: correlation with occupancy of hormone binders

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In the urinary bladder of the toad, *Bufo marinus*, aldosterone increases Na^+ transport and Na,K-ATPase synthesis and decreases tissue resistance. All responses are dose-dependent, saturable events, mediated by hormone-receptor interactions. Comparison of the fractional changes produced by different hormone concentrations with the fractional occupancy of type I (high affinity, low capacity) and type II (low affinity, high capacity) cytosolic and nuclear receptors suggests that a) stimulation of Na^+ transport is probably mediated by both type I and type II receptors and b) the rate of Na,K-ATPase synthesis and change in tissue resistance is related to occupation of type I receptors (parabolic relation). These results suggest that the overall Na^+ transport response to aldosterone is a complex event, produced by a pleotropic action of the hormone in which both type I and type II receptors are involved.

Magnetic particles as a probe for alveolar macrophage function

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Ferrimagnetic particles instilled into the lung are phagocytized by alveolar macrophages. After these particles have been magnetized and aligned by an external field, they produce a remanent field which decays rapidly due to random particle misalignment (relaxation). The relaxation rate, measured by the time required for the remanent field to decay to half of its strength, $T_{1/2}$, was found to be dependent on the time elapsed after particle instillation: $T_{1/2}$ decreased from 24.3±3.7 (SD) min to 3.9±0.7 min within 12 h after particle instillation. This increase in relaxation rate parallels the phagocytosis of particles, i.e. their uptake and progression into the endoplasm. We conclude that changes in the relaxation rate reflect changes in the particles' location and possibly the effect of cytoplasmic fine structure on lysosomal mobility.

Cloned genes are preferentially activated either by transcriptional 'enhancers' or by template DNA replication

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SV40 and polyoma virus (and other viruses) contain a short DNA segment, the so-called transcriptional enhancer, which is essential for efficient expression of the viral early genes. Linkage of these enhancers to some cloned cellular genes, e.g. the rabbit β-globin gene, dramatically increases their expression after transfection into cultured cells. However, not all genes transcribed by RNA polymerase II respond similarly to a viral enhancer element. Certain genes, i.e. the SV40 late transcription unit and the sea urchin histone genes are only moderately activated by enhancers. In contrast, expression of these genes is strongly stimulated by DNA replication (with concomitant template amplification), and this raises the question: Is there a group

of genes which is naturally activated by genomic enhancers, whereas another group is activated by DNA replication?

Effects of aldosterone on surface-exposed (Na^+,K^+)-ATPase and plasma membrane proteins (PMP)

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Aldosterone (A) increases transepithelial Na^+ transport in TBM cells in culture. The effects of A (100 nM, 3 and 24 h) on the incorporation of ^{125}I into surface-exposed proteins were studied using the glucose oxydase-lactoperoxidase procedure (0 °C). The labeling was restricted to the apical surface when the iodination was carried out on confluent cells attached to plastic dishes. In this situation, the α- and β-subunits of (Na^+,K^+)-ATPase were not iodinated, as assessed by immunoprecipitation. Several plasma membrane proteins were labeled but none were modified by A, as assessed by 2D gel electrophoresis. When the iodination was performed on cells in suspension, the labeling was extended to the basolateral surface. In this case, both α and β subunits were readily labeled. Aldosterone increased the expression of (Na^+,K^+)-ATPase ($\sim$ 3-fold) but only after 24 h exposure. By contrast, A modified the expression of several PMPs at the basolateral surface as early as 3 h after hormone addition.

Molecular analysis of *Drosophila* development: the bithorax complex

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The bithorax complex in *D. melanogaster* is a cluster of genes that are involved in the determination and the maintenance of developmental pathways in the thoracic and abdominal segments.
Using cloned DNA probes from the locus (Bender, W., and Spierer, P., in preparation), we have analyzed the transcriptional activity of the part of the locus that is defined by the bxd and pbx mutations. These genetic units are transcribed at low levels early in embryogenesis as a single complex unit of more than 20 kb. A number of different developmentally regulated RNAs are processed from these large transcripts. cDNA clones of some of the RNAs were constructed and isolated, providing more detailed information on the splicing patterns and on the protein coding potential of the genes.

Structure and expression of dispersed transfer RNA genes of *Xenopus laevis*

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The tRNA genes of the frog *Xenopus laevis* are unusual in that they are reiterated 8000 times in the haploid genome. This very high number seems to be dictated by the synthetic demands of oogenesis during which single oocytes accumulate as much as 40 ng of tRNA. The oocyte-type genes appear to be arranged as complex tRNA gene clusters located at just a few chromosomal sites. An example of this type of organization is provided by a 3.18-kb DNA fragment containing 8 tRNA genes that is tandemly-repeated 150-fold at a single chromosomal site. In an attempt to study tRNA genes that may be active only in somatic cells, we have searched for tRNA genes that reside outside this gene cluster, by making use of restriction enzymes that do not cleave the 3.18-kb repeat. A *Bam*HI DNA fragment of 9.1 kb has been cloned in this way. It has very little sequence homology with the 3.18-kb repeat but contains several tRNA genes whose structure and transcriptional properties will be described.

Excision repair defect sensitizes a novel *Drosophila* mutagenicity test

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Drosophila provides in vivo assays that allow to screen chemicals for potential mutagenic activity. An efficient metabolism for xenobiotics in larvae as well as in adults permits the detection of pro-mutagens. In certain assays, the use of tester strains defective in DNA excision-repair had improved the detection capacity for mutagens which induce excisable DNA lesions. A promising short-term test has been devised which is faster and cheaper than the most commonly used sex-linked recessive lethal test. It is based on mutational events induced in somatic cells of larvae which are trans-heterozygous for the marker mutants *mwh* and *flr*. Into this basic system, which proved capable to detect many mutagens and pro-mutagens, the excision-repair defective mutation *mei-9* was introduced. We will show to which extent this change increases the detection capacity of the test system for different classes of chemicals.

A glia derived protease inhibitor modulates neurite extension

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Neurite extension in neuroblastoma cells is an experimental model for studying biochemical events involved in one of the first steps of neuronal differentiation. The fact that macromolecules released by cultured glial cells induce neurite outgrowth indicates the importance of cellular interactions in this phenomenon. The role of proteases in cell migration has been stressed in many developmental systems, including neuronal migrations that occur during the development of the nervous system.
Medium conditioned by glial cells induces neurite extension and also strongly inhibits plasminogen activator or urokinase. The 2 activities copurify. The use of a modified urokinase-sepharose column and of SDS-polyacrylamide gel electrophoresis allows to attribute the activity to a single protein. The biological relevance of the inhibition of neuronal cell-derived proteolytic activity for neurite outgrowth will be discussed in view of the phenomena taking place at the time neuronal migration stops and neurite outgrowth starts.

Localization of *Antennapedia* transcripts in developing *Drosophila* embryos using an improved in situ hybridization method

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Homeotic genes control developmental pathways and the pattern of segmentation in insects. In an effort to elucidate the molecular events involved in the expression of such genes, we have analyzed the tissue distribution of *Antennapedia* transcripts in developing embryos.

Antennapedia transcripts were localized within serial tissue-sections of *Drosophila* embryos by in situ hybridization using cloned c-DNA probes. Since *Antennapedia* transcripts are present at low cellular concentrations it was necessary to extend the sensitivity of the in situ hybridization method. The improved method herein described provides approximately 10–50 times greater sensitivity of signal detection than those that have been previously published.

The transcription unit of the alpha-amylase gene *Amy*-2^a in the mouse

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We have used runoff transcription in isolated nuclei and nuclease S1 mapping of steady state nuclear RNA to define the transcriptional unit boundaries of the mouse α-amylase gene *Amy*-2^a.
Our results indicate that *Amy*-2^a is transcribed exclusively in the pancreas and that RNA polymerase II starts transcription at the cap site and terminates at multiple sites between 3 and 5 kb downstream of the major polyadenylation site. For the generation of the mature 3′-end of the α-amylase mRNA a posttranscriptional processing step is therefore necessary. A similar mechanism has been postulated for the mouse β-globin and adenovirus mRNA (for review see e.g. Darnell, J.E., Nature *297* (1982) 365).
In addition a minor poly(A) containing cytoplasmic RNA species was detected which has an extra 2-kb 3′-nontranslated region and accounts for approximately 1‰ of the major α-amylase mRNA in the pancreas.

Transcripts, genes and bands in 315 kb of *Drosophila* DNA

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We have mapped transcription units, genetic units and polytene chromosome bands in 315 kb of DNA from chromosome section 87D, E of *Drosophila melanogaster*. This region consists of about 14 bands in the polytene chromosome and contains the essential sequences for at least 12 recessive lethal complementation groups (including *rosy* and *acetylcholinesterase*). We have defined 18 discrete polyadenylated RNA species transcribed from nonrepetitive DNA in the region at various developmental stages. There is a generally good correlation between the position of transcription units, bands and complementation groups but with some significant exceptions.

The effects of 'burn toxin' on human erythrocytes and neutrophils

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From early (3rd day) excised human burn eschar a lipoprotein-toxin has been isolated by homogenization, centrifugation and digestion with trypsin and collagenase. Several concentrations of toxin were incubated with washed erythrocytes for different times. It was shown that a significant decrease of the osmotic resistance was direct proportional to the incubation time and/or to the toxin concentrations. However, the separated apoprotein and lipid fractions alone had no effect. By an antitoxic IgG the toxic effect was counteracted. Preincubation of isolated neutrophils with burn toxin reduced the zymosan-stimulated phagocytic capacity as shown by chemiluminescence. This impairment depends also significantly on preincubation time and/or toxin concentration. These results suggest that a specific cutaneous burn toxin exhausts different cellular systems reducing or abolishing vital cellular functions in general.

A time course study of ultrastructural changes induced by EDTA in the rat yolk sac in vitro

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Studies with the Ca^{++}-chelating agent ethylenediaminetetraacetic acid (EDTA) demonstrate structural time-dependent alterations in the epithelial cell organelles. Rat yolk sacs at 9.5 days gestation were cultured in pure male rat serum. After 32 h or 40 h adaptation to this environment, 1 mM EDTA was added to the medium. After 2, 4, 8 and 16 h the yolk sacs were removed and examined in the electron microscope.
No structural alterations were found at the 2-h treatment period. After 4 h, swelling and depletion of mitochondria were the first sign of the calcium reduction. After 8 and 16 h lysis of lysosomes and dilatation of the endoplasmic reticulum were found, whereas the whole cell body remained unaltered. These observations suggest that the calcium chelater acts primarily at the mitochondria and only later at other cytoplasmic organelles. The changes are indicative of membrane instability.

Physiological role of nerve growth factor (NGF) in development and regeneration of central cholinergic neurons?

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Repeated intraventricular injections of NGF into rats during the first postnatal week increased choline acetyltransferase (CAT) activity in hippocampus, cortex and septal area. In adult animals with partial lesions of the septo-hippocampal pathway, repeated injections of NGF during 4 weeks produced a marked recovery of CAT activity in the hippocampus. However, intraventricular injections into newborn rats of anti-NGF antibodies failed to reduce the basal CAT activity in the forebrain areas. The offspring of rats autoimmunized against NGF showed no reduction in CAT activity. Anti-NGF was also without effect on basal CAT activity in cultures of dissociated septal neurons. Therefore, NGF can affect central cholinergic neurons during development and after partial lesions of the pathways. However, NGF does not seem to be identical with an endogenous neurotrophic factor of these neurons.

Isolation of an ecdysone-regulated gene of *Chironomus tentans* by microcloning

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Region I-18C in polytene chromosomes of *C. tentans* is puffed during periods of high ecdysone (Ec) titers. Puff I-18C can also be induced in vitro or in vivo by Ec application. For studies on gene organization, regulation and expression of I-18C its DNA has to be cloned. We excised I-18C regions from salivary gland chromosomes. The DNA

was isolated thereof, EcoRI digested, ligated into a λ-vector and in vitro packaged by microtechniques (collaboration with Edström and Jäckle, EMBL, Heidelberg). The recombinant clones were screened with cDNA derived from polyA$^+$ RNA of animals with high or low Ec titers. 2 clones were found which reacted expectedly. They hybridized in situ to only one chromosome region, I-18C. By restriction mapping and southern analyses it could be shown that their inserts are located very close to each other in the genome. Northern blots revealed that both hybridize, with the same RNA (about 4.8 kb). R-loop mapping located the 5'- and the 3'-end of the gene in the cloned DNA.

Independent lateral mobility of cytochrome bc_1 complex and cytochrome c oxidase in mitochondrial inner membranes

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Immunofluorescence microscopy was used to analyze the distribution of the bc_1 complex (bc_1) and cytochrome c oxidase (aa_3) in Ca^{++} fused inner membranes of rat liver mitochondria. The distribution of bc_1 and aa_3 was determined with FITC and MRITC respectively, after conjugation of these fluorochromes to immunoglobulines (IgG) monospecific for purified heme proteins. At IgG concentrations between 0.02 mg/ml and 1 mg/ml, rings of fluorescence were observed at the periphery of the intact membrane spheres. This indicates a random distribution of both cytochromes in the membrane. Subsequent addition of a secondary IgG directed against either of the 2 primary IgG's led to the formation of separate patches of fluorochromes. These observations indicate that bc_1 and aa_3 diffuse laterally in the plane of the membrane and do so independently of one another.

Scaling mitochondrial volume in heart to body mass

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Heart mass changes in linear proportion to body mass in contrast to metabolic rate which has been shown to increase with the 0.8 power of body mass. Consequently the oxygen consumption per unit body mass of an Etruscian shrew weighing 3 g is some 20 times larger than of a 900-kg cow. As the capacity to carry oxygen by blood is quite similar in most mammals it follows that a unit mass of heart tissue must perform considerably more work per unit time in a small animal than in a large one. The energy to produce tension and hence to do work in heart tissue is derived from aerobic metabolism in the mitochondria of heart muscle cells. One might therefore ask the question how mitochondria are adapted to the size specific metabolic requirements in hearts of various sized animals. We report that the total volume of mitochondria in heart scales to the 0.9 power of body mass; so does the cardiac work rate. We conclude that the size of the mitochondrial compartment closely matches the metabolic requirements of hearts in mammals whose body size span more than 5 orders of magnitude.

Localization of Bowman-Birk inhibitor on thin sections of soybean CV, maple arrow by the gold method

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The Bowman-Birk inhibitor (BBI), a polypeptide of mol. wt 8000, has a specificity directed against trypsin and chymotrypsin. The inhibitor was localized on thin sections of soybean which were incubated with anti-BBI immunoglobulins and 12-nm gold particles labeled with protein A. In cotyledon and embryonic axis, BBI was found in all protein bodies, the nucleus and the cytoplasm. Contrary to the Kunitz trypsin inhibitor (mol. wt 21,500) (Horisberger and Tacchini, Histochemistry, in press), BBI was not present in the cell wall but in the intercellular space. Quantitative data indicated that marking intensity was similar in varieties lacking the Kunitz inhibitor (P.I. 157440 and 196168) but greater to that of Maple Arrow in Norredo and T-102, 2 varieties lacking the lectin. Although this study was not designed to discern the cellular function of protease inhibitors, if any, it may establish criteria consistent with their role in soybean which is unknown at present.

