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PHYSIOLOGIE - PHYSIOLOGY

Contribution of the sympathetic nervous system to the thermic effect of glucose

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Respiratory exchange measurements were performed on 9 subjects for 1 h before and 4 h at plasma insulin concentrations of 90 and 600 μU/ml while 20% glucose was infused to maintain euglycemia. At 2 h propranolol was infused (bolus 3.5 mg, 0.07 mg/min) until 4 h. During steady state conditions glucose uptake (M) was 516 ± 53 and 723 ± 40 mg/min. Metabolic rate (MR) had increased by 0.11 ± 0.02 and 0.16 ± 0.03 kcal/min and plasma norepinephrine (NE) by 0.29 and 0.45 nmol/l. With propranolol M was unchanged, NE increased by 0.35 and 0.33 nmol/l and MR fell by 0.05 and 0.08 kcal/min at the two insulinemias respectively. The increase in MR was correlated with the rate of non oxidative glucose storage before ($r = 0.74$, $p < 0.001$) and after ($r = 0.49$, $p < 0.05$) β-receptor blockade. The energy cost of glucose storage (slope of regression line) was decreased ($p < 0.005$) by propranolol. It is concluded that there is a sympathetically mediated component in the thermic effect of glucose.

Glucose catabolism in the chick embryonic tissues

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Glucose uptake as well as CO_2 and lactate productions were measured in the intact, in vitro developing chick blastoderm, and compared to the values of oxygen uptake (Raddatz and Kucera, Respir. Physiol., *51*, 153, 1983). At the end of gastrulation, glucose represents the main source of energy. Under normoxia, from 25 to 40% of glucose are oxidized, the rest is converted to lactate. The energy consumption of the embryo is comparable to the most active differentiated as well as tumoral tissues. The total ATP produced is estimated at 7–10 $nmol \cdot h^{-1}$. $(\mu g\ protein)^{-1}$. Only 20% of this production is possible under anaerobiosis. In the area pellucida, oxygen and glucose uptakes increase in parallel to growth, and the capacity to oxidize glucose remains unchanged. In the extraembryonic area opaca, the oxygen consumption increases more rapidly than growth parameters, and the efficiency of glucose utilization seems to increase as well.

Enhancement of spindle Ia sensitivity by static γ-axons activated at low stimulation rates and short muscle lengths

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When conventionally classified static γ-axons were stimulated at low rates Ia responses to sinusoidal stretch could be clearly enhanced, provided that mean muscle length was short or intermediate (but within the physiological range) and that any after-effects of preceding fusimotor action were first abolished by a large stretch. The stimulation rates provoking this effect ranged from 5 to 90/s (median 30/s). Increases in Ia sensitivity exceeding passive values by 10% and more were obtained in 65% (> 50% in 45%, > 100% in 21%) of the γ_S-Ia interactions. The size of such changes in sensitivity was not correlated with the excitatory strength of the same γ-fibers nor with the amount of reduction in dynamic index (at 100/s, large ramp stretches). Thus, increases in spindle Ia sensitivity during natural movement may no longer be safely attributed to dynamic fusimotor action, since low-level activation of static efferents may cause the same effect.

Blood flow redistribution during insulin hypoglycemia. A comparative study in dog and rat

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In conscious unrestrained dogs, the major change in blood flow redistribution following the injection of 0.75 UI/kg of insulin is a sharp increase in blood perfusion of adipose tissue, with a large gradient from subcutaneous fat (extreme values −2% and +880%) to deep fat deposits (perirenal values ranging from +125% to +3200%). In conscious rats, injected with a dose of insulin (3 UI/kg) leading to the same level of hypoglycemia and same increase in plasma catecholamines as in dog, a vasoconstriction occurred in adipose tissue, especially marked in the brown adipose tissue. Thus, with the same activation of the sympatho-adrenal system and alteration of metabolic substrates, the final regulatory outcome is completely different in both species. This suggests the existence of an overriding control mechanism which is activated during competing homeostatic drives.

Sodium-dependent transport of phosphate in LLC-PK_1 cells

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Transport of phosphate (P_i) by the epithelial cell line LLC-PK_1 has been studied both in intact cells and in luminal membranes prepared from cells grown upon microcarrier beads. Transport of P_i by subconfluent cell monolayers is dependent on the presence of sodium. Analysis of P_i-uptake in the isolated luminal membranes (8-fold enrichment of apical marker enzymes) identified a sodium-P_i cotransport system. In both systems analysis of the sodium activation of P_i-uptake suggested the involvement of two Na^+-ions in the transport mechanism (app. K_m for sodium: 56 mmoles/l in intact cells and 32 mmoles/l in isolated membranes). Sodium decreases the apparent K_m for P_i of the transport system (at 100 mmoles/l Na^+ the app. K_m was 96 ± 15 μmoles/l in intact cells and 99 ± 19 μmoles/l in isolated membranes).

The results indicate that LLC-PK_1 cells contain a Na^+-dependent cotransport system for P_i localized in the luminal membrane, which is similar to that found in proximal tubular epithelial cells. (SNF Nr. 3.226.082).

Energy metabolism during the post-exercise recovery in man

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A mixed meal (55% CHO, 27% fat and 18% protein) was given to 10 young male volunteers on two occasions: after a 4-hour resting period, and on another day, 30 minutes after completion of a 3-hour exercise at 50% $\dot{V}_{02max}$. Energy expenditure and substrate utilization were measured by indirect calorimetry for 17 h after meal ingestion. The fuel mix oxidized after the meal was characterized by a greater contribution of lipid oxidation to total energy expenditure when the meal was ingested during the post-exercise period, as compared with the meal ingested without previous exercise. During the night following the exercise, the stimulation of energy expenditure seen during the early recovery period gradually faded out. How-

ever, basal metabolic rate measured the next morning was significantly higher (+4.7%), as compared to control values. It is concluded that intense exercise stimulates both energy expenditure and lipid oxidation for a prolonged period of time.

Purification of an insulin secretion promoting factor from rat hypothalamus and plasma

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In previous works, we demonstrated that the hypothalamus of normal rat contains a principle able to stimulate insulin secretion both *in vivo* and *in vitro*. The purification of this factor, consisting in two subsequent gel filtrations and reverse phase HPLC, has permitted its partial chemical and biological characterization: the factor has a molecular weight of 1200–800 D, is thermostable and enzymatically digestible; its action is dose-dependent, not mediated by adrenergic, cholinergic and opioid receptors, it potentiates the insulin secretion promoted by glucose or amino acids. When extracting plasma from normal rats, an insulin secretion promoting activity was found that behaved similarly both upon partial purification and in the bioassay *in vivo* and *in vitro*. It is suggested that both rat hypothalamus and plasma contain factor(s) which could participate to the overall control of insulin secretion.

Dependence of channel conversion at developing motor endplates on contractile activity of the muscle fibers

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At endplates of neonatal rat soleus muscle, the apparent mean open time of the synaptic ion channels changes from about 4 to 1 msec between postnatal days 8 and 18 (Sakmann and Brenner, Nature *276*: 401, 1978). To test whether channel conversion depends on muscle activity the sciatic nerve was crushed at day 7 and endplates were located shortly after reinnervation at day 14 to 15 by recording miniature endplate potentials in response to focal application of hypertonic sucrose solution. No indication of fast gating channels is found at these endplates, in contrast to normally developing endplates where channel conversion is 70% complete at the same age. On the other hand, channel conversion also occurs at endplates of directly stimulated muscle fibers that had been denervated while gating was still slow. It is concluded that conversion of junctional channel gating depends on the contractile activity of the muscle fiber.

Obidoxime chloride action on AChE and AChR in single motor endplates: an electrophysiological study

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When applied iontophoretically at single frog motor end-plates obidoxime chloride (= Toxogonin^R) is shown to have a complex effect on both the AChE and the AChR.
Single short applications of obidoxime alone depolarize very weakly the postsynaptic membrane. When longer applications preceeded a short ACh-pulse, obidoxime exerted a potentiating effect on the ACh-induced depolarization in native end-plates, and an inhibiting effect after having blocked the AChE activity with sarin.
It is concluded that obidoxime on the one side can interact with the AChE by inhibiting its activity and therefore acting as an indirect cholinergic agonist. On the other side it interacts also with the AChR with a low intrinsic activity, acting as a weak direct agonist when applied alone or as a partial antagonist when applied together with the physiological neurotransmitter ACh.

Adaptive response of inorganic phosphate (Pi) transport to low Pi medium in cultured LLC-PK$_1$ renal cells

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Pi transport by the renal epithelial cell line LLC-PK$_1$ was studied in both intact cells and isolated apical membranes vesicles (AMV) after incubation in low and high Pi medium.
Low Pi medium incubation for 20 h leads to a 100% increase in Na-dependent Pi uptake by both cells and isolated AMV. Pi transport kinetics indicates an increase in Vmax in low Pi adapted cells (10.1 ± 3.94 vs 5.73 ± 1.79 nmoles/mg prot) and in isolated AMV (100.0 + 17.1 vs 52.6 ± 17.7 pmoles/mg prot) but no change in apparent Km for Pi and Na^+. Pi transport adaptation in intact cells was already expressed after 1 h in low Pi medium (20% of the effect measured at 20 h). A complete reversal of the adaptive response was obtained 3 h after Pi repletion. The change in Pi transport appears to be specific since the Na^+-dependent alanine cellular uptake was not altered by low Pi medium. In summary these observations demonstrate the existence of an adaptive mechanism for the Na^+-dependent Pi transport in LLC-PK$_1$ cells which appears to be similar to that found in proximal tubular epithelial cells.

Changes in single fiber EPSP's evoked by posttetanic potentiation

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To test the hypothesis that some synaptic boutons are normally silent but can be made active by posttetanic potentiation (PTP), EPSP's evoked by single homonymous spindle afferents were recorded intracellularly from cat extensor motoneurons. Afferents were stimulated by muscle stretch and individual action potentials recorded from intact dorsal root filaments were used to isolate single fiber EPSP's by spike triggered averaging. Averages of 500–3000 EPSP's were recorded before and 1–4 sec after tetani applied to the muscle nerve.
PTP produced amplitude increases in about 60% of EPSP's studied and decreases in the rest. Many EPSP's showed changes in wave shape, chiefly changes in slope of the falling phase. These can be interpreted as changes in the electrotonic distances of active synaptic boutons from the soma of the motoneuron studied. This suggests that some boutons of a single synapse which were silent during normal synaptic activity become active as a result of PTP. Sup. SNF 3.308.0.82 and an A.D. Williams Fellowship.

Is glutamate the transmitter of cerebellar parallel fibers?

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Glutamate (glu) has been implicated as the transmitter between parallel fibers (PF's) and Purkinje cells (PC's). Antagonists of PF-PC transmission should also block glu excitation. In the isolated frog cerebellum, glu, aspartate (asp), kainic acid, quisqualic acid, and N-methyl-D-aspartic acid were iontophoresed while recording extracellularly from single PC's, activated synaptically by electrical stimulation of the PF's and climbing fibers (CF's). Antagonists (γ-D-glutamylglycine, γ-D-glutamylaminomethylphosphonic acid, γ-D-glutamylaminomethylsulfonic acid, 2-amino-5-phosphonovaleric acid, baclofen, and kynurenic acid) were bath-applied. All of these antagonists (0.1 mM) blocked PF-PC evoked spike potentials and amino acid excitations to varying extents. Each reduced asp excita-

tions and left glu excitations unchanged. Higher concentrations (> 1 mM) partially reduced the glu- and the CF-evoked responses.These results suggest that glu is more likely the CF transmitter, and asp is the PF transmitter.

Endurance exercise effects on the respiratory system

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Running training was used to elicit adaptation of the structure and function of the respiratory system of young rats (7 weeks old). Two groups were trained for six weeks, one daily for 25 min. and the other weekly for 10 min., while a third was left untrained as a control. Functional adaptation was assessed by measurements of the aerobic capacity elicited by running ($\dot{V}O_2max$) and the summit metabolism achieved by cold-exposure ($\dot{V}O_2sum$). The mean $\dot{V}O_2max$ of the daily trained and weekly run groups was 28 and 11%, respectively, higher than the control group, with a 58% range of the individual values. No differences were found in $\dot{V}O_2sum$ among the groups either before of after the training period. Thus with training $\dot{V}O_2sum$ is not proportional to and cannot be used as an index of $\dot{V}O_2max$. Evaluation of the structural adaptation of the lung and muscles of these rats is in progress and will be discussed.

Phosphate transport across basolateral membrane of small intestine

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Transport of phosphate (P_i) by the intestine has been throughly investigated at the brush-border level, but it is not known how this anion is transported across the contraluminal membrane. In order to study this, vesicles of basolateral membranes (BLM) from rat jejunum were obtained according to a recently reported technique (Biochem. Biophys. Acta 689: 327, 1982) and uptake of P_i and for comparison of D-glucose (G) into these vesicles was measured. G uptake was not Na-dependent and reduced up to 66% by 100 µM phloretin. P_i uptake was slower than G uptake for up to 10 min but equilibrium values were equal. P_i uptake did not change in the presence of a Na or a K gradient and it was reduced in the presence of AsO_4^- and $SO_4^=$ but not with Cl^- in the medium. Preliminary experiments show that P_i uptake was stimulated by preloading the vesicles with P_i or $SO_4^=$. Thus P_i transport in BLM is not Na-dependent and appears to take place by a carrier-mediated transport mechanism.

In vitro manipulations of the hypothalamo-hypophyseal neuroendocrine system: pars intermedia in development

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The profile of serum pars intermedia pituitary melanophore stimulating hormone (MSH) in pre- and postnatal development shows a pronounced peak four and five days after birth. The decline in hormone levels after this peak is correlated with an increase in the number of catecholamine-containing nerve terminals in the pars intermedia as well as an increase in pituitary dopamine content and functional dopamine receptors (Davis et al., *Neuroendocrinology*, in press). Dopamine is a potent inhibitor of MSH secretion. In order to better assess the functional state of the dopaminergic neurons in development, we have begun in vitro studies on excised hypothalamo-hypophyseal tissue. Electrophysiological, hormonal and biochemical analyses have indicated that as innervation of the pars intermedia proceeds in the early postnatal period, dopamine is liberated from the nerve terminals and causes an inhibition of spontaneous MSH release.

Mammalian retinal function during acid-base changes

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It is known that acid-base balance affects the electroretinogram (ERG), a mass electrical response of the eye to light. Our goal was to determine which acid-base parameter is most important in influencing the ERG. ERGs were recorded in the *in vitro*, arterially-perfused cat eye every 30 seconds during four acid base changes. Three of the changes were done under constant extracellular pH. The amplitudes of the b- and c-waves of the ERG were measured. The important acid-base parameter with regard to the ERG appears to be intracellular pH. Changes designed to produce intracellular acidosis resulted in an initial decrease in b-wave amplitude and an initial increase in c-wave amplitude: changes designed to produce intracellular alkalosis had the opposite effect.

Velocity and position related signals in the frog's ocular motor system

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In frogs, compensatory eye movements are initiated by fast retino-ocular and vestibulo-ocular pathways (carrying velocity signals), and maintained by signals processed by velocity-to-position integrated networks. In contrast to mammals, neuronal signals related to eye velocity are not stored centrally in frogs. This feature greatly facilitates the analysis of the unknown properties of these integrating networks.

Analysis of response patterns of individual, antidromically identified abducens motoneurons or of multiunits of the abducens nerve, evoked by optikinetic or vestibular stimuli shows, that some of these neurons (large motor units) mediate a pure velocity signal, whereas other neurons (medium and small motor units) mediate signals that are composed to variable degrees of velocity plus position commands. From the position related signals, it can be infered that the frog's velocity-to-position integrating networks have remarkably little leakiness (τ ca. 90s) and that they interact linearly.

Comparative susceptibility to gastric ulceration in female Roman high- and low-avoidance rats

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A previous study (Driscoll et al., Physiol. Behav. 31, 225–228, 1983) has shown that male, 6–7 month old RHA/Verh rats, which were individually housed in plastic cages with sawdust bedding and food-deprived (F–D) for 4–5 days, had more pyloric lesions than did their unfasted controls or than their F–D RLA/Verh counterparts. The present study showed this to also be the case for female, 6–7 month old RHA/Verh and RLA/Verh rats treated in the same way, except that the incidence of lesions was generally less than half of that for the male rats tested previously. In addition, certain environmental effects which influenced the lesion scores in F–D male rats, such as being in the same room as the control animals or not, were not evident with the F–D females. Although the genetic differences were essentially the same in the 2 studies, it has also been confirmed that female rats are more resistant to stomach ulceration than are males. (This study was conducted at the Institut für Verhaltenswissenschaft, ETHZ, Zürich).

Serum-free aggregating cell cultures of fetal rat spinal cord

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Aggregating cell cultures of fetal (14 days gestation) rat spinal cord were prepared and grown in a chemically defined medium. Morphological and biochemical examinations of these cultures showed a cellular organization and differentiation comparable to the tissue in vivo. The influence of various tissue extracts, conditioned media extracts, and purified growth factors on the maturation of the cultures was studied. The results suggest that serumfree aggregating cell cultures offer a useful model to study the growth, differentiation and plasticity of spinal cord cells.

Rapid information processing in type A and type B individuals

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From a pool of 255 female subjects, the ten subjects with the highest and the ten subjects with the lowest questionnaire index of coronary-prone behavior (Type A) performed in a single test session a rapid information processing task. It consisted in single digit presentations on a screen in a pseudo-random order, whereby the subjects had to detect if the last three consecutive digits were either even or odd. The speed of presentation was high and automatically adapted to the subject's actual performance. Type A subjects performed less well and tended to a more pronounced reactivity in systolic blood pressure, skin conductance level and vasoconstriction to this task than the Type B individuals. Higher levels of baseline values could be shown for Type A's for pulse transmission time and skin conductance reactions as well as for some FPI-personality subscales.

Dissociation of spatial and taste aversion learning by temporal lobe knife cuts in the rat

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Parasaggital knife cuts between the lateral amygdala and the overlying pyriform cortex produce deficits in conditioned taste aversion apparently identical to those after basolateral amygdala electrolytic lesions (FitzGerald and Burton, Physiol. Behav. *30*, 203, 1983). The effects of these knife cuts on radial maze behaviour were analysed using a fully automated six arm tunnel maze with angled arms. Total activity, position of the first repetition, and number of repetitions to reach all arms were unaffected by these cuts. Reference memory was measured by changing the maze configuration to its mirror image: both lesion and control groups responded identically to this manipulation. We conclude that spatial and taste memory are functionally independent. The anatomical locus for the taste aversion deficits is not clear – we suggest that they may be due to interruption of fibres from the pontine taste area to gustatory cortex, not to amygdala damage (cf. Lasiter, Physiol. Psychol. *10*, 377, 1982).

Some characteristics of K^+ channels in frog erythrocytes

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Potassium channels have been observed in the membrane of frog erythrocytes using the patch clamp technique with both cell attached and inside-out membrane configurations. Seals, typically 2–5 GΩ, were characterised by the presence of a large cell vesicle within the electrode and showed greater stability for positive electrode potentials. The appearance of channels after routine preparation using the Gardos treatment (Gardos, Biochim. biophys. Acta *30*, 653, 1958) confirmed that these channels were calcium activated. Under voltage clamp conditions with isotonic 100 mM K^+ solutions (pH 7.2) in both pipette and cell chamber, the single channel conductance was found to be in the range 80–100 pS (sometimes higher) at room temperature. This is about 5 times larger than that reported for human erythrocytes (Hamill, J. Physiol. 319, 97P, 1981) under similar conditions. A voltage dependence of the channels was observed and in some records up to three independent channels, having the same conductance have been seen.

Acquisition and extinction of one-way active avoidance in adult and senescent rats

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Male rats of ages 6, 19 and 33 mo received 30 trials of acquisition in a shock-motivated, one-way active avoidance paradigm followed on the next day by 10 extinction trials. CS durations of 3 or 10 sec were used to more clearly define the role of motor factors in the expected poorer performance of the old rats. The cumulative reaction times for each subject were fitted to a bent line with interpretable parameters according to a previously described mathematical model. There was a significant difference among age groups. The 33-mo old rats performed poorest, 19-mo old rats intermediate, and 6-mo old rats best. Extinction was evaluated with an analogous mathematical model, but only after the data were adjusted for differences in acquisition. There were no age differences in extinction. Since the pattern of results was similar for the two CS intervals, it seems unlikely that the poorer acquisition of the senescent rats was primarily the result of impaired motor capability.

Effect of coordination between breathing and cycling rhythm on oxygen uptake during bicycle ergometry

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Breath-by-breath computer analysis of oxygen uptake ($\dot{V}O_2$), ventilation ($\dot{V}_E$) and alveolar PCO_2 ($PACO_2$) was carried out in 30 volunteers cycling on an ergometer in 4 runs with a load equal to 50% of their work capacity 170 (Sjöstrand). In 2 runs the subjects were asked to breath synchronously with an acustic signal produced by their cycling, whilst in the 2 other they breathed as they liked. The degree of actual coordination between breathing and cycling was continuously ascertained. Periods of pronounced discoordination, occasionally occurring within runs, were regularly accompanied by an increase of $\dot{V}O_2$. On the other hand, in most subjects $\dot{V}O_2$ decreased with increasing coordination, if runs with analogous $\dot{V}_E$ and $PACO_2$ were compared. This tendency was especially obvious during spontaneous coordination, whereas enforced coordination was sometimes felt as disturbing and led then to relatively high $\dot{V}O_2$. These results suggest that $\dot{V}O_2$ can be reduced by an optimal coordination between cycling and breathing rhythm.

Microcalorimetric study of the Cl^-/HCO_3^- exchange mechanism in the mouse soleus muscle membrane

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SITS (a stilbene derivative) is a known inhibitor of the Cl^-/HCO_3^- exchange mechanism in mouse soleus muscle membrane (Aickin and Thomas, J. Physiol. 273, 295, 1977). In our experiments, 10^{-4}M SITS decreased the steady-state heat pro-

duction rate of mouse soleus muscle by 3.6% under physiological acid-base conditions. The effect developed progressively in about 15 minutes and was only partially reversible. It was abolished in severe respiratory acidosis (pHo = 6.8), but not in extracellular metabolic acidosis of the same amplitude, even after two hours of equilibration. The exact origin of the energy dissipation which is suppressed by SITS is still unknown. The results suggest that, under our experimental conditions, this energy is more likely related to a passive HCO_3^- outward flux through the Cl^-/HCO_3^- exchange mechanism than to an active HCO_3^- inward flux.

Low noise voltage clamp of the cut-open squid giant axon

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The membrane leaflet of a cut-open giant axon was mounted between holes (dia. .3, .5 or 1.0 mm) in the apices of 2 opposing Plexiglas cones which were part of a Ussing type chamber. Lowest noise recordings were obtained using only one large surface Ag/AgCl pellet electrode on either side for passing current and measuring the membrane voltage (Greeff, J. Physiol, *343*, 17P, 1983).

In order to exclude any uncertainty about the membrane voltage due to electrode polarization by the passing current, a conventional clamp circuit was adapted which incorporates a second voltage measuring pair of electrodes. This introduces a feedback loop with extra noise related to the thermal noise of the electrodes and the amplifiers and to the resistance in series with the membrane. Based on a circuit analysis a low noise clamp was developed. Its background noise variance approximately doubled but was still only about 15% of the peak Na-channel variance which was to be measured with this clamp.

Glial cell aggregates: myelin formation in absence of neurons

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Glial cells of newborn rat telecenphalon grown to confluence in monolayer cultures with serum were detached and subsequently cultured as aggregates in a chemically defined medium. Morphological and immunocytochemical investigations of these glial cell aggregates after 2 to 4 weeks showed the presence of differentiated astrocytes and oligodendrocytes and the absence of neurons. MBP-immunostaining and well preserved myelin with normal periodicity (110 Å) were observed. Biochemical measurements after 17 days in culture showed relatively high levels of CNP-ase activity (11417 ± 418 nmoles/mg prot/min) as well as of astroglial GS activity (54 ± 8 nmoles/mg prot/min), whereas neuronal CAT activity was not detectable.

These data indicate that glial differentiation, including myelin formation, occurs in the absence of neurons in these aggregate cultures.

Long term potentiation depends on potassium currents

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The long lasting enhancement of synaptic transmission after a short afferent tetanus (LTP) was studied in the CA1 area of rat hippocampal slices by intracellular recording with CsCl filled electrodes. Through block of K^+ channels by Cs^+ most action potentials were considerably prolonged and after-hyperpolarizations were missing. A short tetanus evoked LTP as recorded by the increase of the dendritic field (extracellular epsp) but failed to increase the intracellularly measured epsp in all 6 pyramidal cells studied. Ca^{++} spikes (in TTX) were much larger (upto 100 mV) than those recorded with KCl electrodes (20–60 mV) and lacking AHPs. Pre- and postsynaptic excitatory signals may thus normally be curtailed by K^+ currents which are subject to modulation by several transmitters with potentially long lasting actions. Classical inhibition is not reduced during LTP (Haas and Rose, J. Physiol. 329, 541–552) but a reduction of Ca^{++} and voltage dependent K^+ currents may well be responsible for this form of synaptic plasticity.

Transport of inorganic anions in rat small intestinal (IV) and rat renal proximal tubular (RV) basolateral membrane vesicles (BLMV)

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Vesicles were isolated by Percoll-centrifugation. Na^+-K^+-ATPase (BLMV) was enriched approx. 10-fold (IV) and 15-fold (RV); brush border membranes, mitochondria and endoplasmic reticulum were not enriched. Sodium independent carrier mediated transport of inorganic anions was documented by transstimulation under 'voltage clamp' conditions. $^{32}PO_4$ uptake was stimulated by preloading with unlabelled PO_4, SO_4, OH and Cl (RV). $^{35}SO_4$ uptake was stimulated by preloading with SO_4, OH and Cl (IV). Occasionally observed stimulations by sodium could be correlated with crosscontaminations by brush border or mitochondrial membranes. It is concluded that IV and RV contain sodium independent anion exchange mechanisms. (SNF 3.226.082).

Peripheral origin of otolith afferent signals relating to the maculoocular reflexes in the frog

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Previous studies have shown that stimulation of the otolith organs by subjecting frogs to static tilts or sinusoidal linear accelerations along their longitudinal or transverse body axis induces torsional or vertical eye movements, respectively. We have now studied the peripheral origin of the afferent otolith signals by comparing the maculo-ocular reflex dynamics of intact frogs with that of animals with bilateral sections of the anterior branch (which includes the utricular afferents), the saccular or the lagenar branches of the VIIIth nerve, respectively.

In the animals with a bilateral section of the ramus anterior no eye movements could be evoked in the dark. The maculo-ocular response dynamics in terms of frequency response in frogs with selective bilateral saccular or lagenar deafferentation was within the limits of control animals.

Our lesion experiments suggest that the utriculus and not the sacculus or the lagena is mainly responsible for the generation of maculo-ocular eye movements.

Action and binding of γ-hydroxybutyrate on neurones of cultured rat central nervous system

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By means of lightmicroscopic autoradiography we have observed binding sites for the GABA catabolite γ-hydroxybutyrate (GHB) on neurones of cultured rat cerebellum and spinal cord. In contrast, glial cells remained unlabelled. ^{3}H-GHB was bound to similar types of neurones as ^{3}H-GABA, although the number of labelled cells by ^{3}H-GHB was considerably smaller than that by ^{3}H-GABA (Hösli and Hösli, Neuroscience Let-

ters, in press). Electrophysiological studies have shown that GHB produces a hyperpolarization which is associated with an increase in Cl^--conductance and which is reversibly antagonized by bicuculline (Hösli et al., Neuroscience Letters *37*, 257, 1983). It is suggested that GHB might influence neurotransmission mediated by GABA or act as neurotransmitter or neuromodulator in the rat central nervous system.

How tight is the coupling of the supplementary motor area (SMA) with the spinal cord in monkeys?

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In previous experiments, it was shown that trains of microstimuli applied to the caudal zone of the SMA elicited twitches, mainly in shoulder muscles. We now have recorded EMG responses from various arm muscles with chronically implanted intramuscular leads. The efferent zones from the SMA appear to frequently involve a number of sometimes disjunctive muscles (e.g. shoulder and forearm muscles). This might be due to a higher degree of collateralization of corticospinal neurones. In further experiments, single micro pulses were injected in efferent zones where train stimuli evoked twitches, in order to investigate post-pulse facilitation of ongoing postural EMG activity. For MI stimulation, post-pulse facilitation was observed in 13 out of 16 tests. The incidence of facilitation in comparable muscles obtained with SMA stimulation was only 7 out of 20 tests. The facilitation latencies obtained from the 2 areas were in the same range (5–7.5 ms). These results indicate that the SMA has some oligo- or even monosynaptic connections with motoneurones, but that the connections are less dense than from MI.

Changes of external and internal pH in acute myocardial ischemia

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Extracellular pH (pH_o) and the transmembrane pH gradient (Δ pH) were measured in guinea-pig hearts during Langendorff perfusion and global ischemia by ion-selective electrodes. During perfusion (25 mM HCO_3^- in perfusate) pH_o was 7.37 ($\pm$ 0.03 SD, 13 exp.) and intracellular pH was more acid (Δ pH: -0.37 ± 0.07, 13 measurements). After interruption of perfusion pH_o gradually decreased by 0.52 pH-units ($\pm$ 0.16 SD, 11) after 10 min and by 0.65 ($\pm$ 0.16 SD, 9) after 15 min. Intracellular pH approached extracellular pH 10–15 min after the onset of ischemia and became more alkaline than extracellular pH after 19–22 min of ischemia (Δ pH: $+0.07 \pm 0.06$ SD, 6). The results indicate a smaller decrease of intracellular pH than of extracellular pH and a reversal of the transmembrane H^+ gradient in early myocardial ischemia.

Effect of 2,3-diphosphoglycerate (DPG) on the relative CO-O_2 affinity of partially saturated human blood

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The aim of the study was to investigate whether DPG modulates the relative CO-O_2 affinity of human whole blood. To this end, the blood samples were either added with citrate phosphate dextrose adenine and dihydroxyacetone (blood A) or with acid citrate dextrose and iodoacetate (blood B); both were tonometered with gases containing 6% CO_2 and O_2 and CO in concentrations insufficient to saturate Hb, the equilibration being accelerated by initial addition of CO as close as possible to the expected equilibrium HbCO. After equilibration and repeated pH adjustments to about 7.4, the DPG/Hb ratio was 1 in A and below 0.1 in B. Total Hb saturation was found to be larger in B, the increase of HbCO being systematically larger than that of HbO_2. Thus, under conditions of partial Hb saturation DPG decreases the relative CO-O_2 affinity; a finding which suggests that attachment of DPG to the Hb-tetramer stabilizes a conformation which has a lower CO-O_2 affinity than other allosteric states.

Callosal projections of primary visual cortex in *Tupaia*

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In three shrews *(Tupaia belangeri)* it has been found that the majority of geniculo-striate projections from both eyes terminate in distinct sublayers of cortical layer IV. This tangential organization opposes the wellknown ocular dominance columns as described for the cat and rhesus monkey.

In the present study we investigated whether in *Tupaia* callosal connections would also differ from those known in the species mentioned above. The organization of a callosal pathway was examined by using retrograde horseradish peroxidase (HRP) transport. HRP was injected unilaterally at various depths and locations within area 17 of adult tree shrews.

Our results show that in *Tupaia* callosal connections exist between the areas 17 with neurons originating above and below layer IV. The projections are not confined to the 17/18 border – as in cats and primates –, but rather extend over a substantial part of the primary visual cortex.

Responses of embryonic cells to electrical stimuli as studied by image analysis using a real-time video processor

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Brief electrical currents were applied by a monopolar electrode to different regions of an intact in vitro gastrulating chick embryo. The evoked mechanical responses of embryonic cells were studied by substracting the image of embryo recorded before stimulation from the images recorded at regular intervals following the stimulus. A single stimulus leads to a phasic contraction starting in the cells surrounding the electrode and then spreading concentrically to distant areas. Such a response is completely reversible. On the contrary, repetitive stimuli, applied at a frequency corresponding to the periodicity of spontaneous cell movements, lead to modifications of cell migrations resulting in a reorganization of embryonic layers: disruption of the extodermal basal lamina and ectopic accumulations of cells in the mesoderm. These results represent an experimental support to the hypothesis of ionic currents as possible epigenetic modulators of the early embryogenesis.

Daily afternoon administration of melatonin does not irreversibly inhibit sexual maturation in the male rat

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Melatonin delayed sexual maturation in the male rat if injected from days 20–40. This was documented at 40 days by markedly lowered plasma levels of testosterone, LH and FSH, pituitary GnRH receptor number and weights of testes and seminal vesicles; but at 80 days of age all parameters studied reflected complete sexual maturation. Male rats were most sensitive to daily afternoon melatonin injections at the beginning of sexual maturation at 20–30 days of age. In rats treated continuously with melatonin from days 20–115, sexual maturation occurred but was delayed by about 20–30 days. Beginning of sexual development was observed at 60 days and full sexual maturation was attained only at 100 days of life. The supression of the pubertal peaks of pituitary GnRH receptor number

and of pituitary and plasma FSH concentrations in treated rats indicate that melatonin probably interferes with the pubertal increase of GnRH secretion. Our results suggest that melatonin represents an important factor for the timing of sexual maturation.