Simian virus 40-induced reprogrammation of cellular gene expression in mouse kidney tissue culture cells

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Abortive infection with simian virus 40 triggers in G_0-arrested mouse kidney tissue culture cells a mitotic response.
Stimulation of overall cellular RNA synthesis (hnRNA, 45S pre-rRNA, 5S RNA and tRNA) began about 4 h after onset of viral T-antigen synthesis and was paralleled by doubling in cellular polyribosome content (A260). In addition, infection induced a marked increase in the synthesis of poly(A)$^+$ host mRNA and a change in size distribution. We presently study the SV40-induced reprogrammation of cellular gene expression by examining the cellular mRNA species synthesized after infection.

Distribution of microtubule associated proteins in rat brain studied by means of monoclonal antibodies

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Brain contains a series of minor microtubule associated proteins (MAPs) which are apparently intrinsic components of the cytoskeleton. To investigate their cellular distribution we have raised monoclonal antibodies against 2 of these proteins (MAP1 and MAP2). Immunocytochemical staining shows that MAP1 and MAP2 are differently distributed in the rat brain. Neither antibody gave detectable staining of glial cells in any of the brain areas examined. Both antibodies stained only nerve cells, although not always with the same pattern. The most striking difference is the strong reaction of antiMAP2 with cerebellar granule cell neurons and the weak reaction of antiMAP1 with the same cells. There are also differences in the intracellular distribution within the same cell, e.g. in Purkinje neurons antiMAP1 reacts with the cell body and the whole dendritic tree whereas antiMAP2 only with the distal dendrites. The differential availability of such MAPs, even within the same cell, suggests a highly localized microtubule organization which could reflect a functional specialization of these cytoskeletal elements.

The DNA inversion system of bacteriophage P1: mapping of the recombinase gene and of the crossover sites

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The phage Pl genome contains an invertible DNA segment (C segment) consisting of 3-kb unique sequences flanked by 0.6-kb inverted repeats. We studied insertion and deletion mutants of P1 derivates and mapped the gene *cin* of the site-specific recombinase within a 0.6-kb DNA segment adjacent to the C segment. The crossover sites *cix* locate at the outside ends of the flanking inverted repeat sequences. The *cin* gene product can also promote recombination between *cix* sites on different replicons resulting in cointegrate formation.

Modulation of vimentin and desmin in rat aortic smooth muscle cells during the evolution of intimal thickening

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The amount and the distribution of vimentin and desmin in aortic media and intima of normal rats and of rats in which the endothelium has been mechanically removed resulting in intimal thickening were studied by means of indirect immunofluorescence and densitometric analysis of SDS-PAGE. 2 weeks after injury, intimal thickening showed a positive immunofluorescent staining for vimentin, but no staining for desmin. 2– months after injury, the intimal smooth muscle cells showed a positive staining for both vimentin and desmin. Densitometric analyses of SDS-PAGE confirmed the evolution of the changes in the proportions of vimentin and desmin; however they showed that small amounts of desmin are present even in intimal thickening 15 days after injury. These findings suggest that the smooth muscle cells which migrate into the intima after injury first contain practically only vimentin, but, as they mature, develop also desmin-containing intermediate-sized filaments.

DNA-repair after UV-C irradiation in lymphocytes of blood donors

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The UV-light induced DNA-repair synthesis was studied in unstimulated lymphocytes from 34 healthy blood donors aged between 44 and 76 years in 2 independent series. Repair synthesis was measured as the incorporation of H^3-thymidine over 2 h incubation period.

In the first control series (17 persons) the total thymidine incorporation rate was 2143 ± 334 cpm/10^6 cells (mean±SD), in the 2nd (17 persons) 1653 ± 1105 cpm/10^6 cells. This synthesis rate was reduced to 512 ± 91 cpm/10^6 cells by hydroxyurea in the first series, to 562 ± 206 cpm/10^6 cells in the 2nd. For the DNA-repair synthesis a dose-dependent relationship resulted between 2 and 16 J/m^2. 2 persons from the first series and 1 from the 2nd had significantly higher levels of DNA-repair synthesis when compared to the 2 control series where no significant difference was found.

Macromolecules as carrier for opiate ligands: binding to rat brain homogenates and synaptosomal fractions

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Covalent conjugates, between the 2 ligands N^{α}-[D-Ala^2, Leu^5] enkephalyl-N^{ε}-maleimidohexanoyl-lysine amide and 2-[2-(p-bromacetamidophenyl)-ethyl]-5,9α-dimethyl-2′-hydroxy-6,7-benzomorphan hydrobromide (BAB) and macromolecules like lactoglobulin, human serum albumin, thyroglobulin and tobacco mosaic virus (TMV) were tested for their action on opiate receptors in synaptosomes and homogenates of rat brain. Displacement studies with tritiated ethorphine showed in the case of BAB that only the TMV complex increases opioid activity in comparison to the free ligand. The decrease of activity of the 3 other conjugates must be due to steric factors resulting from their shapes.

In comparison, naloxone shows a different behavior in synaptosomes and homogenates: in the first, system naloxone is less active than all the conjugates tested and in the 2nd one it is the most active compound. Do the synaptosomes have mainly kappa-receptors?

Nuclear events possibly subserving differentiation in brain neurons

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The differentiation of postmitotic cells, such as brain neurons, requires a) that proliferation be shut off and b) that successive sets of genes be expressed in an orderly temporal sequence. These events are thought to be mediated by nonhistone chromosomal proteins. We have searched for such putative regulatory proteins in differentiating cerebral cortex and cerebellar neurons. In the period investigated, neurons from both the cortex and the cerebellum undergo transition from actively proliferating precursor cells to nondividing, terminally differentiated neurons. We found that conspicuous fluctuations of a number of nuclear proteins occur in striking temporal coincidence with major developmental events. Concomitantly, the chromatin structure is rearranged and, in cortex neurons, DNA synthesis takes place despite apparent absence of cell division. These changes may be involved in the control of either cell proliferation or gene expression.

A new method for the evaluation of autoimmunity in human male infertility

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Testicular biopsies from infertile patients and controls were examined for the detection of autoantibodies against structures of human testis and particularly against differentiation antigens of germ cells. Biopsies were fixed in 3.5% paraformaldehyde, permeated in methanol and embedded in metacrylate (Francklin, R.M., and Martin, M.T., Histochemistry *72* (1981) 173). Detection of autoantibodies in situ was made using FITC-conjugated goat antisera to human Ig, IgG, IgA, IgM and complement before or after embedding. Sections (1 µm) were simultaneously stained with DNA specific dyes (Hoechst 33258) and observed under the light microscope with an appropriate filter sys-

tem. The combination of immune- and DNA-specific staining allows to investigate autoimmune phenomena in testes of infertile patients.

Molecular cloning of the gene encoding the intermediate chain of the human HLA-DR Ia antigens

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HLA-DR is a cell-surface Ia antigen expressed on B-lymphocytes, macrophages and activated T-lymphocytes. DR antigens are encoded in the major histocompatibility complex. They consist of a polymorphic beta chain and a nonpolymorphic alpha chain. A 3rd chain, called intermediate or invariant chain is probably involved in the assembly and the transport of the HLA-DR complex. We have cloned full-length cDNA copies for the alpha, the beta and also for the intermediate chain by hybrid-selection of mRNA and translation in *Xenopus* oocytes. We have also identified and characterized genomic clones for the intermediate chain gene in a cosmide library and established that it is not located in the major histocompatibility complex.

Biochemical and ultrastructural aspects in human breast tissue

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Cell membrane constituents involved in phospholipid methylation, receptors interaction and intimate ultrastructural topography were investigated in human tissues with diagnosed primary carcinoma or benign fibroadenoma. In carcinomas the methyltransferase I, an enzyme located on the cytoplasmic face of the plasmalemma, showed an activity with a 6-fold increase over the level measured in fibroadenoma tissues. Monosialogangliosides GM_3 and GM_2 as well as monosialoganglioside bound-sialic acid were also more abundant in malignant tissues. In contrast, freeze-fracture preparations of intact tissue revealed significant lower intramembrane particle densities on plasma membrane of malignant epithelial cells than in those of benign tissues. An increased activity of the methyltransferase I indicating an increased synthesis and methylation of membrane phospholipids might fit well with the diminished particle density recorded in malignant tissues and suggest an increased fluidity of the plasma membrane.

In vivo activity of cloned mouse H4 histone gene transcription terminator

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Most histone mRNAs lack poly(A) tails but end in a conserved dyad symmetry element. This sequence element is essential but no sufficient for the production of correctly terminated sea urchin H2A gene transcripts in injected frog oocyte nuclei (Birchmeier et al., Cell *28* (1982) 739). To study the sequence requirements for the generation of histone-like 3′-ends in a more closely related, mammalian tissue culture system, we have fused the 3′-half of the mouse H4 gene, with 230 bp of spacer DNA, to the SV40 early promoter. This plasmid recombinant generates RNA with authentic 3′-termini after DNA transfection into Chinese hamster cells. However, a significant number of transcripts traverse the entire histone DNA segment, as judged both by RNA mapping and by assay for the function of an indicator gene (*E. coli* galactokinase) located further downstream. In addition, we have constructed serial deletions from either end of the histone DNA insert and are now testing these mutants for the generation of correct 3′-termini.

Effect of steroid and pituitary hormones on human autologous mixed lymphocyte reaction

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The autologous mixed lymphocyte reaction (AMLR) is a proliferative response of T-lymphocytes to signals from autologous non-T-cells. The AMLR has the attributes of other immune responses and may represent the in-vitro counterpart of important in-vivo immune regulatory phenomena. The AMLR was found decreased in autoimmune diseases as systemic lupus erythematosis (SLE) and rheumatoid arthritis (RA). Both the incidence and course of these diseases are prominent in women and seem to be influenced by pregnancy and oral contraceptives. Steroid and human pituitary hormones were investigated for their capacity to affect the AMLR. Estradiol, progesterone and testosterone were found to depress the AMLR while the polypeptide hormones tested (GH, PRL, LH, FSH, TSH) did not show any influence. It seems possible to distinguish 2 T-cells populations with different steroid sensitivity. This difference appears unrelated with the known subpopulations of T-helper and T-suppressor cells. The possible implications of these findings are discussed.

Monoclonal antibodies to rat liver cytochrome P450b

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Balb/c mice were immunized with purified rat liver cytochrome P450b (P450b) for 1 year prior to fusing their spleen cells with F0 myeloma cells. 42 of 147 hybridoma cultures synthesized antibodies binding to P450b in a solid phase radioimmunoassay. 24 selected cultures synthesized antibodies binding to liver microsomes of phenobarbital treated rats. The majority of the antibodies (predominantly IgG1's) were able to specifically label P450b on western blots performed with purified P450b or liver microsomes. 9 of these antibodies bound to microsomes of chick embryo liver, 6 to human liver microsomes and 9 to rat intestinal microsomes in the solid phase radioimmunoassay. 6 of the cross-reacting antibodies recognized a single 50,000 M_r protein from human liver and/or rat intestinal microsomes on western blots. Based on differences in cross-reactivity we conclude that we have produced at least 3 different types of monoclonal antibodies to P450b. These antibodies will be useful for the elucidation of the evolution and multiplicity of cytochrome P450b.

The snRNA genes of *Xenopus laevis*

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We have cloned genes coding for several small nuclear RNA species from *X. laevis* using a novel cloning procedure. The RNA probe used for screening a *X. laevis* DNA sequence library was prepared by immunoprecipitating small nuclear RNP particles with an Sm antiserum from a

human patient suffering from systemic lupus erythematosus and radioactively labeled by the addition of ^{32}P-labeled polyA tails. The clones obtained were tested for the presence of functional genes by injection into *X. laevis* oocytes. Examination of the clones has shown that, in contrast to other organisms studied (mouse, chicken, man, Dictyostelium), at least some of the *X. laevis* snRNA genes are arranged in tandemly repeated arrays, and that U2 snRNA genes are found closely linked to both U5 snRNA genes and to tRNA genes. A further unusual festure of the *X. laevis* snRNA gene families is that we have so far found no evidence for the presence of snRNA pseudogenes.

Monoclonal and polyclonal antibodies against protein synthesis initiation factor eIF-3 from rabbit reticulocytes: preparation and characterization

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Polyclonal antibodies were obtained from a rat immunized with purified rabbit reticulocyte initiation factor eIF-3. Monoclonal antibodies were obtained by fusion of spleen cells of Balb/c mice immunized with eIF-3 and a nonsecretory mouse myeloma cell line. We show the specificity of the antibodies and their reaction with the antigen in different species and present data on the function and the biosynthesis of eIF-3.

Expression of a *Streptomyces* neomycin phosphotransferase gene in *Escherichia coli* after in vivo DNA rearrangement

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Plasmid pIJ2 carrying the neomycin (Nm) phosphotransferase gene of *Streptomyces fradiae* was ligated in vitro to the *E. coli* plasmid pBR325. *E. coli* K12 cells harboring the resulting hybrid plasmid pTS2 were ampicillin resistant (Ap^R) but Nm^S, and no Nm phosphotransferase activity was detected in extracts derived from them. However, 8 Nm^R clones could be obtained upon selection. They carried plasmids which represented fusion products between 2 pTS2 plasmids. Recombination in vivo had brought one of the Nm phosphotransferase genes to a position downstream of the *tet*-promoter of pBR325. Subcloning experiments indicated that this is the gene copy expressed in *E. coli* and that transcription is initiated at the *tet*-promoter of pBR325.

Immunoreactive sites of SV40 T-antigen blocked by interaction with mRNPs

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In lytic or abortive infection with SV40, about 10% of large T-antigen is present in the cytoplasm, associated with host mRNPs (Michel and Schwyzer, Eur. J. Biochem., in press). T-antigen is isolated and quantitated by immunoaffinity chromatography using saturating amounts of anti-T-serum. The following observations suggest that immunoreactive sites of T-antigen interact with mRNPs and are therefore inaccessible to antibodies. Under the conditions used to isolate mRNPs (isotonic buffer, pH 7.5, $MgCl_2$), cytoplasmic large T-antigen is poorly immunoreactive. Treatment of mRNPs with EDTA/pH9 or EDTA/RNase A increases immunoreactivity up to 5-fold. In contrast, these treatments do not affect immunoreactivity of nuclear large T-antigen. Monoclonal antibody PAb416 (provided by E. Harlow) reacts optimally in the absence of EDTA/RNase, suggesting that it recognizes a site which does not interact with mRNPs. Other monoclonal antibodies are presently tested to define the sites which might be involved in mRNP binding.

Noncoding sequences interrupt a chloroplast gene for a chloroplast protein of *Euglena gracilis*

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We have shown (Rutti et al., FEBS Lett. *134* (1981) 15) that the EcoRI-N fragment of *Euglena* chloroplast DNA codes for a 53 kD soluble chloroplast protein. To define the coding sequences of that protein, we measured the transcribed region by gel electrophoresis of nuclease S_1-resistant RNA-DNA hybrids, using chloroplast RNA and different DNA fragments from cloned EcoRI-N and from the adjacent EcoRI-Q. We show that the size of the stable transcript is about 1.9 kb, 1.7 kb being on EcoRI-N and 0.2 kb on EcoRI-Q. Alkaline gel analysis reveals 3 DNA fragments protected from S_1 digestion by the mRNA, suggesting that the gene is composed of at least 3 exons. *Euglena* chloroplast genome thus contains interrupted protein coding genes as seen in eukaryotes. This is surprising because no interrupted protein genes were found sofar on chloroplast genomes of higher plants and in view of the prokaryotic nature of chloroplasts. Hallick (pers. comm.) showed that EcoRI-N interacts with the bacterial *tuf*-gene, suggesting that the analyzed gene codes for the chloroplast Tu-elongation factor.

Isolation and characterization of DNA sequences at junctions between satellite and nonsatellite DNA from the chromatin eliminating nematode *Ascaris lumbricoides*

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During early cleavage divisions of *A. lumbricoides*, about 25% of the nuclear DNA is expelled from the presumptive somatic cells, thus leading to soma cells which contain considerably less DNA than germ-line cells. In order to elucidate the mechanism of chromatin elimination at the molecular level, DNA sequences containing both satellite and nonsatellite DNA were selected, isolated and amplified by cloning. Regions at junctions between satellite and nonsatellite DNA have been sequenced. We hope that this technique permits to fish out border sequences between the eliminated and the retained portion of the genome in *A. lumbricoides*.

Muscle fiber transformation in the rat

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Unilateral removal of Sternohyoid superior muscle in the rat induced a muscle fiber transformation in the contralateral muscle. This rapid and reversible transformation is characterized - as previously reported - by a massive increase of type-I slow fibers which are almost lacking in control muscles. In addition to earlier histochemical and immunohistochemical evidence the present results clearly show the differences in peptide pattern of the isolated

heavy chains between control and experimental muscles whereas the light chain pattern remains unaffected. It is the myosin heavy chain which is the site of ATPase activity, and therefore, the observed changes support both histochemical and immunohistochemical findings. For a change in light chain pattern it is likely that a much stronger stimulus is needed.

A 39K substrate of tyrosine protein kinases is located at the plasma membrane

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The intracellular distribution of p39, a substrate of several tyrosine protein kinases, was determined by immunofluorescence microscopy. Very similar results were obtained from untransformed and Rous sarcoma virus-transformed fibroblasts. No binding of anti-p39 IgG occurred to intact cells. Following permeabilization of fixed cells, a uniform labeling was observed, fluorescence being most pronounced in membrane ruffles and in areas of cell-cell contact. When detergent-permeabilization preceeded fixation, labeling of a striking reticular lattice was produced. An identical pattern was observed with fluorescent lectins as plasma membrane markers, but not with IgG against several cytoskeletal proteins. We conclude that p39 is, at least in part, located at the cytoplasmic surface of the plasma membrane, where it may participate in mechanical activities of the membrane.

Changes in chromatin structure of the early histone genes during embryogenesis of *Psammechinus miliaris*

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During embryogenesis of the sea urchin *P. miliaris*, the pattern of histone gene expression changes. The genomic DNA clone h22 codes for the major class of early histone genes. We studied the chromatin structure of the h22 genes at various developmental stages using DNase I and micrococcal nuclease (MN). At a time of maximal h22 expression (128 cell stage) the arrangement of nucleosomes on gene coding sequences is not unique. 5′ to the genes, around the TATA box, there are sites hypersensitive to MN and to DNase I, and some sites 3′ to the genes cut by MN in naked DNA are 'protected' in chromatin. At hatching blastula, when h22 transcription is diminished, the MN pattern changes to that of deproteinized DNA, which is the same as the sperm, late blastula and gastrula chromatin patterns. By late mesenchyme blastula, the DNase I 5′ hypersensitivity also disappears. This preferential accessibility or protection of parts of the histone gene chromatin parallels the observed expression of the h22 gene cluster.

One-step purification of the components of Semliki Forest Virus

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By using 3 linear NaCl gradients of different steepness, the components of twice purified (sucrose and tartrate gradients) Semliki Forest Virus can be separated on a SP-trisacryl column (6×60 mm) in 8 M urea, 5% acetic acid, 0.5% triton X-100 and 1 mM dithiothreitol. The RNA eluted with the starting buffer, the membrane proteins E_3, E_2, E_1 between 100 and 350 mM NaCl and the core protein between 350 and 750 mM NaCl. The proteins were electrophoretically pure and displayed only 1 band on SDS-polyacrylamide gels. Separation on urea-triton X-100 gels of the proteins, however, revealed significant microheterogeneities in charge and hydrophobicity. Separation on a Con-A sepharose column showed that the membrane proteins of virions propagated in *Aedes albopictus* cells are eluted with 5 mM α-methyl mannoside.

Nerve growth factor regulates neuropeptides in primary sensory neurons

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Administration of NGF to newborn rats results in a significant increase in both substance P- and somatostatin-content in dorsal root ganglia and spinal cord. The physiological importance of endogenous NGF for the development of substance P- and somatostatin-containing neurons is demonstrated by the effect of anti-NGF antibodies: Application of either purified heterologous or monoclonal anti-NGF antibodies to neonates results in a marked, but temporary reduction of both peptides in spinal ganglia and spinal cord. However, the effects are permanent if the antibodies are injected directly into the embryo. These results indicate that NGF is a trophic agent for substance P- and somatostatin-containing sensory neurons. Since these peptides are present only in subpopulations of dorsal root ganglia neurons it is conceivable that NGF regulates other peptides in sensory neurons as well. This assumption is supported by our immunohistochemical and biochemical findings showing that the content of vasoactive intestinal polypeptide- and cholecystokinin-like immunoreactivity is increased by exogenous NGF in spinal sensory neurons.

Search for the hypothetical protein encoded by the open reading frame of the MMTV long terminal repeat

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The long terminal repeat (LTR) of mouse mammary tumor virus (MMTV) contains an open reading frame (orf) which could code for a polypeptide of 36 kd. An antiserum raised against a peptide of 23 amino acids contained in the 36K protein (synthesized by B. Gutte, University of Zürich) is being used to search for a corresponding antigen in vivo. Immunoprecipitation of products translated from viral RNA in vitro gives polypeptides of 36, 24, 22 and 18 kd as predicted by the position of the initiation codons in the DNA sequence. Mouse mammary gland tissue and various cell lines grown in presence or absence of hormone have been tested for the presence of the protein. The antibody recognizes a band of about 55 kd, specific to lactating mammary gland cells, which could be a modified product of the orf. Other cellular proteins recognized by the antibody appear to be unrelated proteins which have cross-reacting antigenic determinants. Further studies are underway to definitively identify an orf-encoded protein in various tissues and cell lines.

Parvalbumin, a neuronal marker in brain cell cultures

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Brain cell cultures were derived from 14-days-old embryonic mice plated on precoated polylysine dishes and

grown in MEM Dulbecco medium containing 10% fetal calf serum. As evidenced by indirect immunofluorescence about 90% of the cells (several developmental stages have been investigated) exhibiting typical neuronal morphology, were intensively labeled by antiparvalbumin serum. The cells were identical to those marked by the neuronal $\gamma\gamma$-enolase but different from those stained with antisera directed against 2 glial markers S-100 and MBP. The presence of parvalbumin in cell culture extracts was shown by HPLC, 2D gels and immuno-blotting. The combined results establish the Ca^{2+}-binding parvalbumin as a neuronal marker in brain cell cultures.

A plasmid-based test system for in vitro screening and mechanistic studies of mutagenic agents

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Primary mutagenic events which take place on the DNA level can be elucidated by means of a novel plasmid-based test system. Plasmid pBR322 was exposed in vitro to a series of established mutagens (acridine orange, acridine yellow, cis-platinum-II-diammine-dichloride, dimethyl sulfate and triethylenemelamine). Results were topologically and biologically analyzed. Mutagen-caused single- and double-strand breaks, intercalations and adduct formations were assessed by using electrophoretic techniques, cross-link formations by means of differential sensitivity of the rapidly renatured plasmid DNA to the single-strand specific nuclease S1. Biological function impairment was analyzed by transfecting treated plasmids into *E. coli* (amp^s, tet^s) and then scoring the decrease of plasmid-conferred resistance to ampicillin and tetracyclin.
The plasmid-based mutagenicity test system is simple and quick to carry out, and has the additional advantage that the target dosage can be controlled exactly.

Development of afferents to the striate cortex of the tree shrew *(Tupaia belangeri)*

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In cat and monkey the retino-geniculo-cortical projection develops from an initially diffuse to a finally segregated pattern when ocular dominance columns are formed. In *Tupaia* the input from the 2 eyes is not segregated in vertical columns; there is a tangential organization showing overlap as well as segregation of the input from the 2 eyes. We used the transneuronal transport of radioactive amino acids in order to study the development of the projection in *Tupaia*. Our results show that the projection is not diffuse in the early postnatal period when the visual system is still very immature. On the contrary, the pattern of the fibre projection seems to be even more detailed than later on. This phenomenon raises fundamental questions as far as developmental mechanisms are concerned.

Intracellular degradation of newly synthesized collagen in skin fibroblasts from a patient with prolidase deficiency

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Since prolidase deficient patients excrete large amounts of urinary dipeptides with proline or hydroxyproline (Hyp) at the C-terminus, we investigated the extent of intracellular collagen degradation (ID) and the pattern of degradation products in cell cultures. Cells at early confluency were incubated for 24 h in medium containing ^{14}C-proline, serum and ascorbate. Iminoacids and peptides were separated by ion exchange chromatography and ID estimated by the amount of dialyzable Hyp per total Hyp formed. In unhydrolyzed control cultures, most of the dialyzable fraction was in free form (61%). In the mutant culture, dialyzable Hyp was mainly in peptide form and only 21% in free form. After acid hydrolysis, the chromatographic patterns of dialyzable fractions from both cell strains were identical. We concluded that prolidase plays an important role in the generation of free Hyp. However, the extent of ID in prolidase deficient cells was normal i.e. 33% (Steinmann et al., FEBS Lett. *133* (1981) 142).

Immunocytochemical localization of prosomatostatin fragments in maturing and mature secretory granules of pancreatic and gastrointestinal D-cells

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Pancreatic and gastrointestinal D-cells were examined by ultrastructural immunocytochemistry using antisera against somatostatin-28 (SS28) and its N-terminal fragment SS28(1-12), followed by the protein A-gold (pAg) complex. In pancreatic and gastric D-cells incubated with SS28(1-12) antiserum the gold particles produced intense staining of the mature secretory granules but weaker staining of the immature granules associated with the Golgi area, while following SS28 antiserum treatment they accumulated selectively over the population of immature secretory granules. In intestinal D-cells not only SS28(1-12) but also SS28 antiserum produced an intense gold staining over the mature delta granules. These observations show that the relative amounts of immunoreactive sites related to SS28 and its cleavage product SS28(1-12) in maturing and mature secretory granules are different in pancreatic, gastric and intestinal D-cells.

Multiple genes for the polymorphic β-chains of the human HLA-DR Ia-antigens

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HLA-DR is one of the locus of the major histocompatibility complex of man which controls the immune response and codes for highly polymorphic Ia-antigens. The polymorphic component is the β-chain. Several full-length cDNA clones for the HLA-DR β-chain have been isolated from a homozygous individual. 4 distinct cDNA clones, corresponding to 4 DR β-chain genes have been characterized and their structure compared. 3 distinct DR β-chain genes have been isolated from a genomic library made from the same individual. The HLA-DR system is therefore not only polymorphic but also polygenic. Comparison of these 3 genes revealed a remarkable conservation of sequence in the noncoding and flanking regions. Some of these HLA-DR genes have been transferred into animal cells and in certain cases their expression has been documented.

Microtubules in *Dictyostelium discoideum*: immunocytochemistry with polyclonal and monoclonal antibodies

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We used indirect immunofluorescence to study microtubules (MTs) in *D. discoideum*. MTs were not stained with 2 of 4 polyclonal antibodies (ABs) raised in rabbits against dog brain tubulin; the 2 other polyclonal ABs gave a weak to moderate staining. A monoclonal mouse AB against pig brain tubulin (Viklicky et al., Cell Biol. Int. Rep. *6* (1982) 725) also gave negative results, but a monoclonal rat AB against yeast tubulin (Kilmartin et al., J. Cell Biol. *93* (1982) 576) yielded crisp and bright staining. The MT-system undergoes changes related to the division cycle. The elaborate system of cytoplasmic MTs characteristic of interphase disappears at the beginning of mitosis; the spindle is then the only fluorescent structure. During ana-telophase the spindle grows longer and thinner; a complex of astral MTs appears concomitantly and evolves to the interphase system in postmitotic cells.

Regulation of the mRNA for the inhibitor of extracellular cyclic AMP phosphodiesterase of *Dictyostelium discoideum*

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The inhibitor (PDI) is a glycoprotein synthesized during development of *Dictyostelium* which binds to the phosphodiesterase and inactivates it. Following in-vitro translation of RNA and immunoprecipitation with anti-PD serum, newly synthesized PDI can be detected by SDS-polyacrylamide gel electrophoresis and fluorography. PDI can be labeled using ^{35}S-cysteine but not ^{35}S-methionine and can be detected after cell-free translation only after previous acetylation. Purified native PDI blocks immunoprecipitation of the in-vitro synthesized polypeptide. No translatable PDI mRNA was detected in growing cells. mRNA reached a maximal level after 3 h of starvation. In the presence of 1 mM cAMP, the appearance of PDI mRNA was delayed. No mRNA could be detected after the addition of cAMP-S, a slowly hydrolyzed cAMP analogue. Following removal of cAMP-S, the mRNA appeared within 1 h and PDI was secreted after another hour.

Nucleotide sequence of an *Euglena* chloroplast 16S rRNA gene not integrated in a rRNA operon

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The *Euglena gracilis*, Z, chloroplast genome contains 4 16S rRNA genes, 3 of which are integral constituents of functional rRNA operons while the 4th 16S rRNA region maps about 3.1 kbp outside of the operon cluster and has no corresponding large subunit rRNA genes. We have sequenced this extra 16S rRNA gene and its flanking regions. The extra 16S rRNA gene contains 1486 positions and is to 98% identical with the recently sequenced 16S rRNA gene (1491 positions). Its leader contains a cluster of pseudo-tRNA genes quite similar to the leader in the intact operons. Sequence homology stops abruptly 16 positions after the 3'-end of the structural gene. This extra 16S rRNA gene, compared to the operon 16S rRNA gene, shows 21 mismatches, the most important being a deletion of 9 bp which interfers with the formation of a stem-loop structure in a conservative region. All other base changes do not interfere with the secondary structure model of the stable transcript, i.e., it could be a functional rRNA. The leader with the presumptive promoter is conserved, therefore this extra 16S RNA gene might be transcribed.