Calcium-dependent phosphorylation in rat renal brush border membrane vesicles (BBMV)

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BBMV were exposed to γ-^{32}P-ATP either in the presence of saponin or under osmotic shock conditions (transient opening) in order to allow phosphorylation at the cytoplasmic membrane face. In a Ca-free medium, ^{32}P is incorporated into at least 16 polypeptides. When the phosphorylation is made in a Ca-containing medium: a protein of M_r 97 kD is maximally phosphorylated at 0.5 µM free Ca; and a protein of 12 kD is maximally phosphorylated at 10 µM free Ca.
Ca-dependent phosphorylation is inhibited by spermine and cytotoxin I but not by trifluperazine, suggesting the involvement of a membrane bound Ca-phospholipid dependent kinase. Whilst a Ca-calmodulin-dependent phosphorylation was not found, indirect evidence for a Ca-calmodulin-dependent protein-phosphatase was obtained. A role for Ca-dependent phosphorylation in transmembrane Na-dependent phosphate transport in BBMV is however not apparent (SNF 3.226.082).

Pyrazinoate (PZA) transport in renal brush border membrane vesicles (BBMV)

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PZA is an organic anion actively reabsorbed in the mammalian kidney. We investigated its transport mechanisms using rabbit BBMV. In the presence of a Na^+-gradient (out-to-in), a transient accumulation of PZA was induced which exceeded (overshoot) the equilibrium concentration reached when the Na^+-gradient was dissipated. In the absence of a gradient Na^+ itself enhanced the initial rate of PZA uptake. K^+ was unable to produce these two effects. Our data demonstrate the existence of a Na^+-PZA cotransport. Its apparent activator constant for Na^+ was: 58 mM. Its apparent affinity for PZA in the presence of a 90 mM Na^+-gradient was $K_{m\ PZA}$: 2.1 mM; and its apparent $V_{max\ PZA}$ was 2767 pM · mg^{-1} prot · 4 sec^{-1}. Other organic anions competed for this transport mechanism. Lactate (5 mM) inhibited PZA transport by 90%. PZA might be reabsorbed by the Na^+-lactate cotransport mechanism. (Supported by F. Hoffmann-La Roche Stiftung and SNF 3.226.082)

Responsiveness of senescent and adult rats to regulatory challenges

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Temperature and drinking regulation were evaluated in senescent (> 31 mo) and adult (8–10 mo) rats of two strains. Exposure to 5 °C for 4 h produced greater hypothermia in senescent rats, whereas injection of 1.5 mg/kg apomorphine HCl or 1.0 mg/kg oxotremorine sesquifumarate produced comparable maximal hypothermic responses in adult and old rats. However, latency to maximal hypothermia after oxotremorine (but not apomorphine) was longer in senescent than adult rats. Weekly food and water intake, drinking induced by 24-h water deprivation and drinking induced by injection of hypertonic saline (osmotic challenge) were roughly the same in old and adult rats. However, senescent rats drank less after injection β-adrenergic agonist isoprenaline (volemic challenge) than adult rats. There were no apparent strain differences in temperature and drinking regulation, although baselines sometimes differed between strains. These results add to the behavioral and physiological characterization of senescent rats.

Variance analysis of potentiated Ia EPSP's in cat spinal motoneurons

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Fluctuation analysis was performed on aggregate EPSP amplitudes after posttetanic potentiation (PTP) of extensor muscle Ia-motoneuron connections. The amplitude (v) of the unit EPSP and the total number N of synapses given off by the stimulated Ia fibers to each motoneuron studied was estimated using an ensemble averaging technique (Sigworth, J. Physiol. 307: 1980).
10–60 series of stimuli were applied to the muscle nerve. Each series consisted of a conditioning stimulus (10 sec at 500 Hz) followed by 45 test stimuli given at 1–2-sec intervals. The mean amplitude A and variance δ^2 were calculated for the aggregate EPSP's evoked by the n'th test stimulus of all series. The function $\delta^2 = vA - A^2/N$ fitted to the data is expected for N independently acting synapses and an amplitude of the unit EPSP produced by the action of a single synaptic bouton.
In 8 connections the above function fit the data well. Values for v ranged from 40 to 190 µV and for N, from 90 to 350 boutons. Sup. SNF 3.308.0.82

Selective retrograde transport of D-[^{3}H]-aspartate in certain afferents to the rat superior colliculus

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Following D-[^{3}H]-aspartate application in the rat superior colliculus (SC) retrogradely labeled neurons were observed in cortical areas 17, 18 and 18a in cases with superficial injection sites, but extending into auditory, somatosensory and motor areas with deeper injections, and bilaterally in several hypothalamic nuclei in both types of experiments. The parabigeminal nucleus, another important afferent system to SC, however, was not labeled. None of the systems transporting D-[^{3}H]-aspartate showed labeling following [^{3}H]-GABA injections in SC. The labeling in cortico-collicular pathways is consistent with neurochemical data favoring glutamate and/or aspartate as transmitters in these systems; the transmitters in hypothalamo-collicular projections, on the other hand, are unknown.

Release of Ca from nerve by activity

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Previous experiments showed that activity in nerve fibres causes an increased liberation of inorganic phosphate, adenosine, inosine and hypoxanthine (see Maire, Medilanski and Straub, J. Physiol. *323*, 589, 1982). It has now been found that in preparations loaded with ^{45}Ca, activity also leads to an increase in the efflux of ^{45}Ca. The fractional release amounts to 9.4×10^{-6} impulse^{-1} for the first period of activity, and to 7.6 and 3.7×10^{-6} for subsequent periods. The decrease seen for successive stimulations is absent when acidification during activity (see Mullins et al., J. Physiol. *338*, 295, 1983) is prevented by the use of solutions with NH_4Cl/NH_3; further, addition of Ca or adenosine increases Ca release. If the Ca release originates from an intracellular pool of 0.5 m-mole/kg wet weight, the stimulated release would correspond to an efflux of 0.8 fmole · cm^{-2} · impulse^{-1}.

Cytosolic free calcium and parathyroid hormone secretion

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Parathyroid hormone (PTH) secretion is known to be stimulated by low extracellular Ca (Ca_e) and high extracellular K (K_e) concentrations. Measurements of free intracellular Ca (Ca_i) with the fluorescent probe quin-2 provide evidence that Ca_i is related to Ca_e over the concentration ranges of 170 nM to 580 nM and 5 μM to 1.5 mM, respectively. High K_e (60 mM) also lowers Ca_i with Ca_e ranging from 0.5 mM to 1.5 mM, but not in the absence of added Ca_e. In lymphocytes, on the other hand, Ca_i did not respond to comparable changes in Ca_e and K_e.

The time course and stimulability of PTH secretion were similar in unloaded and quin-2 loaded cells. Removal of Ca_e and raising K_e in the presence of Ca_e caused falls in Ca_i associated with raised PTH secretion, while cAMP production remained unchanged. High K_e in the absence of added Ca_e also stimulated PTH secretion but Ca_i and cAMP remained unchanged. In conclusion, while low Ca evoked PTH secretory changes are inversely related to changes in cytosolic free Ca, PTH secretion stimulated by K is not necessarily mediated by changes of Ca_i and cAMP.

Expansion of cortical receptive fields (RF) after systemic or local application of capsaicin

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Capsaicin injected i.p. into neonate rodents provokes a nearly complete, selective loss of peripheral unmyelinated fibres. This is followed by an expansion of RF supplied by myelinated afferents, not only in the spinal cord but also in the cortex (P.D. Wall et al., Nature, *295*, 691–693, 1982). After local application to an adult rat nerve, no fibre loss, no block of conduction occurs, but the terminals of unmyelinated afferents show a decrease in their ability to excite central cells (P.D. Wall et al., Exp. Neurol. *78*, 425–432, 1982).

We now show that, after application of capsaicin on the infraorbital nerve of the adult mouse, cells in the cortical barrelfield also display an expansion of their RF.

Taken together, these findings point to the important role played by unmyelinated fibres in the establishment and maintenance of the connectivity of myelinated afferents with the CNS.

Glucocorticoids inhibit NGF-induced increase in substance P in rat primary sensory neurons

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Endogenous nerve growth factor (NGF) is essential for the development and function of substance P (SP)-containing primary sensory neurons. Treatment of newborn rat with the synthetic glucocorticoid dexamethasone results in a dose-dependent inhibition of the increase in SP both in dorsal root ganglia and in dorsal horn of the spinal cord elicited by exogenous NGF.

These observations support the concept that glucocorticoids are important modulators of specific NGF action not only on sympathetic but also on SP-containing neurons in the rat dorsal root ganglia.

Thermosensitive neurons during waking and sleeping in cats

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Direct anterior hypothalamic-preoptic (AH-PO) cooling or warming was performed in cats, carrying electrodes and thermistors for EEG and hypothalamic temperature recording, by means of thermodes (1.5 mm in diameter, water perfused) placed in the AH-PO region. The animal was restrained in a loose canvas bag and the head was held rigidly but atraumatically in a Kopf apparatus by means of a metal frame fixed on the skull. Tungsten microelectrodes were used for recording the activity of single AH-PO neurons. AH-PO neurons were found which responded to thermal stimulation during waking and synchronized sleep. Responses consisted in either increase-decrease or decrease-increase in firing rate to cold-warm stimuli. In contrast, responses to thermal stimulation were absent or inconsistent during desynchronized sleep. These results are a direct proof of the inactivation of thermoregulatory structures during this stage of sleep.

Ouabain and veratridine induce a β-adrenergic-like stimulation of water flow in toad skin

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Previous work from this laboratory showed that high K induces a marked stimulation of net water flow (Jw) across toad skin. To determine if this effect is related to changes in membrane potential, experiments were carried out with two classical depolarizing agents: ouabain and veratridine. Jw ($\mu l/min \cdot cm^2$) was automatically recorded. At 0.1 mM, Jw was increased from 0.36 ± 0.04 to 0.81 ± 0.10 ($N = 16$, $p < 0.001$) by ouabain, and from 0.44 ± 0.07 to 1.12 ± 0.19 ($N = 9$, $p < 0.01$) by veratridine. This hydrosmotic effect was: a) dose-dependent; b) non-additive to that induced by vasopressin (VP) or isoproterenol; c) unaffected by the VP-antagonist, methohexital (1 mM); d) completely abolished by the β-adrenergic antagonist, propanolol (1 μM). In conclusion, ouabain and veratridine do stimulate water transport across toad skin by a mechanism that appears to involve the triggering of a β-adrenergic pathway.

Stimulation dependent release of 2-aminoethanol from rat basilar pontine gray, in vivo

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The extracellular concentration of 2-aminoethanol (ethanolamine, EA) has been shown to rise in the pigeon optic tectum upon optic nerve stimulation. We report here the stimulation dependent release of EA in another pathway, this time in a mammal.

In the pentobarbital anesthetized rat, the ventral subdivision of the basilar pontine gray was perfused with a bicarbonate buffered Ringer solution by means of a push-pull cannula. The cortico-pontine tract was intermittently stimulated with a bipolar electrode. Effluent fractions obtained before, during and after stimulation were analyzed for EA content by gas chromatographic methods. The resting level of EA was 9.6 ± 1.8 pmol/min, this increased by a factor of 3.6 during stimulation ($p < 0.001$).

Both the compartment of origin and the functional significance of the release are unknown. However, other authors propose, that membrane phospholipid reactions are involved in synaptic transmission; EA, as a polar headgroup of phospholipids, could be part of these phenomena.

Temporal organization of ultrasonic vocalizations in infant wood mice, *Apodemus sylvaticus*

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12 pups were isolated daily from 5 to 15 days of age, for 3 minutes at 20 °C in a small circular area. 4 openings in the side wall of the area led to 4 boxes containing nesting material from various adult conspecifics. The behavior of each pup was continuously monitored. Vocalizations were stored with a Nagra (0 to 150 kHz) tape recorder. Total call production remains stable until day 10 and decreases significantly thereafter. Analysis of intercall intervals reveals a strong bout organization. Bout duration decreases with age both because of increased call rate within bouts and decreased number of calls per bout, while the total number of bouts remains constant. These changes are interpreted in relation with the behavior of each pup in the different parts of the area and with more general maturational steps.

Adaptive thermogenesis during mixed diet overfeeding in man

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After 13 days of a weight maintenance diet (13.72 MJ; 15% Prot, 40% fat, 45% CHO), 5 young male volunteers (15 ± 3% body fat) were overfed at 160% of weight maintenance for 9 days. Overfeeding caused increases in body weight by 4.5%, postabsorptive resting metabolic rate (RMR) by 8% and 24-h energy expenditure (24-EE) by 21%. The thermic effect of food (TEF, 3 meals) calculated over RMR during 16 h increased by 49% but did not change when expressed as a fraction of energy intake (~ 10%). The 2038 kJ increase in 24-EE (i.e. 31% of the excess ingested energy) was explained by the increase in RMR (661 kJ), the increase in TEF (581 kJ) and the cost of physical activity (796 kJ). In conclusion adaptive thermogenesis is mainly related to the stimulation of RMR and the cost of physical activity.

Origin of the H-reflex facilitation before movement initiation in reaction time tasks

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In subjects performing plantar flexions of a foot in a reaction time (RT) situation, the H-reflex is facilitated 100 to 150 ms before movement initiation. The facilitation is specific for the leg which is moved. The two main possible origins of the facilitation are a subthreshold direct activation of α-motoneurons and a removal of a presynaptic inhibition at Ia terminals. If the facilitation is due to a direct motoneuronal activation we assume (1) that the amplitude of the facilitation would always be the same at movement onset, (2) that the slope of the facilitation would be proportional to the slope of the following movement and (3) that RT would decrease if there are motoneurones at discharge threshold during RT. Experimental results point to a removal of presynaptic inhibition since (1) the size of the H-reflex facilitation at movement onset depends on RT, (2) the slope of the facilitation but not the slope of the following movement depends on RT, and (3) RT does not decrease if the muscles are activated tonicly before the reaction signal.

Effects of endurance training on trained and untrained limbs

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An increase in maximal oxygen uptake capacity ($\dot{V}_{O2}$max) after endurance training is not only observed during exercise with trained, but also with untrained limbs (Lewis et al., Eur J Appl Physiol. 44:25–34, 1980). This study was designed to focus on general adaptations to endurance training and on local changes in trained leg muscles and untrained arm muscles. 10 young sedentary men were trained on bicycle ergometers 5 days/week for 30 min over 8 weeks at 85% of their $\dot{V}_{O2}$max. Biopsies of vastus lateralis and deltoideus were taken before and after this training regimen. Leg cycling and arm cranking $\dot{V}_{O2}$max increased 13% and 8%, respectively, and power generated at a lactate level of 4mM increased 27% for leg, but only 2% for arm exercise. These data suggest different mechanisms for the observed increase in $\dot{V}_{O2}$max during exercise with trained and untrained limbs. Currently, we are investigating the muscle biopsies to permit further evaluation of local structural as compared to general changes.

Staining of physiologically identified cells in the cat ventral cochlear nucleus (VCN)

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Comparisons between the regional distribution of functional unit types and morphological cell types have provided indirect evidence for some structure-function relationship in VCN: ‹primary-like› units were associated to ‹bushy› cells whereas ‹on› and ‹chopper› units were hypothesized to correlate with ‹stellate› cells (Bourk, P.h.D. Dissertation, MIT, 1976). To address directly the issue of how close morphological cell types relate to functional unit types, we used intracellular injections of horseradish peroxidase to stain individual neurons after their response properties to tones were determined during intracellular recordings. Three ‹primary-like› units were indeed ‹bushy› cells. However, two ‹on› and one ‹chopper› units were also ‹bushy› neurons. This means that ‹bushy› cells do not exclusively exhibit ‹primary-like› responses. In conclusion, the distinction between ‹bushy› and ‹stellate› cells in VCN does not correspond in any simple way to distinctions between physiological unit types.

Neural activity to the pancreas: stimulation by oropharyngeal, cerebral and duodenal exposure to sugars

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Insulin secretion is known to be neurally modulated via the parasympathetic system. We have recorded, in anesthetized rats, the electrical activity of two abdominal vagal (celiac and pancreaticoduodenal) branches leading to the pancreas, and monitored the changes in discharge frequency induced by sugar stimulation of different sites. The oral cavity was flushed with 10% saccharose; isotonic glucose was infused in the carotid artery, and the duodenum was perfused with 50% glucose. The oral stimulus increased the discharge rate, this effect being a correlate of the ‹early insulin release› phase. The other two stimuli also increased the discharge rate, pointing to the existence of glucose sensors in the central nervous system and/or the intestinal mucosa.

Excitatory interneurons in the spinal circuit of the Mauthner axon in the tench

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Spinal motoneurons (MN) receiving excitatory input directly from the Mauthner axon (MA) form a small portion of the motoneurone pool. The early response in the ventral root output following stimulation of the ipsilateral MA is produced by these MN. The excitatory pathways causing the late response of the same output are still unknown. Two pathways have been proposed: 1) the monosynaptically excited MN act in the capacity of interneurons (IN); 2) there are IN mediating MA excitation to the polysynaptically activated MN. In this study, intracellular recording has been combined with the injection of the fluorescent dye Lucifer yellow-CH into the non-motoneuronal spinal units responding to ipsilateral MA stimulation with a short-latency excitation and to contralateral MA stimulation with a postsynaptic inhibition. This approach allowed the histological identification of intersegmental IN in the spinal circuit of the MA and the mapping of their anatomical relationship to the MA and MN.

Functional denervation of brown adipose tissue in rat fasted at thermoneutrality

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The level of brown adipose tissue metabolic activity is sympathetically controlled. The role of innervation on the tissue's metabolic capacity as tested in presence of norepinephrine is compared in vitro in intact or in surgically denervated tissue from fed or fasted rat acclimated either at 22 °C or at 30 °C. In fed animal, denervated tissue has the same matabolic capacity as the intact tissue. However, the metabolic capacity of tissue from rat kept at 30 °C is half of that obtained in rat kept at 22 °C. Tissue from 30 °C fasted rat shows, in both intact and denervated samples, the same decreased capacity. In fasted rats kept at 22 °C, however, the metabolic capacity of the denervated tissue is only 60% of that of control. These results suggest that, in fasted rat, at thermoneutrality, the neural input to the tissue is suppressed whereas at 22 °C a neural tonic activity persists due to cold stimulus.

Vestibular end organ vs vestibular ganglion lesions in the albino rat. Are the effects different?

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It is known that after an end organ lesion the proximal portion of the vestibular nerve survives for at least several months. But is there still spike activity in the afferent fibers, and if so, is it functionally significant? We recorded single units in the vestibular ganglion. There was almost no resting activity during the first 2–7 h, and very little compared to controls at 1, 2, and 14 d after an end organ lesion. Behavioral signs of unilateral end organ lesions (spontaneous ocular nystagmus and head tilted damaged side down) largely abated during the first day. Afterwards, nystagmus continued to subside, but head tilt generally increased again between 1 and 6 d after surgery. The effects of ganglion lesions (which are known to cause rapid nerve degeneration) were quantitatively similar, except that the secondary head tilt increases were on the average larger. Thus, the two kinds of lesions are functionally equivalent, at least during the initial stage of recovery.

Decrease in exogenous free fatty acid utilization in brown fat from diabetic rats

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Brown fat from streptozotocin-diabetic rats has a decreased heat production, which was attributed to a decreased capacity for fatty acid β-oxidation (Seydoux et al., 1983). The maximum amplitude of reduction of the flavoproteins of the acyl-CoA dehydrogenase pathway in response to nerve stimulation is similar in control and diabetic rats, whereas this amplitude is reduced (to 20% of control) during octanoate addition in the latter. The utilization of octanoate can be partially restored (60% of control) by in vitro incubation of the tissue with insulin (200 μU/ml). The biochemically determined specific activities of the enzymes of the palmitoyl-CoA β-oxidation are decreased by 50% in homogenates from diabetic rats as compared to control. The correlation between the spectrometrical and biochemical determinations in the tissue from the same diabetic rats is direct only when the spectrometrical signal is measured in the presence of insulin. These results indicate that in intact brown fat from diabetic rats there could be a limitation of exogenous fatty acid utilization prior to acyl-CoA β-oxidation.

Effects of Harmaline on active ion transport across bovine tracheal epithelium

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The effects of Harmaline, an hallucinogenic drug known to modify the electrical and ion transport properties in various epithelia, were studied on bovine tracheal epithelium. When applied to the luminal side of the epithelium, Harmaline at low concentrations (10^{-6} to 10^{-5}M) stimulated the short-circuit current (I_o) by 5% to 15%. Larger concentrations (5.10^{-5} to 2.10^{-3}M) induced a dose-dependent inhibition of I_o up to 100% ($ID_{50} = 5.10^{-4}$M). When the drug was applied to the serosal side, the same pattern of response was observed but larger doses were needed. Harmaline 8.10^{-4}M on the luminal side reduced the active sodium absorption by 81% (control value 3.6 ± 0.3 μEq $h^{-1}cm^{-2}$, $n = 7$) and reduced the active chloride secretion by 52% (control value 3.2 ± 0.3 μEq $h^{-1}cm^{-2}$, $n = 7$). The kinetics of the decrease of I_o elicited by Harmaline revealed three components, with half times of 0.34 ± 0.02, 2.2 ± 0.2 and 15.2 ± 1.1 minutes ($n = 11$).

Effects of propranolol on the endogenous microvibrations of the body

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In 25 medical students with and 26 without beta-receptor blockade, changes of whole-body microvibrations (MV) in the three space directions were quantitatively analyzed immediately before an examination situation and during the ensuing vacation (control). Striking differences were found for J_z, the rectified impulse in the vertical axis, which correlates with the cardiac output: The propranolol group showed similar low J_z values in both measurements, whereas in the group without propranolol the pre-examination values were significantly increased, and fell during the ensuing vacation. Propranolol was without effect on muscle tone, reflected in J_y, the sideward MV-component, since both groups had identical mean pre-examination values, which decreased during the control period, but only in the non-treated subjects. Conclusion: MV are mainly due to the cardiac activity.

Structure and function of an osmosensory organ, the osphradium, in the sea snail *Aplysia californica*

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The osphradium (Merton, Abh. Senchend Naturforsch. Ges. **36**: 445, 1920) is one of the rare organs where structure and function of osmoreceptors can be studied. The epithelium facing the osphradial ganglion (OG) is brush-bordered and contains processes that emerge from OG cell bodies. They may be the osmosensory endings, and their relationship with epithelial cells is currently observed by EM. A large (150 µm) neurone was discovered in OG that appeared to project towards the abdominal ganglion (AG); its function is still unknown. Electrophysiological recordings in R15 of AG show typical bursting firing patterns only when the branchial nerve between OG and gills is sectioned. Phasic firing is then inhibited by superfusion of the osphradium with hypotonic (50% diluted) sea water, and by 1–10 µM 5-HT. The functional identification of the neurotransmitter (e.g. 5-HT) of osmosensitive primary afferents will allow for its (immuno)cytochemical detection in osmoreceptors.

Dopaminergic influences on transstriatal impulse transmission

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In chloralhydrate anaesthetized rats, the electrical stimulation of different areas of the cerebral cortex elicited mainly depressing responses in the electrical activity of single cells in the globus pallidus. The reduction of striatal dopaminergic transmission by systemically applied neuroleptics (Haloperidol, Fluphenazine) lead in 75% of pallidal cells to an increase or decrease of the cortically evoked depression. Increasing striatal dopaminergic transmission by electrically stimulating the substantia nigra was followed by mainly an attenuation of cortically evoked depression in pallidal cells. The results demonstrate a considerable dopaminergic influence on transstriatal impulse transmission, as measured in the responses of pallidal neurons to electrical stimulation of the cortex. Reduction of striatal dopamine receptor stimulation appears to increase the rythmicity of pallidal responses.

Stimulation causes an increase in [ATP] in honeybee drone retina

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A traditional paradigm for the control of mitochondrial respiration in cells is that work (e.g., ion pumping) causes a *decrease* in [ATP], and this decrease leads to stimulation of respiration. Tsacopoulos et al. (Nature, 301, 604, 1983) have suggested that another mechanism can operate in drone retina. We have now measured [ATP] in 300 µm slices of retina rapidly frozen at chosen times after a 40 ms light flash. [ATP], assayed with luciferase, increased within 0.5 s and returned to baseline in about 10 s. The maximum increase was at about 2 s and was 1.4 ± 0.1 (S.E.) times the dark concentration. During repetitive stimulation (5 min at 0.5 Hz with 2.4 log units attenuation) steady state [ATP] was indistinguishable from the dark level. Subsequent exposure to anoxia until the receptor potential was abolished reduced [ATP] to one half: perhaps at this time there was no ATP in the photoreceptors (which contain many mitochondria) and the normal [ATP] in the glia (which contain very few mitochondria).

Corticofugal influence on single unit activity in the medial geniculate body (MGB) assessed by cortical cooling

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Single unit spike trains were recorded simultaneously from a microelectrode array advanced in the MGB of nitrous oxide anesthetized cats. Each unit was studied before during and after the cooling of the ipsilateral auditory cortex. Only 38 units recovered comparable firing rates after the cooling periods and are considered here. During cortical cooling, the spontaneous firing rate of MGB units increased (14%), decreased (68%) or stayed constant (18%), and autocorrelograms showed an increased tendancy to bursting activity (40%). Responses to noise bursts were modified during cortical cooling for most units and the peaks in the PSTH were enhanced (41%) or reduced (31%). The ratio of the PSTHs peaks over the spontaneous firing rate usually increased during cooling. Thus the cortex can affect differentially both the spontaneous and the acoustically driven activity.

Enzyme-linked immunosorbent assay (ELISA) for measuring endogenous NGF

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We report here the development of a modified double antibody sandwich ELISA for measuring endogenous nerve growth factor (NGF). Polyclonal goat antibodies against 2.5S mouse NGF were adsorbed to polystyrene multiwell plates. After adding the NGF-sample, the second antibody, a specific monoclonal IgG_{2a}-antibody was applied. This antibody-complex was detected by a peroxidase-labelled immunoglobulin and quantified by the change in the optical density of the reaction product, using ortho-phenylendiamine as a substrate.
The sensitivity of the assay is 3 pg/well, corresponding to 0.1 fmol NGF. We have used this technique to measure the endogenous NGF content in sympathetic and sensory ganglia and their respective target tissues under different experimental conditions.

Adaptive changes of Na^+-gradient dependent basic amino acid transport across rat intestinal brush border membrane

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Although it is well known that basic amino acids are actively absorbed by the intestinal muccosa a general accepted view about the driving force does not exist.
Therefore uptake of L-arginine and L-lysine into brush border membrane (BBM) vesicles isolated from small intestine of rats fed either a high protein (HP) or a high carbohydrate (HC) diet was studied under conditions of an inwardly directed transmembrane NA^+-gradient or under Na^+-equilibrium conditions. Under Na^+-gradient conditions L-arginine and also L-lysine uptake displayed the overshoot phenomenon typically associated with active transport processes into BBM vesicles, and the overshoot in group HP exceeded that in group HC significantly. It is concluded from these results that a transmembrane Na^+-gradient is able to energize active basic amino acid transport across the intestinal BBM. Furthermore the transport mechanism of the intestinal BBM for basic amino acids appears to adapt to the protein content of the diet.

Pathways of bilaterally reciprocal inhibitory actions of Mauthner axon system

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Mauthner axons (MA) represent a pair of giant nerve fibers which are known to exert a hyperpolarizing effect upon each other. Fluorescent studies have shown that by injecting the MA with Lucifer Yellow it is possible to label commissural axons (identified by dye-coupling) occurring at a spatial frequency of about one coupling site at Mauthner axon collateral (MAC) per spinal segment. Gap junctions between proximal collaterals of interneurons and MAC were found at about the same frequency. F-type, symmetrical synapses have not been verified in a large sample of MAC. Thus direct postsynaptic inhibition of MA is not likely (in keeping with the absence of conductance change at the MA membrane). However, F-type synapses have been consistently identified to insert on proximal collaterals of interneurons which are connected to MAC via gap junctions. This finding suggests that interneurons may provide postsynaptic inhibition to motoneurons and inhibitory interneurons of the opposite side.

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Structural studies on human galactosyltransferase (lactose synthetase A protein)

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Soluble galactosyltransferase (GT) purified from human milk by affinity chromatography remained undegraded in presence of PMSF or EDTA. GT moved as a broad band in the region of 55 kD on SDS/PAGE, whereas an extensive microheterogeneity of GT was observed by isoelectric focusing (IEF). The pI values of the isoforms from different batches isolated by preparative IEF varied within 0.2 pH units. The isoforms thus prepared had corresponding pI values on analytical refocusing. Cleavage of sialic acid by neuraminidase produced a cathodic shift and reduced the microheterogeneity of the native enzyme from 13 to 6 isoforms. Neither sulfate nor phosphate substitution could be detected. In contrast to milk GT, GT released from HeLa cells had a single peak on IEF. Affinity chromatography on Con-A-Sepharose and Lentil-Lectin-Sepharose, respectively, produced a bound fraction and a non-bound fraction of milk GT. Amino-terminal sequence analysis carried out on native milk GT revealed two identical sequences one being shorter by 3 amino acids; a high proportion of hydrophobic amino acids in this sequence was detected.

Sidechain dynamics of two aromatic amino acids in pancreatic phospholipases. A ^{2}H-NMR study

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In order to investigate the molecular details of the phospholipase A_2 activity, in particular, the dynamics of the interface recognition site and the active site, two different amino acids were replaced by their partially deuterated analogs: Trp_3 is known to be involved in the interaction with lipid-water interface and Phe_5 is part of the hydrophobic cavity surrounding the active site.

Deuterium NMR is a powerful method for obtaining dynamic information on the deuterated molecule. Solid state NMR spectra of all lyophilized phospholipases indicated that the aromatic rings were immobilized. In the other extreme, T_1- and T_2-relaxation time analysis of water dissolved enzymes revealed different dynamic properties for different amino acids: Phe_5 remained rigidly fixed in the bovine enzyme. Trp_3 at the same protein was rotating freely. Trp_3 of the bovine phospholipase A_2 was held fixed in the dissolved enzyme, but the side chain started to rotate freely upon binding of the enzyme to a lipid-water interface.

Hormonal regulation of rat liver fructose 1,6-bisphosphatase by a redox mechanism?

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Fructose 1,6-bisphosphatase (fru-P_2ase) isolated under non-reducing conditions looses part of its inhibition by AMP (K_i 370 µM vs. 140 µM) and exhibits 2 fast reacting thiol groups less than the enzyme isolated in the presence of dithiothreitol (J. Biol. Chem. *257*, 4552, 1982). The hypothesis was examined that gluconeogenic hormones could activate the enzyme by a redox mechanism. The enzyme was purified from isolated hepatocytes by immunoaffinity chromatography. AMP and fructose 1,6-bis-phosphate were added to protect the fast reacting thiols. The disulfide groups in the protein were quantified by radioactive labeling with iodoacetic acid subsequent to carboxymethylation of the free thiols. Treatment of hepatocytes with glucagon or an α-adrenergic agent did not cause measurable changes in the number of disulfide groups associated with fru-P_2ase as compared to untreated controls. Regulation of fru-P_2ase by gluconeogenic hormones does not appear to be mediated through a redox mechanism.

Photolabeling of the membrane attack complex of complement

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The membrane-restricted photoactivatable probe 3-(trifluoromethyl)-3-(m-iodophenyl) diazirine (TID) was utilized to study the insertion of complement proteins into lipid bilayers. The purified complement proteins C5b-6, C7, C8 and C9 were added to vesicles containing the probe and irradiated after assembly of C5b-6, C5b-7, C5b-8 intermediate complexes. Thereby, mostly C5b became labeled. In the final C5b-9 complex, however, more than 90% of the label was attached to C9. When C9 was cleaved by thrombin into two hemolytically active fragments, only one of the fragments was labeled, supporting the amphiphilic structure of C9. If C9 was polymerized by Zn^{2+} to heterogeneous tubular complexes in the absence of C5b-8, C9 was also heavily photolabeled indicating spontane-

ous lipid insertion of polymerizing C9. Fully closed C9 tubules could be separated from unclosed tubular C9 polymers by SDS-PAGE. The photolabel predominantly labeled the closed tubular form suggesting that tubule closure is important for the stable insertion of C9 polymers into lipid membranes.