Transformation of mice with an in vitro mutated mouse α-globin gene

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In order to allow the detection of a newly introduced homologous gene in a transgenic organism a strategy was devised whereby a cloned mouse α-globin gene was mutated in vitro by insertion of linker DNA in its 3' untranslated region. The mutated versions are shown to be expressed at a level comparable to the unmodified gene in a transient expression assay in HeLa cells. Furthermore, we show that the artificially introduced mutations are sufficient for the easy identification of a) the transcripts of the mutated genes in a mixture containing up to 95% wild type mRNA, and b) the newly acquired mutant genes in transgenic mice and in their offspring. Preliminary data suggest that the newly acquired homologous genes are very poorly if at all transcribed in the transgenic mice. We are currently investigating the possible reasons for this reduced expression.

Induction of differentiation of human T-lymphoid leukemia cells with phorbol-12-myristate-13-acetate

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Phorbol-12-myristate-13-acetate (PMA) induced a reduction of cell growth and profound phenotypic changes which were detected by murine monoclonal antibodies against human T-lymphocytes specific for discrete stages of T-cell differentiation (OKT series). After 4 days of treatment of CEM cells with 15 nM PMA, 71–98% of the cells expressed reactivity with OKT3 and OKT8 antibodies, whereas reactivity of the cells with OKT4 and OKT6 was markedly reduced (1–4%). These results indicate that treatment of CEM cells, with PMA can cause the cells to express a phenotype that resembles that of a mature suppressor T-cell.

DNA sequences and proteins of the minute virus of mice

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We have determined the nucleotide sequence of the DNA of the lymphocyte-specific immunosuppressive virus MVM(i) and we compare it with that of the fibroblast-specific MVM (Astell et al., private communication). As suggested by our previous restriction site mapping, the 2 viruses have very similar sequences with a few differences. Distinctive sequence features were seen near the viral 5'-end including a region with very high adenine plus thymine content, which could be an origin of replication of the viral DNA.

We isolated replicative-form DNA from infected cells using detergent but no protease. This DNA was cleaved with HaeIII enzyme and the fragments separated by gel electrophoresis using protease-treated DNA as a control. 2 DNA bands from the 2 ends of MVM DNA were absent from the

pattern when protease treatment was not done, suggesting that these fragments contain very tightly bound protein.
A similar result has been reported for the parvovirus H_1 (Revie et al., PNAS *76*, 5539).

Ribonucleotide reductase activity in cold- and heat-sensitive mammalian cell cycle mutants

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A heat-sensitive (hs, multiplying at 33 °C, arrested in G_1 at 39.5 °C) and a cold-sensitive (cs, multiplying at 39.5 °C, arrested in G_1 at 33 °C) clonal cell cycle mutant isolated from the same clone (K 21) of the P-815 murine mastocytoma line were tested for ribonucleotide reductase (RR) activity, using cells made permeable to nucleotides. After shift of mutant cells to the nonpermissive temperature, RR activity decreased and low levels (i.e. less than 10% of those observed at permissive temperature) were attained within 16–24 h in hs and after 2–4 days in cs mutant cells. These changes with time of RR activity are similar to those of thymidine kinase (Schneider et al., Experientia *37* (1981) 660) and of numbers of DNA-synthesizing cells. RR activity of revertants obtained from hs and cs mutants was comparable to that of wild-type K 21 cells.

Effects of DNA de novo synthesis inhibitors on mammalian neurulation in vitro

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The central nervous system (CNS) is the first system to develop in mammals. A technique has become available (New, D., Biol. Rev. *53* (1978) 81) which permits the detailed study of normal and abnormal morphogenesis of neurulation in vitro. Rat embryos were cultured in the presence of methotrexate (MTX) or azathioprine (AZ) from the beginning of neural plate formation to the definitive CNS primordium stage in order to determine which neural tube regions are the most sensitive to chemicals which interfere with DNA de novo synthesis.
Both drugs caused concentration-dependent effects on overall embryonic development. However, concentration thresholds were found where embryonic development remained unchanged, but where the fore- and hindbrain regions were severely affected. This indicates that during neurulation cell divisions and/or cell movements are more pronounced in the fore- and hindbrain regions, and that these regions are, therefore, more susceptible to chemicals affecting normal DNA synthesis.

DNA polymerase α-, β-, and γ-activities in a cold-sensitive mammalian cell-cycle mutant

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A cold-sensitive (cs, multiplying at 39.5 °C, arrested at 33 °C) clonal cell-cycle mutant isolated from the murine P-815 cell line was tested for DNA polymerase α-, β- and γ-activities. After shift of mutant cells to 33 °C, polymerase α-activity decreased to approx. 15% of the initial value within 6 days, whereas less than 2% of cells were in S-phase already after 2 days. Polymerase β- and γ-activities exhibited a maximum at 2 days, and at 5–6 days, values were 20% (β) and 100% (γ) of the initial activity. After return of arrested cells to the permissive temperature, polymerase α-activity increased with nearly the same time course as the number of DNA-synthesizing cells, i.e. with a lag phase of approx. 20 h, while polymerase β- and γ-activities decreased for 2–3 days before increasing again. In wild-type cells, polymerase α- and γ-activities did not change significantly after shift from 39.5 °C to 33 °C, while polymerase β-activity decreased by approx. 50%.

Sequence analysis of the chloroplast ribosomal spacer of *Chlamydomonas reinhardii*

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The sequence of the 1.7-kb chloroplast ribosomal spacer of *C. reinhardii* between the 16S and 23S rRNA genes has been determined. This spacer contains the genes coding for tRNA ile and tRNA ala as in the chloroplast ribosomal units of Tobacco, maize and *Euglena* and in at least 3 ribosomal spacers of *E. coli*. These 2 tRNA genes do not contain introns in contrast to the corresponding genes of higher plants. The tRNAs ile and ala are 82 and 97% homologous resp. to the corresponding *Euglena* tRNAs. However no significant sequence homology exists between the remaining portions of the spacer of *C. reinhardii* and those of higher plants and *Euglena*.

Selective uptake and transneuronal transport of wheat-germ agglutinin in the central nervous system

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^{125}I wheat-germ agglutinin (WGA) was injected into the eyes of turtles and pigeons. In turtles, all primary visual stations were heavily labeled due to *anterograde* axonal transport of WGA from the retina. In addition, there was consistent labeling of some caudal mesencephalic cells which indicates *retrograde* transport of label. *Transneuronal* transport was observed in anterograde-anterograde and anterograde-retrograde direction. In another species, the pigeon, however, the primary visual stations, and the isthmo-optic nucleus (ION) known to project to the retina, were labeled weakly and only after huge amounts of WGA injected. In contrast, horseradish peroxidase labeled the ION quite heavily. *Selective uptake and/or transport* of WGA was observed also in turtles, where injections into the cerebellar cortex showed retrograde labeling in the parallel fibre system but scarcely any transport in the mossy fibre afferents.

Cell wall and membrane associated forms of acid phosphatase in yeast

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Acid phosphatase (a. pase) of yeast is a cell surface enzyme that, if tested with nitrophenylphosphate (NPP) as substrate, seems to be mainly located in the cell wall. The following observations indicate that there exists also a plasma membrane-bound form of a. pase that is however enzymatically inactive against NPP: 1. A Triton extractable glycoprotein fraction reveals fingerprint patterns that are very similar to those of the cell wall-bound enzyme and partial amino-acid sequences of tryptic peptides of the triton extractable protein(s) match with the DNA sequence of the structural gene of a. pase. 2. In plasma membranes having essentially no NPPase activity we can detect proteins that do react with antibodies raised against purified cell wall a. pase. There is no evidence that the membrane-bound a. pase is a kinetic precursor of the cell wall a. pase.

Under different physiological conditions the 2 forms of a.pase are differentially expressed (Schweingruber and Schweingruber (1982)).

Tubulin and tubulin genes from African trypanosomes

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African trypanosomes are the causative agents of human sleeping sickness. We are presently characterizing tubulin proteins and tubulin genes from *Trypanosoma brucei*.
A genomic library has been constructed in phage lambda 1054. The library was screened with chicken cDNA probes for tubulin genes, and recombinant phages were isolated and analyzed. The genomic arrangement of tubulin genes was deduced from southern blot hybridization of total genomic DNA. Results from both lines of experimentation were in good agreement. In conclusion, the large majority of the multiple α- and β-tubulin genes of *T. brucei* are arranged within one tightly packed cluster of alternating α- and β-genes.

Cleavage of a membrane receptor is required for the translocation of polymeric IgA antibodies across epithelia

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The receptor mediating transport of IgA antibodies across epithelia is identical with a transmembrane precursor (mSC) of secreted secretory component (SC). During transport, the IgA receptor is cleaved into a membrane anchorage peptide and a secreted peptide (secreted SC) which remains bound to IgA dimer. Monoclonal antibodies have been produced with specificity against both domains of membrane SC. One antibody recognizes the IgA binding site of the secreted domain, a 2nd one the 15 RD cytoplasmic extension of mSC. Studies on the biosynthesis of SC in mammary glands revealed that synthesis of mSC is rapid, glycosylation is completed within 30 min, most mSC expressed at the cell surface is occupied by IgA antibodies, and the appearance of mature secreted SC as a result of cleavage of membrane SC is demonstrable after 60 min. Cross-reactivity of the monoclonal antibody with rat liver mSC allows the dissection of the IgA transport process by cell fractionation.

The study of cell lineages during neuroectodermal differentiation using monoclonal antibodies as probes

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S/D rats have been immunized with superior cervical ganglia, dorsal root ganglia (DRG) and adrenal medulla taken from newborn mice. After the fusion of the rat splenocytes with a mouse myeloma line (45.6.TG.1.7.5 from E.A. Kabat's laboratory, Columbia Univ.) according to Milstein and Köhler (1976), we have used the ELISA technique in order to screen for PNS specific hybridomas. About 10 monoclonal antibodies (MABs) have been further tested in cryostatsections of mouse tissue, using the indirect immunofluorescence technique. One MAB is of particular interest as a DRG specific intracellular cell marker in newborn mice. It can be detected as early as day 10 (E 10) of gestation. So far, E 8 sections have not been stained with this MAB. A further characterization of this cell marker and the antigen by using the western blot technique is in progress.

Changes in the pattern of synthesis of axonal proteins concomitant with synapse formation

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Fetal chicken sensory ganglia cells were cultured in the center compartment of the 3-compartment cell culture chamber devised by Campenot (PNAS *74* (1977) 4516). The axons grown into the side compartments were cocultured with spinal cord cells. 4 days after plating, the spinal cord cells showed synaptic input upon extracellular stimulation of the sensory ganglia axons. Newly synthesized axonal proteins, metabolically labeled with [^{35}S]methionine (added to the somas), had similar patterns in 2-dimensional SDS-polyacrylamide gel electrophoresis in the presence and absence of postsynaptic target cells. However, one protein disappeared and a small number of proteins newly appeared in the presence of synapse forming target cells. Studies of the biochemical properties and functions of these proteins may further our understanding of the development and organization of the nervous system at the molecular level.

Different types of antennal sensilla in *Drosophila* project into different glomeruli of the brain

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Cobalt fills show that multineuronal sensilla on the 3rd antennal segment of *D. melanogaster* predominantly project into 10 antennal glomeruli identifiable by their size, shape and location in the brain. Most of the glomeruli are innervated from both antennae, but a few receive exclusively ipsilateral input. Every sensory fibre appears to terminate only in one specific glomerulus. Each type of sensillum at a particular location is connected with a specific set of glomeruli. With one exception, projections from all sensillar types consist of an ipsilateral and a bilateral component. Fills from the same sensillar type located at different sites yield similar patterns, suggesting that the projections observed reflect predominantly the type of sensillum rather than its location on the antenna. Accordingly, individual glomeruli might represent functional units, each receiving a characteristic combination of antennal input.

Morphology and meiosis of bovine oocytes taken from ovaries collected after slaughter

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The use of ovaries collected after slaughter as a source of large numbers of immature oocytes gave us the opportunity to study the variable morphology and the capacity for in-vitro maturation of such a material. Each oocyte (20-30 per ovary) was scored by virtue of its surrounding cumulus mass and its morphology was described (after 24 h incubation) in terms of the properties of the ooplasm, the presence or absence of an ooplasmic membrane and a polar body. The capacity for spontaneous in vitro maturation was investigated by means of culturing the oocytes and subsequent staining for chromosomes. These experiments revealed that a compact cumulus mass, a dark, finely

granulated ooplasm and a smooth, intact membrane were essential features for an oocyte to mature in our culture system. Besides, these experiments demonstrated the wide range of the resulting meiotic configurations, namely 35% at a germinal vesicle stage, 15% at diakinesis and 15% at metaphase II (35% could not be clearly classified).

Assessment of micronuclei in blood erythrocytes of intact and splenectomized rats

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In order to investigate the role of the spleen in eliminating micronucleated erythrocytes, intact and splenectomized rats were treated i.p. with single doses of 0.5 mg/kg triethylenemelamine (TEM) on postnatal day 20. The 2 corresponding control groups were treated with the solvent (water) only. Micronuclei were assessed in polychromatic blood erythrocytes 24, 48 and 72 h after treatment. In both treatment groups effects were most pronounced after 48 h. The micronucleus rate was, however, considerably higher in the splenectomized rats (12.0%) than in the intact rats (6.4%). This tendency was also present in the control groups, where the incidences of micronuclei were 1.0 and 0.2%. These results indicate that in rats micronucleated erythrocytes are eliminated by the spleen. This elimination process, may, at least in part, be responsible for the low micronucleus rates usually found in the blood erythrocytes of rats in comparison to those in the bone marrow erythrocytes.

In-vitro transcription of SV40 chromosomes

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We are using in-vitro transcription with Hela cell extracts to determine the mechanism by which SV40 chromosomes give primarily late type RNA, while in naked SV40 DNA the early promoter is preferentially active. We think that this specificity is not due simply to a structural feature of the viral chromosome. When soluble factors are removed from the SV40 chromosomes by gradient centrifugation, their transcription pattern is altered. The existence of diffusable factors modulating transcription is also suggested by the observation that extracts of SV40-infected cells can increase the activity of the late promoter on in-vitro transcribed naked SV40 DNA. The factor does not seem to be T-antigen since removal of free T-antigen and T-antigen-containing chromosomes from SV40 chromatin preparations has little effect on the amount of late transcription. We also prepared chromatin from SV40 virions, using an EGTA disruption method, and studied its structure and properties as a template for transcription in vitro.