Shape change of human erythrocytes monitored by laser turbidimetry

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Shape changes of human erythrocytes induced by incubation with dimyristoylphosphatidylcholine (DMPC) liposomes were followed by laser turbidimetry. The transmission of the cell suspension as well as the light scattering at an angle of 90° to the incident beam were continuously recorded. During the first 15 minutes of incubation, transmission, transmission noise and light scattering noise increased significantly, then decreased to their original levels after approx. 30 minutes and decreased further to reach steady levels after 60 minutes. Control erythrocytes (incubated without DMPC) showed no such changes. Light scattering theory suggests that red blood cells, during the DMPC-induced conversion to symmetric echinocytes and sphero-echinocytes, pass through an intermediate state which is flatter (more asymmetric) than the original discocyte.

The use of chemical labels and proteases for the study of the architecture of the chromatophore membrane of the photosynthetic bacterium *Rhodospirillum rubrum* G-9

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The chromatophore membrane of *Rhodospirillum rubrum* contains two types of pigment-protein complexes, namely the reaction center (RC) with three polypeptides (H, M, L) and the light-harvesting complex with two polypeptides (LHP, α- and β-chain). All these polypeptides were labelled in their hydrophilic or hydrophobic domains depending on the chemical label used. Moreover it was observed which parts of the polypeptides are accessible to proteases. By these investigations insight into the organisation of the polypeptides with respect to the membrane was obtained. As the amino acid sequences of the LHPs are known, the exact determination of the sites labelled chemically or cleaved by proteases was possible. With newly developed unspecific hydrophobic markers it is hoped that the conformation of the membrane integral domains of the LHPs can be elucidated.

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Antibodies to synthetic peptides as probes of acetylcholine receptor structure

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The entire sequence of the four polypeptide chains (α, β, γ, δ) of the nicotinic acetylcholine receptor (nAChR) have recently been deduced from the cDNA sequences. However, the molecular weights calculated from this data, in particular that of the alpha-chain, differ markedly from those determined by SDS-gel electrophoresis, suggesting that the chains might be post-translationally cleaved with removal of the C-terminus. We have synthesised the proposed 12 aminoacid C-terminal peptide of the alpha-chain and raised polyclonal antisera to it. These bind to native and denaturated nAChR and membrane-bound nAChR. Immunoprecipitation and SDS-gel electrophoresis followed by immunoblotting clearly show that this sequence is an integral part of the molecule and exists on the mature alpha-chain.

Chemical characterization of RNAse Phy I

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Few endoRNAses are *specific* and even T-1 cleaves other pairs than -GpN- given no other opportunity. Conversely, hnRNA-processing in vertebrates shows an exquisitely selective recognition mechanism. Three RNAses distinguish U from C and two, used in sequencing, Phy I, *minus-C* (JBC *253*, 437, 1978) and Phy M, *U + A*, arise in *Physarum polycephalum*, a mold with nacked nuclei. We characterize Phy I as a typical glycosylated, acidic lysosomal RNAse. It dimerizes, undergoes conformational transition at pH 5–7 and like all RNAses, moves anomalously on SDS-gels; systematic Fergusson tests led to 24.5 kdal. AA analyses, CNBr- and tryptic peptide maps show 4 di-S bonds, 4 Trp (none in RNAse A) and, unlike T-1, 9 Lys + 2 Met, aside from 5 His. Although extreme dilution and scarcity precluded detailed chemistry, cleavage rates *vs.* pH and peptide compositions led to a probable Glu/His push-pull mechanism, similar to T-1. Discrimination of Pyr is dominated by electrostatic effects at the =NH of C. *Residual* split-rates at CpA (sole susceptible pair) vs ApC drop sharply with deprotonation of C.

The structure of an electron transfer complex: Cytochrome c binding site on cytochrome c peroxidase revealed by differential modification of carboxyl groups

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Cytochrome c peroxidase (CCP), a hemoprotein from yeast, catalyzes the electron transfer from cytochrome c to H_2O_2. To localize on CCP the recognition site for Cyt c, which is known to contain negatively charged groups, we used the method of differential chemical modification. CCP either alone or bound to Cyt c was modified with a trace amount of carbodiimide and [^{3}H]taurine whereby carboxyl groups were amidated according to their reactivity. The ^{3}H-labeled peroxidase was modified with [^{35}S]taurine under denaturing conditions and the reactivity of individual aspartate and glutamate residues was determined from $^3H/^{35}S$ ratios after degradation. In the electron transfer complex residues 33, 34, 35, 37, 221, 256, and 290 of CCP are approximately 5 times less reactive. Based on this data and with the knowledge of the three-dimensional structure of CCP, we can define the binding site for Cyt c on the surface of CCP.

Ammonium-stimulated potassium efflux from duckweed (*Lemna minor* L.)

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A remarkable net efflux of potassium was observed after addition of ammonium to a duckweed culture grown on nitrate. This effect strongly depended on the ammonium concentration (final concentrations in the culture medium: 1–30 mM). The net potassium efflux did not depend on the anion added with the ammonium (chloride, sulfate or nitrate). No major potassium release was caused by the addition of Li^+, Na^+, Rb^+ or Cs^+, but the potassium uptake rate was affected by these ions. The ammonium-induced potassium efflux was markedly reduced, if an inhibitor of glutamine synthetase (methioninesulfoximine) was added together with ammonium. These data

suggest that the assimilation of ammonium in the cells by glutamine synthetase stimulated the net potassium efflux. It remains open in which way NH_4^+-influx and K^+-efflux are linked together.

Differential modification of Cd-metallothionein by iodoacetamide

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Mammalian metallothioneins (MT) contain 20 cysteines in a total of 61 amino acid residues and bind 7 Cd and/or Zn ions. The metal is bound in two clusters composed of 3 and 4 metal-thiolate complexes located at the N- and C-terminal half of the chain, respectively. Cluster formation has now been followed by differential modification of cys with ^{14}C-iodoacetamide in MT reconstituted with increasing amounts of Cd. When more than 2 moles Cd/mol MT are bound, the cys from residues 33 to 50 are less strongly labelled than the remainder, suggesting that the 4 Cd-cluster is formed first. The subsequent formation of the 3 Cd-cluster is initiated at the N-terminal end of the chain. Maximum protection of all cys from alkylation is attained only after addition of the full complement of 7 Cd. The extent of labelling indicates that the ligands of the 3 Cd-cluster are twice as accessible to iodoacetamide as those of the 4 Cd-cluster, suggesting greater kinetic lability of the former. The unequal reactivity of the clusters may be linked to MT function.

Determination of protein secondary structure from patterns of distance constraints observed by NMR in solution

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Once sequence-specific assignments are available for the cross peaks between backbone and C^β-protons in the 2-dimensional nuclear Overhauser enhancement (NOESY) spectrum, visual inspection of NOESY spectra for certain characteristic cross peak patterns can be used for delineating the polypeptide secondary structure. Here, stereochemical studies of regular secondary structures and statistical investigations of high resolution single crystal structures of globular proteins are used to assess extent und uniqueness of the secondary structure identifications by this method. A novel computer graphics presentation for polypeptide backbone conformations will be used to illustrate the results obtained.

A single subunit P700 reaction center of a thermophilic cyanobacterium

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The thermophilic cyanobacterium *Mastigocladus laminosus* grows in hot springs and controlled laboratory cultures under a wide range of environmental conditions. Thermophilic cyanobacteria are the only organisms with a thermophilic photosynthetic system of the higher plant type. Earlier, we reported on the purification of the photosystem I reaction center from the thermophilic cyanobacterium *Mastigocladus laminosus*. The subunit composition and some photochemical properties were compared with those of photosystem I reaction centers from green algae and higher plants. In this communication we describe the isolation and characterization of a single subunit photosystem I reaction center of the thermophilic cyanobacterium *Mastigocladus laminosus*. Depending on denaturing conditions, the subunit, isolated in 0.1% SDS, appears on SDS-PAGE in the 70 kDal or in the 50 kDal region and contains bound chlorophyll in both forms. The 70 kDal form shows all the activities of an intact reaction center.

Phosphorylation of platelet membrane glycoproteins

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Platelets were incubated for 60 min at room temperature with 1 mCi/ml of [^{32}P]-sodium orthophosphate. The labelled platelets were either directly solubilized in the presence of enzyme inhibitors or after activation with thrombin. The solubilized platelets were analyzed by two-dimensional gel electrophoresis. Proteins were detected by silverstaining and [^{32}P]-phosphoproteins by autoradiography. In order to identify glycoproteins which were phosphorylated, hydrophobic phase partition, lectin affinity chromatography and immunoprecipitation with anti-platelet glycoprotein antibodies were performed. The two-dimensional gel electrophoresis patterns obtained with phosphorylation and with surface specific carbohydrate labelling of platelets were also compared. These results all indicated that GPIbβ is the principal phosphorylated glycoprotein on human platelets and that GPIIb and IIIa were not phosphorylated under the conditions used. No striking changes were observed in glycoprotein phosphorylation after thrombin activation of platelets.

The mechanism of electron transfer between hemoproteins: the cytochrome c peroxidase – cytochrome c system

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The structure of the cytochrome c peroxidase·cytochrome c electron transfer complex is now known in remarkable detail. In particular we have defined the intermolecular interface of the complex both by computer modelling and actual experiment. An important feature of the complex is that the heme edges must be 16 to 18 Å apart and that, therefore, direct electron transfer between hemes is not possible. However, we find that the imidazole group of His 181 of peroxidase is located between both hemes and near to the center of the intermolecular interface where it may serve as a bridging group in electron transfer. Destruction of this residue by photooxidation blocks electron transfer but does not intefere with formation of the electron transfer complex. Another histidine residue, His 52, acts as an acid-base catalyst in electron transfer from the heme iron of peroxidase to H_2O_2.

'Golden blot' – a new way for specific, sensitive and rapid staining of gel replicas

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Protein A bound to colloidal gold is used for cytochemical localization of antigens. We have modified this method for detection of proteins on 'Western blots'. The procedure involves the following steps: 1) electrophoretical separation of proteins, 2) transfer to nitrocellulose, 3) incubation with polyclonal antibodies, and 4) detection of immune complexes with protein A-gold. Monoclonal antibodies can also be used. The amount of antigen detectable is 10–50 ng. Sensitivity comparable with autoradiographs can be reached by subsequent silverstaining. The advantages of the 'golden blot' are: 1) rapidity in performance, 2) radioactivity is avoided, 3) no overstaining/understaining of major/minor bands, and 4) the staining is stable. The protein A-gold complex is quickly and easily prepared. The 'golden blot' does not require any special equipment.

Regulation of adenosine 5'-phosphosulfate sulfotransferase (APSSTase) and nitrate reductase by L- and D-cysteine in *Lemna minor* L.

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We have shown before that addition of 0.5 mM L-cysteine to the culture medium caused a rapid decrease in APSSTase activity of *Lemna minor*. With 0.5 mM D-cysteine APSSTase remained constant or increased slightly, with 1 mM there was an increase of 100% of the original activity. Nitrate reductase activity was decreased by 16 and 80% after 20 h with L- and D-cysteine, respectively. Taken together with the fact that reduced nitrogen sources increase APSSTase activity our results show that D-cysteine has the same effect as a reduce nitrogen source or a poor sulfur source. L-cysteine acts to a smaller extent like a reduced nitrogen compound but represents a good sulfur source.

Structural studies of calmodulin and related Ca^{2+}-binding proteins by hydrophobic labeling and fluorescence spectroscopy

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Calmodulin belongs to a family of highly homologous proteins which contain 1–4 Ca^{2+}-binding domains of similar properties. Ca^{2+}-binding to these proteins induces large conformational changes (Klee and Vanaman., Adv. Prot. Chem. *35* (1982) 213; Krebs Cell Calcium *2* (1981) 295) exposing hydrophobic sites a shown for calmodulin (La Porte et al., Biochemistry *19* (1980) 3814). Recently we developed a highly sensitive autoradiographic method to identify these sites (Krebs et al. Biochemistry 1983, in press) by using the hydrophobic photoreactive probe $\{^{125}I\}$-TID, originally developed to label selectively intramembrane segments of transmembrane proteins (Brunner and Semenza, Biochemistry *20* (1981) 7174). We extended these studies to calmodulin-related proteins and compared the results with those obtained by fluorescence techniques.

Structural differences between isoenzyme subunits β_1 and γ_1 of human liver alcohol dehydrogenase

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The dimeric human enzyme alcohol dehydrogenase occurs in multiple forms which exhibit distinct electrophoretic mobilities and catalytic properties. The isoenzymes $\beta_1\beta_1$ and $\gamma_1\gamma_1$ were isolated from human liver of Caucasian origin by affinity chromatography and ion exchange chromatography. Peptides were prepared by cleavage of the carboxymethylated proteins with trypsin and CNBr. They were purified by exclusion chromatography and reverse phase HPLC. Their primary structures suggest that out of 373 residues, β_1 differs from γ_1 at 21 positions. All differences are compatible with one base mutations. Five mutations lead to charge differences which could account for the different electrophoretic mobilities and catalytic properties. Comparison with isoenzyme differences in other species leads to new conclusions about isoenzyme development.

Analogues of cytochrome c modified at the invariant residue Tyrosine 48 prepared by semisynthesis

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I have recently described a strategy for preparation of semisynthetic cytochrome c (C.J.A. Wallace and K. Rose, Biochem. J. *215* (1983) 651), involving chemical modification of residues within peptide fragments containing only one example of that residue. Analogues containing independent modifications of the two arginine residues of cytochrome c were prepared in this way.

We have now prepared analogues modified at the invariant residue, Tyrosine 48, by the same strategy. One of these, with the tyrosine o-acetylated, is completely N-ε-acetimidylated, to avoid amino group acetylation. Acetimidyl cytochrome c has biological properties very similar to those of the native protein (C.J.A. Wallace, Biochem. J. *217* (1984) 595). The other, with tyrosine iodinated, required no such amino protection.

We present the results of physicochemical and biological studies of the analogues, and our conclusions on the functional role of this residue.

Enzymatic and ultrastructural organization of pericellular membranes in human leukemic cell lines

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Enzymatic and ultrastructural properties of plasma membrane were studied on human lymphoid leukemia cell lines. Subcellular fractions obtained from REH-6, Nalm-1 and Raji cell lines by isopycnic centrifugation were assayed for the markers enzymes: γ-Glutamyltranspeptidase,5'Nucleotidase,(Na-K)Mg-Adenosine triphosphatase, Alkaline phosphatase, Alkaline phosphodiesterase, Glucose-6-phosphatase and β-N-Acetylglucosaminidase. The ultrastructural topography was investigated on freeze-fracture preparations of intact cell by recording particle density on the protoplasmic and external face of both plasma and nuclear membranes. In conclusion, enzymatic data seem to correlate with the stage of differentiation, as supported by the enrichment of the enzymatic equipment of the plasma membrane and the trend toward homogeneous distribution profiles of the markers enzymes. However the ultrastructural topography does not correlate: indeed, no characteristic particle density for the different cell lines was found.

Biophotocatalysis based on semiconducting powders

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Biophotocatalytic systems using semiconducting powders as light energy harvesters are presently studied in our laboratory. One of them, called the LIES system (Light Induced Enzymatic Synthesis) based on the immobilization of redox enzymes at the surface of semiconducting particles has been developed in collaboration with D.O. Hall and K.K. Rao, University of London King's College. Various hydrogenases have been immobilized on the surface of TiO_2 anatase or CdS particles. Under band gap irradiation of the support, electrons can be transfered from the conduction band of the semiconductor to the catalytic site of the enzyme. Enzyme-catalyzed H_2 evolution due to the oxidation of an electron donor via the photoexcited semiconductor can then be observed. The catalytic properties of this light energy converter will be discussed, as well as the problem of the electron transfer between the surface of the support and the active site of the enzyme.

Preparation of a glucagon derivative for the semi-synthesis of altered glucagon sequences

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Semisynthesis is the process whereby natural peptides are used as ready-made intermediates in the preparation of altered sequences. The amino-terminal histidine residue of glucagon plays an important role in the biological activity of the hormone. Chemical alterations at this position should therefore give information on the interaction of glucagon with its target cells. In order to synthesise analogues of glucagon with altered amino-acid residues at position 1 of the molecule it is first necessary to protect the side chain of lysine 12. Conditions have been established for the preparation of [ε-Msc-Lys-12]glucagon and its purification by ion-exchange chromatography. The modification was confirmed by end-group determination and the purity investigated by reversed phase HPLC. Histidine-1 was then removed by the Edman reaction leaving the side-chain protection intact. This derivative can then be used for coupling to amino acids producing glucagon analogues with a sequence altered specifically at position 1 of the molecule.

Occurrence of ATP-dependent protease in the procyclic form of *Trypanosoma brucei*

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The major energy source of *Trypanosoma brucei* procyclic form is the metabolization of aminoacids coming from the blood meal in the tsetse gut. It was then interesting to search for proteases acting on denatured proteins. 31000 × g supernatant of *T. brucei* homogenates showed protease activity toward/^{14}C/ methylated-casein. This proteolytic activity is stimulated 20 to 25% by the addition of 5 mM ATP. Similar activation is obtained by ADP, while GTP, UTP, CTP were unefficient. The basal and the stimulated hydrolytic activities are PMSF-sensitive indicating the involvment of serine proteinase(s).

Characterization of the low affinity kainic acid binding site solubilized from pigeon cerebellum

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The pigeon cerebellum contains a large number of kainic acid (KA) binding sites (B_{max} = 118 pmol/mg protein). Isotopic dilution experiments indicate the presence of a high (K_d = 30 nM) and a low affinity (K_d = 330 nM) site. In crude membrane preparations binding to the low affinity site shows positive cooperativity (n = 2.2). Triton X-100 at 0.25% solubilizes preferentially the low affinity site, whereas at 1% Triton both the low and high affinity sites are solubilized. The K_d for KA was 20 nM and 440 nM respectively for the solubilized high and the low affinity site. The positive cooperativity (n = 1.5–2) and the pharmacological specificity of the low affinity site were retained in solution. Gel filtration chromatography resulted in a six-fold enrichment of the low affinity site and gave a molecular weight of about 440000. Isoelectric focusing experiments suggested a pI of 4.8–5.5 for the low affinity KA binding site.
sponsor: Kim Quang Do

Isolation and characterization of a specific inhibitor peptide of M-LDH

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From lyophilized human urine an inhibitor of M-LDH reassociation was isolated using Bio-Gel P-2 columns and RP-18 HPLC. This peptide was identical to that previously reported in human liver by: inhibition kinetics, heat stability, specific susceptibility to proteolytic digestion and the elution profiles from two different chromatography systems.
We finally succeeded to obtain a degree of purification of one single peak on RP-18 HPLC. Hydrolysis of this preparation followed by dansylation and subsequent analysis of the dansyl-amino acids on HPLC confirmed the peptide nature. However, dansylation followed by hydrolysis and HPLC revealed no N-terminal dansyl-amino acid. Accordingly negative results with LAP digestion as well as during three Edman cycles strongly suggest that the N-terminus of this peptide is blocked.

Iron-sulfur hydratases: the involvement of an Fe-S structure in hydro-lyases

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The presence of an Fe-S cluster in Aconitase (AH) has been reported. We provide evidence that Fe-S structures play a functional role in some hydro-lyases (besides their function in electron transferring systems). A protein with similar properties, maleic acid hydratase (MH), has been purified to homogeneity from rabbit kidney and contains a single 4Fe-4S cluster when active. Transferred hyperfine interactions between ^{1717}O-labeled substrates of either AH or MH and the cluster are shown, establishing a direct role of the cluster in the enzymatic reaction. Furthermore six different Fe-requiring hydro-lyases have been induced in either *Ps. fluorescences* or *E. coli*. EPR studies on partially purified extracts show the involvement of an Fe-S cluster in most proteins investigated, some undergoing rapid cluster degradation. Similar degradation on MH is due to conversion of the 4Fe-4S cluster (active form) to a 3Fe-3S via an intermediate 3Fe-4S structure. Hydratases active on some related substrates do not contain a Fe-S cluster, allowing clues about requirements of the involvement of a Fe-S cluster in hydratases.

Oxidation of C1q with chloramine T. Comparison with 'normal' C1q

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The iodinating agent chloramine T (CT) has potent oxidating properties. We studied the effects of oxidation with CT prior to iodination versus iodination with lactoperoxidase on purified human C1q, a subcomponent of the first component of complement. Treatment with CT resulted in reduction of binding sites and affinity for complexed IgG, whereas the binding properties towards heparin and fibrinogen were unchanged. Ultracentrifugation, SDS-PAGE and ion-exchangechromatography (FPLC) showed no differences between the two C1q preparations, whereas a higher amount of diiodotyrosine and an undefined compound, probably iodohistidine, was found in the preoxidized C1q. We conclude that preoxidation of C1q with CT led to alterations in the globular part of the molecule, the binding site for complexed IgG, as reflected in the increased availability of tyrosine and probably histidine residues for iodination. The collagen-like part of the molecule is less affected and permits adequate binding of heparin and fibrinogen.

Immunogenic surface proteins of *Leishmania major* during mouse infection

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Promastigotes of the parasite *Leishmania major* were surface labeled with ^{125}I and disrupted by Dounce homogenisation. The subcellular components of the cell were analysed by equilibrium sedimentation in sucrose gradient. Fractions were tested for ^{125}I incorporation. Pellicular membrane fractions were found to contain the vast majority of the label. In the pellicular membrane a single protein (MW ~ 60 kD) was strongly labeled.

Experimental leismaniasis was induced in inbred mice and sera were collected after 3 months of infection. These sera were tested on the sucrose gradient fractions containing the various organelles of the parasite by 'Western blotting'. The highest antigenicity was found in the fractions containing soluble proteins and/or small vesicles. Pellicular membranes and flagella showed relatively little antigenicity. The significance of the low immunogenicity of the external surface proteins of this parasitic protozoan is being investigated.

Precursor of a periplasmic protein in a *Pseudomonas* sp.

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The *myo*-inositol binding protein (MI-BP) of *Pseudomonas* sp. JD34 is a 30 Kd periplasmic protein which has been isolated and purified in our laboratory. We have searched for a cytoplasmic precursor of this BP using short ^{35}S methionine pulses followed by chase and immunoprecipitation. After gel electrophoresis we observed a 32 Kd band which decreases upon chase whereas the mature form increase slightly. In order to slow down the maturation process we tried inhibitors efficient in *E. coli* for similar process, like TAME, phenethyl alcool or procaïne. Phenethyl alcool and procaïne were unefficient but TAME showed a marked inhibitory action which is relieved when removed. The bands of the presumed precursor and mature protein were cut out and digested with V8 *Staphilococcus aureus* protease according to Cleveland et al (JBC 252 p. 1102–1106 (1977)). The autoradiography of the fingerprints showed a nearly total identity giving the evidence of the 32 Kd protein of being the precursor of the MI-BP.

Characterization of low titer, naturally occurring antibodies to the major integral membrane protein of human red blood cells

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Naturally occurring antibodies were isolated from IgG of single healthy blood donors by immunoadsorption on their own red cell band 3 protein (autoantibodies, AAB) or from pooled IgG (SRK) (antibodies, AB). The purified material represented a fraction of $2 \pm 0.5 \times 10^{-5}$ ($n = 10$) of the IgG applied to the immunosorbent. AAB and AB were specific to band 3 protein as revealed by immunoblotting. Their binding to bands 4.2, 5, and 6 was quantitatively similar as with whole IgG and IgG depleted of AB or AAB. Thus, any IgG binds unspecifically to previously SDS denatured bands 4.2, 5, and 6, while binding to band 3 is due to natural antibodies, not complexed to antiidiotypic IgG. AB showed binding to the 65 K, but not the 38 K chymotryptic fragment of band 3. Band 3 protein contained 4 antigenic peptides, generated by V8 protease, which were also present in the 65 K fragment. One antigenic peptide was exoplasmic, one transmembrane and both hydrophobic, while the other two were labeled from neither side.

Cloning vectors that autoamplify in the stationary phase of bacterial growth of *Escherichia coli*

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We have constructed cloning vectors based on the replicon of plasmid ColD-CA23. Vectors pKT234, pKT252 and pKT254 contain two, pKT235 three antibiotic resistance genes, and several unique restriction endonuclease cleavage sites for gene cloning. Most of these sites are located within the antibiotic resistance genes and thereby allow the use of 'insertional inactivation' for the identification of hybrid molecules. These vectors undergo autoamplification when cultures of host bacteria enter the stationary phase of growth, dramatically increase their copy numbers and therefore significantly elevate the expression of cloned genes as a result of consequent increase in gene dosage. The auto-amplification of these vectors is of considerable interest for high expression of the cloned genes.

Fatty acids, the major fuel of the heart. Intracellular transport as studied by electron spin resonance (ESR)

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Heart cells are strongly dependent upon fatty acids as an energy source. However the mechanism of transport of these compounds from the blood capillaries to the intracellular compartments has remained unsolved until now. The data obtained in the present study are consistent with a model of transport mediated by a specific fatty acid binding protein which is present in all the cardiac cells including the capillary endothelium, the intercellular spaces and the myocytes. In the latter, the protein is compartmentalized, 77.5% being localized on the myofibrils, 7.1% in the spaces surrounding the mitochondria and 15.4% in the mitochondria. This gradientlike distribution allows the coexistence of multi species of the protein by self-aggregation. A strong modulation of the energy production by the mitochondria is predictable, if we consider that only one of these aggregated species is able to transfer fatty acids to the mitochondrial β-oxidative system.

Amino-acid sequences of linker-polypeptides from phycobilisomes of the cyanobacterium *Mastigocladus laminosus*

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The phycobilisome (light harvesting antenna) of the cyanobacterium *Mastigocladus laminosus* consists of three major phycobiliproteins (allophycocyanin, C-phycocyanin, phycoerythrocyanin) and of several minor phycobiliproteins and colourless linker-polypeptides.

Two colourless linker-polypeptides were isolated and their amino-acid composition as well as their amino acid sequences were determined. They exhibit a length of about seventy and eighty residues, respectively. Both peptides are very hydrophilic and basic. Their high contents of the amino-acids serine, threonine, glutamine, asparagine, lysine and arginine suggest that they interact highly specifically with phycobiliproteins via ionic and hydrogen-bonds.

Specific binding to isolated acetylcholine receptor of a short naja toxin with an open disulfide bridge

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Derived from the major toxin of *Naja naja samarensis* (NT) by chemical reduction, a renaturation intermediate with native-like conformation, 3S-S bonds and 2 blocked cysteines was isolated. Its affinity for the acetylcholine receptor (AChR) was evaluated. The fully reduced toxin was oxidized by air in the presence of GSSG at pH 7.1, 37 °C and the products were blocked with iodoacetate. Three sets of molecular populations with 3 disulfide bonds were isolated (NT^3I, II, III). NT^3III was incubated at equilibrium with a mixture of cysteine and cystine and the fraction corresponding to a native-like conformation, as determined by HPLC and gel filtration, was isolated by ion-exchange chromatography. Its appearent K_D for the AChR, i.e. $K_D = 1.1 \pm 0.2.\ 10^{-9}$M and the position of its blocked cysteines were determined. The information present in the amino-acid sequence of the toxin is thus sufficient to generate a stable, biologically active structure, even without a complete S-S framework.

Cryoenzymological investigation of the catalytic mechanism of aspartate aminotransferase (AspAT)

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Conditions were elaborated that kept AspAT active in various cryosolvent mixtures. The overall reaction rate with the substrates glutamate and oxalacetate was estimated as a function of temperature by measuring the consumption of oxalacetate at 280 nm. The apparent activation energy increased from 18 kcal/mol above 4 °C to about 30 kcal/mol at −30 °C. At −40 °C the conversion from the pyridoxal to the pyridoxamine form in the presence of cysteine sulfinate could be followed by recording absorption spectra (240 to 550 nm) in intervals of seconds. Coincidence of the disappearance of the pyridoxal form with the appearance of the pyridoxamine form ($k \sim 0.19\ min^{-1}$) with an isosbestic point at 344 nm was observed. Although no intermediate could be identified so far, the present results suggest that cryotechniques might render enzyme-substrate intermediates of AspAT accessible to X-ray analysis.

A soluble form of acetylcholinesterase in human caudate nucleus

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Acetylcholinesterase (AChE) occurs as soluble enzyme or in association with membranes and basal lamina and exists in multiple molecular forms. Sørensen et al. (J. Neurochem. *39*, 1050, 1982) have investigated the properties of the predominant AChE species in human caudate nucleus, i.e. the membrane bound detergent soluble DS-AChE (80% of total activity). The remaining activity comprises a salt soluble AChE (SS-AChE), which was purified by affinity chromatography. It sedimented at 10.6 S and detergents are neither bound nor necessary for catalytic activity. These findings well distinguish SS-AChE from DS-AChE. On the other hand the two enzymes are both tetramers, with subunit molecular weights of about 66000, and in tandem crossed-immunoelectrophoresis SS-AChE showed a reaction of identity with DS-AChE. It is concluded that in brain the membrane bound and soluble tetrameric forms of AChE are closely related.

Purification and characterization of prothylakoids and prolamellar bodies from oat etioplasts

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When oat is grown in darkness, the proplastids of the leaves develop into etioplasts. These organelles contain internal crystalline structures, the prolamellar bodies and flat membranes, the prothylakoids. These structures are devoid of chlorophyll and active photosystems. So far, the purity and characteristics of the two fractions are still controversial. Intact etioplasts were purified by Percoll gradient centrifugation. Prothylakoids were separated from prolamellar bodies by sonication and sucrose gradient centrifugation. The two fractions were analyzed for protein, pigment and lipid content and enzyme activities. Both contained the same lipid classes. No saponins were detected. CF1-ATPase, protochlorophyllide reductase and duroquinol-cytochrome b552 oxidoreductase activities were found mostly in the prothylakoid fraction whereas ferredoxin-$NADP^+$ reductase activity was equally distributed between the two fractions. These results will be discussed in terms of the biogenesis of chloroplasts.

The functional significance of a 45 Kd T cell membrane glycoprotein in cell proliferation

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A heavily glycosylated 45 Kd T human cell membrane protein is characterised in detergent lysate and isolated membrane fractions. By peptide fragment analysis it is the product of one gene locus and the strong heterogeneity is due to posttranslational modification. It is located in specialised membrane regions which contain also glycolipids. It is hydrophobically anchored in the membrane of the resting cell, but is shed in soluble form early in polyclonal cell activation. A derivatisation in the shedding is suggested by a new cleavage site in peptide fragment analysis and a small molecular weight difference of the deglycosylated peptide backbones. A monoclonal a-45 Kd protein-antibody specifically enhances cell proliferative responses in partial polyclonal activation suggesting that the 45 Kd protein is a growth regulation receptor. It is proposed that the shedding is obligatory for the progress through the cell cycle. A complex mitogenic membrane site may exist which includes the 45 Kd protein and other receptors.

Alkaline earth cation specificities of small membrane carriers

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An investigation has been made of the kinetic and thermodynamic aspects of complex formation between alkaline earth cations and the following antibiotics: calcimycin (a pyrrole ether); virginiamycin S, mikamycin B, plauracin and viridogrisein (all depsipeptides). The measurements were carried out in a medium of water/methanol (30:70, w/w) which is presumed to mimic the polarity of membrane interfaces.

The deprotonated protolytic states of these antibiotics form predominantly 1:1 cation complexes. Virginiamycin S_1, as an example for the depsipeptides, exhibits a pronounced alkaline earth cation specificity {logK values: 3.30(Mg^{2+}), 2.50(Ca^{2+}), 1.80(Ba^{2+})} which is absent in case of calcimycin {logK values: 4.0(Mg^{2+}), 4.1(Ca^{2+})}. The nature of the coordinating groups has been identified. In the case of the depsipeptides an analysis has been made of the contribution of individual amino acid constituents to the thermodynamic parameters measured.

Fragmentation of calmodulin by controlled proteolysis

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Different fragments of calmodulin (CaM) can be obtained by limited proteolysis depending on whether the fragmentation has been performed in the presence or absence of Ca^{2+}. By using trypsin CaM is split essentially in two halves (1–77; 78–148), provided CaM is in the Ca^{2+}-bound conformation. These fragments can be separated by help of reverse phase-HPLC. The obtained fragments showed heterogeneity if analyzed by gel electrophoresis using 6M alkaline urea gels. Final purification was achieved by using DEAE-HPLC resulting in the following pure fragments as identified by partial sequence analysis of AA-composition: 75–148, 76–148 and 78–148 and, most probably, 1–74 and 1–75. In the absence of Ca^{2+}, CaM is split by trypsin into the fragments 1–106 or 1–90 (depending on the conditions) and fragment 107–148. These fragments have been purified and identified by similar methods as described before. The ability of these fragments to stimulate the erythrocyte Ca^{2+}-ATPase has been tested and the regions of calmodulin essential for the activation of this enzyme have been identified.

Immunoradiometric assay (IRMA) for quantitative determination of a cutaneous human burn toxin in plasma of severely burnt patients

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An IRMA for burn toxin in human plasma was elaborated. The calibration curve ($y = a + bx$) was obtained by adding 0,5, 10, 20, 30, 40 µg/ml burn toxin isolated from human eschars to a standard plasma pool. An individual unspecific influence of plasma was eliminated by restricting 6 dilutions for both the standard (control) and the specimens to the range of 60%–100% plasma where this effect remains constant. By linear regression one obtains for the patients' plasma $y' = a' + b'x'$ where a' is the unspecific influence of this unknown plasma. After subtraction of the a (standard) and/or a' (specimen) respectively from the corresponding experimentally obtained curve we can calculate the unknown toxin concentrations in the specimens. The daily level ranged between 5–25 (low) and 80–230 (high) µg/ml with undulating patterns (day 1–7).

Removal and recombination of the two regulatory light chains in myosin

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In isolated myosin the first of the two regulatory light chains (R-LC) is digested 5–10 times faster by chymotrypsin (CT) than the second one. Treatment with EDTA and DTNB removes only one of the two R-LC per myosin. Interaction between the two globular head portions seems therefore to protect one of the two R-LC from proteolytic attack and from removal. In actomyosin, where both myosin heads bind to actin in rigor, this steric hindrance is abolished and both R-LC are equally susceptible to proteolytic attack and both can be removed completely by treatment with CT, EDTA and DTNB. The actin activated Mg-ATPase of myosin free of R-LC is 45% (s.d. ± 13% in 10 experiments) of the normal one. The Ca-ATPase of myosin free of R-LC is unchanged. Complete recombination with R-LC in the presence of Mg2+ and ATP fully restores the actin activated ATPase. The R-LC thus seem to affect the interaction of themyosin heads with actin during the hydrolytic cycle.

Spatial structures for BPTI in the crystal and in solution from distance geometry calculations

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Recent experience indicates that spatial structures of non-crystalline proteins can be determined from nuclear magnetic resonance measurements of intramolecular distance constraints. A promising approach for the structural interpretation of the NMR data is distance geometry calculations. Here we compare BPTI structures computed with a new distance geometry algorithm from several different sets of data. The first set consisted of the upper bounds on 60 interatomic distances between sequentially nonadjacent residues, which were observed by NMR. The second set included, in addition, about 100 short-range NMR constraints. To determine the relative advantage of quality versus quantity of data, additional sets of either more precise or more numerous distance bounds were extracted from a high resolution crystal structure.

Specific chemical modifications at the carboxy terminal acyl function of peptide hydrazides

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The activation peptide of bovine trypsinogen Val-Asp_4-Lys, which provides the sequence for recognition by enterokinase, was modified at its α-carboxy group to both the hydrazide and the t-butyloxycarbonyl hydrazide by trypsin-catalysed reaction (Jones, R.M.L. and Offord, R.E. (1982) Biochem. J. *203*, 125–129). Conditions were developed for the conversion of Val-Asp_4-Lys hydrazide to the corresponding peptide alcohol via reduction of the azide with sodium borohydride. The terminal carboxyl of the activation peptide was also converted to the proline amide derivative; use of the t-butyloxycarbonyl hydrazide allowed subsequent differential protection of the amino groups prior to coupling to proline amide by the azide method. The suitability of these derivatives of the activation peptide for direct conversion to a powerful and specific peptide aldehyde inhibitor of enterokinase is discussed, along with the potential application of such inhibition in relation to the inappropriate activation of pancreatic zymogens in acute pancreatitis.

Synthetic peptides duplicating putative polymorphic determinants of HLA class I

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Multiple differences in amino acid sequences distinguish individual HLA antigens. Thus, serologically polymorphic determinants of HLA can be conceived on the basis of sequence comparison and intrinsic hydrophobicity of the polypeptide chains. The corresponding polypeptide, KVKA-HAHTVRVDLGTLRG, duplicating the sequence 66–83 of HLA-A2, was synthesized by solid phase technique. After partial purification by ion-exchange chromatography, the peptide was coupled with glutaraldehyde to RSA. Antibodies were raised against this conjugate in mice and rabbits. The antisera were tested by ELISA assays on immobilized peptides or on intact F2B cells (typed HLA-A1, 2; B7, 8). Immunoblots of SDS gels from membrane protein extracts showed a single labelled band at 45Kdaltons. Immunoprecipitation of ^{35}S-Met labelled membrane proteins was carried out. A complement

mediated cytotoxicity assay on pretyped PBL cells was used to determine the specificity of the antisera for the different HLA of class I.

Structural and functional properties of a designed 24-residue DDT-binding polypeptide

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Recently we described the design and synthesis of a 24-residue polypeptide that was capable of binding the insecticide DDT (R. Moser, R.M. Thomas, and B. Gutte, FEBS Lett. *157*, 247 (1983)). DDT binding by the designed peptide was specific as an analogue of this peptide possessing the same amino acid residues in a random sequence bound DDT ~ 100 times less strongly. Antibodies raised against the designed peptide also reacted weakly with the analogue indicating that they were probably directed toward the DDT binding site. Preliminary Raman spectroscopic studies showed that the designed peptide adopted largely β-structure as proposed earlier. In the presence of the 24-residue DDT-binding polypeptide the DDT-modifying activity of heme was increased more than 100-fold. The DDT-binding peptide-heme mixture may represent a model system to study the evolution of an enzyme.

Antibody-independent activation of Cl, the first component of complement, by cardiolipin

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Human heart mitochondria (HHM) activate the classical pathway in an antibodyindependent manner. Since trypsinized HHM still activated complement, we tested the ability of mitochondrial lipids to activate Cl. Reconstituted human Cl with iodinated Cls is activated at 37°C by liposomal preparations (Lp) containing various amounts of cardiolipin (CL), cholesterol (Chol) and phospholipids. After SDS-PAGE, Cl activation is determined by the radioactivity transferred from intact Cls to the Cls heavy chain. The results show: a) Lp containing CL but no Chol activate Cl only if the relative amount of CL exceeds 60%; b) however, Lp containing 20% CL and increasing amounts of Chol activate Cl with already 20% Chol, whereas Lp lacking CL fail to do so. Clq binding studies indicate that Lp containing CL but no Chol, which fail to activate Cl, do bind Clq. These data show that CL incorporated into Lp binds Clq. The subsequent activation of Cl requires either a critical level of CL or a decrease of membrane fluidity by Chol.

Structural studies on calmodulin, an intracellular Ca^{2+}-binding protein, by using different NMR-techniques

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Calmodulin is an ubiquitous Ca^{2+}-binding protein (M_r = 17.000) which plays a pivotal role in most intracellular Ca^{2+}-dependent processes (Klee, C.B. & Vanaman, T.C. Adv. Prot. Chem. 35, 213, 1982). It contains 4 Ca^{2+}-binding sites which are composed of 2 helical parts enclosing the Ca^{2+}-binding loop, a view which is based on the crystal structure of the highly homologous Ca^{2+}-binding protein parvalbumin (Kretsinger, R.H. C.R.C. Crit. Rev. Biochem. 8, 119, 1980). Calmodulin has a highly flexible structure which can adopt different conformations due to the binding of Ca^{2+} (Krebs, J. Cell Calcium 2, 295, 1981). The protein has been studied under a variety of different conditions using various NMR-techniques (Forsén, S., Krebs, J. et al. FEBS Lett. 117, 189, 1980; Krebs, J. & Carafoli, E. Eur. J. Biochem. 124, 619, 1982; Guerini, D., & Krebs, J. FEBS Lett. 1983, in press). The results will be summarized and discussed with respect to the functional properties of this protein.

Cytosolic cAMP-dependent protein kinases and protein kinase C of three estrogen-dependent and three estrogen-independent human mammary cell lines

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The soluble cAMP-dependent protein kinases of three estrogen receptor-containing (MCF-7, ZR-75-1, and T47D) and three estrogen receptor-lacking (BT-20, MDA-MB-231, and HBL-100) human mammary cell lines were determined by DEAE-cellulose chromatography and photoaffinity labeling with 8-azido-/^{32}P/cAMP. The comparison revealed no correlation between estrogen receptor presence or absence with specific cAMP-dependent kinase holoenzyme ratios, but a greater heterogeneity of cAMP-binding proteins was observed in estrogen receptor-containing cells. Protein kinase C (Ca^{2+} and phospholipid-dependent protein kinase) was detected by DEAE-cellulose chromatography in all six cell lines.

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Structure and intermolecular interactions of a synthetic DNA duplex in solution by ^{1}H NMR methods

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The decadeoxynucleotide nonaphosphate dGpCpApTpTpApApTpGpC was synthesized by the phosphotriester method in liquid phase. This DNA fragment was studied in its duplex form by ^{1}H NMR spectroscopy. A DQF-COSY spectrum was used to assign all nonexchangable protons of the pyrimidine bases and part of the sugar protons. With the use of phase sensitive NOESY spectra sequence-specific assignments were obtained for all protons, except for some H5′ and H5″ resonances of the deoxyribose moieties. These resonance assignments provide the basis for elucidation of the spatial structure of this decadeoxynucleotide duplex, structural comparison with related DNA fragments, and studies of intermolecular interactions between this DNA fragment and substrates.

Sarcoplasmic reticulum ATPase: Investigations on the state of aggregation, Triton X-100 binding and on tryptic fragmentation using HPLC-technique

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(i): Purified preparations of sarcoplasmic reticulum ATPase from fast skeletal muscle were solubilized with different detergents (2 mg/mg protein). Only SDS, Triton X-100 and myristoylglycerophosphocholine were able to completely monomerize the protein, whereas in $C_{12}E_8$ or deoxycholate containing solutions a mixture of monomers, dimers and large aggregates were obtained. (ii): Applying the method of Hummel and Dreyer it could be shown, that the protein in the E_1-conformation (Ca^{++} bound to the protein) binds less Triton X-100 than the protein in the E_2-conformation (no Ca^{++} bound). (iii): The four tryptic fragments of the ATPase were separated in SDS containing solutions and it is demonstrated that the calcium binding site is located in the fragment with a molecular weight of 25000.

Topology and biogenesis of the Lyt-2/3 antigenic complex of cytolytic T lymphocytes

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The Lyt-2/3 antigens of cytolytic T lymphocytes (CTL) have been shown to contribute to the stabilization of the interaction between CTL and target cells. This complex of membrane proteins is composed of three polypeptide chains of 37 Kd, 32 Kd and 28 Kd. Their mode of association with the membrane and their topology has been studied by using different cell labeling methods in conjunction with immunochemical techniques. All three polypeptides have a transmembranous orientation. The rate of synthesis and the identification of the molecular precursors of the Lyt-2/3 complex was further investigated by pulse-chase experiments combined with endo-H and endo-F digestions. The results suggest that the 37 Kd and the 32 Kd polypeptides are N-glycosylated. In addition, both these polypeptides are expressed at the cell surface in an endo-H sensitive form thus indicating the simultaneous presence of non-processed and processed carbohydrate moieties.

N-terminal amino acid sequence of FDP aldolase from *Drosophila melanogaster*

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FDP aldolase from *Drosophila m.* and rabbit brain aldolase have a similar spatial structure, as shown by the ability of their subunits to form stable hybrid quaternary structures (Eur. J. Biochem. *31*, 423, 1972). The mammalian and insect aldolases have also homologous sequences. Thus, FDP aldolase from *Drosophila* and rabbit muscle aldolase exhibit > 70% identity in the central 100 residues and > 90% in the active site tryptic peptide. Extension of these studies to the N-terminal segment (116 residues) of the chain have now shown that in this portion 62% of the residues are identical with rabbit muscle aldolase but that in the first 20 residues there is only little homology and that in the insect enzyme the N-terminus is acetylated.
In rabbit muscle aldolase, the region from residue 60–100 is in dispute (Lai *et al.*, Science *183*, 1204, 1974; Sajgo *et al.*, Acta Biochim. Biophys. Hung. *9*, 239, 1974). Our data on *Drosophila* aldolase are very close to the sequence reported by Lai *et al.*, thus supporting their assignments.

Solubilization and purification of the superoxide-forming oxidase from human neutrophils

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The superoxide-forming oxidase was solubilized and purified by affinity chromatography over a red agarose column. Plasma membranes were obtained from a sonicate of neutrophils activated with opsonized zymosan by centrifugation through a sucrose discontinuous gradient. Solubilization of the superoxide-forming activity was accomplished by extraction with a mixture containing lubrol PX, sodium deoxycholate and glycerol, followed by a centrifugation at 100000 g. The activity of the soluble enzyme was chromatographed over a column of red agarose. The 4 best preparations of red agarose eluate generated 15,1 ± 1,3 SE μmol/min/mg protein, with a 7% yield and a 80 fold purification over the total sonicate. On SDS gels, the enzyme dissociated into subunits of molecular weight 32 and 66 kd. Spectroscopy suggested that at least one of the components of the oxidase was a b-type cytochrome with a molar ratio of cytochrome to enzyme of approximately 1 to 1. This value is consistent with the notion that the cytochrome is an integral part of the superoxide-forming oxidase.

Electrostatic effects in water accessible regions of proteins

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A simple point charge model has been used to calculate electrostatic effects in water accessible regions of proteins. In order to account for the shielding effect of the dielectric medium, an empirical dielectric permittivity function of charge separation is proposed. This function has been parametrized on the basis of data obtained from experimental and theoretical studies of the ratio of the first to the second dissociation constant of bifunctional organic acids and bases. In addition, the reduction of the solvent's accessibility to charged groups due to steric effects arising from the bulk presence of the biopolymer has been considered.
This approach is tested by calculating shifts in dissociation constants (ΔpK) in a number of enzymes. The reasonable agreement between calculated and observed ΔpKs, i.e., lysozyme: calc. ΔpK = 1.0, obs. ΔpK = 1.1, shows that this model, with an accounting of solvent and ionic effects is able to rationalize the observed free energy changes.

Inhibition of cytosolic and mitochondrial rat liver adenylate kinase by (+)-catechin

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Cytosolic as well as mitochondrial adenylate kinase from rat liver was found to be inhibited by the flavonoid (+)-catechin. Using the cytosolic enzyme and 1.2 mM ATP + 0.8 mM AMP as substrates, 30% inhibition was observed at 2 mM and 50% at 5 mM (+)-catechin. Reducing the concentration of AMP or, alternatively, of ATP did not change the inhibitory action of (+)-catechin, suggesting a non-competitive type of inhibition. This was confirmed by kinetic analysis. The inhibitory effect of (+)-catechin on mitochondrial adenylate kinase was similar to the one observed with the cytosolic enzyme althoug some kinetic differences may exist. It is suggested that the inhibition of adenylate kinase by (+)-catechin is involved in the hepatoprotective action of this drug, possibly by increasing the cytosolic ATP content.

Ascorbic acid uptake in adrenal glands

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The adrenal glands (AG) of mammals contain a higher concentration of ascorbic acid (AA) than any other tissue. Whereas AA is known to participate in hydroxylation reactions in the medulla, its role in the cortex is still unknown. In guinea pigs orally administered 1-^{14}C-AA appears very fast in the plasma, somewhat slower in the liver with a radioactivity peak after 2 hours and in the AG the labelled AA increases during the 24 hours of observation. 93% of the AA can be found in the ultrafiltrate of the cytosol of the AG. The mechanism of the AA uptake has been further studied in porcine adrenal cells in vitro. The uptake is temperature dependent with a maximum of 42 °C and is negligible below 20 °C. The activation energy calculated from the temperature curve is 28 kcal/mol. Glucose or lactate/pyruvate is needed for maximum uptake which is in contrast to granulocytes where glucose inhibits the AA transport. KCN inhibits the uptake, but NaF is without effect. Substitution of K for Na leads to a complete inhibition of the uptake. The V_{max} of the uptake is achieved at a [Na]/[K] ratio of 80 mM/65 mM.

The Ca^{2+} and Mg^{2+} dependence of the leukotriene formation in eosinophils

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We have shown (Hoppe-Seyler's Z. Physiol. Chem. *364*, 1029 (1983)) that horse eosinophils stimulated with the ionophore A23187 (10 µg/ml) generate leukotrienes (LT), which can be separated by ion-pair HPLC into LTB_4, LTC_4, LTD_4 and their stereoisomers and that the respiratory burst plays an important role in the LT formation. Because the burst is Ca^{2+} and Mg^{2+} dependent, we studied the role of extracellular [Ca^{2+}] and [Mg^{2+}] in the LT generation. In ionophore-stimulated cells, reduction of [Ca^{2+}] or [Mg^{2+}] to 0.6 mM and 0.5 mM respectively almost suppressed LT formation. In cells stimulated with arachidonic acid (50 µg/ml) the omission of Mg^{2+} at normal [Ca^{2+}] (0.9 mM) had no effect on the LT formation. Assays with normal [Mg^{2+}[(0.8 mM) and reduced [Ca^{2+}] (0.6 mM) showed a strongly decreased LT formation. Our results suggest that the release of arachidonic acid from phospholipids in eosinophils is Ca^{2+} and Mg^{2+} dependent, and that the LT formation is Ca^{2+} dependent but Mg^{2+} independent.

Natural killer cells share an antigenic determinant with the myelin associated glycoprotein: Possible role in a human demyelinating disease

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The monoclonal antibody anti-Leu 7 (HN Kl) which reacts with a 100-kd differentiation antigen defining a subpopulation of human granular lymphocytes with natural killer (NK) and K cell function cross reacts with the myelin associated glycoprotein (MAG). This integral membrane component of both CNS and PNS myelin has a molecular weight of 100-kd and is the antigen for human monoclonal IgM antibodies in a chronic demyelinating neuropathy. Patients with neuropathy and antibodies to MAG exhibit markedly reduced numbers of Leu-7 antigen positive cells in peripheral blood ($8.7 \pm 1.3\%$ compared with $17.3 \pm 1.5\%$), while total T-cell numbers are normal. These data demonstrate that a myelin glycoprotein shares antigenic determinants with an NK cell surface antigen and provide the first evidence that cross reactive cell surface antigens are the target for an autoimmune response in a human demyelinating disease.

Structural analysis of the mouse Lyt-2/3 antigen complex

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The mouse Lyt-2/3 antigen complex, a glycoprotein specific for functional T lymphocytes, consists of three polypeptides linked by disulphide bonds with apparent molecular weights of 37 Kd, 32 Kd and 28 Kd. These subunits are surface expressed and glycosylated. Tryptic digests of the individual subunits were analysed by high performance liquid chromatography on a reverse phase column and by two dimensional peptide mapping. The results indicate that the 37 Kd and 32 Kd are structurally similar. The difference in their apparent molecular weights observed on SDS-PAGE might result from differences in glycosylation or other post-translational events. In contrast, 28 Kd component appears to be a different polypeptide.

Kinetic properties of reduced cytochrome P-450

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In the reduced, carbon monoxide bound form, cytochrome P-450, isolated from phenobarbital induced rat liver microsomes, exists in an equilibrium with its inactive form P-420. The interconversion between the two enzyme forms is influenced by the aggregation state of the protein. Carbon monoxide binding kinetics to P-450 and P-420 have been investigated by stopped flow and flash photolysis spectrophotometry. For both forms the kinetics of CO-association can be described as a biphasic process with rate constants of $1.7 \cdot 10^7$ $M^{-1}s^{-1}$ and $4.5 \cdot 10^6$ $M^{-1}s^{-1}$ for P-420 and $1.5 \cdot 10^6$ $M^{-1}s^{-1}$ and $4.7 \cdot 10^5$ $M^{-1}s^{-1}$. The off-rates for CO are 138 s^{-1} and 13.9 s^{-1} for P-420 and 1.2 s^{-1} and 4.0 s^{-1} for P-450. These results are in contrast with previous reports showing oligophasic processes for CO-binding kinetics to P-450. (Gray, R.D. J. Biol. Chem. *253*, 4364, 1978; Gray, R.D. J. Biol. Chem. *257*, 1086, 1982; Gray, R.D. J. Biol. Chem. *258*, 3764, 1983; Debey, P., Douzou, P. FEBS-Letters *39*, 271, 1974). The large differences found in the reaction velocities with carbon monoxide between P-420 and P-450 seem to be correlated with the geometry of the heme-pocket and with the proximal ligation state of the theme-iron.

Parvalbumin-like protein in human epidermoid carcinoma cells

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Transformed cells are characterized by high levels of calcium and Ca^{2+}-binding proteins (e.g. calmodulin). It is suggested that some of these might contribute to their rapid proliferation and motility. In the present study three cell-lines deriving from human carcinomas of the larynx and tongue were analyzed for the presence of the Ca^{2+}-binding protein parvalbumin. Applying the indirect immunofluorescence technique, most tumor cells but not normal epithelial cells were intensely stained by anti-parvalbumin. The staining was inhomogeneous, and sometimes even an association of this antiserum with filamentous structures was observed. Extracts of these cultures, when analyzed by 2D-PAGE, contain a protein with identical M_r of 12K and similar pI of approx. 4.7 to parvalbumin (pI 4.9). Both proteins, however, differed in their hydrophobicities and peptide maps. Structure and function of this parvalbumin-like tumor protein will be further investigated.

Binding sites on bacterial cytochrome c_2 for photosynthetic reaction center and mitochondrial cytochrome bc_1-complex

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Isolated cytochrome c_2 from Rhodospirillum rubrum is able to form electron transfer complexes with the photosynthetic reaction center (physiological electron acceptor) as well as the mitochondrial cytochrome bc_1-complex (non-physiological electron donor). By comparing rates of incorporation of radiolabelled reagent, we identify those lysine residues of cytochrome c_2 which are shielded by complex formation and, hence, are involved in binding. In mitochondrial cytochrome c, a common recognition site for redox partners is formed by a cluster of positively charged lysine residues around the heme edge (Rieder, R. and Bosshard, H.R. (1980) J. Biol. Chem. *255*, 4732–4739). Preliminary results suggest that a similar cluster is important for binding of cytochrome c_2 to the reac-

tion center. This result substantiates the well known evolutionary conservatism of c-type cytochromes at the level of function.

Viral polypeptide (VP) specificity of polyclonal or monoclonal antibodies induced by immunization with human rotavirus antigens

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Sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by immunoblotting was used to study the serum antibody responses to the human Hochi rotavirus antigens in mice and a rabbit immunized with purified viral particles or in mice immunized with an enriched preparation of VP7, the putative 'neutralizing' antigen. This analysis revealed strong antibody reactions against VP6 (41 kD) and against unidentified antigens of lower molecular weight (16, 17 and 21 kD), while the response to VP7 (32 kD) was weak and the response to VP1 (117 kD) and VP2 (95 kD) not detected. All monoclonal antibodies derived from a mouse immunized with Hochi viral particles were directed at VP6 antigen. We thank Dr. H. Hilpert and Dr. H. Brüssow for providing us with the purified rotavirus particles and Nestlé S.A., Vevey, Switzerland, for financial support.

Amino acid sequence determination using combined gas liquid chromatography–mass spectrometry of peptides

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The title method was optimised recently (Rose et al., Biochem. J. 215, 1983, 261–272) to be applicable at the 2–10 nmol level; it has been used successfully in the characterisation of a truncated form of gamma-interferon which possesses full antiviral activity (Rose et al., Biochem. J. 215, 1983, 273–277) and to characterise the N-terminal regions of two viral proteins which are Nα blocked and therefore inaccessible to the Edman degradation (Rose et al., Biochem. J., in press).
Further applications of the title method will be presented.

The immune response to synthetic peptides coupled to carrier proteins

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Antibodies to synthetic peptides are an important research tool, but little is known about the immune response to peptide-carrier conjugates. We have investigated the response in rabbits and rats to several conjugates of peptides from the nicotinic acetylcholine receptor (nAChR).
50–90% of antibodies were IgM, this being independent of the number of injections. Antibody affinities were 10^6–10^8M depending on the peptide used. Titres (up to 10^6M) were dependent on the degree of conjugation. A high proportion of antibodies reacted with the native nAChR.
In contrast to other hapten-conjugates, immunological memory was observed.

Isocitrate lyase in germinating soybean

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In plant seeds, the action of the glyoxylate cycle is essential to the net synthesis of carbohydrates from reserve lipids. Isocitrate lyase (EC 4.1.3.1), which catalyses the cleavage of isocitrate into succinate and glyoxylate, is specific to this cycle. Its *in vivo* activity may be used as a direct measurement of the occurrence of this peculiar metabolic pathway. Using germinating soybean (*Glycine max.*, var. Maple arrow), we present the evolution of isocitrate activity, which is best explained by induction phenomena. Also, the enzyme from 5-day germinated cotyledons war purified using conventional techniques, and various physico-chemical and reactional characteristics were determined. Its relative molecular mass is ca. 100000, and the K_m for isocitrate is equal to 1.75 mM.

Determination of enkephalins by HPLC with electrochemical detection (EC) in biological material

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Based on HPLC-EC we have developed a sensitive method for the simultaneous determination of Leucine-enkephalin and Methionine-enkephalin (Leu-enk and Met-enk) in biological material.
Using a two column-switching technique the two peptides are separated and electrochemically detected in less than 10 minutes. Rat brain tissue is homogenized in acetone-aqueous HCL and the clear supernatant, after blowing off the acetone with a stream of nitrogen, is injected on the column (20 µl). On the first column (anion exchange) both peptides coelute simultaneously but are separated from the large void peak. The second column (RP 18), connected over a switching valve with the first column, separates Met-enk from Leu-enk, which are detected by EC at a potential of +1V. For quantification, peak heights are compared with those of known amounts of standard peptides. The levels found in various regions of the rat brain correspond well with levels reported in the literature using different procedures. Due to its simplicity and specificity it is an attractive alternative to existing methods.

A channel ligand binding site on the purified GABA/benzodiazepine receptor complex from bovine cerebral cortex

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A GABA/benzodiazepine receptor complex has been solubilized and purified from bovine cerebral cortex by a novel procedure using the zwitterionic detergent CHAPS. A high affinity binding site for the presumed channel ligand [^{35}S]TBPS was co-purified with the high affinity binding sites for GABA and benzodiazepines which have been shown to reside on the same physical structure (E. Sigel, F. A. Stephenson, C. Mamalaki and E. A. Barnard (1983) J. Biol. Chem. 258, 6965–6971). The dissociation constant for [^{35}S]TBPS was 80 ± 30 nM. The ratio of [^{35}S]TBPS to [^{3}H]flunitrazepam binding sites in the purified complex was 0.25–0.58. [^{3}H]flunitrazepam binding was stimulated by GABA and pentobarbital in a dose dependent manner. Analysis by SDS-PAGE showed the identical two subunit pattern as previously reported for a purified preparation that did not display any of the properties that indicate the presence of a channel.

Heterobifunctional crosslinking of bacteriorhodopsin by 4-azido-azobenzene-4′-isothiocyanate, a site-directed cleavable photoreagent

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4-Azido-azobenzene-4′-isothiocyanate, a chromophoric heterobifunctional reagent is utilized for site-directed structural exploration of bacteriorhodopsin in purple membranes. The reagent with an approximate linking range of 14 A, contains the advantageous feature of reversible cleavability by virtue of its azo bridge. Selective covalent arylthiocarbamylation of the

protein is attained in the dark reaction (arylisothiocyanate binding site = Lys 41). Crosslink formation is induced by photoactivation of the heterofunction (arylazide). Fragmentation (CNBr, thermolysine) of the modified protein yields labeled peptides which are susceptible to dithionite reduction. The chemical identity if the reversibly cleaved peptides is correlated with the established amino acid sequence of bacteriorhodopsin.

Acetylcholinesterase in amniotic fluid detected by enzyme antigen immunoassay

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For the prenatal detection of neural tube defects, the determination of acetylcholinesterase (AChE) has proven to be superior to the determination of α-fetoprotein. The determination has been performed either by an electrophoretic technique (qualitative) or with a spectrophotometric method. We here present an enzyme-antigen-immunoassay for the determination of AChE in amniotic fluid, both normal and pathological. The determination takes place in micro-titer plates, and utilizes an antibody to human erythrocyte membrane AChE (Nørgaard-Pedersen et al. Clin. Chem. 29: 1061, 1983). The method is simple, inexpensive and the results are quantitative. Further it has a large potential for being automated. With a sample material of 1400 normal amniotic fluids, we demonstrate, that the AChE content of these samples have a mean value, that is independent of the gestational age. In no cases false positive results were obtained.

Partial sequence determination of the human $C\bar{1}s$ heavy chain and identification of the peptide bond cleaved during activation

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Purified single-chain Cls proenzyme was converted to active two-chain $C\bar{1}s$ with self-activated $C\bar{1}r$. The reduced and alkylated chains were separated on DEAE-Sephacel. CNBr-cleavage of the heavy chain (M_r 57,000) yielded at least five fragments (with a M_r ranging between approx. 470 and 30,000). They were characterized by N-terminal sequence analysis. The C-terminal CNBr-fragment (44 residues) was completely sequenced.

From BNPS-Skatole cleavage of reduced and alkylated Cls proenzyme a fragment was isolated which makes the overlap between the heavy and light chain regions and which contains the peptide bond cleaved during activation to $C\bar{1}s$. The results show that this is an Arg-Ile bond and that under standard conditions of activation no peptide material is liberated from this portion of the molecule. The sequence data and homology with two-chain serine proteases indicate a single interchain disulfide bond in $C\bar{1}s$.

Somatostatin (SRIF) lowers the cytosolic free Ca^{2+} concentration in clonal rat pituitary cells (GH_3 cells)

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Changes in the cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$, have been proposed to mediate the regulation of the secretion of pituitary hormones by hypothalamic peptides. Using an intracellularly trapped fluorescent Ca^{2+} probe, quin2 (Tsien, R.Y., Biochemistry *19*, 2396, 1980), $[Ca^{2+}]_i$ was monitored in GH_3 cells. SRIF lowers $[Ca^{2+}]_i$ in a dose dependent manner ($K_{app} = 2 \times 10^{-9}$M). At 10^{-8}M, SRIF decreases $[Ca^{2+}]_i$ from a prestimulatory level of 120±9 (SEM) to 98±10 nM. This small but significant ($p < 0.001$) effect on $[Ca^{2+}]_i$ is observed within 10 seconds after SRIF addition and $[Ca^{2+}]_i$ remains lowered for several minutes. Concommitantly SRIF causes hyperpolarization of GH_3 cells. SRIF does not inhibit the rapid rise in $[Ca^{2+}]_i$ elicited by thyrotropin releasing hormone (TRH) and can still cause a decrease in $[Ca^{2+}]_i$ in the presence of TRH (10^{-7}M). The lowering of $[Ca^{2+}]_i$ by SRIF indicates a possible mechanism for its action to supress pituitary hormone secretion.

Triiodothyronine (T_3) favors ornithine decarboxylase induction by parathyroid hormone (PTH) in cultured bone cells

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Calvaria cells of newborn rats seeded in 1% FCS and grown for 6 days in medium containing 1 g/l of HSA had properties of osteoblasts: a high alkaline phosphatase activity and an ornithine decarboxylase induction by PTH. T_3 (6 days) did not enhance cell replication, but dose-dependently (at 0.1 nM and at 1 nM) increased ornithine decarboxylase activity of the cultures. Moreover, cells grown in the presence of T_3 showed a greater response to PTH and to dibutyryl cyclic AMP than cells grown without T_3. T_3 effects were specific for bone cells (no effects in primary rat fibroblasts) and evident only after many hours. – Our data directly support the hypothesis based on in vivo observations, namely that thyroid hormones sensitize bone to actions of PTH.

A purified GABA receptor from brain tissue: biochemical and immunological characterization

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A GABA/benzodiazepine receptor complex was purified from bovine synaptic membranes by affinity chromatography.

i) The receptor fraction contained high affinity binding sites for GABA ($K_D = 13.8$ nM for ^{3}H-muscimol) and benzodiazepines ($K_D = 2.5$ nM for ^{3}H-flunitrazepam). The number of both sites was similar. ii) A low affinity binding site for GABA ($K_D = 0.1$ µM) was revealed by the modulation of benzodiazepine receptor binding by GABA. The affinity for diazepam (an agonist) was enhanced, whereas that for the β-carboline βCCM (an inverse agonist) was reduced in the presence of GABA. In contrast, the affinity for Ro 15-1788 (an antagonist) was unchanged by GABA. iii) On SDS-PAGE three bands were present with apparent MW of 50K, 55K and 62K daltons. iv) Monoclonal antibodies against the purified receptor complex were raised. They immunoprecipitated both the GABA- and benzodiazepine receptor. Thus, the binding sites for GABA and benzodiazepines reside on the same structural entity.

Formation of lipid-protein bilayers by micropipette guided contact of two monolayers

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Bilayers of a few µm^2 area were formed from proteoliposomes or native membrane vesicles [Schürholz, Th. and Schindler H. FEBS Lett. 152, (1983) 187–190]. First, two monolayers are generated from vesicles. In contrast to the earlier technique [Schindler, H. FEBS Lett. 122, (1980) 77–79] the two monolayers are brought into local contact by use of a micro-pipette to form a bilayer. Membrane channels of Matrix-Protein and of Colicin A exhibit normal properties in these bilayers. Apart

from avoiding solvents completely and of using any kind or mixture of vesicles as starting material there are two main virtues to this strategy: (1) Current resolution is very high, a few tenth of a pA at 150 μs time resolution, and (2) transport processes can be studied in dependence of surface pressure between 28 and 48 mN/m. Instead of using two monolayers one can dip the pipette into a vesicle suspension. When removing the pipette again a monolayer is formed at the tip by self-assembly from vesicles. The latter procedure is technically less involved but it does not allow to directly control the surface pressure in the two monolayers.