Binding sites for monoclonal antibodies on SV40 large T-antigen

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Limited tryptic proteolysis cleaves SV40 large T-antigen into a number of fragments, which have been mapped by amino-acid sequence analysis (Schwyzer et al., J. biol. Chem. *255* (1980) 5627). We have used this cleavage technique to determine the binding sites of monoclonal antibodies PAb402, 416, and 423 (Harlow et al., J. Virol. *39* (1981) 861). T-antigen fragments are separated by electrophoresis and transferred to nitrocellulose strips. Antibody binding to the fragments is revealed with peroxidase- or ^{125}I-labeled 2nd antibody. The results show that the binding sites for PAb416 and 423 are located near the N- and C-termini of T-antigen, within amino-acid residues 1-130 and ~ 590-708, respectively. PAb402 cannot be mapped in this way, because it does not bind SDS-denatured T-antigen. However, it protects a specific region of native T-antigen (amino acids ~ 450-520) from tryptic cleavage. The mapped antibodies will be used to study the topography of T-antigen mRNP complexes (see abstract by Michel et al.).

Action of aldosterone (A) and thyroid hormone (T_3) in regulating pleotropic responses in the toad urinary bladder

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In the toad bladder, T_3 had no effect on baseline Na^+ transport but antagonized the mineralocorticoid action of A. Tissues were treated with 80 nM A and/or 60 nM T_3 (control; T_3; A; A + T_3) and then labeled for 1 h with ^{35}S-methionine. We used 2D gel as described by O'Farrell to examine whether the antimineralocorticoid action of T_3 might be mediated by alteration of the rate of biosynthesis of specific proteins. After 18 h exposure to A the basal rate of synthesis of at least 3 proteins was increased, while the rates of synthesis of others were decreased. T_3 alone had a distinct pattern of induction and repression of several proteins. When both hormones were added simultaneously, 2 of the 3 proteins induced by A alone were selectively repressed in the presence of T_3. We conclude that a) both T_3 and A have distinct hormonal domains of response and b) the 2 domains overlap at least for 2 aldosterone-induced proteins which are selectively repressed by T_3.

Role of gravity on cell growth

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We report on the effect of high-g on growth and locomotion of different cell types in vitro. This study makes part of a program of ground-based investigations supporting 3 experiments with cell cultures performed in the Spacelab. Human lymphocytes, chicken embryo fibroblasts, sarcoma Galliera, HeLa and Friend leukemia cells were grown at high-g (between 2 and 10 × g) in a centrifuge at 37 °C. The growth rate was determined by incorporation of ^{3}H-thymidine and by cell counting. In all instances cell growth is increased by 20-30% at high-g compared with the 1 × g controls. Experiments with Friend cells show that the consumption of glucose in the medium is approximately equivalent at high-g and 1 × g. Cell locomotion has been investigated on substrates coated with colloidal gold: Movements of HeLa cells are remarkably reduced at 10 × g. We interpret this results as follows: High-g environment prevents cells from normal locomotion and cells are capable to reallocate part of the energy spent for migration in favor of proliferation.

Metastasis, migration and homotypic adhesion of B16 melanoma cells selected through nuclepore filters

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Cells selected through 2-μm-pore filters were compared with the Fl parent line and clone Fla in the wounded monolayer assay. The decrease in cell numbers/0.1 mm distance from the wound line was calculated after 16-h incubation. Comparing cell number decrease, filter pene-

tration values (3 µm), and metastasis in percent, we obtained: Fla 9.1/16/28, Fl 5.9/102/55, No. 10 5.0/336/55 and No. 4 3.6/538/79 respectively. Time lapse cinematography showed elevated and same motility in selected lines No. 10 and 4 versus Fl. It further revealed that not only motility but also cell-cell adhesion influence the spread into the wound area. When aggregates of lines Fla and Nr. 4 were sheared by pipetting, Fla showed a 3-fold higher adhesion than Nr. 4. High motility alone may not lead to higher metastasis but has to cooperate with other factors such as reduced adhesion. The low metastatic rate of Fla supports the concept that high homotypic adhesion can be a limiting factor during early events when cells leave a primary tumor.

The ontogeny of a mouse cell surface antigen: from the ovary through embryogenesis to the adult intestine

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A monoclonal antibody E9 has been raised against embryoid bodies of a multipotential mouse teratocarcinoma OTT2289. The monoclonal antibody is not only specifically reacting with the surface of endodermal cells from OTT2289 derived embryoid bodies but also with 2 teratocarcinoma derived endodermal tumors TDE 274 and TDE 305. During mouse embryogenesis, the antigen recognized by this antibody is first detected on unfertilized eggs and on the zona pellucida. It is also present on fertilized eggs, embryos at 2- to 8-cell stages, and morulae but disappears on the trophectoderm of blastocysts. The antigen is found, albeit at weak concentration, on the inner cell mass (ICM) of blastocysts and, at high concentration, on the primitive endoderm surrounding the ICM. In the postimplantation embryo at days 7-8 of gestation, the antigen is detected exclusively on the visceral endoderm and continues to be associated with this cell layer of the yolk sac at day 9 of gestation. In the adult mouse, endodermal derivatives such as the intestinal duodenum retain their reactivity to the specific antibody. We propose that this antigen first appears within the ovary, remains expressed during early embryogenesis, and becomes limited to the endodermal cell lineage. Further investigation on the molecular characterization of this antigen should reveal its possible function during mouse development.

A novel double-line staining pattern of skeletal muscle M-lines upon incubation with monovalent anti-M-creatine kinase antibodies

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Incubation of chicken skeletal muscle fibers with anti-M-creatine kinase (CK) IgG and with an excess of anti-M-CK Fab fragments leads to heavy decoration of the M-line and to removal of the electron-dense M-line structure (Wallimann et al., PNAS *75* (1978) 4296), respectively. On the other hand, incubation with low concentrations of anti-M-CK Fab does not extract but rather decorate the M-line giving rise to a distinct 2-line staining pattern. The 2 decorated lines appearing in the middle of the A-band are spaced axially 42-44 nm apart and correspond most likely to the 2 M4 and M4′ m-bridge rows described by Sjöström and Squire (J. Microscopy *111* (1977) 239). It is concluded that the muscle-specific form of creatine kinase, MM-CK, contributes mainly to the electron density of these M4 and M4′ m-bridges within the M-line structure. This specific labeling pattern is a further demonstration that CK is an integral part of the M-line.

The myofibrillar localization of creatine kinase (CK) is isoenzyme-specific

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The presence of M-line bound CK in cultured myogenic cells is demonstrated by removing the unbound sarcoplasmic CK through permeabilization with Triton X-100 and extensive washing of the cells prior to immunofluorescence staining. When stained with antibodies specific for M-CK subunits these cells exhibit bright fluorescence within the M-line region of myofibrils. Anti-B-CK incubation, in contrast, results in a weak, diffuse fluorescence at the Z-band. Even though these cells contain appreciable amounts of B-type CK, specific fluorescence at the M-line is never observed with anti-B-CK antibody thus ruling out the presence of BB- or MB-type CK at this location. Therefore the presence of CK within the M-line structure of myogenic cells which contain all 3 CK isoenzymes seems to be restricted to the MM-type isoenzyme. MM- and BB-CK refer to the muscle and brain type of creatine kinase, MB-CK represents the hybrid form.

Abnormal cilia and ciliary elements in microvilli in cells of a xenografted human germ cell tumor

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Aberrations of the 9+2 arrangement in cilia and sperm flagella include variations in the number of the 9 peripheral microtubular doublets and/or the 2 central microtubules, as well as absence of related structures such as dynein arms, spokes, nexin and others. In this study, ciliated cells of a testicular teratoma were analyzed ultrastructurally after growth of small fragments in nude mice. The cilia displayed aberrations in the number of the peripheral doublets similar to nonneoplastic 9+2 structures. The frequency of ciliary aberrations per cell, however, seems to be much higher in these tumor cells. Interestingly, in microvilli of these ciliated cells, single peripheral doublets were detected. Obviously, cilia can serve as structural markers in tumor differentiation. The presence of ciliary elements in microvilli raises questions concerning early ciliary differentiation and tumor specific formation of cilia.

Implantation site and expression of the invasive phenotype in human colon tumor xenografts

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2 human colon tumor lines, Co115 and Co112, were implanted in situ in teh colonic wall of NIH:II nude mice. 2 human melanomas were used as comparative (skin-derived) tumors and implanted both into the gut (GI) and s.c. Colonic tumors invaded the regions of the colonic wall and mimicked the original pattern of the patient tumor. Colonic tumor cells invaded and grew within lymphatics, veins and infiltrated smooth muscle cell layers (as documented by light and electron microscopy). Invasion/infiltration were minimal for s.c. implanted tumors. ^{3}H-Thymidine and tumor size data suggested that GI-tumors, initiated from a small number of tumor cells. In view of a possible modulatory effect by tissue sites on invasive ability, the expression of tissue plasminogen activator (PA) and urokinase-like PA was determined in thyocyanate extracts of both sc versus GI implanted tumor samples.

Chemiluminescence (CL) measurement: a sensitive method for the study of cell surface antigens and Fc receptors

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Antibodies directed against viral and nonviral cell surface antigens stimulate CL in mouse spleen cells (J. Immun. Meth. *47* (1981) 295). CL reflects the generation by the cells of various radical species and can be monitored in a liquid scintillation spectrometer in the presence of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). The antibody Fc portion is responsible for the stimulation of CL. Consequently, 2 kinds of antibody recognition phenomena can be studied: with antibodies bound to antigens, the activation of Fc receptors (FcR) by Fc, and with compatible Fc/FcR systems, antibody specificities and antigens can be analyzed. Using 'indicator' cells (e.g. polymorphonuclear leucocytes, PMN) to provide Fc/FcR-dependent CL generation, antigens on the surface of CL-neg. cells and antigens in virus preparations can be investigated. With bovine PMN as 'indicator' cells, surface antigens of bovine herpesvirus type I could be detected by CL measurement using 100 infected lung cells.

Anemia induces precocious transition from larval to adult globin synthesis in *Xenopus laevis*

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Hemoglobin transition in *X. laevis* is a useful model to study developmental gene regulation. Analysis by in-situ hybridization of globin mRNA in circulating erythroblasts of anemic *Xenopus* larvae has revealed at least 2 distinct populations of cells expressing either larval or adult globin genes; at stage 59 already 95% of the cells have been found to contain adult globin mRNA. In contrast, quantitation of larval and adult globin mRNAs, isolated from normal animals of the same stage, has revealed a 20-fold lower content of adult sequences as compared to anemic animals. Analysis of the globins from anemic larvae by triton X-100-urea gel electrophoresis has disclosed precocious appearance of adult globins between stages 56 and 62, but much lower amounts of adult globins in normal animals at the corresponding stages. Since anemia-induced globin transition precedes morphological transformation we conclude that different mechanisms may be operative in the induction of globin transition and morphological metamorphosis.

Chromatin structure in the active Balbiani ring 2 (BR 2) of *Chironomus* salivary glands

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Electron microscopic and biochemical studies indicate that in *Xenopus laevis* oocytes (Labhart and Koller, Cell *28* (1982) 279) and in *Dictyostelium discoideum* (Ness, Labhart, Banz, Koller and Parish, J. molec. Biol., submitted) the active nucleolar chromatin consists mainly of free DNA and the components involved in transcription and its regulation. As a model for an RNA polymerase II transcribed gene we studied the BR 2 region in salivary glands of *Chironomus tentans* larvae under conditions of heavy transcription, as well as in an established cell line not expressing the BR 2 gene. In this gene the restriction enzymes tested had no access in the cell line nuclei and digestion with micrococcal nuclease revealed a nucleosomal structure. On the other hand in salivary gland nuclei the restriction enzymes tested cleaved the BR 2 chromatin and micrococcal nuclease generated no DNA fragments of discrete sizes. These data are compatible with our earlier findings on transcribed ribosomal genes.

Lipoprotein from *E. coli* envelopes: secondary structure and interactions with other outer-membrane proteins

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Lipoprotein is a small, abundant polypeptide which is linked covalently at its N-terminus to 3 fatty acyl chains, and at its C-terminus to peptidoglycan. The latter bond occurs in $\frac{1}{3}$ of the 750,000 copies/cell. The rest exists in free form. Its arrangement in the envelope is unknown. Previous purifications in SDS at 100 °C yielded a protein in α-helical configuration. Since this converts most proteins to that form, I have isolated free lipoprotein by selective extraction (pH 5) in octyl-POE and purified it by DEAE chromatography, isoelectric focusing and gel filtration. Circular dichroism confirmed helical structure. OmpA protein, purified as byproduct, yielded spectra typical for β-sheets. Previous findings of mixed α- and β-structures may be due to contamination with lipoprotein, as the 2 proteins copurify in most steps. When isolated individually from 2 strains lacking either gene product, they associated spontaneously. Antibodies against lipoprotein are used to probe its interaction with OmpA protein and other outer-membrane components.

Mixed-disulfide TMV peptide conjugates for the fluorescent labeling of receptors

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We had shown (1981) that ternary conjugates of rhodamine, tobacco mosaic virus (TMV) and α-melanotropin interact specifically and almost irreversibly with receptors on Coudman S-91 mouse melanoma cells in culture. Potentially they can be used for localizing receptors and studying their dynamics. Their application would yield more information (e.g. on internalization of receptor complexes), if the bonds between peptide and virion could easily and specifically be broken. To study this hypothesis, conjugates with disulfide links between peptide and virion were prepared. We describe the preparation of N^{α}-sulfomercaptovaleryl-α-melanotropin, its biological properties as an agonist, and its conjugates with derivatives of TMV. These conjugates display, as expected, enhanced pharmacological potency and physical receptor binding. The fluorescent virion can easily be detached from the cell surface by reducing agents. Results on recoptor dynamics (internalization) will be presented.

Promutagens detected by a rapid test with *Drosophila* somatic cells

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Somatic mutation and recombination testing was used to assess the activity of a number of mutagens and promutagens in *Drosophila*. The utility of any submammalian test depends on its capacity to detect also those compounds

which become mutagenic upon metabolic activation. We evaluated a test system which in addition appeared advantageous because it is inexpensive and straightforward in application. Larvae, transheterozygous for 2 recessive wing hair mutations *(mwh+/+flr)*, were fed with the chemicals. Mutational events lead to mutant clones in the wings of the adult fly that appear as *mwh//flr* twin spots or as single spots, either *mwh* or *flr*. Significant frequencies of spots were induced by aflatoxin B_1, DEN and procarbazine. Thus, *Drosophila* larvae possess the different metabolic activation systems which are required for the conversion of these substances into their respective genotoxic metabolites.