Electron transfer from reduced ferredoxin to the chloroplast fructose 1,6-bisphosphatase via the ferredoxin/thioredoxin system

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Using EPR technique we have looked at the oxidation of ferredoxin (Fd) reduced anaerobically by dithionite and excess dithionite removed by NAD, after addition of ferredoxin-thioredoxin reductase (FTR) alone, or FTR, thioredoxin f (Th) and fructose 1,6-bisphosphatase (FBPase) together. When adding anaerobically an equimolar amount of FTR to reduced Fd, the EPR signal of reduced Fd disappears completely indicating a transfer of electrons from Fd to FTR. In the presence of only catalytic amounts of FTR and Th reduced Fd is completely oxidized by an excess of FBPase, but very little oxidized without FBPase. Oxidation of reduced Fd correlates with either FTR or FBPase activity. Titration studies of the electron transfer between the different proteins are discussed. Our results provide evidence for a net electron transfer from Fd to the FBPase responsible in vivo for the light activation of the FBPase by reduction of disulfide bridges on the enzyme.

Calcium, calmodulin and the oxidation of exogenous NADH by higher plants mitochondria

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Mitochondria from higher plants are able to oxidize exogenous NADH at high rates, via a dehydrogenase which is located at the outer surface of the inner membrane and not linked to the first energy-coupling site. The NADH oxidase from potato tubers mitochondria shows a specific dependence on calcium, in addition to a non-specific stimulation by cations. The electron flow from NADH to oxygen, exogenous cytochrome c or duroquinone, but not to ferricyanide, was strongly inhibited by EGTA and markedly stimulated by calcium. Calmodulin antagonists like chlorpromazine and phenothiazine were strong inhibitors of the NADH oxidase activity. This suggests that the effect of calcium on the activity of the NADH dehydrogenase could be mediated by calmodulin. However, the NADH duroquinone oxidoreductase, solubilized by Triton X-100, was not retained on either phenothiazine-Affi gel or calmodulin-Sepharose affinity columns. The possible direct or indirect regulation of NADH oxidation by calmodulin will be discussed.

Characterization of microtubular protein from *Trypanosoma brucei*

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Cytoskeletal microtubules of *Trypanosoma brucei brucei* do not disrupt under conditions used to solubilize other tubulins, e.g. those from brain. Solubilization of most tubulin was achieved by extensiv sonication. Tubulin was then partially purified from crude trypanosomal cell extracts by taxol-induced polymerization. The resulting microtubular structures were identified by electron microscopy. SDS-gel-electrophoresis revealed two major proteins of 52000 and 56000 Da, which cross-reacted immunologically with the alpha- and beta-subunits of bovine brain tubulin. Peptide patterns generated from the trypanosomal alpha-and beta-subunits by chemical cleavage with N-chlorosuccinimide and enzymatical cleavage with staphylococcal protease V8 were closely related to each other.
The trypanosomal alpha-tubulin is posttranslationally modified *in vivo* by the reversible addition of a tyrosine residue at its C-terminus.

Hydrophobic labeling of the membrane binding domain of acetylcholinesterase from *Torpedo marmorata*

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The hydrophobic domain of the membrane bound form of acetylcholinesterase from the electric organ of *Torpedo* was labeled with the photoactivatable reagent 3-trifluoromethyl-3-(m-[^{125}I]iodophenyl) diazirine ([^{125}I]TID). By limited digestion with proteinase K this amphiphilic enzyme could be converted to a catalytically active hydrophilic, amphiphile independent form. Native and proteinase K treated [^{125}I]TID-labeled acetylcholinesterase were subjected to SDS-gel electrophoresis. In the native enzyme radioactivity was associated with the catalytic subunit (apparent M_r 67000) whereas the proteinase K treated form (apparent M_r 66000) had lost the radioactively labeled peptide which appeared at approximately 3000 D. Thus only the proteinase K sensitive membrane binding segment was labeled with TID. This hydrophobic peptide could be isolated by gel filtration on Sephadex LH-60.

Regulation of glyceollin accumulation in soybean hypocotyls by sulfhydryl groups

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Accumulation of the phytoalexin, glyceollin, in hypocotyls of etiolated soybean seedlings was induced with various sulfhydryl binding (SH) reagents. Dithiothreitol effectively reduced accumulation of glyceollin elicited by non-covalently bound SH-reagents. Accumulation of glyceollin induced by SH-reagents and their reactivity with either L-cysteine or SH-groups in buffer extracts from soybean hypocotyls were not strictly correlated. Results clearly indicate that glyceollin synthesis can be regulated by interaction with SH-groups located mainly at the outer surface of the plasmalemma.

Apoenzyme-catalyzed model reactions for enzymic transamination

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The apoenzyme of aspartate aminotransferase catalyzes the reaction Pyridoxal + Aspartate⇄Pyridoxamine + Oxalacetate. The nonphosphorylated cofactor acts as dissociable substrate rather than as prosthetic group (Wada H., and Snell E.E., JBC *237*, 127, 1962). For this model system continuous spectrophotometric assays have been worked out which allow the two half-reactions of transamination to be monitored separately. The rates of the forward and the back reaction are 0.01% and 0.005%, respectively, of that of the reactions of the holoenzyme. Preformation of the internal aldimine by reacting apoenzyme with pyridoxal before adding aspartate does not increase the initial rate of the forward reaction. However, preformation

of the external aldimine between pyridoxal and aspartate results in a 1.7-fold increase in the initial rate. Apparently, in this model system formation of the external aldimine by transaldimination is not faster than its de novo formation.

Calcium calmodulin-dependent protein phosphorylation in *Neurospora crassa*

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A calcium calmodulin-dependent protein kinase activity has been partially purified by calmodulin-Sepharose affinity chromatography from the soluble fraction of *Neurospora crassa*. The calcium calmodulin-dependent kinase activity can only be detected in the calmodulin-Sepharose affinity column eluate, obtained by calcium chelation in the presence of high ionic strength. The phosphorylated peptide has an apparent molecular weight on SDS-polyacrylamide gel of 47.000 Dalton. Maximal phosphorylation is obtained after 10 min at 30 °C in the presence of calcium and calmodulin. The half maximal activation of the phosphorylation is obtained in the presence of sub-micromolar and micromolar concentrations of calmodulin and calcium, respectively. This kinase activity was enhanced about seven fold by calmodulin.

Similarities between human and rat leukocyte elastase and cathepsin G

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Both human leukocyte elastase and cathepsin G have been implicated in a wide variety of diseases such as emphysema, ARDS, shock, thus suggesting a potential therapeutic value for elastase or cathepsin G specific proteinase inhibitors. Since the rat is a likely test animal for such drugs we compared relevant properties of human and rat neutrophil neutral proteinases. Both species of elastase and cathepsin G displayed similar specificity toward various natural (plant and animal) proteinase inhibitors and also toward peptide substrates and a serine proteinase specific reagent. Such overlapping specificity implies similar reactive center sequences and sequence homology between extended substrate/inhibitor binding regions (P_5–P_1) of the respective proteinases. This apparent homology leads us to conclude that inhibitors which were to be effective in rat models where elastase and cathepsin G play a pathogenetic role, would probably also become useful in the treatment of related human diseases.

2D NMR studies of metallothionein

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Rabbit metallothionein-2 (MT) is a small protein (61 residues) containing 7 diamagnetic metal ions (Cd^{2+}, Zn^{2+}). The protein contains 20 cysteines; these complex the 7 metal ions in two metal-thiolate clusters. Isotopically pure ^{112}Cd-MT or ^{113}Cd-MT can be prepared by reconstitution. In addition to two-dimensional ^{1}H NMR techniques, heteronuclear ^{1}H-^{113}Cd NMR techniques were applied to aid structure elucidation. In the ^{1}H NMR spectrum a large proportion of the resonances have been attributed to residue types, and partial sequential assignments have been made. Spin-spin couplings between ^{113}Cd and the C^βprotons of 19 cysteines have been observed, with ^{3}J values of $\sim$ 5 to 75 Hz. Heteronuclear ^{1}H-^{113}Cd correlation experiments were used to establish connectivities between Cd^{2+} ions and individually assigned cysteines, which also provide further information about the overall spatial structure of the protein.

Covalent crosslinking of cytochrome c to cytochrome c peroxidase

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Cytochrome c peroxidase and cytochrome c were crosslinked by a carbodiimide to a covalent 1:1 complex. The complex was cleaved with CNBr and fragments were separated by gel permeation chromatography and identified according to molecular weights and amino acid compositions. After digestion of CNBr fragments with trypsin in the presence of SDS, tryptic peptides were separated by a combination of gel filtration and high pressure liquid chromatography. Crosslinked peptides of the two hemoproteins were identified by determination of amino-terminal residues and amino acid compositions using the dansylmethod. Crosslinking of cytochrome c occurred to acidic residues in the sequences CCP 30–48, CCP 76–90, and CCP 213–226. These data localize the binding site for cytochrome c within the three-dimensional structure of cytochrome c peroxidase.

Assignment of the ^{1}H-NMR spectrum of toxin II from *Anemonia Sulcata* (ATX II)

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ATX I is a peptide of the venom of *Anemonia sulcata* (we thank Dr. G. Wunderer, Munich, for a gift of ATX II). The sequence of the 47 amino acid residues is known, but no three-dimensional structure has been determined. To investigate the conformation in solution we use ^{1}H-NMR. First we determine the sequence-specific assignments for the ^{1}H-NMR spectrum. Compared to previous assignment strategies, which relied exclusively on two-dimensional (2D) correlated spectroscopy (COSY, SECSY) and 2D nuclear Overhauser enhancement spectroscopy (NOESY), additional, new 2 D-NMR methods are used, such as relayed-COSY, double-relayed-COSY and double quantum-COSY. Furthermore, phase sensitive spectra are recorded with much higher digital resolution. This advanced methodology allows identification of the complete spin systems (including amide protons) for a larger proportion of the amino acid residues prior to sequential assignments with the use of NOESY.

Immunochemical identification of aldo/keto reductases in human tissues

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Aldose reductase (AR), glucuronate reductase (GR) and carbonyl reductase (CR) comprise a family of cytosolic, monomeric, NADPH-dependent enzymes with wide specificity for the reduction of carbonyl groups to the corresponding alcohols. Antibodies against the homogeneous human enzymes were raised in rabbits and purified by ammonium sulfate precipitation, DEAE-cellulose and immunoaffinity chromatography. Monospecific antibody preparations against each of the 3 reductases were obtained as assessed by immunodiffusion, crossed immunoelectrophoresis and immunoisoelectric focusing and by ELISA. Crossed immunoisoelectric focusing of extracts from brain liver and kidney revealed at least 7 antigenetically identical molecular forms of CR with isoelectric points between pH 5 and 9, 3 forms of AR in the pH range 5–6 (brain and kidney only) and a single form of GR at pH 5.3. This method provides a fast and sensitive means for the discrimination of aldo/keto reductases in tissue extracts.

The uptake and metabolism of lysophosphatidylcholine by *Saccharomyces cerevisiae*

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Different forms of a phospholipase B capable to split all phospholipids extractable from yeast were found in the plasma membrane, mitochondria, cell wall and growth medium of *S. cerevisiae* (Witt, Biochim. biophys. Acta *711*, 403, 1982). Lysophospholipids are converted to a small extent into the corresponding diacyl-compounds by all forms of the enzyme. In order to obtain information about the function of the enzyme – especially about the acylation of lysophospholipids in vivo – lysoPC, [1-^{14}C]palmitoyl was added to the medium of growing yeast. Within 1 h 72% of the applied ^{14}C was found in the lipid extracts of the yeast cells (53% phospholipid with a preferential labelling of PC and 47% neutral lipid). Further in vitro experiments with the purified enzymes and plasma membrane vesicles support the assumption, that the phospholipases not only catalyse the release of fatty acids, but also may take part in the remodelling of intracellular membrane phospholipids.

Regulation of glucose transport in the heart of normal rat

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Glucose transport in the perfused rat heart is stimulated by glucose, perfusion pressure or insulin. Glucose transport was correlated to the number of transport systems (i.e., transporters) using the cytochalasin B binding technique. In plasma membrane, number of transporters is, basal: 6.1, 15 mM glucose: 19.2, 10 mU/ml, insulin: 24.7 and higher perfusion pressure (100 mmHg): 37.1 pmole/mg protein. These effectors provoke simultaneously a decrease of the number of transporters measured in the microsomal membrane fraction, basal: 39.2, glucose 15 mM: 19.5, insulin: 18.1, higher perfusion pressure: 6.2 pmole/mg protein. As the total number of transport systems (plasma membrane + microsome) is constant under these different conditions, the results suggest that the effectors tested provoke an increase in glucose transport via an augmentation of the number of transporter in the plasma membrane. This is achieved by a translocation of the transporter from an intracellular pool to the plasma membrane.

Bacterial phosphotransferase system: characterization of monoclonal antibodies directed against the glucose-specific enzyme II

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The bacterial phosphotransferase system (PTS) mediates active transport and concommittant phosphorylation of sugars, sugar chemotaxis and regulation of the bacterial metabolism. It comprises two cytoplasmic proteins and a number of sugar-specific transmembrane permeases (enzyme II). We have purified the glucose-specific permease (II^{Glc}) of the PTS. Purification included the following steps: solubilization of membranes in polydisperse octyloligooxyethylene, isoelectrofocusing, chromatofocusing and glycerol gradient centrifugation. Monoclonal antibodies were prepared against the purified protein. A panel of six monoclonal antibodies is characterized with respect to the following properties: (1) Inhibition of II^{Glc} dependent phosphorylation of glucose *in vitro*. (2) Interference with ^{32}P-enolpyruvate dependent phosphorylation of II^{Glc}. (3) Discrimination between the structurally homologous and functionally exchangeable II^{Glc} of *E. coli* and *S. typhimurium*. (4) Epitope specificity.

ZELL- UND MOLEKULARBIOLOGIE
BIOLOGIE CELLULAIRE ET MOLÉCULAIRE
CELL AND MOLECULAR BIOLOGY

Improvements of low-temperature embedding media Lowicryl®

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The hydrophobic resin Lowicryl HM20® (Carlemalm et al., J. Microscopy *126*, 123, 1982) can be used at temperatures down to −45 °C in the embedding of biological samples for electron microscopy. A development of this medium has given a new resin, 'HM23', which allows for embedding down to at least −80 °C, such that an increase in the preservation of biological structures may be possible. The hardening by photo-polymerization required a new photo-initiator – benzyl dimethyl acetal – to compensate for the loss of reactivity. In the same way a new hydrophilic resin, 'K11M', has been extrapolated from the Lowicryl K4M®. The viscosity, linked to the association of hydrophilic functions (e.g. hydrogen bonds), puts the limits of use at −60/−65 °C. For photohardening, the initiator benzoin methyl ether can be kept.

A transposable element involved in the chromatin diminution process of *Ascaris lumbricoides?*

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The DNA eliminating nematode *A. lumbricoides* contains a DNA satellite which is mostly eliminated from the presumptive somatic cells during early cleavage divisions. In order to elucidate the mechanism of chromatin diminution at the molecular level, a clone containing both satellite and non-satellite DNA sequences was isolated from the germ line DNA library and analyzed. Sequencing studies revealed great homology between the 122 bp satellite monomers and the consensus sequence of the satellite DNA known to be eliminated. The 11 last bp of the satellite cluster are repeated 5 kb downstream in the non-satellite DNA, a region which is extremely rearranged between germ line and soma. The 5 kb putative transposable element is present roughly 50 times in both the germ line and somatic genome and may be involved in the process of chromatin diminution.

Manipulation of the intracellular Mg^{2+} level of PMBN sensitized *E. coli* by A23187 ionophore

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It was recently discovered that a polymyxin B derived nonapeptide (PMBN) sensitizes gram(−) bacteria to many kind of hydrophobic antibiotics (Vaara and Vaara, Nature *303*, 526, 1983). We found, that PMBN sensitizes *E. coli* B cell also to A23187 and induces Mg^{2+} leakage. It is dependent on the extracellular Mg^{2+} level: In the presence of 0–1 mM, 5 mM and 20 mM $MgCl_2$ in the medium the total intracellular Mg^{2+} contents (after efflux was completed, about 10 min) were less than 10%, 50–70% and 90–100%, respectively. These observations, together with results of the A23187 dose-experiments support the hypothesis, that in *E. coli* B the free Mg^{2+} concentration is about 20 mM. During the Mg^{2+} efflux we observed a temporary increase of the K^+ content possibly due to counter influx. The efflux can be halted by adding adequate amounts of Mg^{2+} to the outside medium. The consequences of such 'dumpings' on cell growth and phage multiplication are studied presently and results will be reported.

Characterization of an ecdysone-regulated gene and its flanking regions

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As previously reported we had isolated the ecdysone-regulated gene, I-18C, from Chironomus tentans by microcloning. In order to obtain DNA fragments comprising the whole gene as well as 5′-upstream sequences (of a putative regulatory importance) a λ-library of total genomic DNA was screened with the microcloned material. A recombinant clone was isolated with a C. tentans-DNA insert which extends approx. 6 kb 5′-upstream and 5 kb 3′-downstream from the coding region (4.6 kb) of the I-18C gene. The restriction map of this fragment corresponds with that of the microcloned fragments as well as with that previously established by Southern analysis of larval DNA. R-loop measurements confirmed the existence of a large exon at the 3′-end. Base sequence analyses were also carried out.

Heat shock proteins of *Drosophila melanogaster* are constituent of both nuclear and cytoplasmic RNP particles

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Exposure of *Drosophila melanogaster* tissue culture cells to 37 °C induces the synthesis of several new polypeptides HSP 22, 23, 26, 27, 68, 70 and 84. During the heat shock these proteins (except HSP 84) were found associated with nuclear RNPs. At 25 °C following a 1 hour heat treatment they migrate back to the cytoplasm where they are recovered as RNP particles. HSP 22, 23, 26 and 27 copurify with a 20S preexisting RNP complex similar to the 'prosome' described by others (Schmid *et al.*, 1984). HPS 68, 70 and 84 may be part of different cytoplasmic RNPs of smaller size (10–15S). Reinduction of the heat shock induces the migration of the HSPs back to the nucleus. The results presented here suggest that the HSPs may have a function at the level of the processing of the RNA during the heat shock and at the level of the transport of RNA to the cytoplasm during the recovery period following the heat treatment.

Effects of guanyl nucleotides on hormone-sensitive [^{3}H]-Gpp(NH)p release from human platelet membranes

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Applying the method of Michel and Lefkowitz (J. biol. Chem. *257*, 13557, 1982) to measure the release of [^{3}H]-Gpp(NH)p from prelabelled human platelet membranes, we have found that both GDP (100 μM) and GTP (10 μM) alone stimulated release of the tritiated non-hydrolyzable GTP analog, while the two nucleotides GDP and GTP in combination caused an inhibition of the release. Release was stimulated by 10 μM prostaglandin E_1 and by 100 μM epinephrine in the presence of GTP, but was decreased to below the basal level when GDP was included in the incubation mixture. Furthermore, the stimulation by epinephrine was reversible; later addition of GDP restored the hormone-stimulated release to the basal level. These results cannot be explained by a model of competition among the three nucleotides for a single, non-cooperative binding site.

Training-induced biochemical and structural changes of contractile proteins in single human muscle fibers

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The pattern of myosin light and heavy chains and the ATPase activity of single muscle fibers were studied before and after a 9 weeks period with high-intensity training of short-duration. The analysis was carried out in aliquots of histochemically typed and pooled fibers from lyophilized muscle tissue.

After training, the densitometric scan profile of a 1 D-gel of type I fibers became more similar to the one found in type II fibers, indicating a training-induced modification of some components of the contractile proteins. The specific activity of the myofibrillar ATPase in type I muscle fibers showed a slight increase after the training period.

It is concluded that high-intensity training of short duration partly leads to a transformation of muscle fiber types at the level of the molecular structure of the contractile proteins. These changes in the phenotype of fibers is most probably due to a different gene expression induced by specific environmental factors.

Quarternary structure of the precursor of mitochondrial aspartate aminotransferase

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The mitochondrial isoenzyme of aspartate aminotransferase (a_2 dimer, subunit MW 44.5 kDa) is synthesized on cytosolic free polysomes as a precursor (pre-mAspAT) of higher MW (ΔMW 2.5 kDa; JBC *257*, 3339, 1982). In order to determine the quarternary structure of the extramitochondrial form of the enzyme, pre-mAspAT labeled with ^{35}S-Met was synthesized in a reticulocyte lysate programmed with chicken liver free polysomes. The postribosomal supernatant was centrifuged on a linear sucrose gradient. Pre-mAspAT was detected in the fractions by immunoprecipitation, SDS-PAGE, and fluorography. Its major portion migrated in a MW range around 80 kDa; a minor fraction was found in the high MW range of the gradient (MW > 300 kDa). Apparently, at the concentration present in the reticulocyte lysate pre-mAspAT exists predominantly as a dimer. However, an association of monomeric precursor with another protein cannot be excluded.

Localization of sialyltransferase by immunofluorescence in bovine fetal kidney fibroblasts

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Purified bovine sialyltransferase (ST) was used for raising a polyclonal antiserum in rabbits. Specificity of antiserum was tested by inhibition of ST activity and ELISA.
Primary cultures of fetal calf kidney fibroblasts were cultured to subconfluency and processed for immunofluorescence using rabbit anti-human galactosyltransferase (GT) and rabbit anti-bovine ST antisera. In permeabilized cells, GT was localized in a typical Golgi-like juxtanuclear structure, whereas ST was predominantly found in peripheral cytoplasmic vesicles. On the cell surface, ST was found in one to two focal areas per cell. Staining intensity for ST was highest in isolated cells and diminished upon confluency.
These results demonstrate a different localization of GT and ST and, thus, argue against the concept of multiglycosyltransferase systems. In addition, they suggest a transmost (or GERL) localization for ST.

Mapping and fine structure analysis of a vaccinia virus late gene

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Vaccinia is a large DNA virus that replicates in the cytoplasm of infected cells and utilizes its own RNA polymerase for the transcription of its genes. A combination of hybridization selection of RNA, *in vitro* translation and immunoprecipitation was used to map a gene coding for a major late structural polypeptide on the vaccinia virus DNA.
The DNA sequence analysis of the gene and its 5'-flanking region revealed some interesting features. For example, the region upstream of the mRNA start site is extremely A + T rich (approximately 80% for the first 80 base pairs) but does not contain a 'good' TATA box. Furthermore, there are less than 10 nucleotides between the cap site and the initiation site of translation.
Together with sequence information of early genes obtained by others, this suggests that vaccinia virus might have its own regulatory elements of gene expression.

Consequences of inhibited redistribution of cell surface molecules on immunological functions

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Inhibition of redistribution of cell surface molecules by short treatment (10″) with low concentrations (0.05%) of glutaraldehyde abrogated the capacity of murine P-815 tumor cells to function as targets in syngeneic and allogeneic T cell-mediated killing. This functional loss occured despite the successful recognition of H-2^d-characteristic class I H-2 antigenic specificities by monospecific anti H-2 antisera and successful lysis upon addition of complement. P-815/glut.-cells effectively stimulated killer T cells from allogeneic spleen cell populations after in vivo priming. However, they failed to induce killer T cell activity in primary allogeneic mixed lymphocyte-tumor cell cultures. Under syngeneic conditions all stimulating capacity, primary and secondary was lost after glutaraldehyde treatment. Possible conclusions concerning nature and functional mechanism of the tumor antigenic structures will be discussed.

Expression of measles virus-specific RNA species in acute and persistent infections

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DNA clones of 3 MV-specific mRNAs (Rozenblatt et al., J. Virol. *42*, 790, 1982) and of 4 different genome regions (Billeter et al., Virology, in press) yielding probes for all six defined viral genes were used to detect viral RNA species by Northern blot analysis. In acutely infected cells and rat brain, in addition to the 6 major polyadenylated mRNA species and the genome-length RNA, 4 minor RNA species, presumable read-through products of two adjacent genes, were detected. From their hybridization specificity and size the MV gene order 3'N, P, M(F, H)L 5' was deduced. In some persistently infected cell lines additional small RNAs (D1 RNA) accumulated. Specimen from SSPE cases showed an excess of multisized minus-strand RNA over mRNA species; in one case almost no P- and M-mRNAs were detected whereas P-M-read-through product accumulated. The same was found in a rat brain infected with SSPE-MF virus.

Neural differentiation in mouse teratocarcinomas

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The transition in teratocarcinomas of embryonal carcinoma (EC) cells to neural-like cells was analysed by two-dimensional gel electrophoresis. The analysis was carried out in *in vivo* propagated sublines derived from one multidifferentiating teratocarcinoma OTT6050, but arrested at different stages in development: OTT2158 forms only embryoid bodies (EB), whereas both teratocarcinoma-derived neuroblastomas TDN2151 and TDN2283 homogeneously express neural differentiation. In an attempt to identify developmentally relevant proteins, the set of proteins appearing during differentiation was compared with patterns found in developing normal mouse tissues, i.e. brain and limbs, from day 8½ to 15 of gestation. Three different subsets were identified by this comparison: 1. proteins which appeared only in the neurogenic tumours, 2. proteins which appeared in both neurogenic tumours, but were present also in both normal tissues, and 3. proteins, which were also present in the developmentally related normal tissue, but not in the unrelated one.

Some α-amylase genes of CE mice exhibit unusual structural features

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The aim of our studies is to elucidate the molecular basis for the differential regulation by insulin of the various *Amy*-2 α-amylase genes in the pancreas of CE mice. At least 5 different *Amy*-2 alleles, whose mRNA differ by about 1% in sequence, are expressed. Gene counting experiments using a probe specific for the first exon indicate the presence of more than ten copies/genome. In contrast, a gene internal probe discloses only 6 *Amy*-2 gene copies. The reason for this discrepancy is revealed by analyzing 4 different alleles that have been isolated from a genomic cosmid library. They all have a duplicated 5' terminus, which contains at least 200 bp of the 5' flanking region, the entire first exon and portion of the first intron. These sequence elements lie 5.5 kb upstream of the *Amy*-2 genes and have the same orientation. No transcripts initiating at these duplicated 5' termini are detected by polymerase mapping. The mechanism responsible for the functional differences between *bona fide* and orphan 5' termini is being investigated at the present time.

Competition by monosaccharides for zymosan binding sites on the macrophage cell surface

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Evidence is accumulating that the macrophage cell surface mannose/N-acetylglucosamine glycoprotein receptor may be important in induction of lysosomal enzyme secretion. It has previously been shown that secretion induced by the yeast cell wall preparation zymosan can be inhibited by mannose and that this inhibition is due to effects of mannose at the cell surface, presumably by competing for receptor binding sites. Other compounds which may compete for the mannosyl receptor such as mannose-6-phosphate, N-acetylglucosamine and 2-deoxyglucose are shown here to inhibit zymosan binding to mouse peritoneal macrophages. L-fucose (100 mM) and the polysaccharide mannan (2.5 mgs ml^{-1}) were found to have no effect on binding. In general these compounds which inhibit zymosan binding also inhibit zymosan-induced lysosomal enzyme secretion providing further evidence for the role of the mannose/N-acetylglucosamine receptor in zymosan binding and initiation of secretion.

The Na^+ channel on the apical membrane of epithelial cells. Possible involvement of epitopes of the catalytic subunit of (Na^+,K^+)-ATPase

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Toad bladder epithelial cell lines exhibit in culture transepithelial Na^+ transport (SCC) mediated by aldosterone regulated (Na^+,K^+)-ATPase. The surface expression of the enzyme α (αS) and β (βS) subunits was studied in monolayers. With aldosterone, SCC increased (2 fold in 90 min) with a fall in tissue resistance (R). In unfixed control monolayers, the 2 subunits were restricted to the basal membrane whereas on 0.5 µ frozen sections, α but not βS was detected on the apical membrane. With aldosterone (90 min), αS epitopes increased on the apical membrane of some ells, and by 24 h on the basal membrane of all cells. The α, but not βS epitopes were recovered from the apical membrane selectively radioiodinated and immunoprecipitated. Our data suggest that the apical αS epitopes share homology with the αS of (Na^+,K^+)-ATPase and play a role as Na^+ channels.

Subcellular localization of various β-glucuronidase forms in mouse liver

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We showed earlier that the presence or absence of the microsomal form of β-glucuronidase affected the processing of two structurally different lysosomal enzyme forms. The interrelations of the various forms during processing are studied in two mouse strains containing or lacking the enzyme in the microsomes by the analysis of subcellular fractions obtained by isopycnic and Percoll gradient centrifugation of homogenized liver. In C57Bl/6 liver, β-glucuronidase was found in three fractions, i.e. the microsomal, the lysosomal and a not yet identified fraction close to the mitochondria. The microsomal and lysosomal enzymes, as identified by isoelectric focusing, were found only in the respective fractions. In the yet undefined fraction all three enzyme forms were found. By radioactive labelling and cell fractionation of C57Bl/6 (containing microsomal β-glucuronidase) and of C57Bl/6 YBR Eg° liver (lacking it), the importance of the microsomal accumulation of β-glucuronidase for the processing of the lysosomal enzyme(s) is examined.

In vitro transcription of mouse immunoglobulin genes in homologous cell-free extracts

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Mouse immunoglobulin (Ig) genes (rearranged Igκ light chain and Igμ heavy chain) have been transcribed *in vitro* with concentrated whole-cell extracts from mouse B-cell hybridomas. The transcription products are visualized as R-loop molecules and analyzed in the electron microscope. Specific RNA-polymerase II dependent (α-amanitin sensitive) transcription initiation sites have been localized in the 5′ region of the Vκ and the Vμ genes. Additional transcription initiation sites have been detected in the introns, 5′ to the Cκ and the Cμ gene. Competition experiments indicate that the hybridoma extracts contain specific factors which are involved in the formation of stable preinitiation complexes.

Preliminary experiments (carried out in collaboration with A. Iglesias, A. Traunecker and G. Köhler, Basel Institute for Immunology) suggest that efficient *in vitro* transcription of Ig genes in these homologous cell extracts requires the presence of the Ig enhancers.

In vitro transcription of *Ascaris* ribosomal RNA genes in a homologous cell-free system

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An efficient *in vitro* transcription system which utilizes cloned *Ascaris* ribosomal RNA genes and cytoplasmic extracts from *Ascaris* oogonies has been established. Since this system is completely resistant to high concentrations of α-Amanitin (up to 1 mg/ml), we conclude that transcription is mediated by RNA polymerase I. Run-off assays and S_1 nuclease protection mapping experiments show that RNA is transcribed from a unique site of rDNA, corresponding to the 5′-end of the *in vivo* ribosomal RNA precursor. This initiation site was also localized at the same position (both *in vivo* and *in vitro*) for clones of all three *Ascaris* rDNA size classes. Run-off products obtained with different truncated rDNA fragments indicated that the promotor is located in a region between the Cla I (−72) and the Hinc II (+138) site. Upstream and downstream sequences flanking this 210 bp region do not affect the specificity of *in vitro* transcription initiation.

Lectin-gold cytochemistry reveals dark cell heterogeneity along rat kidney collecting ducts

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Dark cells of collecting ducts are believed to be involved in urinary acidification and/or K^+ reabsorption. To examine the glycocalyx of these cells, we applied lectins complexed to colloidal gold, to semithin sections of Epon-embedded kidney, and to thin sections of Lowicryl K4M-embedded tissue. When a lectin whose main specificity is for N-acetyl D-galactosamine was used (*Helix pomatia* lectin), a marked regional heterogeneity in dark cell labeling was seen. Dark cells from the cortex and outer stripe of the outer medulla had heavily-labeled apical plasma membranes and cytoplasmic vesicles. In contrast, dark cells from the inner stripe and the inner medulla were either negative or weakly-labeled. Apical membranes of adjacent clear cells were stained in all parts of the collecting duct. These results show that, in terms of their glycocalyx composition, dark cells do not represent a homogeneous population along the length of the collecting duct.

Molecular organisation of the light-harvesting polypeptides of *Rhodospirillum rubrum*

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Detailed studies concerning the structural organisation of the two light-harvesting polypeptides from *Rs. rubrum* within the membrane and at the membrane surface are possible since two essential requirements are fulfilled:
i) the primary structures of the light-harvesting polypeptides from the wild type bacterium S1 as well as from the carotenoidless mutant G-9$^+$ were determined in our laboratory;
ii) defined membrane vesicles can be prepared in a preparative scale: chromatophores (inside-out v.) and sphaeroplasts (right side-out v.).
Chromatophores and sphaeroplasts from *Rs. rubrum* S1 and G-9$^+$ have been treated with proteases. Protein-chemical analyses of the chloroform/methanol extracted remaining membrane-bound light-harvesting polypeptide fragments provided information on: i) transverse and lateral organisation of the α- and β-antenna subunit; ii) bacteriochlorophyll binding regions; iii) tentative anchor points of the carotenoid molecule.