PHARMAKOLOGIE - PHARMACOLOGIE - PHARMACOLOGY

Neurohumoral regulation of adrenocortical ornithine decarboxylase

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Administration of apomorphine (APM) to rats increases adrenocortical ornithine decarboxylase (ODC, EC 4.1.1.17) activity. This effect is prevented by the dopamine antagonist haloperidol or by hypophysectomy (Almazan et al., Neuropharmacology *21* (1982) 631). The neurohumoral mechanism responsible for the induction of ODC by APM has been further explored here. Some rats were subjected to hypophysectomy, section of the spinal cord, or section of the brain at various levels. ACTH injection to hypophysectomized rats increased ODC activity. Section of the spinal cord at T_2 level or formation of a hypothalamo-pituitary island elevated basal adrenocortical ODC activity and potentiated the APM-mediated induction. On the other hand, complete diencephalic transection decreased both the endogenous and APM-induced ODC activity. These results suggest a) APM acts at the level of the hypothalamus to increase adrenocortical ODC activity; b) the effect is mediated by the release of ACTH; c) peripheral and extrahypothalamic neural influences modulate the mechanism.

Theobromine (Tb) distribution in the pregnant rat and the fetus and the impairment of its metabolism due to pregnancy

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Pregnancy was shown recently to affect theophylline (rat) and caffeine (human) metabolism. In order to demonstrate the effects of pregnancy on Tb metabolism, [8-^{14}C]Tb (4 mg/kg) was administrated orally to unfasted rats at the 18th day of gestation.

Fecal excretion amounted to 31% of the dose while 60% was recovered in the urine and only 1.3% as expired CO_2.

Tb crossed the placenta and 6, 15, 36 and 48 h after the administration 9, 3, 1 and 0.2% of the dose was respectively found in the fetuses. At any time, Tb concentration was shown to be in equilibrium between the blood, brain and liver of the fetus and the blood of the pregnant rat.

Compared with nonpregnant rat, urinary excretion of unchanged Tb increased in the pregnant rat from 47±4% to 74±3% of total urine radioactivity while 6-amino-5[N-formylmethylamino]1-methyluracil decreased from 35±4% to 22±2%.

Thus, similarly to theophylline, pregnancy impaired Tb metabolism in the rat leading to an increased urinary excretion of the unchanged dimethylxanthine.

Clonidine withdrawal in SHR

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We have studied changes in the cardiovascular and sympathetic nervous systems following cessation of chronic clonidine treatment (osmotic minipump, 0.1 $mg \cdot kg^{-1}$ in 24 h, i.v.) of SHR bearing permanently implanted intraaortic cannula. Arterial pressure (AP) and heart rate (HR) were lowered by clonidine; following cessation of pump function, AP and HR returned rapidly (within 16 h) to pretreatment values, whereas lability (expressed as (variability/average) %) of both AP and HR rose to 3 fold control values. Such lability increases resemble those seen after electrolytic or chemical lesion of hindbrain areas such as nucleus tractus solitarii. This was corroborated by the fact that a) tyrosine hydroxylase activity was increased in certain hindbrain nuclei during withdrawal, b) that the K_d for 3H-clonidine binding to cerebral cortex membranes was increased, and c) withdrawal from treatment with 0.5 $mg \cdot kg^{-1}$ increased the B_{max} for clonidine binding in the SHR cerebral cortex.

Biochemical effects in emetine cardiotoxicity

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In an experimental rat model emetine, at therapeutic doses of 1 mg/kg day, induced electrocardiographic changes comparable to those observed in human patients. Concomitantly biochemical changes developed. The effects could be correlated with emetine concentrations in heart tissue and serum. Upon prolonged treatment (19 doses), concomitant with the accumulation of emetine in heart tissue (70-fold increase from dose 1-19), heart mitochondrial oxidative phosphorylation (67% of control) and mitochondrial creatine kinase (75% of control) decreased in activity and the creatine content of mitochondria increased (150% of control), reflecting a loss in semipermeability of the inner mitochondrial membrane. Heart tissue content of total adenine nucleotides (74% of control), especially ATP (65% of control), decreased, resulting in a reduced protein synthesis in heart tissue (76% of control) and heart mitochondria (71% of control). Similar effects of emetine were seen in liver and kidney tissue and the mitochondria thereof.

Triazolam concentration-effect relationship in healthy volunteers

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The short plasma half life makes triazolam an interesting model drug for studying the relationship between pharmacokinetic and pharmacodynamic effects of a benzodiazepine. Doses of 0.25 mg were given p.o. to 6 healthy volunteers. Triazolam plasma concentrations were assessed at intervals for 6 h by capillary GLC with ECD. Subjects also repeated a psychological test battery consisting of measures for mood, for recognition and recording of visual information, and for psychomotor performance 11 times within 6 h. Only the digit symbol substitution test and card sorting according to numbers showed significant impairment in performance roughly in proportion to plasma concentrations. Performance had a tendency to return to baseline before the plasma concentrations were below 0.5 ng/ml, suggesting learning effects. These data in healthy volunteers represent a standard of comparison for the study of the responsiveness of the brain to triazolam in various diseases.

The disulfiram-alcohol reaction (DAR) revisited

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Disulfiram (Di) inhibits the acetaldehyde (A) dehydrogenase, producing an accumulation of A in blood after alcohol ingestion. Since a precipitous blood pressure (BP) fall represents the most dangerous result of DAR, we reassessed the role of A in producing cardiovascular changes. In 5 alcoholics, pretreated with daily doses of 400 mg Di during 3–6 days, the fall in systolic and diastolic BP exhibited a close relationship (R_s 0.9 and 0.9, respectively) with peak A levels, measured by GLC. Since A levels are largely dependent on A oxidation, we measured A oxidizing capacity (AOC) in erythrocyte suspensions. Whereas in 9 normal volunteers and 7 patients with nonalcoholic liver disease, AOC was 4.7±SD 1.1 and 4.2±1.7 nmoles A/ml min, respectively, 6 alcoholics treated during 5–10 months with Di (1.2 g per week) were characterized by a marked decrease in AOC (0.9±0.7; $p < 0.001$ compared to volunteers or patients). Our data suggest that AOC may represent a useful parameter for assessing Di compliance; conceivably, it may also predict severity of the DAR.

Renal uptake of cadmium

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Cadmium accumulates in renal cortex where it elicits tubular lesions. Our studies attempted to characterize the luminal and peritubular uptake of Cd. Left kidneys of anesthetized rats were exposed. Microinjections of samples (10 nl) containing ^{109}Cd (1 mM) and ^{3}H methoxyinulin were carried out in proximal or distal tubules, and urine samples were collected serially. Inulin recovery was nearly complete. The following fractions of the total Cd microinjected in proximaltubules were recovered: 20.8±4.0% ($CdCl_2$, n=17), 17.7±3.9% (Cd-L-cys 0.8 mM complex, n=6), 46.4±6.3% (Cd 1 mM L-cys 5 mM, n=11), 49.0±10.8% (Cd 1 mM L-cys 10 mM, n=5), 98.8±2.8% (Cd-DTPA chelate 1 mM, n=6). Distal microinjections allowed total cadmium recovery. To investigate peritubular uptake of Cd, droplets (66 nl) containing ^{109}Cd were streaked on the left kidney surface 10 min later there was 7.5±1.3% (n=7) recovery of ^{3}Hinulin from both kidneys but none of ^{109}Cd. I.v. infusion of L-cys (6.6 µmoles/min 100 g b.wt) slightly enhanced ^{109}Cd recovery. Application of Cd as chelate with DTPA allowed marked increase in Cd recovery but there was no transepithelial secretion as shown for ^{14}C-PAH. I.v. administration of DTPA did not modify recovery of Cd.

Dopamine content and synthesis in rabbit retina: a pharmacological study with LCEC

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A reverse phase and cation exchange liquid chromatography coupled with electrochemical detection (LC-4, BAS) was used to measure the dopamine content of rabbit retina. Retinae were quickly isolated and sonicated at 0 °C in 200 µl 0.1 M $HClO_4$ containing 20 ng DHBA. 40 µl aliquots of crude supernatants were injected for LCEC analysis. Peaks for DHBA (retention time = 7.9 min) and dopamine (retention time = 14.7 min) were measured at sensitivity 10 nA/l V full scale and with electrode potential of +0.75 V. Standard solutions containing 2, 4 or 8 ng per 40 µl of both DHBA and dopamine were injected for quantification of endogenous dopamine. Alternatively, supernatants and standard solutions were extracted with alumina for prior purification of dopamine. Dopamine levels detected with LCEC in rabbit retina were 3.97±0.23 (SEM) ng per mg protein. This method will thus be used for assessment of dopamine metabolism after various pharmacological manipulations (Sovilla and Schorderet, Life Sci. *31* (1982) 2081).

Development of diurnal rhythms of central dopamine-, serotonin- and spirodecanone-binding sites and motor activity in the rat

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The binding of ^{3}H-spiperone to its 3 high affinity sites (dopaminergic D_2, serotonergic S_2 and spirodecanone site) was determined in forebrain homogenates of 14-, 30- and 88–90-day-old male rats at different times of the day. Diurnal variations were seen in the spirodecanone site from postnatal day 15, in the D_2 and S_2 sites at the age of 30 days. Each site showed a different diurnal rhythm, moreover, the rhythms of the D_2 and S_2 sites differed between immature and adult animals. Differences were also seen in diurnal variations of motor activity between the age of 30 and 88–90 days. The variations in dopaminergic D_2 sites and motor activity showed a reciprocal relationship during the dark phase at both developmental stages. These data suggest a link between the dopaminergic D_2 site and motor activity which is evident throughout ontogeny.

Assessment of glyceryl trinitrate release from Nitroderm® TTS by digital plethysmography

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The rate of release of glyceryl trinitrate in vivo from Nitroderm® TTS was assessed in 6 healthy normal volunteers. Instead of measuring plasma concentrations the pharmacological effects of glyceryl trinitrate were followed by digital plethysmography. Each experiment consisted of the establishment of a dose-response curve which was followed

by a 1-h application of Nitroderm® TTS (20 cm^2) and a 2nd dose-response curve. The investigations were repeated after pretreatment of each subject with 0.4 mg pindolol i.v. The results show a satisfactory overall consistency of the data and indicate a release rate of 4.4±SD 1.7 µg/min of bioavailable glyceryl trinitrate during the first of application of this therapeutic system.

Induction of cytochrome P450 in liver cell culture correlates with physicochemical properties of pyridine-type inducers

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The pyridine derivatives nicotinamide and metyrapone are inducers of cytochrome P450 (P450) in chick embryo liver cell culture (Biochem. Pharmac. *31* 1735). Using this system, we have now studied 12 other pyridine congeners-homologs and regioisomers of pyridylalkanamides. We found a direct correlation between increasing length of the side chain (which increases lipophilicity) and potency. The potency of the regioisomers was as follows: γ-position > β-pos. > α-pos. Relative differences in potency were much greater than differences in efficacy. Type II-binding spectra were obtained using these compounds and liver microsomes. The potency to induce P450 was directly correlated with binding affinity. These binding affinities are influenced by the isomeric position of the side chain in relation to the ring nitrogen. These studies thus demonstrate that the physicochemical properties of substituted pyridines exert major effects on their ability to induce P450.

Effects of oxotremorine on central dopamine metabolism in RHA/Verh and RLA/Verh rats

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This study follows another study (Martin et al., Experientia *38* (1982) 756) which showed that RLA/Verh rats exhibited stronger tremor, chromodacryorrhea and hypothermia responses to injections of oxotremorine (OXO) than did RHA/Verh rats. Striatal dopamine (DA), HVA and DOPAC (DA metabolites) and hypothalamic DA and DOPAC were measured in samples taken 0, 10, 20 and 30 min after i.p. injections of 1.0 mg/kg OXO. 5 male rats of each line were used for each time interval. Both time-dependent increases and genetic differences were seen in striatal DA metabolism following OXO injections, with the RHA/Verh rats showing stronger increases than the RLA/Verh rats. Only time-dependent increases in DA metabolism, however, were found in the hypothalamus, without any signs of genetic differences. Peripheral vs central effects of OXO, and possible involvement of the cholinergic system, may be inferred in the results of both studies.

Freeze fracture of presynaptic membrane during transmission of a single giant impulse in *Torpedo* electric organ

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Dissected prisms of *Torpedo* electric organ were treated with 10^{-4} M 4-aminopyridine (to prolong discharge) and quickly frozen before, during and after a giant electric discharge induced by a single field stimulus. Freeze-fracture replicas of the presynapse were quantitatively assessed to determine: a) the number of exocytotic pits and b) the number of intramembrane particles (IMP). During discharge (30–60 msec poststimulus), the number of pits (average = 0.69/μm^2) was not found different from that in resting and postdischarge (570–600 msec) conditions, while P- and E-face IMP larger than 10 nm increased reversibly ($p < 0.001$): 233 (0 msec); 473 (30–60 msec) and 252 (570–600 msec), (IMP/μm^2, P-face); 103, 235 and 97 (IMP/μm^2, E-face); these changes seem directly related to the ACh release since they were not detected when stimulation was carried out in presence of low Ca^{++} and high Mg^{++}. Their relationship to exocytosis, however, remains unsettled.

Circadian variations of neurotransmitter binding in 3 age groups of rats

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During a 24-h period H_3-spiroperidol, H_3-QNB and H_3-Gaba binding were measured in striatum, hippocampus and cerebellum of male Füllinsdorf-Wistar rats of 3 age groups. Significant variations over the 24-h period were found in 3-month- and 12-month-old rats. With the exception of H_3-Gaba binding in cerebellum circadian variations were not significant in 24-month-old rats. Maximum binding occurred during daylight (11–15 h) in young and aged rats, but during the night (23 h) in adult animals. The 24-h averages for H_3-spiroperidol and H_3-QNB binding in striatum and for H_3-Gaba binding in cerebellum showed a significant reduction with age. H_3-QNB binding in hippocampus of the aged rats was slightly increased. It is interesting that these age-related changes are not always reflected in the values at each time point.

Effects of preincubation in microbial mutagenicity studies of coffee

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Aqueous extracts of instant coffee were examined in the *Salmonella*/microsome assay using TA 100. The weak mutagenic effects of coffee per se (Aeschbacher et al., Fd Cosmet. Toxic. *18* (1980) 605; Nagao et al., Mut. Res. *68* (1979) 101) could be reproduced. In the preincubation assay a dose-related mutagenic effect was found between 5 and 30 mg coffee per plate. As a result of the addition of active S-9 or S-105 fractions of rat liver homogenate, the mutagenic effect disappeared (inactivation of direct acting mutagenic activity by mammalian metabolism). Interestingly also the preincubation at 37 °C of coffee solution without S-9 led to a spontaneous loss of the mutagenic activity within 6 h. In the presence of N_2 this chemical degradation of the mutagenic compound(s) was slowed down. Our results clearly demonstrate that standard bacterial mutagenicity tests cannot be used for human risk estimation without additional experiments.

Evaluation of long-acting opiate antagonists: effects on morphine-induced analgesia and motor activity in 2 strains of mice

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To assess the antagonistic potency of naloxazone (NXZ) and β-chlornaltrexamine (CNA) 2 strains of mice were used, in which morphine either induces analgesia (DBA/2) or increases locomotion (C57 Bl/6). The antagonists were applied s.c. 24–120 h before morphine (10 mg/kg, i.p.). The minimal dosages which completely antagonized morphine-induced analgesia and locomotion during 24 h were: for NXZ 50 and 100 mg/kg, for CNA 0.8 and 6.2 mg/kg, respectively. NXZ turned out to be a less feasible antagonist, since with 100 mg/kg the effect disappeared after 48 h, although the toxic range was reached (10–40% of mice died). In contrast, the minimal dosages on CNA were fully effective for 72 h in DBA and 48 h in C57 mice, and were 16- and 4-fold below the toxic limit. CNA was more potent in antagonizing the morphine effect in DBA mice. This might be due to differences in the composition and dynamics of the opiate receptor populations of the 2 strains.

Effects of diphosphonates (APD, EHDP) on inflammation and bone turnover in adjuvant arthritis rats

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APD (disodium-3-amino-1-hydroxypropane-1,1-diphosphonate) exerts beneficial effects in RA and Paget's disease (Bijvoet et al. Arthr. Rheum. *23* (1980) 1193). The action of APD, EHDP, diclofenac and D-penicillamine on adjuvant arthritis has been compared. Lewis rats were treated with *M. butyricum* on day 0, and drugs given from day 0, 5times weekly. Hindpaw swelling, ESR, Ca^{2+}-, urea-, alk.phosphatase levels of serum, and hydroxyproline content in urine were determined. APD EHDP and diclofenac reduced the 1st and 2nd lesions, although onset of activity of diphosphonates was delayed. D-penicillamine was ineffective. Similarly, a dose dependent inhibition of ESR, alk.phosphatase and urinary hydroxyproline was seen with APD. Conclusion: APD efficiently reduces bone turnover and inflammation in adjuvant arthritis of rats and is more active than EHDP at the same dose levels. The pharmacological pattern differs from that of NSAIDs like diclofenac. Treatment with D-penicillamine is ineffective.

Conformational changes of adrenocorticotropin-peptides upon interaction with lipid membranes

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Infrared attenuated total reflection spectroscopy (IR-ATR) was used to study conformational and topological aspects of the interaction between 2 adrenocorticotropin fragments and dioleoyl phosphatidylcholine membranes. Corticotropin- (1–10)-decapeptide, $ACTH_{1-10}$, was found to exist as a rigid antiparallel pleated sheet structure in dry membranes. In aqueous environment, it completely escaped from the lipid. This dominant preference for the aqueous phase is a possible explanation for the very low biological potency of $ACTH_{1-10}$. On the other hand, the very potent corticotropin-(1–24)-tetracosapeptide, $ACTH_{1-24}$, was firmly incorporated into dry and wet membranes. In aqueous environment, part of the molecule entered the bilayer and adopted a helical structure with the axis oriented perpendicularly to the bilayer plane. Our experiments clearly demonstrate that the observed specific membrane interaction critically depend on the presence of the address segment $ACTH_{11-24}$. This could explain the pharmacologically observed potentiating effect of the 'address'.

Effects of guanine nucleotides and divalent cations on ^{3}H-clonidine binding in bovine retina

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Bovine retina membranes contain a large number of alpha$_2$-adrenergic receptors. ^{3}H-Clonidine binding (CLB) to these receptors has a high affinity ($K_d = 0.3$ nM, H. Bittiger et al., Nature *207* (1980) 645). This binding is only very slightly inhibited by GTP or Gpp (NH)p (10%), and the inhibition cannot be attributed to a change in either B_{max} or K_d. Neither Mg^{++} nor Mn^{++} stimulate CLB either in the presence or absence of GTP, but CLB can be inhibited by monovalent cations in the order: choline$^+ > NH_4^+ \gg Na^+ = Li^+ > K^+$. CLB in systems in which alpha$_2$ receptors inhibit adenylate cyclase activity is inhibited by GTP and Gpp (NH)p, and this inhibition can be reversed by Mg^{++} and Mn^{++}. The absence of effects of GTP and divalent cations on retinal CLB suggests that these binding sites are not coupled with the nucleotide binding protein or adenylate cyclase.

Transient decrease in β-adrenergic binding sites after isoproterenol treatment

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Short-time agonist treatment of β-adrenergic receptors in intact C_6-cells induces a transient decrease of β-adrenergic binding sites as determined with the antagonist [^{3}H]CGP 12177. The reduction of the binding sites is independent of ATP, Ca^{++} and monovalent cation gradients. The reappearance of binding sites is strictly dependent on intact cells. Decreasing the incubation temperature decreases the velocity of reappearance in a nonlinear relation. The reappearance of binding sites is inhibited by high intracellular Ca^{++} activity, increased lysosomal pH and by decreased $K^+/Na^+/H^+$ gradients. The results obtained are in agreement with the hypothesis that intracellular recycling of β-adrenergic receptors is one step in the transient desensitization of the receptor complex.

'Stretched attend posture', an ethological element sensitive to conflict reducing drugs

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The ethological element 'stretched attend' to a conspecific, reflects conflict between approach and avoidance (Grant and Mackintosh (1963)). In a nonsocial situation this element (i.e. SAP = stretched attend posture) was used to examine drug effects. 1 h after single oral drug administration, adult, male naive mice were individually placed on a perforated, plastic platform, previously rubbed with foreign male urine and boluses. The frequency and duration of SAP and other acts were recorded for 2 min. It was found, that diazepam (0.1–10 mg/kg) and chlordiazepoxide (3–20 mg/kg) or phenobarbital (3–30 mg/kg) reduced SAP dose-dependently and increased immobility or the element

'go forwards in SAP'. SAP was only reduced in untreated mice after several exposures to the test situation. Chlorpromazine and imipramine did not influence SAP. The results suggest, that measurement of SAP provides a simple test for conflict reducing properties of drugs, which dispenses with the use of noxious stimuli and prior training.

Effects of amyotrophic lateral sclerosis sera on cultured cholinergic neurons

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Dissociated monolayer cultures of chick ciliary ganglion neurons have been used to study the effects of control and amyotrophic lateral sclerosis (ALS) sera. This ganglion contains 2 populations of cholinergic neurons; one innervates striated muscle and can be classified as 'motor-neuron-like'. The cultured neurons survive and extend neurites for a minimum of 2 weeks in a standard tissue culture medium containing 10% heat-inactivated human serum. 3 parameters have been examined to assess the functional properties of the cells when cultured in the presence of control or ALS sera for 8–12 days: neuronal survival; the enzyme, choline acetyl-transferase; the synthesis of ^{3}H-acetylcholine using ^{3}H-choline as a precursor. ALS sera (25 samples tested) cause a small decrease in these 3 parameters as compared to control sera (24 samples tested) but this difference was not statistically significant. We are presently testing the effect of ALS sera on the levels of CAT in dissociated spinal cord cells in culture.

Chronic methamphetamine: phase shifts in feeding and lateral hypothalamic receptor rhythms

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Motor activity, feeding and drinking in the rat are confined mainly to the dark phase. Chronic methamphetamine shifts these rhythms into the light phase. When saline is infused into the lateral hypothalamus (LH), the circadian feeding rhythm is maximal at the beginning of the dark phase (Margules et al., Science *178* (1972) 640). We have observed a similar rhythm of spiroperidol and imipramine binding in the LH. In contrast, when noradrenaline is infused into the LH, a bimodal rhythm is induced, with peak feeding at the end of the light and at the end of the dark phase (op. cit.). A similar bimodal rhythm of binding to β-adrenergic receptors was found in the LH. Methamphetamine shifted binding of all 3 ligands to the end of the dark phase: although body weight was reduced 24 h mean food intake and 24 h mean binding was not. Thus these receptor rhythms in the LH appear to be involved in the timing rather than the homeostasis of feeding.

^{3}H-Glycogenolysis in vitro: interactions between VIP and monoamines in cerebral cortex and retina

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VIP is contained in bipolar, intracortical neurons; when applied to mouse cortical slices it promotes the hydrolysis of ^{3}H-glycogen newly synthesized from ^{3}H-glucose (Magistretti et al., PNAS *78* (1981) 6535). A similar glycogenolytic action is displayed by norepinephrine (NE) which is contained in afferent projections to cerebral cortex. In order to test the possibility of a functional complementarity between noradrenergic afferents and VIP-containing intracortical neurons, we have initiated the pharmacological characterization of the nature of the interactions between VIP and NE on cortical ^{3}H-glycogen. Initial results indicate that the effects of VIP 0.1 and 1 nM are potentiated by NE 500 nM. We have observed that intact rabbit retinae also synthesize ^{3}H-glycogen, which can subsequently be hydrolyzed by VIP 500 nM. Thus it may be possible to study also in retina, where VIP and the monoamine dopamine are contained in subclasses of amacrine cells, the interactions between VIP and monoaminergic systems.

Effect of apomorphine on monoamine systems in specific rat brain regions

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In the course of studying the role of dopamine (DA) in the locus coeruleus (LC), we have previously shown that there is not a significant DA cell body population in the LC (Neurosci. Lett. *30* (1982) 297) and that there is a DA input from the A10 region to the LC (Brain Res. (1983), in press). In this study, apomorphine was administered at 2 different doses (0.004 and 1 mg/kg i.p.). Monoamine levels were determined in the LC, raphé dorsalis and A10 region 30 min later. A dose-dependent increase of the 5-HT and 5-HIAA levels was observed in the 3 structures. A similar effect was observed on the A10 DA content. These results tend to support the existence of serotonergic and dopaminergic interactions between these 3 structures.

Uptake of pyrazinoate (PZA) by isolated, nonperfused proximal renal tubules of rabbit

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S_1, S_2 and S_3 segments of proximal tubules from superficial nephrons were dissected and incubated (30 min, pH 7.4, 37 °C) in oxygenated Ringer's solution containing bovine serum albumin, and ^{14}C-PZA ($2 \cdot 10^{-4}$ M), an organic anion. PZA concentrations in tissue water (microphotographic tubular volume multiplied by water concentration in tissue) or in incubation medium were calculated from dpm and PZA specific activity (62 mCi/mM). Uptake was expressed as the tissue water/medium ratio of PZA concentrations (T/M). The uptake (mean±SE, (n)=number of tubules) was 3±0.7 (22) in S_1, 21±2 (24) in S_2, and 9±2 (14) in S_3. Probenecid added to the medium at 10^{-4} and $5 \cdot 10^{-4}$ M decreased the uptake by only 50% and 60% respectively. At 10^{-6} M it stimulated the uptake by 20–30%. P-Aminohippurate (PAH) decreased the uptake by 50% and 70% when added at 10^{-4} and 10^{-3} M respectively, whereas 10^{-5} M ouabain decreased it by 90%.

Solubilization of the 5-hydroxytryptamine carrier protein of human blood platelets

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In blood platelets and brain synaptosomes imipramine appears to be a specific marker (binding constant $Kd \sim 2 \times 10^{-9}$ M) for the carrier protein (imipramine-binding protein, IBP) transporting 5-hydroxytryptamine (5HT) through the plasma membrane. The binding capacity for ^{3}H-imipramine of the plasma membrane of human platelets is diminished in mental depression. In the present work membranes of platelets of healthy persons have been

presolubilized with digitonin (10 mg membrane protein/ml + 1 vol. 2% digitonin), and a soluble, high mol.wt form of IBP (mol.wt > 1,000,000) was obtained. Desaggregation of this complex by further addition of 7 vol. 2% digitonin caused a lowering of the mol.wt to 150,000 as determined on sepharose 6B. This preparation still showed specific binding of ^{3}H-imipramine in the nanomolar range. Attempts to further purify and characterize IBP by affinity chromatography and photoaffinity labeling are under way.

Kainic acid and central control of the cardiovascular system

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It has been suggested that glutamate is a neurotransmitter of baroreceptor afferents. We injected the rigid glutamate analogue kainic acid (which readily crosses the blood brain barrier) into freely moving normotensive rats. A dose of 9 mg · kg^{-1} kainic acid i.p. produced falls in blood pressure and heart rate with nadirs at +25 min (104±4–59±4 mm Hg and 424±14–274±23 bpm). Similar changes occurred in anesthetized rats and following i.v. injections. Baroreceptor reflex following i.v. phenylephrine was impaired after kainic acid administration. Bilateral electrical destruction of the nucleus tractus solitarii abolished kainic acid-induced depressor effects.

Drug-induced changes in nerve fiber calcium content

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Measurements of Ca in desheathed rabbit vagus nerves were made by a method which allows a continuous monitoring of the ^{45}Ca content of the preparation (Jirounek et al., J. Physiol. Lond., in press). The results show a rapid equilibration of ^{45}Ca with the exchangeable Ca poll of the nerve. An increase of this cellular Ca is observed on removal of extracellular Na or after addition of micromolar concentrations of the Ca ionophore A23187, but only the former is reversible. Application of some neuroleptic drugs (trifluoperazine, flupenthixol) which are known to inhibit the calmodulin-dependent fraction of the Ca-ATPase activity, produce a slight increase of the cellular Ca. This effect is generally enhanced in low extracellular Na concentrations. Ca entry antagonists, such as Co or verapamil, decrease the Ca content of the preparation.

Inhibition par un neuroleptique de la libération d'acetylcholine (ACh) à la jonction nerf-électroplaque

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Les neuroleptiques sont connus pour leurs effets secondaires anti-cholinergiques. Nous avons utilisé l'organe électrique de la torpille, une préparation homologue de la jonction neuro-musculaire, pour tester si les neuroleptiques sont capables de bloquer la transmission cholinergique par une action directe.

La trifluopérazine (1–50 μM) inhibe la transmission nerf-électroplaque, en déprimant la libération d'ACh provoquée par stimulation. Le mécanisme de cette inhibition semble être un blocage des flux entrants de calcium dans la terminaison nerveuse.

D'autres neuroleptiques (halopéridol, clozapine, chlorpromazine, dropéridol) exercent un effet similaire. La dépression de la libération d'ACh par les neuroleptiques dans le système nerveux central est d'ordinaire attribuée à un effet indirect, via des neurones dopaminergiques. Nos résultats suggèrent qu'il pourrait s'agir également d'une action directe sur les synapses cholinergiques.

Brain renin substrate (BRS) in spontaneously hypertensive rats (SHR-sp): effect of captopril (CAP) treatment

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BRS was measured in SHR-sp with and without CAP treatment to study the possible contribution of the brain renin angiotensin system to the hypertension in these rats. BRS varied from 0.37 (cortex) to 2.96 pmoles ANG I/mg prot. (ant. hypothalamus). It was increased in the cortex, hippocampus and cerebellum of 9-week-old SHR-sp as compared to Wistar Kyoto (WKY) rats, but was decreased in the ant. hypothalamus of SHR-sp. Chronic CAP treatment kept SHR-sp normotensive. BRS was suppressed in plasma but was stimulated in the ant. hypothalamus of SHR-sp and WKY rats under CAP treatment. CAP reduced the BRS content in hippocampus of SHR-sp and in brainstem of WKY rats respectively. There was no change in septum and post. hypothalamus. In conclusion, the BRS distribution in the brain of SHR-sp differs from that of WKY rats and may partly be regulated in a different manner as shown by the CAP-induced changes.