Characterization of the region of mouse mammary tumor virus (MMTV) DNA required for glucocorticoid regulation of transcription

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Transcription of MMTV DNA is stimulated by glucocorticoid hormones. In transfection experiments using chimaeric DNA molecules in which the coding sequence of the herpes simplex thymidine kinase (tk) gene was under transcriptional control of MMTV, we demonstrated that MMTV DNA sequences between −105 and −204 upstream of the initiation site of viral transcription are required for glucocorticoid stimulation (EMBO J. *2*, 1423, 1983). In order to test if these sequences, or more, can confer glucocorticoid responsiveness to a heterologous, normally non-regulated promoter, we replaced the MMTV promoter in the above constructions with that of tk. Upon transfection of these plasmids into Ltk$^-$ cells, tk$^+$ cell clones were obtained, in which the effect of glucocorticoids on tk transcription was studied by the S1 mapping technique.

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

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The chlorophyll a/b light-harvesting protein complex (LHC-II) of pea contains two major phosphorylated polypeptides of 27 kD and 25 kD. Amino acid analysis, serological comparisons and proteolytic digestion have established that the two proteins are structurally related, but it was not possible to show the intramolecular location of the structural difference. Partial DNA sequencing data on the 27 kD polypeptide has recently been established. (Coruzzi G., Chua N., (1983) J. biol. Chem. 258, 1399–1402). In order to define the whole sequence and to study the nature of the difference between the two LHC-II polypeptides we fragmented the complex by chemical cleavage. Obtained peptides were separated and sequenced. Homologies of the sequences found with the data from DNA sequencing were studied. Further attempt was made to establish the nature of the N-terminal blocking group of the two proteins.

Correlation between fibrinolytic activity and implantation site of human colon carcinomas xenografted to nude mice

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Human colon tumors exhibit a benign type of growth when grafted subcutaneously (sc) to the nude mouse but are highly invasive when implanted orthotopically in the gut wall (gi). In view of a modulatory effect of the graft site on invasive potential, sc and gi grafts were analyzed for the contribution of urokinase-type (u-PA) and of tissue-type (t-PA) plasminogen activators to total fibrinolytic activity (FA). Samples were extracted in 0.1% Triton X-100, run on SDS-PAGE followed by zymographic detection on fibrin-agar underlays. FA, u-PA and t-PA were determined using ^{125}I fibrin plates and antibodies. PA activities normalized to human tissue content (LDH isoenzyme assay) and protein level decreased significantly from patient tumors to sc grafts. Two human colon lines were implanted sc and gi: the Co112 gi versus grafts showed an increase in u-PA, the Co115 gi grafts, not the sc, expressed human t-PA activity.

The accumulation of mRNAs encoding house keeping functions in several mouse tissues

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Using recombinant DNA technology we have studied the synthesis of mRNAs that are shared by all tissues. While the four most abundant mitochondrial mRNAs each account for more than 1% of polyadenylated mRNA in most tissues, the housekeeping mRNAs specified by nuclear genes are moderately abundant to rare. Ten recombinant cDNA plasmids have been isolated and characterized that are complementary to mRNAs which are specified by nuclear genes and accumulate from 0.006 to 0.4% of polyadenylated mRNA in L cells. Using these cloned sequences as probes we have performed run-off transcription experiments in isolated nuclei and kinetic labeling experiments. Our results indicate that differential mRNA stability in the cytoplasm appears to be a major mechanism in determining the differential cellular concentrations of the ten housekeeping mRNAs studied. Whereas the rare mRNAs reach similar concentrations in all tissues, the moderately abundant mRNAs accumulate to much higher concentrations in rapidly growing cells than in nondividing cells.

Cloning of developmentally-controlled *Xenopus laevis* genes that crosshybridize with *Drosophila* homeotic genes

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In *D. melanogaster* homeotic mutations define genes which are involved in the specification of segment identity during development. Recently Garber et al. (EMBO J. vol.2 *11* (1983)) have cloned the cDNA of the Antennapedia (Atp) gene from *D. melanogaster*. A fragment of this cDNA clone (903G) contains a sequence of about 200 bp called the homeo box which crosshybridizes with other Dros. homeotic genes such as Ultrabithorax (Ubx) and Fushi Tarazu (ftz). This box contains a conserved protein coding sequence (W. McGinnis, M. Levine, E. Hafen, A. Kuroiwa and W.J. Gehring, in prep.) using the homeo box of Atp to screen a genomic library of *X. laevis*. It has been isolated two clones (λAC_1 and λAC_2) which crosshybridize with the homeo box of Atp, Ubx and ftz. A Northern blot of poly A + mRNA from different embryonic stages of *X. laevis* was probed with a 5.5 kb fragment of λAC_1, which contains the crosshybridizing region. Two RNAs homologous

to this probe first appear in late gastrula and increase up to the tailbud embryo stage. This cloned DNA sequence could be involved in the control of development in vertebrates.

Effects of intraocularly injected kainate on the retinopetal neurons in chick embryos

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We found that intraocularly injected kainate (KA, 20–40 nmols) selectively lesions the inner half of the inner nuclear layer (i.e. the amacrine sublayer) of the chick embryo's retina to an extent which varies according to the stage of development. Following the injections (at 13–15 embryonic days) most of the retinopetally projecting neurons within the isthmo-optic nucleus (ION) die, suggesting that during development they depend for survival on the amacrine cells (their probable targets). However, retrograde labelling with peroxidase from the eye, long after the KA injection, shows that the so called 'ectopic neurons', scattered around the ION, survive the treatment, possibly because they have different targets. This survival is surprising since the ectopic ION neurons had previously been found to resemble the orthotopic ones in most respects.

Hepatitis B virus transcription

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The structure of Hepatitis B virus transcripts was investigated in the infected liver and in cell lines containing HBV DNA. The major surface (S) gene transcript is identical in these two systems: it is 2.1–2.2 kilobases (kb) in length, unspliced and its promoter does not contain a «TATA» recognition sequence but a region of homology with the SV40 late promoter (Cattaneo et al., Nature *305*, 336 (1983)). The major S transcript initiates within the so-called pre-S region and not upstream of it, thus ruling out the possibility that the 25000 d Hepatitis B surface antigen (HBsAg) is cleaved from a 41000 d precursor. The 5′ ends of a second transcript, covering the X region, are heterogeneous. The HBV polyadenylation signal is situated within the core antigen gene, implying that expression of core antigen depends on transcripts not processed/polyadenylated at this signal. Indeed such transcripts were produced at different levels in the infected liver and in cell lines.

Myomesin and M-protein in developing muscle

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Two distinct M-line proteins from chicken muscle were identified by monoclonal antibodies: Myomesin with a M_r of 185K and M-protein with a M_r of 165K. Myomesin and M-protein are only present in crossstriated muscle but not in smooth muscle or tissue not containing muscle cells; M-protein however is absent from slow muscle fibers. During myogenesis of chicken embryonic breast muscle cells in vitro, Myomesin is already detectable in cells cultured for one day whereas M-protein was not found until the third day in culture. During the development of embryonic pectoral muscle and heart in ovo, Myomesin accumulation also preceeds M-protein. Myomesin seems to exist in multiple forms and appears to be expressed in early skeletal muscle development as well as in heart as isoproteins with higher apparent M_r. The possible functions of these proteins will be discussed.

A 140 base pair DNA segment from plasmid R1 acts as an origin of replication and promotes *recA*-independent site-specific recombination

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A circular form of Tn*2350*, an IS*1* flanked kanamycin resistance transposon carried by plasmid R1, was isolated and shown to be capable of autonomous replication. Essential replication functions of this plasmid (pTn*2350*) are contained within a 140 bp region that has been sequenced. This 140 bp segment acts as an origin of replication since it allows autonomous replication of a plasmid composed only of this sequence and the tetracycline resistance gene of pBR322. In addition this sequence promotes three kinds of *recA*-independent recombination events (fusion, deletion and inversion) that characterize other site-specific recombination systems. When cloned on pBR322, a single copy of this sequence permits multimer formation, in a *recA* strain. Two copies permit either deletion or inversion of the intervening region, depending on their respective orientation. DNA gyrase seems to be involved because the inversion rate in a plasmid carrying sequences in opposite orientation varies in different independently isolated spontaneous nalidixic acid resistant strains (*gyrA* mutants).

Rat monoclonal antibodies against differentiation and transformation markers in murine basophil/mast cells

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Using malignant and non-malignant basophil/mast cell lines that we developed recently (Ball et al., Differentiation, *24*, 74, 1983) and bone marrow cells as immunogens, we have obtained and purified fourteen monoclonal antibodies against cell surface determinants. Seven reagents react with subpopulations of bone marrow cells and four of them also bind to basophil/mast cells, whereas the other three do not. The other seven antibodies react with basophil/mast cells only. Among the reagents that bind to basophil/mast cells, six display a reactivity with malignant cells different from that observed with non-malignant cells. Preliminary evidence indicates that the binding of three antibodies to malignant basophil/mast cells is increased following exposure of the cells to conditioned media known to contain interleukin-3, a lymphokine essential for the in vitro proliferation of non-malignant basophil/mast cell lines.

Partial characterization of cells from *Xenopus laevis* 'lymphoid' tumor

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In *Xenopus* we have isolated a spontaneous 'lymphoid' tumor which can be transmitted by tissue transplantation. To determine the exact cell type at the origin of the tumor, the surface membranes of tumor cells were examined for the presence of Ig and Fc receptor molecules. Cell suspensions of induced tumor tissues, originating in a large number of animals, were evaluated by immunofluorescence and SDS-PAGE of cell surface iodinated proteins. Results attest to the heterogeneity of the cells. An average of 32% of them carry Ig on their surface. Most of these molecules, being of IgM isotype, are reversibly bound to the cell membrane and can be dissociated by acid pH or overnight culture. The serum origin of these cytophilic Ig's has been confirmed by the analysis of electrophoretic mobility of their heavy chains. Fluorescent staining, using either heat aggregated IgM or antigen-complexed IgY, revealed the presence of structures homologous to the mammalian Fc receptor molecules on the surface of 16% of cells in the first case and of 32% in the second.

Characterization of the mobile genetic element IS30

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The nucleotide sequence of IS30, a residential insertion sequence of E.coli, is 1221 bp long and has 26 bp inverted terminal repeats with 3 bp mismatched. In transposition IS30 has a pronounced target specificity and generates a 2 bp duplication at the site of insertion. IS30 has no significant DNA sequence homologies with IS1, 2, 4 and 5, the other sequenced E.coli residential elements. The largest open reading frame could encode a protein of 383 amino acids. The same frame on the opposite strand does not contain a large open reading frame. The amino acid sequence of the putative transposase has no homologies with the possible products of the largest open reading frames of IS1, 2, 4 and 5. The open reading frame is preceeded by a Shine-Dalgarno sequence and a sequence with homologies with the consensus sequence for transcriptional promoters. A fragment of IS30 which includes this sequence and part of the open reading frame has been shown to possess promoter activity in two systems.

Study of protein-RNA interactions and of their possible biological roles in viral systems

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Protein-RNA interactions were studied in the phage R17 (a gift of R. Gesteland), the oncovirus RSV and in SV40 infected cells. Intact virus was irradiated with UV light which links the tightly bound proteins to the RNA. The resulting RNP was purified and Interacting protein and RNA sequences analyzed. We demonstrated the tight binding of coat protein to R17 RNA and of RSV P12 and DNA polymerase to the 70S RNA. Two coat protein binding sites were detected on R17 RNA and mapping close to the initiation codon of the A and L genes, respectively. In RSV, P12 binds tightly to twelve sites on the RNA located in the dimer linkage structure and close to the splice sites. Viral DNA polymerase is bound to the primer $tRNA^{trp}$. In SV40 infected cells large T antigen was found to be associated with RNA in a form resistant to detergents. The T-antigen RNA appears to originate from the non coding and repeated elements of the cellular genome. Biological implications of these viral protein-RNA interactions will be discussed.

Structure and transcription of different forms of SV40 chromatin

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Electron microscopy of SV40 virions disrupted with EGTA showed a circular nucleoprotein structure with ~ 60 beads. SV40 chromatin from infected cells has only 24 nucleosomes. The disrupted virions contained as much histone and capsid protein VP_1 as intact virions. The structures were longer than SV40 chromatin, closer in length to bare SV40 DNA suggesting that the histones are not in a normal nucleosomal structure. Their DNA was accessible to staph. nuclease, yielding protected fragments similar to nucleosomes but still containing both histones and VP_1. Topoisomerase I treatment of disrupted virions did not change the linking number of the extracted DNA indicating an absence of torsional stress.
On transcription of disrupted virions in vitro the SV40 early promoter was the strongest one. In contrast, with SV40 chromatin isolated from infected cells the late promoter was the most active. This property did not correlate with the presence of T-antigen, but was lost on treatment with NaCl at a concentration high enough to destroy chromatin structure.

Electrophysiological activity of sympathetic neurons infected directly with herpes virus suis or rabies virus

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A method has been developed allowing the direct inoculation of either herpes virus suis (HVS) or rabies virus in the superior cervical sympathetic ganglion (SCG) of the anaesthetized rat. The rats show the same symptoms as after inoculation into the anterior chamber of the eye. The infection of the neurons is demonstrated by immunofluorescence and electron microscopy. The infected ganglia were excised after various lengths of time and their electrophysiological activity tested in vitro. The HVS does elicit spontaneous electrophysiological discharges similar to those appearing after intraocular incoulation. However, the rabies virus does not cause abnormal spontaneous bursts, though the neurons can still be stimulated by excitation of the preganglionic nerve. If the preganglionic nerve was cut at the time of direct inoculation with HVS, the abnormal spontaneous discharges can still be recorded. Thus the spinal neurons are not involved in this electrophysiological activity, though its presynaptic origin has been proven.

Choline acetyltransferase, a possible marker for the membrane of the nerve endings of the electric organ of *Torpedo*

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The electric organ of *Torpedo* receives a profuse and purely cholinergic innervation. Synaptosomes isolated from this electric tissue were disrupted by hypotonic shock; the synaptosomal plasma membranes (SPM) were then purified. A general problem encountered during SPM purifications is the absence of a specific marker for these membranes. In the synaptosomes isolated from the electric organ, we found that the enzyme choline acetyltransferase (ChAT) was present in 3 forms: a soluble (cytoplasmic) form (60–70%), a form associated with membranes through ionic interactions (about 20%) and a membrane-bound form (about 10%). The membrane-bound form appeared to 'copurify' with SPM and therefore might be a marker for these membranes. The purified SPM were used as immunogen into Balb/c mice and monoclonal antibodies (McAB) were prepared. Preliminary assays indicated that of the McAB obtained, 5 produced a 40 to 60% inhibition of the soluble as well as membrane-bound ChAT.

Serum-free medium for clonal growth of hemopoietic progenitor cells

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A serum-free, methylcellulose containing medium has been developed which supports colony formation by immature murine hemopoietic progenitor cells. The medium consists of a 3:1 mixture of an enriched Dulbecco's modified Eagle's medium (EMED) and a modified Ham's F-12 nutrient mixture (FMED), supplemented with 1% w/v bovine serum albumin, 0.9% methylcellulose, human transferrin (320 µg/ml), insulin (9 µg/ml), nucleosides, trace elements, hemin, linoleic acid, cholesterol and additional L-glutamine. Mouse bone marrow cells, cultured for 9–10 days in this medium plus stimulatory factors (serum-free conditioned medium from WEHI-3b cells and partially purified human urinary erythropoietin), will form colonies composed of granulocytes, macrophages, erythrocytes or megakaryocytes, as well as colonies containing various combinations of these cell types. This system will be useful for studies on the regulation of hemopoietic cell differentiation using purified factors, hormones and progenitor cells.

Herbicide resistance in *Chlamydomonas reinhardii* results from a mutation in the chloroplast gene for the 32 kilodalton protein of photosystem II

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We have isolated and sequenced *psbA*, the chloroplast gene for the 32 kDal protein, from both wild-type and herbicide-resistant algae (selected and characterized by P. Bennoun). The coding regions of the gene are contained in five exons. The only difference between the exon nucleotide sequences of the wild-type and mutant *psb*A is a single TA to GC transversion which results in a predicted amino acid change of serine in the wild-type protein to alanine in the mutant. Both whole and broken-cell preparations of the mutant alga show a resistance to the effects of herbicide. The 32 kDal protein from wild-type cells, but not mutant cells, binds 10^{-7} M azido-(^{14}C) atrazine. We suggest that the alteration in the 32 kDal protein is the molecular basis for herbicide resistance in the *C. reinhardii* mutant.

Biochemical characterization of a factor which has a similar biological activity to erythropoietin

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Erythropoietin (Ep) is considered to be the primary regulator of the terminal stages of red cell differentiation. However, an Ep-like activity was found in mouse spleen cell conditioned media (Fagg, Nature *289* (1981) 184). This unexpected finding raised the question of whether the spleen cells produced Ep itself or another factor. High performance liquid chromatography has enabled us to analyse relatively small amounts of material with high resolution. Parallel analyses were made of spleen cell conditioned medium, mouse Ep and human Ep. There was little difference in the ionic strength at which the biological activities eluted from an anion exchange column. However, they could be separated using reverse phase chromatography. Molecular sizing gave the following apparent molecular weights: Ep-like activity 15,000; Mouse Ep 64,000 and Human Ep 56,000. These and additional experiments indicate that Ep-like activity is biochemically distinct from Ep of mouse or human origin.

RNA polymerase II loading on MMTV after hormone treatment

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After transfection of mouse Ltk$^-$ cells with recombinant bacteriophage DNA containing a complete proviral copy of mouse mammary tumor virus (MMTV) with its flanking cellular sequences, the newly acquired MMTV proviruses are transcribed in a hormone regulated fashion. After hormone treatment of the cells in tissue culture (10^{-6}M dexamethasone) we isolated the nuclei and elongated the nascent RNA chains *in vitro*. By hybridization to unlabeled DNA the number of RNA polymerase II molecules transcribing MMTV DNA and flanking mouse DNA was determined. The increase in RNA polymerase II loading on MMTV DNA after hormone treatment was about 10-fold, which corresponds to the 10-fold increase in stable MMTV mRNA. When DNA sequences which are resonsible for hormone-receptor binding and for the increased MMTV mRNA levels were deleted in the MMTV LTR no increase in RNA polymerase II loading was observed. Hormone responsive transcription was not only detected from the transfected MMTV DNA but also from the mouse DNA sequences adjacent to the 3′ end of the provirus.

Dissection of the fusion process of Semliki Forest Virus (SFV) Infected *Aedes albopictus* cells (C6/36). II. Monensin (M)

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Cell-cell fusion from within can be induced at pH *6* within 30 min in C6/36 16 h after infection with SFV. The general assumptions for the fusion process are given in the preceding abstract. These imply that the individual events of fusion are experimentally separable. Here we show that M, a ionophore for monovalent cations, is an effective tool for this purpose. M, added in pH 6-medium, blocks fusion immediately and as long as it is present. However, its action is reversible. If monolayers are exposed to pH *6*-medium containing M and then brought into pH *7*-medium without M, fusion follows. Therefore, by using M, the initial step which may represent a conformational change of viral spike proteins induced by low pH can be kept latent and separated from the following pH independent, visible membrane fusion.

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

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The import of the higher MW precursor of mitochondrial aspartate aminotransferase (pre-mAspAT) into mitochondria is completely blocked by treatment of chicken embryo fibroblasts with the uncoupler CCCP (JBC *257*, 13334, 1982), the ionophore valinomycin or with antimycin A. Under these conditions pre-mAspAT is rapidly degraded ($t_{½} \sim 5$ min). The rate of degradation is not markedly decreased by inhibitors of lysosomal degradation (50 µg/ml leupeptin, 50 µg/ml antipain, 50 µg/ml chymostatin, 10 mM ammonium chloride, and 30 µM monensin). However, after homogenization of the cells pre-mAspAT is no longer attacked, although it remains highly susceptible to cleavage by exogenous proteases. Conceivably, the degradation of pre-mAspAT in fibroblasts depends on intact intracellular structures. If the cells are treated with the cationic fluorescent dye rhodamine 123 (100–200 µg/ml) not only the processing but also the degradation of pre-mAspAT is strongly impaired.

Molecular analysis of the esc-locus of *Drosophila*

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The extra sex combs (esc) gene of *Drosophila* provides two aspects that are of interest to us. First, it is known to regulate the genes of the bithorax complex and hence represents a system suitable to study the mechanism of the control of gene expression. Second, it exhibits a maternal effect, and its targets are genes known to specify segment identity. Therefore, it may inform us about the mechanisms and logic of early determinative events during Drosophila development.

We have microdissected the region defined by the esc^{10} deletion from giant chromosomes and cloned about 250 kb by subsequent screening of a genomic library. *In situ* hybridization of biotinylated probes to salivary gland chromosomes and whole genome Southern analysis have confirmed that the cloned DNA is confined to the esc^{10} deletion. We are in the process of identifying the gene on the basis of other esc mutants and by searching for transcripts that exhibit the expected developmental profile.

Activation of the *N-ras* oncogene in a patient with acute myeloblastic leukemia

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We are interested in the relationship between activated oncogenes and their potential role in human leukemogenesis. We found that DNA derived from bone marrow cells from a patient with freshly diagnosed acute myeloblastic leukemia induced foci of transformed cells using the NIH3T3-transfection assay. Southern blot analysis identified the activated oncogene as the *N-ras* gene. To approach the question whether *N-ras* activation in this patient was a somatic event, the transforming human gene was cloned in lambda together with *N-ras* alleles originating from a fibroblast culture derived from the same patient. The cloned oncogene was highly transforming in NIH3T3 cells, while the fibroblast-derived copies were negative. Experiments are in progress to identify the nature of the *N-ras* activation of the nucleotide level.

Is lysosome movement an actin-based mechanism? A cytomagnetometric study

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When cultured macrophages, containing magnetic particles, are brought in a magnetizing field, these particles are magnetized and aligned. Upon removal of the magnetizing field the particles produce a remanent field measurable above the cells with a magnetometer. This field decays with time due to progressive random misalignment of the particles (relaxation). Since these particles are confined to secondary lysosomes it is the rotation of these lysosomes which causes relaxation. Normally the remanent field decayed by 70% within 20 min. If the cells were fixed with glutaraldehyde the remanent field stayed constant. Inhibition of the energy supply (cold, uncoupler FCCP) could significantly slow the decay (decay $< 50\%$). Cyt. B, known to desintegrate the microfilament system was found to have the same effect, whereas colchicin, known to disrubt the microtubular system, did not change the relaxation. Cytomagnetometry thus provides evidence that lysosome movement is an energy dependent, actin-based mechanism. (Supported by SNSF grant 3.130-0.81).

In vitro biosynthesis of Na,K-ATPase

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Size-fractionated or total mRNA from cultured epithelial cells (A_6 and TBM cells) was translated in reticulocyte or wheat germ systems and the synthesis of α- and β-subunits (α, Sβ) of Na,K-ATPase was detected by monospecific polyclonal antibodies. Sα was recovered as a 96K protein in absence and presence of microsomes (RM). This protein was not released from RM by high salt or pH 11 but apparently totally digested by proteinase K. Sβ was synthesized in the absence of RM as a 32K protein like in tunicamycin-treated intact cells. Synthesis of the 32K protein was blocked by purified signal recognition peptide (Meyer D.I., TIBS 7 (1982), 320) in the wheat germ system. Addition of RM yielded a 42K core-glycosylated form protected against proteinase K. These results suggest that i) no higher molecular weight precursor forms exist for Sα or Sβ, ii) Sβ is co-translationally inserted into RM and core-glycosylated and iii) unlike Sβ, Sα follows an unusual pathway of membrane insertion.

Sequence homologies within the 5'end region of the four estrogen-controlled vitellogenin genes in *xenopus*

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In oviparous vertebrates vitellogenin, the precursor of the major yolk proteins, is synthesized in the liver of mature females under the control of estrogen. We have established the primary structure of the 5'end region of the four isolated estrogen-controlled vitellogenin genes in Xenopus. A comparison has been made with the 5'end region of the chicken vitellogenin gene (Walker et al.; 1983, The EMBO Journal Vol. 2, No. 12). The results demonstrate that exon sequences are more highly conserved than intron sequences. Furthermore, significant blocks of homology are found between the 5'end flanking regions of the genes analyzed suggesting that they play a role in the control of the transcription machinery. Therefore, it will be important to test if some of them are involved in the hormone-regulated expression of the vitellogenin genes.

Collagen II induced arthritis raises acute phase serum amyloid protein (SAP) in mice

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Injection of collagen type II into DBA-1 mice produces a slowly onsetting polyarthritis (Courtenay et al., Nature 283:666 (1980)). We have assessed the immunological, arthritic and SAP responses in DBA-1/J mice after intradermal administration of 0.1 mg cartilage collagen in CFA. Type II collagens of bovine, chicken or rat chondrosarcoma (RCS) origin all evoked arthritic symptoms though RCS collagen was the weakest arthritogen. 1α, 2α, 3α and M collagen from RCS as well as HMW collagen from chicken were poorly immunogenic and did not produce arthritis. SAP levels significantly increased in arthritic mice and correlated with periarticular tissue swelling. Joint destruction as judged by X-ray photography was delayed by some days, and usually correlated with SAP. No clear correlations between anti collagen IgG and SAP or other parameters of arthritis could be found although antibodies were higher in arthritic than non arthritic animals. We conclude that SAP measurements are useful parameters for monitoring arthritis in mice.

Structure and expression of *Chlamydomonas* nuclear genes for the small subunit of the chloroplast enzyme ribulose bisphosphate carboxylaseoxygenase

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Chloroplast function is dependent on both chloroplast and nuclear genes. An example of this cooperation is ribulose bisphosphate carboxylaseoxygenase which is composed of only two kinds of subunits: the large subunit (LS) is encoded by the chloroplast while the small subunit (SS) is made in the nucleocytoplasmic compartment and imported into the chloroplast. From a recombinant library of *C. reinhardii* DNA in phage lambda EMBL3, genes for the SS were isolated by screening with synthetic oligonucleotide hybridization probes (provided by A. Chollais, Biogen SA). I have analyzed the sequence and organization of two closely linked copies of the gene. The recombinant clones also provide specific hybridization probes to investigate the coordinate expression of the nuclear SS genes and the LS gene in the wild type and in LS mutants (Spreitzer, Goldschmidt-Clermont and Rochaix, this volume).

Evidence for seven loci for human class II histocompatibility antigen β chains

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Following our initial cloning of cDNA for the β chain of HLA-DR and -DC, we have isolated and analyzed cDNA and genomic clones for β chain genes of all 3 subregions of the human MHC class II region, DR, DC and SB. Direct structural comparison has indicated the existence of 5 different DR β chain genes in a heterozygous individual, implying *3* distinct DR loci. Two of these are separated by only 24 kb of DNA. The HLA-DC subregion was shown to contain *2* distinct β chain loci. Finally, in the SB subregion, we have cloned 2 separate β chain genes, one of which is flanked by α chain genes. The total number of class II β chain genes in man is thus 7, whereas in the mouse I region only 2 complete β chain genes (I-Eβ and I-Aβ) have been identified. Extensive sequence comparison between human β chain genes of different subregions, and between human and mouse β chains, permits an analysis of the evolutionary relationship of these genes, and of the structural basis for the extensive polymorphism.

Evaluation of cadmium concentration in chick embryos

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To determine what quantity of cadmium could be incorporated in hens eggs after immersion in cadmium solutions, we elaborated an equipment allowing simultaneously treatment of 40 samples, and constituted of two gas-heated sand baths, two stands for cooling down solutions and an evacuation system for toxic vapors. The method, based on mineralisation by wet way, consisted in desintegrating experimental 17 days-old embryos in a HNO_3–H_2O_2 mixed solution. After heating and evaporating, cadmium quantity in the remnant was determined by atomic absorption spectrophotometry. The reliability of our technique was tested by studying embryos injected with a known quantity of $Cd(NO_3)_2$. It showed no loss of cadmium. This method allowed a good evaluation of Cadmium content in embryonic organs. Cadmium concentration was very high in the urogenital tract, nervous system and heart, while muscles and eyes were less contaminated.

Sendai virus-induced chemiluminescence (CL): where does the light come from?

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On contact with its receptors in phagocytic cells, Sendai virus triggers an immediate, short-lived burst of CL that reflects the generation of unstable oxygen species. Using the chemiluminogenic probes, luminol and lucigenin, we have attempted to locate the light-generating reactions. Luminol-dependent CL in bovine polymorphnuclear leucocytes was superoxide dismutase (SOD)-resistant but inhibited by azide. In contrast, lucigenin-dependent CL was SOD-sensitive and azide-resistant. Together with the sensitivity of both types of CL to inhibition by ETYA, but ot by aspirin and indomethacin, our results can be interpreted as follows: a) luminol-dependent CL is mediated by myeloperoxidase, b) lucigenin-dependent CL reflects $^{-}O_2^{\cdot}$ generation and c) the lipoxygenase reaction (known to be insensitive to azide), is not a direct source of CL but exerts a regulatory effect on the NAD(P)H oxidase that reduces O_2 to $^{+}O_2$.

Neuroectodermal antigens detected by monoclonal antibodies (Mab) to human neuroblastoma cells

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Neuroectodermal derived tumors have been shown to express common differentiation antigens. Mab to a great variety of these antigens may provide a better understanding of the restricted expression of such molecules and their presence in relation with the developmental stage of the tumor. Following immunisation with neuroblastoma cell lines IMR-32 and LAN-1, mouse Mab have been raised. We selected 21 clones strongly reactive to neuroblastomas while non reactive to 6 control human tumor cell lines by binding assay. The majority of Mab showed variable reactivity against 6 gliomas, 6 melanomas, 1 retinoblastoma and 4 T cell lines. 2 Mab presented a reactivity restricted to neuroblastoma cells.

By immunoperoxidase staining (IP) of frozen tissue sections, preliminary results reveal absence of binding to normal brain by 8 Mab and staining of neuroblastoma, melanoma or glioma tissues. The exact localization of the antigens and the specificity for neuroectodermal tumors is currently tested by IP on a large panel of different tissues.

DNA binding properties of two different SV40-T antigens

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SV40-T antigen, a multifunctional phosphoprotein, has a general nonspecific affinity for DNA and a highly specific affinity for the viral *ori* region. Experiments in two different systems indicated that nonspecific and specific DNA binding are two distinct properties of T antigen possibly serving different biological functions. 1) Complete dephosphorylation of purified D2-T antigen by acid phosphatase resulted in a greatly reduced affinity for the viral *ori* region without affecting the nonspecific DNA binding activity. 2) SV80-T antigen is shown to bind nonspecifically to DNA but to have lost (by mutation) the specificity for the wild type viral *ori* sequences. We have tested, by fusion of SV80 cells to permissive monkey cells and by direct DNA binding studies with the integrated viral sequences cloned into plasmids, the possibility that the SV80-T antigen specifically recognizes its own template. The results clearly disprove this and indicate that specific DNA binding is not required for the maintenance of the transformed state of these cells.

The regulation of α-amylase gene expression

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We have determined the boundaries of the pancreatic α-amylase transcription unit *Amy*-2^a of mouse. Mapping of *in vitro* elongated nascent transcripts to *Amy*-2^a restriction fragments demonstrates that transcription initiation occurs at the cap site and that polymerases terminate transcription 2 to 4 kb dowsteam of the poly A site. S1 nuclease mapping of steady state nuclear RNA suggests that termination occurs at multiple sites. *Amy*-2^a expression *in vivo* is under control of a strong promoter which is active exclusively in the pancreas. Thus, the *Amy*-2^a, and the strong parotid gland-specific promoter of *Amy*-1^a exhibit absolute tissue-specificity. In contrast, the weak promoter of *Amy*-1^a has a limited tissue-specificity since it is active in liver, parotid gland and pancreas. Our results demonstrate that α-amylase mRNA synthesis in these three tissues is regulated predominantly at the transcriptional level. The requirements for the tissue-specific modulation of the

Amy-2[a] promoter are being elucidated by transient expression of intact and truncated *Amy*-2[a] genes in an exocrine pancreatic cell line.

Identification of human intestinal microvillus membrane (MVM) hydrolases by monoclonal antibodies and expression of these enzymes in the colonic carcinoma cell line Caco 2

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With the ultimate goal of elucidating the number and types of MVM hydrolases and the mechanisms of their biosynthesis we have produced monoclonal antibodies to MVM. Antibodies to 3 different disaccharidases and 4 peptidases were characterized and used for immunoisolation of the corresponding antigens from human intestines. Immunoelectronmicroscopic localization of sucrase-isomaltase in intestinal biopsies revealed intracellular antigen in the Golgi complex, in apical vesicles and in lysosomes in addition to the MVM. No labeling was found associated with the basolateral membrane suggesting that newly synthesized sucrase-isomaltase directly is transferred from the Golgi complex to the MVM and bypasses the basolateral membrane. Several MVM enzymes also were expressed in Caco 2 cells which allowed to study their biosynthesis by using subcellular fractionation in conjunction with immunoprecipitation.

Mammalian Z-DNA-binding proteins

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DNA in the left-handed Z-conformation is a potentional regulatory signal punctuating the genome. This conformation may be expected to be induced and/or stabilized by Z-DNA-binding proteins. A search for Z-DNA-binding proteins was therefore undertaken in mammals (Kuenzle et al., Cold Spring Harbor Symp. Quant. Biol. *48*, 1983, in press). Nuclei were prepared from rat cerebral cortex neurons, the proteins were extracted, labeled with ^{14}C by reductive methylation and fractionated by affinity chromatography on tandemly arranged columns of immobilized polynucleotides in the B- and Z-conformation. B-DNA was represented by poly (dG·dC)·poly(dG–dC) and Z-DNA by poly-(dG–Br^{5}dC)·poly(dG–Br^{5}dC). The retained proteins were eluted and analyzed by 2D gel electrophoresis followed by fluorography. Three proteins of pI 6.5/M_r 60–70K with preferential affinity for Z-DNA were detected. Thus, Z-DNA-binding proteins occur in mammals.

Z-DNA and eukaryotic tRNA gene transcription

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In vitro transcription of a variant methionine tRNA gene of *Xenopus laevis* is inhibited by natural 5′ flanking sequences with Z-DNA potential. This effect can be mimicked by inserting synthetic 8 bp tracts of alternating pyrimidine and purine residues upstream of this gene and of a *X. laevis* tyrosine tRNA gene. For example, transcription is inhibited by d(CG-CATGCG) but not by a *Bam*HI linker, d(CGGATCCG). When present on plasmid DNAs at physiological levels of negative supercoiling, both the natural and synthetic tracts bind an antibody specific for Z-DNA. We shall report our attempts to determine the minimum number of alternating pyrimidine and purine residues required for this antibody binding, and the maximum distance over which such Z-DNA tracts can inhibit tRNA gene transcription.

The detergent-resistant domain of T lymphocyte plasma membranes

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In the plasma membrane of murine T lymphoma cells, a select set of surface glycoproteins consisting of the Thy-1 glycoprotein (25 Kd) and 2 other acidic glycoproteins of 40 and 50 Kd is shown to 1) segregate in a light density population of plasma membrane vesicles upon sub-cellular fractionation and 2) remain associated to specific membrane proteins upon non-ionic detergent extraction of purified plasma membrane vesicles. These associated surface glycoproteins and membrane proteins can be separated from detergent-solubilized membrane components by gel filtration and further purified by isopycnic sedimentation in sucrose. Such detergent-resistant proteins thus form a complex that might represent both a structural and functional membrane domain, given its content of select surface glycoproteins with potential receptor properties.

Mesenchymal rat cells stimulate growth of their transformed counterpart in vivo and in vitro

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The influence of normal cells on growth of transformed cells was investigated in the nude mouse system and in soft agar cultures. Normal cells were isolated from subcutaneous granulation tissue of untreated rats. The transformed counterpart derived from fibrosarcomas induced chemically in the same subcutaneous tissue. The cells were cultured under physiological pO_2.

In vivo and in vitro, normal cells, added in ratios of 10:10 to 1:1000, stimulated growth of transformed cells. In vivo the lag time for the formation of subcutaneous nodules was reduced. In vitro the clone size was enlarged when compared with the corresponding cultures without addition of normal cells. The number of transformed cells in the inoculum which leads to detectable growth in the two systems can be reduced by a factor of 10^3 when the cells are mixed with normal cells.

We conclude that mesenchymal rat cells are able to release growth stimulating factors to which in vivo tranformed cells are responsive.

Regions of c-*mos* (rat) oncogene necessary for transformation

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The cellular homologue (c-*mos* (mouse)) of the transforming gene, v-*mos*, of Moloney sarcoma virus (MoSV) causes transformation of cells when activated by promoters. The c-*mos* genes of rat or human origin never gave rise to stable transformation of cells in similar experiments. We sequenced c-*mos* (rat): its base sequence is 93% homologous to c-*mos* (mouse). The open reading frame encodes a polypeptide of 339 amino acids. Comparison of all c-*mos* genes allowed us to define domains on c-*mos* that are evolutionary conserved and that are implied in protein kinase activity. Comparison of restriction maps of v-*mos* and c-*mos* (rat) made it possible to construct two hybrid *mos* genes, that were linked to the MoSV promoter. In order to determine sequences necessary for stable transformation of cells the constructs, as well as plasmids containing truncated *mos* genes, were transfected on mouse fibro-

blasts or cotransfected on Ltk$^-$ mouse cells with the HSV TK-gene. The c-*mos* constructs were integrated as tandems and remained unmethylated.

A selective blockade of viral mRNA translation is involved in the interferon type specific antiviral state of mouse cells towards influenza viruses

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In mouse cells, a gene locus is responsible for the antiviral action of IFN α/β against influenza viruses (alleles Mx^+ and Mx^-). IFN α/β renders Mx$^+$ cells resistant against influenza virus replication and induces a unique protein of Mr 72,000 (p72). The IFN-mediated Mx action does not affect uncoating or primary transcription of the virus, but inhibits translation of functional viral mRNAs. We are investigating the precise mechanism(s) of this selective inhibition. Polyadenylated viral mRNAs were quantified by hybridisation to radioactive genomic RNA, followed by a separation of hybrids by affinity chromatography on oligo(dT). The transport of viral mRNAs from the nucleus into the cytoplasm was not affected by IFN α/β. The entry of viral mRNAs into the polysomes and the localisation of p72 are under investigation.

Cell wall architecture of *Schizosaccharomyces pombe* and *Saccharomyces rouxii*

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Using TEM and SEM, cell wall polysaccharides of *S. pombe* and *S. rouxii* were detected immunocytochemically by the gold method. On thin sections of *S. pombe*, a yeast dividing by fission, β-glucan and α-galactomannan were found interwoven in the cell wall. Galactomannan was associated with the secondary septa, glucan with the primary septum. In opposition to galactomannan, glucan was detected on the cell surface only on wall newly generated by fission but not on walls growing by extension. Thus glucan is involved in the fission process. In *S. rouxii*, an osmotolerant budding yeast grown in the presence of 18% NaCl, β-glucan interspersed with α-mannan in cell wall and septum. Chitin was present only in the septum and the bud scar. Mannan was found to overlay glucan on the cell surface and glucan and chitin on the bud scar.

Leading and lagging strand forms of mammalian DNA polymerase α holoenzyme

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DNA polymerase α holoenzyme from calf thymus (Hübscher et al. EMBO J. *1*, 1513, 1982) has been separated into four functional forms upon chromatography on DEAE cellulose and termed A, B, C and D. Peak A possesses in addition to the DNA polymerase α a DNAdependent ATPase, topoisomerase II and 3'–5' exonuclease activity. Peaks B, C and D contain with DNA polymerase α activities of primase, topoisomerase II and 3'–5' exonuclease. Furthermore, in peak B a 5'–3' exonuclease is detected. Peaks C and D are the DNA polymerase α's with the best activities on long ss DNA's such as parvoviral or primed M13 DNA, and they contain DNA methylase. The peak C is more salt stable than the other three forms. This is suggestive for a leading-(A) and three lagging-strand replicase forms (B, C, D). We propose that (i) B might represent the DNA polymerase α form for primer removal and gap filling, (ii) D is the DNA polymerase α holoenzyme described earlier (op. cit.) and (iii) C form could be a functional holoenzyme fitting in a higher order structure such as the chromatin.

Site-specific recombinase gene *cin* and its product for the inversion of the C segment of bacteriophage P1

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Inversion of the 4.2 kb C segment flanked by 0.6 kb inverted repeats on the bacteriophage P1 genome is mediated by the P1 encoded site-specific *cin* recombinase. The *cin* gene lies adjacent to the C segment and the C inversion cross-over sites *cix*L and *cix*R are at the external ends of the inverted repeats. The *cin* structural gene consists of 561 nucleotides and terminates at the inverted repeat end where the *cix*L site is located. Only two nucleotides in the *cix*L region differ from those in the *cix*R and they are within the *cin* TAA stop codon.
In order to overproduce the *cin* recombinase the *cin* promoter which had been localized by transposon mutagenesis was replaced by the lambda P_L promoter. The *cin* gene product was identified as a protein of monomer molecular weight 21,000. It was purified partially by making use of its DNA binding properties.

Progress toward cloning of cDNA coding for mitochondrial aspartate aminotransferase

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Poly(A)$^+$ mRNA isolated from chicken liver free polysomes was fractionated by ultracentrifugation on a sucrose gradient. The fractions containing mRNA coding for the higher MW precursor of mitochondrial aspartate aminotransferase (preAspAT; JBC *257*, 3339, 1982) were detected by *in vitro* translation and immunoprecipitation of the precursor. The message sediments at 16 S and probably contains extended noncoding regions. The peak fraction was enriched about 20-fold in preAspAT mRNA and has been used to construct a cDNA expression library in the plasmid vector pUC_8. The library consists of about 3,000 independent cDNA clones. The average lenght of the cDNA inserts is $\sim$ 500 bp. Colony screening on nitrocellulose filters using anti-mAspAT antiserum and ^{125}I-protein A showed no positive clones at a detection level of 1 ng per colony. Preliminary hybridization experiments with a mixture of small synthetic oligonucleotides, designed on the basis of the amino acid sequence are promising.

Localization of *Rhodopseudomonas viridis* photosynthetic proteins

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The photoreceptor units of *Rps. viridis* can be isolated in a native state using non-ionic detergents. Structurally both isolated and native units contain a central core surrounded by a halo of proteins subdivided into twelve subunits (Stark et al., submitted). Evidence is presented suggesting an allocation of reaction center (RC) to the inner core and light harvesting (LH) to the outer ring.
(1) The predominant LH proteins sterically can only be accommodated in the outer ring.
(2) Cross-linking with a 12 Å surface-specific reagent forms aggregates of B1015-α or β-extending past tetramers.
(3) Iodination of isolated units labels hydrophobically-situated tyrosine residues in B1015-β which, in native membranes, are inaccessible.
(4) Electron microscopy of monoclonal antibody-labelled membranes shows an enhanced signal from the central core region when RC-specific sera are used.

Regional distribution of calcitonin gene-related peptide (CGRP) and calcitonin (CT) binding sites in the rat brain

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CGRP is a neuropeptide derived from the CT-gene by Rosenfeld et al. (Nature *304*, 129, 1983). Binding sites for CGRP and CT, as assayed by displacable binding of radioiodinated synthetic rat CGRP and salmon CT, were found on crude membranes. CGRP in amounts as low as 10^{-9} M inhibited the binding of [125-I]CGRP to the membranes, whereas 200 times higher amounts of salmon CT were required to achieve a similar inhibition of the binding. The ratio of ID_{50}-values between CGRP and CT, on the other hand, was 15. Specific binding of radioiodinated CGRP and CT was highest in the midbrain-thalamus and the brainstem. The number of CGRP binding sites was similar in the neocortex, cerebellum and spinal cord, which contained a negligible number of CT binding sites. A comparable topographical distribution was obtained by autoradiography. Our results suggest different physiological roles of CGRP and CT in the mammalian brain.

Microtubules during cleavage of normal and naked eggs of a dipteran insect

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The eggs of the dipteran insect *Heteropeza pygmaea* lack a chorion, remain enveloped by the follicular epithelium, and continue to grow and elongate during embryogenesis. Naked eggs, i.e. eggs lacking the follicular epithelium, remain spherical and can attain the blastoderm stage. The occurrence of microtubules (MT) has been studied in cleaving normal and naked eggs. The distribution of MT is similar in normal and naked eggs. MT are numerous in the periphery of young eggs and in association with cleavage furrows appearing after the 2nd cleavage division. Blastoderm cells contain a well-developed microtubular frame. MT are also numerous in the intercellular bridges joining the blastoderm cells with the central part of the egg and in the periphery of the egg yolk. It is suggested that the microtubular frame is responsible for the formation and maintenance of the blastoderm architecture by progressively establishing a kind of scaffold, even in the absence of a bordering and form-giving envelope.

Molecular genetics of symbiotic nitrogen fixation *(nif)* genes of the soybean root nodule bacterium, *Rhizobium japonicum*

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The genes for nitrogenase *(nifDK)* and nitrogenase reductase *(nifH)* were cloned, and found to be unlinked on two separate operons. DNA sequences were established for all three genes including their promoter regions, and functionally important sequences were identified. Interspecies sequence comparisons suggest that *nif* genes are of ancestral origin. Fusion of the genes to *E. coli* promoters allowed their expression in *E. coli* and the assessment of their gene products by two-dimensional electrophoresis. Genetically defined *nif*$^-$ mutants were obtained by general and site-directed Tn*5* mutagenesis, and by generating deletions. The biochemistry and physiology of the mutant phenotypes was investigated, and the morphology of the infection process was studied by electron microscopy.

Carbonic anhydrase and acid phosphatase isoenzymes in dorsal root ganglia of mouse and chicken I. Methodological approach

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The activity of carbonic anhydrase (CA) and acid phosphatase isoenzymes (API) was examined by light and electron microscopy. After perfusion with 1.5% glutaraldehyde and 1% formaldehyde in 0.1 M cacodylate buffer, cryostate or vibratome sections of ganglia were incubated in Hansson's medium for CA. The specificity of the cytoenzymatic reaction was controlled by use of an inhibitor (acetazolamide) and by immunocytochemistry with anti-CA-II-antibodies. After postfixation with osmium-ferrocyanide at low pH, thin sections revealed the ultrastructural characteristics of the reactive cells in both mice and chicks.

API activity was detected by incubation in selective substrates: thiamine monophosphate chloride, phosphorylcholine chloride, D-ephedrine phosphate. The specificity of the reaction was tested by use of NaF or L-tartrate as inhibitors. The reaction was observed in the Golgi apparatus of small neurons in mouse but not in chicken.

Effects of estrogen on the transcription of the vitellogenin and albumin genesin *Xenopus laevis*

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Estrogen induces in the liver of male Xenopus frogs a high amount of the four vitellogenin mRNA's, whereas the same hormone reduces the level of albumin mRNA's. By in vitro transcription in isolated nuclei prepared from liver cube cultures, we now demonstrate that transcription of the four vitellogenin genes is coordinately induced by estrogen. Using the same approach we show that estrogen does not alter the constitutive transcription of the albumin genes. Quantitation of the albumin precursor mRNA reveals that the amount of these RNA's remains unchanged after estrogen treatment. These data indicate that estrogen does not reduce albumin gene transcription but rather decreases the half-life of the albumin mRNA. This contrasts to the wellknown stabilization of the vitellogenin mRNA's by estrogen.

Characterization of a prohead core component of bacteriophage T4

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The prohead core of phage T4 contains, among other proteins, a 17,000 molecular weight species, the 17K protein. We have raised antibodies against the protein purified from proheads by preparative SDS gel electrophoresis. Using these antibodies we have shown that: a) The protein is coded on the phage genome; b) in infected cells it can first be detected 10 minutes after infection (at 37 °C) at the time when late protein synthesis starts; c) during phage maturation the protein is proteolytically processed to give a species of molecular weight 13,000, the cleavage depends on the activity of gp21, the T4-coded prohead protease; d) the processed protein can be found in mature phage particles.

Using a helper phage which allows the expression of T4 late genes cloned into small plasmids we have found a plasmid containing the 3,200 base pair *Hind*III fragment spanning genes 20 to 22 that expresses the 17K protein.

Translational control of *Drosophila* heat shock response

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Heatshock (hs) leads to the induction of seven major hs mRNAs which are selectively translated into heat shock proteins, whereas the synthesis of non-hs proteins ceases. The mechanism of selective translation of hs mRNA is being examined by manipulating the cloned genes and reintroducing them into the germline of the flies by using the P-transposon as a vector. Since hs mRNAs have long leader sequences which might mediate selective translation, deletions have been introduced into the leader sequences of the smallest hs gene, hsp22. Deletions of the putative control sequences should lead to the synthesis of mRNA that is not translated during heatshock. The possibility that any mRNA transcribed during hs acquires some modification responsible for selective translation, the mRNA coding region of a non-hs gene (Adh) has been fused to a hs promoter. The transcription of this hybrid Adh gene should be hs inducible. The translatability of the resulting mRNA can be deduced from Adh activity.

Dissection of the fusion process of Semliki Forest Virus (SFV) infected *Aedes albopictus* cells (C6/36). I. Temperature

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Cell-cell fusion from within can be induced at pH *6* within 30 min in C6/36 16 h after infection with SFV. The consensus is: (1) infected cells aggregate at pH *7* (2) *low pH* leads to a conformational change of viral spike proteins (3) the *transition* event itself or a *new* conformation triggers the fusion. This implies that it should be experimentally possible to separate the individual fusion steps. We show: (1) Infected cells in Spinner culture at pH *7 aggregate* but never fuse. (2) Below 17 °C at pH *6* C6/36 monolayers will not fuse. (3) Monolayers kept at 4 °C and pH *6* for as long as 150 min remain unfused but will fuse within 30 min when brought to 28 °C and pH *7*. Thus by using different temperature levels we can discriminate an initial step (conformational change) occurring at 4 °C and pH 6 from the pH-independent membrane fusion.

In Semliki Forest Virus (SFV) infected *Aedes albopictus* cells (C6/36) pH 6 leads to an irreversible activation of a fusogenic state

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SFV infected C6/36 cells fuse if the pH of the medium is lowered to 6. Viral infection is a prerequisite for this fusion from within. The pH change probably induces a conformational change of viral spike proteins in the plasma membrane. Then the question arises whether it is the conformational *transition* per se or the *final* state which triggers the fusion. Minimal exposition to pH *6* tested to affect a fusion at pH *7* is 1 sec. Fusion was thus initiated. If *uninfected* cells stained with the vital stain toluidine blue are deposited on *infected* unstained monolayers at different times after lowering the pH, the preformed syncytia will still fuse 120 min later with the uninfected cells, even under conditions where formation of new viral proteins was blocked with cycloheximide. These results clearly demonstrate that the final conformation contains the fusogenic activity and once formed is stable.

The light-harvesting chlorophyll a/b protein complex: biochemical characterization and three-dimensional structure

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The light-harvesting chlorophyll a/b protein complex (LHC) isolated by detergent solubilization from pea chloroplasts occurs in a monomeric and in an oligomeric form. The monomer contains two polypeptides of 25 and 27 kD mol. wt. Peptide mapping suggested extensive homology but also indicated significant differences between the 25 and the 27 kD polypeptides. Upon dialysis against salt solutions, LHC forms extensive, well-ordered two-dimensional crystals of space group P321 with a 125 Å unit cell. A three-dimensional map of LHC was obtained by processing images of tilted, negatively stained two-dimensional crystals. At a resolution of 16 Å, the map showed two LHC trimers in the unit cell related by two-fold symmetry. Each complex exposed different structural detail on both crystal surfaces which indicated that LHC is an asymmetrical membrane protein. The three-dimensional map may represent the structure of LHC of all green plants.

The stability of a nucleoprotein complex on the ribosomal DNA of *Physarum* is cell cycle dependent

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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.

We have investigated the chromatin structure of the rDNA in two 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 nucleases and analyzing the purified DNA by blot hybridization.

We have localized 4 homologous sequences in the central spacer, inaccessible to several restriction enzymes as well as micrococcal nuclease and DNAseI. Standard deproteinisation abolishes this protection. The protected regions correspond to about two nucleosomes in length and are found in mitotic and G2 phase of the cell cycle. Exposing G2 nuclei to 2.5M NaCl has little influence on protection, exposing mitotic nuclei to the same conditions, however, destroys the effect.

Cloning and in situ localization of the transcripts of the *fushi-tarazu* gene in *Drosophila*

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In the course of our study of the *Antennapedia (Antp)* locus, we found that one *Antp* exon has cross homology to the *fushi-tarazu (ftz)* gene, which is located 28 kb to the left of *Antp*. Homozygous *ftz* embryos die before hatching and show only half the number of body segments. The reduced number of segments is due to the fusion of the anterior portion of one segment with the posterior portion of the next segment. We have shown by Northern blot analysis, that *ftz* encodes a 1.9 kb polyA$^+$ RNA which is expressed only from blastoderm to gastrula stages in early embryogenesis. Using the method of *in situ* hybridization of cloned DNA to RNA in tissue sections, we found that the transcripts are located in 7 discrete stripes of cells along the anterior-posterior axis of wild-type embryos at the blastoderm stage.

Mitotic behavior of chicken nuclear antigens studied by monoclonal antibodies

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As a first application of our monoclonal antibody library against chicken nuclear antigens we have carried out an immunofluorescence study on the behavior of these antigens during fibroblast cell division. While a few antigens are associated with condensing chromosomes, most proteins become diffusely distributed throughout the entire cell during metaphase. Many of these latter antigens remain diffusely distributed during anaphase and re-enter the newly forming nuclei in telophase. Certain antigens, however, begin to display a strikingly 'patchy' distribution in anaphase, suggesting the formation of a relatively large supramolecular complex, e.g. a vesicular structure. This 'patchy' distribution persists in the cytoplasm for some time past the re-formation of daughter nuclei in telophase. The nature of the antigens showing this particular mitotic behavior and the reason for their temporary extranuclear sequestration are currently under study. (C.F.L. is supported by an ETH doctoral grant).

A library of monoclonal antibodies against chicken nuclear antigens

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Monoclonal antibodies were raised against nuclear extracts prepared from chicken embryonic tissues. Antibodies reacting with nuclear antigens were selected by immunofluorescence microscopy and the corresponding antigens were characterized by immunoblotting and immunoprecipitation assays. Among the antibodies obtained, some discriminate between different lamin proteins and stain distinctly the nuclear envelope. Other antigens are localized either throughout the entire nucleus, or appear to be confined to discrete subnuclear compartments, e.g. nucleoli. To provide some information on the potential involvement of selected antigens in transcription (t) and/or replication (r) events, we have studied the distribution of individual antigens in representative cell types, i.e. fibroblasts (t+, r+), myotubes (t+, r−) and adult erythrocytes (t−, r−), as well as their potential nucleic acid-binding properties. (C.F.L. is supported by an ETH doctoral grant).

Replication of pSC101: Analysis of plasmid encoded functions

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The plasmid pSC101 is the only plasmid whose replication has been shown to be dependent upon the activity of the product of the *dnaA* gene of *E. coli*. This gene product is absolutely required for the initiation of chromosome replication in *E. coli*. In a attempt to determine what plasmid encoded functions are involved in pSC101 replication, insertion mutants have been isolated. They define three replication functions: i) a minimal region of 450 bases ii) a 200 base pairs segement encoding a regulatory function that lies within the minimal replication region and iii) a region encoding a trans acting function. This transacting function has been shown to be a protein of 35 kD. Analysis of protein fusions with the enzyme β-galactosidase allowed us to determine the control of the expression of the trans acting function. The protein is autoregulated on the transcriptional level. Mutants with altered control of protein expression have been isolated. These results and some important features of the sequence of the replication origin will be discussed.

Membrane properties of human breast carcinoma cell lines

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In a current study on the human mammary gland, we investigated wether cells derived from human breast carcinomas espress and modulate their membrane properties during in vitro culture. Variations of enzymatic (methyltransferase, 5'-nucleotidase, -glutamyltranspeptidase), biochemical (expression of sialogangliosides) and ultrastructural (particle density distribution on freeze-fracture preparations) properties of the plasma membrane were recorded on MCF-7 cells and other mammary strains cultured in the presence or not of polipeptidic hormones. On the basis of the indicated parameters, it appears that carcinoma cell lines display membrane properties which are similar to those expressed by epithelial cells of primary breast carcinoma.

Cleavage of chromatin-bound histone Hl by α-chymotrypsin as a probe for higher order structures of chromatin

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In order to elucidate the location of histone Hl in chromatin fibers, soluble rat liver chromatin fragments, depleted of non-histone components, were digested with α-chymotrypsin (CT) under conditions where chromatin adopts different structures. CT cleaves purified rat liver histone Hl at a preferential site located in the globular domain producing a N-terminal fragment (CT-N) and a C-terminal fragment (CT-C). The CT-C fragment was detected under conditions where chromatin fibers were unfolded or distorted. No stable CT-C fragment was detected under conditions where fibers are observed in electron microscope. We conclude that in chromatin fibers the preferential cleavage site for CT is protected. These data support models in which Hl is located close to the axis of the chromatin fiber. It is postulated that the hydrophobic globular domains are involved in contacts between neighbouring Hl-molecules which are thought to form a polymer.

Far upstream sequences modulate the rate of transcription of sea urchin histone genes in vivo

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The rate of transcription of sea urchin histone H2A and H2B genes is modulated in vivo by sequence elements located far upstream from the site of transcription initiation. The modulator of the Psammechinus miliaris H2A gene has been localized between positions −111 and −164 by measuring the transcriptional activity of deletion mutants and multiple point mutants in the frog oocyte system (Grosschedl et al., Nucl. Acids Res. 11, No. 23, 1983). This region shows a striking sequence homology (14 out of 17 bq) to the Moloney murine sarcoma virus enhancer. The −150 region of the H2B gene contains a partially overlapping 12 bp homology in opposite orientation when compared to the H2A modulator sequence. If this pre-H2B region is deleted, H2B transcription is reduced by a factor of 5–10. We are currently refining our analysis of the H2B modulator by Bal 31-resection and linkerscanning of the −150 region.

Surface membrane polarity in epithelial cells: Basolateral Na^+,K^+-ATPase shares antigenic site with an apicaly localized K^+-ATPase in the distal colon

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Na^+,K^+-ATPase is an accepted marker of the basolateral membrane in polarized epithelial cells. A monoclonal antibody to cultured small intestinal crypt cells was found to recognize the catalytic α-subunit of Na^+,K^+-ATPase. Immunocytochemical labeling with this antibody confirmed the basolateral localisation of Na^+,K^+-ATPase in the rat small intestine. However, in the distal colon immunoreactivity also was found on the apical pole of the cells. Apical brush border membranes of the distal colon were purified and the crossreacting protein was isolated by immunoprecipitation. It had the same apparent molecular weight as the α-subunit of Na^+,K^+-ATPase but was not associated with a β-chain. Enzyme measurement in the immunoprecipitate suggested that this protein was a K^+-ATPase. ATPases may respresent an interesting new system for studying the biogenesis of surface membrane polarity in epithelial cells.

Molecular cloning of the recessive oncogene lethal (2) giant larvae of *Drosophila melanogaster*

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Mutations in the *1(2)gl* locus of *Drosophila melanogaster* profoundly affect the development of the third instar larva and give rise to lethal neoplasms of the imaginal discs and the brain hemispheres. We have isolated chromosomal segments from the *1(2)gl* locus (21A, chromosome 2L). Based on the comparison between wild type DNA and mutant DNA from 21 alleles and on the analysis of the transcripts throughout the cloned region, we have defined a transcription unit which is disrupted by all the *1(2)gl* mutants. The developmental profile of expression of the two RNA (6 and 4.5 kb) made by this transcription unit fits well with the effective lethal periods of *1(2)gl* mutant alleles, which also coincides with the peaks of cell proliferation in the developing fly. Based on the above evidence, we believe we have localized the *1(2)gl* gene, a recessive oncogene of *Drosophila*.

The onset of response to whisker stimulation in mouse barrelfield (BF), a deoxyglucose (DG) and cytochromoxidase (CO) study

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Three groups of 3 albino mice each (6 and 12 day old, and adults, ICR stock) were restrained and given [C-14]DG i.p.; on one side, mu-metal bits had been glued to 3 whiskers in one row and to a single whisker in another; all others were clipped. Stimulation was with magnetic field bursts (6.7/s) at ca. 100 Gauss for 45 minutes. Increased DG-uptake in layer IV of the contralateral BF was first found on day 12. Adults had columns of high uptake with halos of decreased activity. CO activity, high in barrels already on day 6, was stimulus-independent.

Is absence of DG-uptake at day 6 due to functional immaturity of the pathway or to low sensitivity of the DG-method employed?

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It is RSV protein p12 and not p19 that binds tightly to RSV RNA

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The interactions between Rous Sarcoma virus RNA (RSV)* and the viral proteins in the virus have been analysed by Sen and Todaro (1977) using U.V. light irradiation; they showed that the major protein U.V. cross-linked to the viral RNA was p19 as identified by polyacrylamide gel electrophoresis. We report here that it is not viral protein p19 but p12 that binds tightly to RSV RNA upon U.V. irradiation of the virus. Therefore the binding sites of the viral protein along RSV RNA that we have previously characterized (Darlix and Sphar, 1982) should be now correctly attributed to p12 and not p19.

SV40 T-antigen complexes present in polyribosomes and 20-80S mRNPs are interchangeable

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In SV40-infected cells, a fraction of the newly-synthesized large T-antigen molecules rapidly associated with small 20-80S mRNPs and after a delay of about 10 min also with polyribosomes. The association of large T-antigen with 20-80S mRNPs containing poly(A)-rich mRNA was confirmed by centrifugation in discontinuous sucrose gradients in which T-antigen exhibited an apparent density of 1.2–1.3 g/ml, corresponding to that of RNPs. Furthermore, treatment of mRNP/T-antigen complexes with EDTA/RNase A released monomeric 5-6S T-antigen. That the two types of T-antigen complexes present in polyribosomes and in small mRNPs are related was shown by reversible inhibition of protein synthesis: Increased ionic strength of the culture medium led to a rapid dissociation of polyribosomes and the release of free mRNP/T-antigen complexes. Restoring isotonicity resulted in a reinitiation of protein synthesis which was paralleled by an increased transfer of small mRNP/T-antigen complexes into reformed polyribosomes.

Cytological sub-division of S-phase in the female Chinese hamster cell line 19/1

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Irradiation or chemical treatments cause perturbation of the cell cycle in dividing cell populations. Statements about cellular sensitivities are not valid if perturbed cells can not be identified and selected by scoring. The replica banding technique of Kim et al. (Cytogenet. Cell Genet. *15*, 363, 1975) allows the location of cells within subphases of the DNA synthesis period on the basis of distinctive chromosome banding patterns in the next metaphase after BrdU application (Savage and Bhunya, Chromosoma (Berl.) *77*, 169, 1980). Parallel monolayer cultures of our line 19/1 were treated with 10 µg/ml BrdU during 10 hrs, covering the whole S-phase. Cells were fixed after intervals of 2 h Colchicine treatment. After scoring 50 metaphases per sample and location of bands, a computer programm was developed to estimate frequencies of time-specific banding patterns. They were found on chromosomes No. 1, 2 and X.

Analysis of chloroplast genes coding for the ribosomal proteins S12 and S7

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Chloroplast ribosomes are of prokaryotic nature (70s). Extensive sequencing studies were done on the rRNA genes level,

but very little information exists about genes coding for chloroplast ribosomal proteins. While analysing the *Euglena gracilis* chloroplast *tuf*A gene (EF-Tu protein) (NAR *11*, 5877, 1983), we found upstream and in close vicinity of the *tuf*A gene the genes coding for the proteins S12 (*rps*12) and S7 (*rps*7). The three genes are arranged in cluster similar to the situation in *E. coli*. The same gene cluster was found in the chloroplast genome of *Glycine max*. The Euglena proteins S12 and S7 have calculated Mr of 13828 and 17831 and match the sequence of the *E. coli* S12 and S7 proteins to 68% and 38%, respectively. Sequence comparison allows to identify highly preserved domains probably involved in ribosome assembly and/or translation processes.

G0-G1 transition of rat hepatocytes after promoter treatment in vivo detected by nuclear chromatin changes

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Phenobarbital (PB) treatment in vivo causes a shift in rat liver hepatocytes from G0- to G1-phase of the cell cycle, as demonstrated by the quinacrine dihydrochloride (QDH) nuclear staining method (Cancer Letters, *19*, 1983:253). This study was extended to examine the reversibility of the PB effect and to test whether other liver tumor promoting substances such as butylhydroxytoluene (BHT), clofibrate (CLF) and progesterone or liver carcinogens such as N,N-diethylnitrosamine (DEN) cause a similar shift. Liver smears and isolated nuclei from treated animals were stained with QDH and the degree of nuclear chromatin brightness was measured with a cytofluorometer. The PB effect (treatment for 4 weeks with 1 g/l in drinking water) was reversible, taking 4 weeks for recovery. Liver nuclear samples from rats treated for 4 weeks both with progesterone and BHT showed a decrease in fluorescence intensity signifying the shift from G0 to G1, whereas samples from CLF- and DEN-treated rats did not show this alteration.