Effects of sleep promoting 'Factor S', muramyl dipeptide (MDP) and L-cycloserine on the sleep of rabbits

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An extract from brainstems of cattle, containing sleep promoting 'Factor S', was tested in normal rabbits and in animals with an increased brain GABA concentration, due to i.v. administration of 30 mg · kg^{-1} of the GABA-transaminase inhibitor L-cycloserine. The effect of 12 GBE (gram brain equivalents) of brain extract i.c.v. was compared with that of 30 and 100 nmoles · kg^{-1} MDP i.v. and with that of an i.v. administered standardized pyrogen. The brain extract did not affect Rapid Eye Movement Sleep (REMS), but augmented nonREM-sleep (NREMS) in the 3rd to 5th h after administration. L-Cycloserine augmented both NREMS and REMS in parallel to the increase of the brain GABA concentration. MDP raised body temperature and augmented NREMS, but abolished REMS and induced head shakes. The pyrogen also raised body temperature and diminished REMS, but did not affect NREMS. L-Cycloserine strongly potentiated the effects of brain extract and MDP.

Subcellular distribution of phospholipids (PL) in DMI-treated cultured human fibroblasts

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Desipramine (DMI) is a lysosomotropic drug. Chronic drug treatment of cultured fibroblasts induces a granular morphology and a 3–5 times increased PL content. Cellular localization of DMI and PL was studied in acute and

chronically DMI treated fibroblasts by subcellular fractionation on a Percoll Gradient. Cultures were incubated with 10 µM DMI for 9 days. 2 h before harvesting the cells, DMI treated and control cultures were labeled with ^{3}H-DMI. Fibroblasts were lysed by trituration in 0.25 M sucrose. After centrifugation at 1000 × g the supernatant was separated at 25,000 × g for 10 min into a crude microsomal and a crude mitochondrial/lysosomal fraction, that were centrifuged on Percoll Gradients. Fractions were analyzed for PL, ^{3}H-DMI, protein and marker enzymes. ^{3}H-DMI and increased PL were associated with lysosomal enzyme containing vesicles of lower density than lysosomes in control cells, indicating a common intralysosomal localization of the drug and the PL.

Pipratecol, a vasodilator drug: HPLC technique for quantification in plasma and its use for pharmacokinetic study in dog

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Extraction of pipratecol (PIP) [(dihydroxy-3,4 phenyl)-1 (methoxy-2 phenyl)-4 piperazinyl-1)-2 ethanol] (constituent of Hydrosarpan 711®, Lab. Servier, France) from plasma with organic solvents gave poor recovery due to the very low organic/water partition coefficient of PIP and its instability at acidic or alkaline pH. XAD-2 resin extraction of PIP by column at pH 7 with water/methanol gradient gave 95% recovery from plasma. An internal standard [(dihydroxy-3″,4″phenyl)-1′methyl)-1(pyrimidine 2″)-4 piperazine] was used for the HPLC separation on an RP-18 column with 45% acetonitrile/55% 0.025 M phosphate buffer (pH 3) and electrochemical detection (+1.15 V). The pharmacokinetics of PIP was studied in female dogs after oral administration of six 5-mg tablets. The drug was rapidly absorbed ($t_{1/2ka}$ = 0.17 h), distributed into a large central compartment (V_D = 105 L), with 0.34 mg/l peak plasma level, and rapidly eliminated ($t_{1/2B}$ = 2.1 h). These results are in accordance with the rapid pharmacodynamic action of the drug.

Ultrasound analysis of newborn rats in behavioral toxicology

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Subtle teratogenic effects on the CNS are difficult to detect in newborn rats. Methods of behavioral teratology which are based on neuromotor performance yield measures which are hard to quantify. Ultrasound vocalization (USV) of newborn rats was evaluated as a possible alternative. The advantage of this test system is an objective quantification of a reliable phenomenon.

The USV of 3 litters (4 males and 4 females) was recorded with dedicated hardware and a minicomputer. A control litter was compared with litters treated with 5 mg/kg d for 7 d s.c. hexachlorophene and saline respectively.

The USV of isolated pups was recorded for 1 min at 22 °C every day from day 6 until the USV ceased (day 19). Each USV was registered as records (start time, a sample of the main frequency every 5ms and endtime).

The treated rats showed altered duration, quantity, interval and frequency of the USV indicating a pronounced developmental retardation.

The usefulness of vitamin B2 as a marker for compliance in antidepressive therapy

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About 20-40% of the patients show low compliance. In a research project, where depressive patients were treated for 3 weeks with 150 mg amitriptyline (At) daily, it was necessary to get objective information about the compliance of the patients. Therefore, they were medicated with special tablets containing 150 mg At and 15 mg vitamin B2 (RO 4-1575/063), At and nortriptyline were analyzed in urine by GC. The presence of vitamin B2 in urine was examined visually by its fluorescence, but also photometrically and fluorospectrometrically. For comparison, studies with healthy subjects medicated with vitamin B2 were performed. The results show that visual appreciation does not reflect truly the actual presence o vitamin B2 as assayed by physical methods. Urine collected in the morning, i.e. 12 h after medication, does not inform reliably about compliance, which may be explained by the short half-life of vitamin B2. Finally, compliance may be most efficiently tested in comparing the vitamin B2 contents in urine collected before and during 4 h after medication.

CNS-effects of caffeine (C) in cirrhotics (Cir) measured by an extended Flicker fusion (De Lange) test

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In Cir, sleeplessness, anxiety, tachycardia and tremor are often noted. Because of the similarity to 'caffeinism' and the known impairment of C plasma clearance (from 148±SD 34 ml/min to 40±19; n = 23) in Cir, we proposed that the syndrome results from methylxanthine (MX) intoxication. To test this hypothesis, the CNS effects of C were measured in 8 Cir and 5 volunteers using the De Lange procedure, a test measuring thresholds of visual perception for modulation of average luminance at given frequencies (3-40 Hz), resulting curves evaluated by estimating AUC. In all subjects, AUC was closely correlated ($r = -0.89$) with fasting C plasma levels. 1 h after oral C (4 mg/kg), volunteers exhibited only mild and variable CNS effects, AUC increasing from 8.5±0.9 to 9.0±0.8 ($p < 0.05$); in Cir AUC increased consistently from 6.4±1.9 to 7.6±1.9 ($p < 0.005$) suggesting enhanced MX effect. We conclude that the De Lange procedure represents a reproducible, noninvasive test for assessing CNS effects of xenobiotics in man.

β-Adrenergic and EGF receptors comigrate on a sucrose gradient

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Human astrocytoma cells (1321N1) possess both EGF (epidermal growth factor) and β-adrenergic receptors. Separation of membrane fragments on a sucrose gradient shows that both receptors occur on the gradient in a fraction, which contains plasma membrane. When cells were preincubated at 37 °C with either EGF or isoproterenol - a β-adrenergic agonist - the corresponding receptor was distributed in fractions: a) in a plasma membrane fraction, b) in a vesicular fraction. Incubation of cells with EGF together

with isoproterenol resulted in a comigration of both receptors on the gradient. However, EGF did not induce a comigration of the β-adrenergic receptor in the absence of isoproterenol.
These results are in agreement with the hypothesis that agonist treatment of the β-adrenergic receptor induces an internalization of the receptor similar to the well-established internalization of the EGF receptor.

Circadian rhythm of imipramine binding in the rat suprachiasmatic nuclei

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The suprachiasmatic nuclei (SCN) of the anterior hypothalamus, implicated in circadian rhythm generation, contain high concentrations of serotonin (5HT). The antidepressant imipramine binds with high affinity to brain homogenates at specific sites associated with 5HT uptake.
Specific binding of imipramine in SCN punches was highest at the end of the dark and lowest at the end of the light phase in 2 studies. Chronic methamphetamine treatment and 1 week withdrawal abolished the imipramine binding rhythm, which had partially returned after 4 weeks withdrawal. The timing of peak imipramine binding is compatible with other SCN studies that indicate a decrease of metabolic 5HT activity at lights on and an increase at lights off. If methamphetamine can abolish this rhythm, and at the same time induce internal desynchronization in behavioral rhythms, then 5HT metabolism in the SCN may be important in the as yet unknown neurochemical mechanisms of circadian rhythm generation and maintenance.

Importance of genetic factors for ethanol-induced alterations in liver plasma membrane (LPM) properties in mice

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Mechanisms of alcoholic liver disease are ill defined. We evaluated the following hypotheses: a) hypermetabolic state of the liver due to increased NaK-ATPase activity, b) alterations in LPM lipids and c) genetic predisposition. 2 mouse lines with different sensitivities towards the hypnotic effect of ethanol designated long sleep (LS) and short sleep (SS) were fed a liquid ethanol diet for 30 days. Ethanol intake reached 30 g/kg day, increasing hepatic triglyceride levels 3-fold. Following ethanol feeding in LPM fractions: a) NaK-ATPase was significantly increased to 26% above control in LS only, b) alkaline phosphatase activity was 2-fold increased in both lines ($p < 0.01$), c) free cholesterol and phospholipid content was unaltered, d) cholesterol ester increase was more pronounced in SS (1.5 in LS vs 4-fold in SS). Thus, ethanol induces specific alterations in liver plasma membrane structure and function which are influenced by genetic factors.

GENETIK - GÉNÉTIQUES - GENETICS

Structure and transcription of the *Antennapedia* locus of *Drosophila*

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Mutations at the homeotic locus *antennapedia* result in an altered developmental program of particular cells in *Drosophila*. In heterozygous adults the antenna is transformed into a 2nd leg, whereas the homozygous embryos show a transformation of the 2nd and 3rd thoracic segment towards the 1st segment. We have cloned this gene in order to study its function at the molecular level. Chromosome rearrangements associated with *antennapedia* phenotype were mapped at the DNA level. By Northern blot analysis of mRNAs and the isolation of cDNA clones the composition and number of transcripts from the *Antennapedia* gene have been studied and compared over the course of development.

Excision repair defect sensitizes a novel *Drosophila* mutagenicity test

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Drosophila provides in vivo assays that allow to screen chemicals for potential mutagenic activity. An efficient metabolism for xenobiotics in larvae as well as in adults permits the detection of pro-mutagens. In certain assays, the use of tester strains defective in DNA excision-repair had improved the detection capacity for mutagens which induce excisable DNA lesions. A promising short term test has been devised which is faster and cheaper than the most commonly used sex-linked recessive lethal test. It is based on mutational events induced in somatic cells of larvae which are trans-heterozygous for the marker mutants *mwh* and *flr*. Into this basic system, which proved capable to detect many mutagens and pro-mutagens, the excision-repair defective mutation *mei-9* was introduced. We will show to which extent this change increases the detection capacity of the test system for different classes of chemicals. (Postdoctoral Res. Fellowship, Univ. Witwatersrand, Jbg., SA; SNF grant 3.670-0.80).

Aristolochic acid: an old drug is a mutagen

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Extracts from the leaves and roots of *Aristolochia clematitis* L. have been used as medicines since the ancient Egyptian and Greek times. The most important active ingredient is aristolochic acid, a nitrogen containing, water insoluble compound which is neither an alkaloid nor a glycoside. This compound was fed to 72-h-old *Drosophila* larvae which were trans-heterozygous for the 2 genetic wing hair markers multiple wing hairs and flare (*mwh+/+flr*). The wings of surviving adults showed genetically marked, induced cell clones proving a mutagenic activity of the aristolochic acid. This result, together with a positive Ames

test (Robisch et al., Mutation Res. *105* (1982); 201-204), induction of chromosome aberrations and SCEs in human lymphocytes (Abel, unpubl.) and the observation of multiple carcinomas in treated rats (BGA, Pharm. Z. *126* (1981) 1373) indicates that aristolochic acid is a genotoxic carcinogen. (SNF grant 3.670-0.80).

DNA-repair after UV-C irradiation in lymphocytes of blood donors

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The UV-light induced DNA-repair synthesis was studied in unstimulated lymphocytes from 34 healthy blood donors aged between 44 and 76 years in 2 independent series. Repair synthesis was measured as the incorporation of H^3-thymidine over 2-h incubation period. In the 1st control series (17 persons) the total thymidine incorporation rate was 2143±334 cpm/10^6 cells (mean±SD), in the 2nd (17 persons) 1653±1105 cpm/10^6 cells. This synthesis rate was reduced to 512±91 cpm/10^6 cells by hydroxyurea in the 1st series, to 562±206 cpm/10^6 cells in the 2nd. For the DNA-repair synthesis a dose-dependent relationship resulted between 2 and 16 J/m^2. Two persons from the 1st series and one from the 2nd had significantly higher levels of DNA-repair synthesis when compared to the two control series where no significant difference was found.

Assessment of micronuclei in blood erythrocytes of intact and splenectomized rats

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In order to investigate the role of the spleen in eliminating micronucleated erythrocytes, intact and splenectomized rats were treated i.p. with single doses of 0.5 mg/kg triethylenemelamine (TEM) on postnatal day 20. The 2 corresponding control groups were treated with the solvent (water) only. Micronuclei were assessed in polychromatic blood erythrocytes 24, 48 and 72 h after treatment. In both treatment groups effects were most pronounced after 48 hours. The micronucleus rate was, however, considerably higher in the splenectomized rats (12.0%) than in the intact rats (6.4%). This tendency was also present in the control groups, where the incidences of micronuclei were 1.0 and 0.2%. These results indicate that in rats micronucleated erythrocytes are eliminated by the spleen. This elimination process, may, at least in part, be responsible for the low micronucleus rates usually found in the blood erythrocytes of rats in comparison to those in the bone marrow erythrocytes.

Promutagens detected by a rapid test with *Drosophila* somatic cells

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Somatic mutation and recombination testing was used to assess the activity of a number of mutagens and promutagens in *Drosophila*. The utility of any submammalian test depends on its capacity to detect also those compounds which become mutagenic upon metabolic activation. We evaluated a test system which in addition appeared advantageous because it is inexpensive and straightforward in application. Larvae, trans-heterozygous for 2 recessive wing hair mutations *(mwh + / + flr)*, were fed with the chemicals. Mutational events lead to mutant clones in the wings of the adult fly that appear as *mwh//flr* twin spots or as single spots, either *mwh* or *flr*. Significant frequencies of spots were induced by Aflatoxin B_1, DEN and Procarbazine. Thus, *Drosophila* larvae possess the different metabolic activation systems which are required for the conversion of these substances into their respective genotoxic metabolites. (SNF, grant 3.670-0.80).