Adherence and metabolic activity of stimulated human PMN on biological surfaces

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High concentrations of formylated chemotactic peptides stimulate granulocyte (PMN) adherence on petri dishes accompanied by intense release of secondary granule constituents, e.g. transcobalamine (TC), and activation of the hexosemonophosphate shunt (HMPS). We examined basal and stimulated PMN adherence and metabolic activity on different biological surfaces.

On human fibronectin, PMN adhesion was increased from 3% to 23% with only little to moderate HMPS activation and TC release. On human endothelial cell monolayers (ECML) and human fibroblasts, we observed poor stimulation of adhesion as well as minimal metabolic activation. Extracellular matrix, prepared from ECML, had the same low adhesive property as cellular surfaces.

By scanning EM's an impressive congruency between the degree of cellular spreading, depending on the surface coat, and metabolic activation was demonstrated.

Therfore, biological surfaces possess low adhesive properties protecting them from cytotoxic damage of stimulated phagocytes.

Oxytocin receptors on sheep myometrium cells: low affinity binding

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Myometrium cells were prepared by a combined tryptic/collagenase treatment of myometrial layer obtained from young ovine uteri. The cells were cultured for 3 to 14 days. The cultures did not contain any visible fibroblastic elements. Cells were incubated for 90 min with tritium labelled oxytocin (T-OTC, 24 Ci/mmol); conc. range was 10^{-11} to 5×10^{-4} M. After quick centrifugation in Eppendorf cuvettes, cells were washed with a cold medium and solubilized for scintillation counting in a tissue solubilizer. Non-dissociable oxytocin fraction was estimated by repeated displacement of bound T-OTC fraction by 'cold' oxytocin. Analysis of the binding isotherm indicates at least two fractions of binding sites, the high affinity population with $K_{diss} \approx 10^{-9}$ mol/l (less than 10% of the total binding sites), and the low affinity population with $K_{diss} \approx 10^{-7}$ mol/l. The circumstantial evidence resulting from pharmacological experiments favours the hypothesis that the low affinity fraction represents the oxytocin receptors. Supported by SNSF, No. 3.705-80.

Effects of butyrate on cell differentiation of cell-cycle mutants and wild-type murine mastocytoma cells

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The degree of cell differentiation of heat-sensitive (hs, arrested at 39.5°C) and cold-sensitive (cs, arrested at 33°C) mutants and wild-type (WT) cells of the P-815 mastocytoma was assessed by determination of histamine (H) and 5-hydroxytryptamine (HT) content and of numbers of metachromatic granules per cell. Without butyrate, the increase in granule number of cs cells at 33°C was more pronounced than the increase in H and HT, whereas in hs cells at 39.5°C, an increase of H and HT, but not of granules was observed. Granule formation may thus be dissociated from amine production. Butyrate caused a marked increase in amine content, but not in granule number of WT and hs cells. In cs cells at 39.5°C, butyrate induced an increase in granule number and amine content comparable to that observed in cs cells arrested at 33°C in the absence of butyrate, suggesting that butyrate may act by a mechanism similar to that of intracellular factors that are operative in cs cells at 33°C.

In anemic *Xenopus* larvae the liver and the peripheral blood cells are the main sites of erythropoiesis

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In *Xenopus laevis* the switch from larval to adult globin gene expression coincides with the replacement of larval erythrocytes by adult ones. To study regulation of globin gene expression, it was important to localize putative sites of erythropoiesis in larval stages. Therefore incorporation of ^{3}H-thymidine was followed in kidney, spleen, liver and circulating blood after induction of anemia by phenylhydrazine. Determination of the labelling index showed selective stimulation of ^{3}H-thymidine incorporation by erythroid-like cells in liver and circulating blood with a peak 3 days after induction of anaemia, representing a 20-fold increase over controls. In this stage mitotic cells were abundant in the peripheral blood, suggesting a close correlation between DNA synthesis and cell proliferation. In situ hybridization with labelled larval globin cDNA revealed the presence of larval globin mRNA in putative erythroid cells. We conclude that the liver and peripheral blood cells are the main sites of erythropoiesis in larval *Xenopus*.

Parvalbumin in cross-reinnervated and denervated muscles

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Cross-reinnervation of the extensor digitorum longus (EDL) muscle by the soleus (SOL) nerve leads to the well known transformation towards a slow muscle. Nine weeks after the operation, quantitative analysis of the Ca^{2+} binding protein parvalbumin by high performance liquid chromatography shows a threefold reduction of parvalbumin in the cross-reinnervated EDL muscle. Denervation of the EDL muscle, which leads to an increase of the half-relaxation time, was followed by a 20% decrease of the parvalbumin concentration within 4 days, prior to any changes of the myofibrillar ATPase. Following self-reinnervation of the EDL muscle normal parvalbumin concentrations are reached after 9 weeks. These experiments strongly support the view that parvalbumin is involved in the relaxation of rat fast skeletal muscles. Since parvalbumin changes precede histochemical changes (as in the case of denervation), this protein may also be a sensitive marker for early stages of neuromuscular disturbances.

Myelin basic protein and glial fibrillary acidic protein in the CNS of *mld* mice

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Myelin basic protein (MBP) synthesis is repressed in myelin deficient *(mld)* mice until 60 days of age. Thereafter, the MBP content of myelin and the number of myelinated fibers increase. At early myelination stages, in *mld* mice, a few myelin sheaths and oligodendrocytes contain MBP as shown by immunocytochemistry. Astrocytes have larger and more extended branches in *mld* than in control mice. Their cell body and processes are intensely immunostained for glial fibrillary acidic protein (GFAP). After 60 days, the number of myelinated fibers increase in *mld* mice and, in contrast to normal sibs, a few oligodendrocytes are still immunostained for MBP. In spite of the improved myelination observed in adult *mld* mutants, the extended network of astrocytic branches remains unchanged. These results confirm that immature *mld* mice can synthesize some MBP and incorporate it into myelin. Furthermore, in adult mutants, oligodendrocytes are still actively synthesizing MBP and producing myelin.

Site-specific susceptibility of *Dictyostelium* ribosomal genes to an endogenous nuclease

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An endogenous nuclease cuts the palindromic ribosomal DNA *Dictyostelium discoideum* close to the start of transcription, at the end of the transcribed region, and about 2,100 bp upstream from the transcription start. Nuclei or nucleoli are attacked by the enzyme both during organelle preparation and during subsequent incubation. Additional digestion with DNase 1 or with S1 nuclease did not reveal additional hypersensitivity. When supercoiled plasmid DNA containing the S' end of the gene and upstream regions was mixed with isolated nucleoli, the DNA was nicked but there were no double stranded cuts.
The ribosomal DNA is attacked by the endogenous nuclease not only in exponentially growing cells, where the transcribed region and upstream sequences are nucleosome free, but also in differentiating cells, where the bulk of the ribosomal genes are arranged in nucleosomes.

Expression and regulation of an artificial yeast *TRP*-gene cluster in yeast and *E. coli*

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Molecular cloning of all 5 *TRP*-biosynthetic genes of *S. cerevisiae* made it possible to unify them on a single plasmid pME554, which is designed as yeast/*E. coli* shuttle vector. Expression and regulation in *S. cerevisiae* of genes *TRP2, 3, 4,* and *5* was found to be very similar if located either on the plasmid or on the chromosomes; gene *TRP1* was expressed poorly however on the plasmid. In *E. coli* strain W3110 *ΔtrpEA2* (*trp*-operon deleted) plasmid pME554 allowed for half maximal growth rates if the first or the last intermediate (anthranilic acid or indole) were added. Accordingly poor expression of the first and the last enzyme of *TRP*-biosynthesis was observed; the other enzymes were expressed reasonably well. A strong repressive effect on 4 of the 5 yeast *TRP*-genes was found in *E. coli* after addition of tryptophan. Homology between sequences of the *E. coli trp*-operator and sequences in the yeast *TRP3* and *TRP5* promoters was found.

Myelin basic protein is already expressed in undifferentiated glial cells

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Basic protein (BP) is a biochemically well characterized major constituent of both central (CNS) and peripheral (PNS) nervous system. Light microscopic immunocytochemical investigations revealed that BP antiserum stains specifically developing oligodendroglia (CNS), Schwann cells (PNS) and myelin (CNS/PNS). To define the distribution of BP in imature CNS tissue at a developmental stage of premyelination, we used an immunoelectronmicroscopic procedure (Omlin et al., J. Cell Biol. *95*, 242, 1982). Teased optic nerves of newborn rats were treated according to the immunoperoxydase technique. Deposits of BP immunoreaction products were localized in processes of undifferentiated glial cells, which did not present fine structural characteristics of oligodendrocytes. The cytoplasma membrane of these processes was immunostained, too. We suggest that these BP^+ cells represent precursors of myelinating oligodendroglia. Since BP could act as a kind of glue at stages during myelination, the function of this early synthetized BP remains unknown.

Permeability of rat caudal artery in spontaneous and experimentally induced lesions

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Permeability to horse-radish peroxidase (HRP) of lesions of the caudal artery developing spontaneously with age in male Wistar rats was evaluated by electronmicroscopy and compared with that of lesions induced at different times after ligature, pinching and internal scraping of the artery. The arterial wall does not contain HRP when 1., internal elastic lamina (IEL) is absent and endothelium damaged (spontaneous lesions, early after ligature and pinching) 2., IEL is maintained and endothelium missing (early after scraping) 3., IEL is absent and endothelium regenerated (spontaneous lesions, later after ligation and pinching). By contrast, HRP is present in the intima when IEL is maintained and endothelium is regenerating or has recently regenerated (edge of some spontaneous lesions, later after scraping, areas between ligatures later after ligation). We conclude, that an increase in permeability to HRP is a phenomenon related to renewal of the endothelial cells and the presence of IEL.

Isolation of transcription complexes from *Dictyostelium discoideum* nucleoli

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The actively transcribed ribosomal genes in *D. discoideum* lack a nucleosomal structure. The aim of this work was to identify proteins associated with the transcribed region and two methods were employed. (1) Chromatin fragments were separated on non-denaturing polyacrylamide gels following micrococcal digestion of nucleoli. One of the fragments contained DNA (50–80 bp in length) exclusively from the transcribed region as well as RNA and protein. (2) Electronmicroscopy showed that heparin treatment removed all nucleosomes and RNA-binding proteins from nucleoli, leaving naked DNA and rows of transcription complexes. This material was isolated on sucrose gradients. Proteins in the transcription complexes isolated by the two methods were examined and compared with purified RNA polymerase I.

Chromatin structure of a single copy developmentally regulated gene in *Dictyostelium discoideum*

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Ribosomal genes lose their nucleosome structure when actively transcribed in *D. discoideum* and we wished to discover whether genes transcribed by polymerase II behaved similarly. A cDNA clone derived from mRNA coding a 33 kd protein was used as a probe. Northern blotting showed the gene is developmentally regulated, transcription reaching a maximum after 6 h of development and mRNA levels remaining constant until differentiation is complete. Nuclei from various developmental stages were treated with micrococcal nuclease, the fragments transferred to DBM paper and hybridized with the probe. The inactive gene was organized into nucleosomes, but following activation nucleosome structure was lost.

Stage-specific protein synthesis and surface antigen expression in correlation to DNA replication during early mouse embryogenesis

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Fertilized mouse eggs were treated with cytochalasin B which prevents cellular division but allows chromosomal replication to continue. Consequently, the eggs became tetraploid and more and more polyploid. After incorporation with ^{35}S-methionine, protein synthesis in the eggs was analysed by 2-dimensional PAA gel electrophoresis. The protein patterns expressed by polyploid eggs no longer resembled those of fertilized control eggs but were similar to those of normally cleaving embryos of equivalent age and nuclear replication. Eggs were also treated with aphidicolin to study the feature of protein synthesis after blocking both, cleavage and replication. In parallel, the stage-specific appearance of surface-antigens was analysed by immunofluorescence.

Retrograde axonal transport for detection of peripheral projections of dorsal root ganglion (DRG) cells in chicken

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Wheat germ agglutinin coupled with horseradish peroxidase (WGA-HRP) was used as retrograde tracer to identify which types of DRG neuron innervate a definite peripheral target. Lyophilised particles of WGA-HRP were inserted within the sartorius muscle or the tendon of gastrocnemius. 24 hrs later, chickens were fixed by perfusion and corresponding lombosacral ganglia were sectionned with a vibratome. Peroxidase activity was detected with Tetramethyl benzidine for light microscopy or Diaminobenzidine followed by a postfixation with K-ferrocyanide-OsO_4 for electron microscopy. A limited number of DRG cells showed the reaction in GERL or in lysosomes scattered throughout the perikarion. After insertion of WGA-HRP in the muscle, most reactive cells consisted of small neurons with a well developed endoplasmic reticulum. After insertion in the tendon, WGA-HRP was mainly recovered in large neurons.

Immunoglobulin gene enhancer elements

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Recently, a lymphocyte-specific enhancer has been identified downstream of the joining region in immunoglobulin heavy chain genes. Now we show direct evidence for a functionally similar enhancer within the large intron of the kappa light chain gene of the mouse. The kappa enhancer is, however, less active than the heavy chain gene enhancer. No enhancer was found within a cloned λI light chain gene. To analyse the enhancer activity during lymphocyte differentiation, we have linked these enhancers to different test genes and studied their expression in various lymphoid cell lines. Surprisingly, the heavy chain gene enhancer is highly active in Pre-B-cells and even in T-lymphoma cells, in contrast to the low activity of the endogenous heavy chain genes in these cells. It therefore appears that the activation of immunoglobulin genes during lymphocyte differentiation is a multistep process dircted by enhancers as well as other (yet to be defined) components.

Recent advances in cell-cell adhesion in rat fibroblasts

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In a study on the molecular processes which play a role in the intercellular adhesion and contact inhibition in normal and transformed cells, we investigated the function of Ca^{++} and Mg^{++} and membrane proteins in cell-cell adhesion in rat fibroblasts.

The results obtained through an appropriate alteration of the adhesion assay medium, demonstrate that the substitution of one of the two cations with corresponding equimolecular concentration of the other one is not able to restore the optimal adhesion. Adhesion kinetcs obtained by treating with cross-linking reagents show that the inhibitory effect on the cell-cell adhesion of two imides (DMA and DMS) is due to an alteration of the motility of membrane proteins engaged in the adhesive phenomenon. This has also been confirmed by some experiments on single cells kept at 0°C for different times, a condition in which result a stiffening of the structural order of the cell coat.

Guanine nucleotide-binding protein induced by interferons in mouse cells: strain distribution and inheritance of inducibility

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Interferons (both type I and type II) are able to induce in mouse cells a set of proteins with high binding affinity for guanine nucleotides (Stäheli et al., J. Virol. *47*, 563, 1983). Using

cells from various inbred mouse strains we have found strain variations in inducibility of synthesis of one of these proteins. A major guanine nucleotide-binding protein of Mr 65,000 (GBP-1) was inducible in cells from 5 out of 20 mouse strains tested, namely A/J, BALB/c, C3H, CPB-TK and 129/J. Cells from 15 strains, including A2G and DBA/2, failed to synthesize GBP-1 in response to interferon although induction of several minor GBPs was the same as in the first group of mice. Analysis of F_1, F_2 and BC-1 offspring of reciprocal crosses between A/J and A2G as well as between A/J and DBA/2 mice showed that inducibility of GBP-1 was inherited as a single autosomal semidominant allele.

Identification of mRNA molecules that carry repetitive sequence elements in stage 40 *X. laevis* embryos

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We have previously described repetitive sequences which are transcribed in stage 40 *X. laevis* embryos. The repetitive sequence transcripts are detected in cytoplasmic polyadenylated RNA, suggesting that they could be present on mRNA molecules. In order to identify such putative mRNAs, we carried out hybrid selected translation experiments with two different cloned repetitive sequences. These experiments show that each of the two sequences selects by hybridization a set of different mRNAs which are translatable *in vitro* into different proteins. One of the repetitive sequences selects mRNA molecules coding for at least 16 different proteins. The second repetitive sequence selects mRNA molecules coding for at least 5 different proteins. All of these mRNAs are rare in stage 40 embryo RNA. We are presently in the process of analyzing RNA from different sources in order to determine whether the presence of these mRNAs is specific for this developmental stage.

MAP4, a novel class of microtubule associated proteins in neural tissue

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A novel class of high molecular weight (HMW) proteins in rat brain microtubules was detected using monoclonal antibodies. The antigen (MAP4) was present in microtubules and enriched in preparations of MAPs. It was not detectable in isolated brain intermediate flaments or actomyosin or in other subcellular fractions. As determined by solid phase immunoassay and SDS-gel immunoblots, MAP4 was found only in central nervous system. Immunocytochemical staining showed that MAP4 is located in nerve cells, where it is present in both axons and dendrites. This pattern resembles those previously found for MAPs 1 & 2 with respect to the neuronal localization (G. Huber & A. Matus, J. Neuroscience, in press), but differs from them in that MAP4 is distributed throughout the nerve cell cytoplasm whereas MAPs 1 & 2 are both concentrated in dendrites. These results provide further evidence that HMW MAPs are a heterogeneous group of proteins which are variously distributed between and within the cells of the central nervous system.

Golgi apparatus subcompartments revealed with lectin-gold complexes

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The subcellular distribution of mannose/glucose, galactose, fucose, sialic acid and GalNAc residues was investigated on ultrathin sections from low temperature Lowicryl K4M embedded intestine by the use of lectin-gold complexes (Roth, J., J. Histochem. Cytochem. *31*, 547 and 987, 1983). Nuclear envelope, rough ER and its transitional elements as well as a few cis-Golgi cisternae and lysosomes were positive for mannose as visualized by ConA. Galactose, fucose and sialic acid residues detected with Ric. communis I lectin, Lotus tetragonolobus lectin and Limax flavus lectin, respectively, were found only in certain trans-Golgi cisternae, associated vesicles, lysosomes and the plasma membrane. GalNAc residues localized with Helix pomatia lectin were found in a few cis and trans-Golgi cisternae but were absent from medial Golgi cisternae. These cytochemical data demonstrate further the existence of glycosylation subcompartments in the Golgi apparatus.

Analysis of a rRNA gene on its way to obliteration

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It is known that the circular chloroplast DNA from *Euglena gracilis* Z, contains three tandemly arranged rDNA regions (5'-ptRNA^{Ile}-ptRNA^{Trp}-16S rRNA -tRNA^{Ile}-tRNA^{Ala}-23S rRNA, 5S rRNA – interoperon spacer) and an extra 16S rDNA region which is about 3.2 kbp away from the first regular operon. From EM-studies it became evident (Koller and Delius, FEBS Letters 140, 198, 1982) that this 3.2 kbp DNA fragment contains several interesting structural features. We have now totally sequenced this fragment which contains a major ORF coding for a protein of 405 aminoacids. This ORF is flanked by two short inverted repeats. On either side we find two direct repeats. One is adjacent to the 3' end of the extra 16S rRNA gene, the other one is located within a 615 bases stretch, homologous to a DNA fragment of the regular rDNA which contains the 5S gene and adjacent parts of the interoperon spacer. Considering the various elements of this mosaic we may postulate that a transpositional event occurred in the past, introducing a protein coding gene in the rDNA region, leaving intact the 16S rRNA gene and parts of the interoperon spacer including the 5S gene, but completely eliminating the genes for tRNA^{Ile}, tRNA^{Ala} and the 23S rRNA.

Transcriptional activation of rabbit β-globin genes in *Xenopus* oocytes by a crude lysate from rabbit erythropoietic cells

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Cloned rabbit β-globin genes are actively transcribed in transfected cells (Dierks et al. cell *32*, 695 (1983)) but are transcriptionally inactive when injected into the oocytes without the vector sequences. It appears that the oocyte lacks some factor required for the activation of β-globin gene transcription.
In order to evidence such gene specific transcription activators, rabbit bone marrow cells have been fractionated on Percoll gradients, and S100 extracts have been prepared from early and late stages of erythropoietic cells. Cytoplasmic injection of S100 from erythroblasts results in the activation of β-globin gene transcription in the oocyte. These early hematopoietic cells which express their globin genes thus seem to contain some as yet not characterized element capable of selectively activating β-globin gene transcription also upon transfer into a heterologous environment.

Immunolocalization of determinants of photoreceptor outer segments in the frog retina

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Synchronous membrane shedding from photoreceptor outer segments (OS) is triggered by the transition from dark to light.

The shed material is subsequently phagocytosed (phagosomes) and degraded within the retinal pigment epithelium (RPE). In order to follow more closely the phagocytic pathway of individual OS components within the RPE, we have produced antisera in rabbits against purified OS from *Rana ridibunda* and *Xenopus borealis*. Immunofluorescence microscopy on methacrylate embedded sections shows OS and phagosomes specifically stained by both antisera. As determined by immunoblotting, the antiserum against OS of *Rana* contains antibodies against 4 different protein components, two of which were identified as rhodopsin and phosphodiesterase. The different antibodies purified by affinity chromatography, allows to track specifically defined OS proteins and to test their possible implication in phagocytic and exocytotic events within the RPE. (SNSF grant 3.390.0.82)

A segment of the human interferon-*a*1 promoter mediating viral induction of alien genes

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We previously found that a segment of 117 nucleotides preceding the cap site suffices for inducibility of the IFN-*a*1 gene. To indentify the 3′ border of the relevant sequence, we constructed a set of hybrid promoters in which 3′ truncated IFN promoters and 5′ truncated β-globin promoters were combined in various fashions and joined them to the β-globin transcription unit. Mouse LMTK$^-$ cells were permanently transformed with the modified genes by TK-linked transformation. Correctly initiated β-globin transcripts from induced and uninduced cells were determined by quantitative S_1 mapping. The results show that an IFN promoter segment extending to position −65 (relative to the IFN CAP site) is sufficient to mediate viral induction of transcription when placed 56, 78 and even 109 nucleotides upstream of the β-globin cap site.

Sequence comparison between the prototype minute virus of mice and its immunosuppressive variant

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We have compared the nucleotide sequence of the lymphocyte-specific immunosuppressive virus (MVMi) with that of the fibroblast-specific (MVMp) published by C. Astell et al. Out of 4708 nucleotides compared we found 3.3% changes, involving mostly transitions from T to C and A to G. In the potentially coding part of the genome, 75% of changes occurred in the third position of the codon, 12% in the first and 13% in the second.

The potential amino acid changes represented 2.4%, most of which do not change the class of amino acid encoded. We observed an additional amino-acid in MVMi between positions 3519 and 3528 changing the amino-acid chain from Ile-Pro in MVMp to Met-Asn-Ser in MVMi. We are now constructing hybrid viruses between MVM(p) and MVM(i) to test their immunosuppressive phenotype in a mixed leukocyte culture in order to determine the parts of the genome conferring the immunosuppressive phenotype to MVMi.

Organization of the transcriptional unit of a human class II histocompatibility antigen: HLA-DR heavy chain

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A total of 5724 base pairs of a recombinant phage DNA containing a human HLA-DR heavy chain gene including flanking regions has been analyzed. The regions corresponding to all the exons were identified. The sites of initiation of transcription and polyadenylation were also determined. A large intron of 2399 base pairs separates the first exon containing the 5′ untranslated region and the signal peptide from the second exon containing the N-terminal peptide domain. Conserved sequence elements upstream of the TATA box and within the first intron may be involved in the lymphocyte-specific expression of the gene.

The cephalopod embryo, a suitable system for the *in situ* recording of cell migration

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The morphogenetic importance of ordered cell movements in developing systems makes the Cephalopod embryo particularly interesting as it is a suitable system in which to study basic problems of cell migration *in vivo*, such as locomotory behaviour, cell-cell and cell-substrate interactions. Our investigations on the pre-organogenetic embryo of *Loligo vulgaris* are performed by cutting a window in the ectoderm and using time lapse micro-cinematography to record *in situ* the migration of individual mes-entodermal cells. Cell and substrate morphology changes are observed using the S.E.M. We found that a) cell divisions go on during migration; b) cells move individually and not in aggregates; c) cells migrate into a performed lumen; d) marked morphological changes in the substrate indicate its active role in adhesion, motility and possibly orientation of the migrating cells.

Monoclonal antibodies directed against synthetic peptides corresponding to the *ras*-oncogenes

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We are isolating monoclonal antibodies directed against selected sites of the gene products of the *ras*-oncogenes. Of particular interest are the regions around amino-acid 12 and 61, since it has been shown that single base pair mutations affecting these aminoacids activate the normal *ras*-genes into transforming oncogenes (1–3). We have obtained 27 hybrids producing monoclonal antibodies directed against octapeptides containing glycine (normal) or valine (activated allele) at position 12. Of these, 3 have been characterized further. By ELISA testing one of them was found to be specific for glycine, one reacted specifically with valine, while the third one recognized both peptides equally well. We have also obtained 116 hybridoma cultures producing antibodies directed against three hexapeptides containing glutamine at position 61 (normal) and leucine or lysine (activated alleles), respectively. All 116 reacted equally well with all three peptides. Immunoprecipitation and western-blot experiments are in progress to determine the usefulness of these monoclonal antibodies in discriminating cells containing activated *ras*-genes from normal cells.

1. Tabin, C.J. et al., Nature 300, 143–149 (1982)
2. Yuasa et al., Nature 303, 775–779 (1983)
3. Taparowsky, E. et al., Nature 300, 762–765 (1982)

Effect of pyridoxal (PL) deficiency on cytosolic (c) and mitochondrial (m) aspartate aminotransferase (AspAT) in chicken embryo fibroblasts

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In chicken embryo fibroblasts cultured in the presence of PL, cAspAT accounts for 15% and mAspAT for 85% of the total AspAT activity. These values remained unchanged over four consecutive passages. Under these conditions no apoenzyme was found. When the cells were grown under PL deficiency,

20% of mAspAT was present as apoenzyme in secondary cultures. This value was decreased, however, after further passages. Apoenzyme of cAspAT was never detected. PL deficiency resulted in a twofold increase of total mAspAT protein relative to total protein, whereas cAspAT was decreased steadily with each passage and was no longer detectable in tertiary cultures. Additional glutamine enhanced the effect of PL deficiency, i.e., up to 40% of mAspAT was found to be in the apoform in tertiary cultures. However, cAspAT was not influenced by addition of glutamine. The present results show that the two isoenzymes are differentially affected by PL deficiency.

The mouse α-amylase loci: structure and expression

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We have isolated a long contiguous stretch of DNA (106 kb) spaning the *Amy*-1^a locus and part of the *Amy*-2^a locus. The single copy *Amy*-1^a gene is separated from a member of the *Amy*-2^a oligo gene family by 23 kb of nontranscribed spacer DNA. *Amy*-2^a genes are exclusively expressed in the pancreas. *Amy*-1^a has two promoters of different strenght. The weaker one is utilized by all alpha-amylase producing tissues (parotid, liver, pancreas). The approximately 40 fold stronger one is exclusively active in the parotid. In parotid, the weak promoter is already active at birth. The stronger one, however, is only induced during weaning (10 to 20 days after birth). Upon transfection of *Amy*-1^a into mouse L-cells, only the weak promoter is functional. These results suggest that a transcriptionally competent chromatin structure is sufficient for the activity of the weak promoter. In contrast, the strong, parotid specific promoter appears to require tissue-specific factors for its activity.

Primary structure of the light harvesting protein C-Phycoerythrin from *Fremyella diplosyphon*

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C-Phycoerythrin is a red, water soluble pigment-protein complex and a component of the phycobilisome, the large light harvesting antenna of cyanobacteria. Its maximal absorbance is at 562 nm and it transfers light energy via C-Phycocyanin (CPC) and Allophycocyanin (APC) to the reaction centres in the thylakoid membrane. C-Phycoerythrin was separated into two subunits, α and β, 18000 and 19000 D molecular weight, resp. The six chromophores are linear tetrapyrroles (phycoerythrobilin), covalently bound to cysteine residues. Large fragments of the chains were prepared by cleavage with chemical and enzymatic methods and separated by gel filtration, ion exchange chromatography and HPLC. The amino acid sequence was determined by sequenator analysis with polybrene as carrier. The primary structure was compared with CPC and APC from *Fischerella*. Inserted peptides were recognized, due to additional chromophores or genera specific differences between *Fischerella* and *Fremyella*.

The 34 kd cellular protein phosphorylated by the Rous Sarcoma Virus transforming gene product is associated with a 8S ribonucleoprotein particle

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The subcellular localization of a 34 kd protein, substrate of pp60^{src} tyrosine kinase was investigated by biochemical fractionation of uninfected chicken embryo fibroblasts; the majority of the protein fractionated with the crude membrane pellet with 25% being found in the cytoplasm. If approximately half of the 34 kd protein behaved as a peripheral membrane protein, the remainder was even more tightly bound; the only treatment capable of almost completely release 34 kd from the membranes was 1 mg/ml of heparin. We have partially purified a 7–8S containing 34 kd ribonucleoprotein particle; this RNP had a M_r = 150 to 200 kd and a buoyant density after fixation of 1.38 g/cm^3 which corresponds to an RNA/protein ratio of 1:3.5. The results presented here suggest that 34 kd may be associated with membranes together with an RNA.

Actin isoforms in fibroblastic tissues and smooth muscles in vivo

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Actin is a cytoskeletal protein present in every eukariotic cell and is mainly involved in cell motility and contractility. Two dimensional gel analysis shows that there are three isoforms of actin: α, β and γ. We have investigated the pattern of these isoforms in various fibroblastic and smooth muscle tissues of human and non human origin. Fibroblastic tissues, such as derm or tendon, only contain the β- and γ-isoforms, β being predominant over γ. On the other hand, smooth muscle tissues, such as the uterine or the tracheal muscle, show a large heterogeneity of patterns that allows to distinguish between different muscle types. This study provides a way to distinguish fibroblastic tissues from smooth muscle tissues by their actin isoform pattern. This distinction may be useful in the study of differentiation and pathological phenomena.

Psoralen-crosslinking of DNA as a probe for the structure of the active nucleolar chromatin

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We have used psoralen to crosslink the ribosomal DNA of Dictyostelium in purified nucleoli and nuclei. The DNA from the non-transcribed spacer shows a very low extent of crosslinking, whereas DNA from the coding region is heavily crosslinked. The electrophoretic mobility of restriction fragments from the inactive region was not altered, however, the mobility of the fragments from the active region decreased continuously with increasing times of crosslinking. Electron microscopy showed that the transcription units were not displaced by the psoralen treatment. Furthermore, in crosslinked soluble rat liver chromatin the histones were not displaced. We conclude that the extent of psoralen crosslinking of chromatin DNA is diagnostic for the structur of inactive and active chromatin. Our observations show for the first time unambiguously, that within these active ribosomal transcription units the nucleosomes are absent.

Biosynthesis of the IgA dimer antibody receptor

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Transmission of IgA_2 across epithelia is mediated by a receptor [secretory component (SC)]. Using 3 antibodies directed against different domains of SC, we examine its processing in the lactating rabbit mamary gland. SC is synthesized as a coreglycosylated transmembrane protein on the RER. Pulsechase experiments reveal the time course of SC maturation in the Golgi, as demonstrated by the aquisition of Endo H resistance and a concomittant increase in Mr (30–60 min). The subsequent routing of SC to the basolateral plasma membrane,

where IgA_2 binding and endocytosis occur, the cleavage of the membrane anchoring domain of SC and the exocytosis from the apical plasma membrane of IgA_2 bound to the ectoplasmic domain of SC takes place rapidly (30–60 min). Thus maturation in the Golgi may represent the rate limiting step in SC routing. SC may exist in several conformational states which are processed at different rates.

Carbonic anhydrase and acid phospatase isoenzymes in dorsal root ganglia of the mouse. II Application to determine the neuronal subclasses

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Carbonic anhydrase (CA) and acid phosphatase isoenzyme (API) activities were studied in the T13, L2 and L5 ganglia. On the basis of ultrastructural and cytoenzymatic criteria, 6 subclasses of neurons were considered. Three subpopulations of large neurons (A; ca. 36%) were revealed according to the intensity of CA reaction in the perikaryon and nucleus: 1) strongly reactive cells (8%), 2) moderately reactive cells (16%), 3) nonreactive cells (12%). All the satellite cells were positive except in one subclass of small neurons (B). After staining with anti-CA II antibodies in adjacent section the immunocytochemical reaction was found in the same neurons but not in satellite cells. API reaction was found in most small neurons (B; ca. 51%); 2 subclasses were identified by ultrastructural characteristics. A third discrete subpopulation of small or very small neurons (13%) does not react with API or AC.

Differential modulation of the synthesis of axonal proteins of dorsal root ganglia by peripheral and central neuroglial cells

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Chicken embryonic dorsal root ganglia (DRG) were grown in a compartmental cell culture system that allows selective examination of the axonal proteins. Peripheral or central neuroglial cells were co-cultured with the DRG axons. The axonal proteins synthesized under these different environmental conditions were examined by metabolic labeling and 2-dimensional SDS-polyacrylamide gel electrophoresis. Computerized quantitation of the axonal proteins revealed that peripheral and central neuroglial cells modulate the synthesis of axonal proteins of DRG neurons differentially. Plasticity in the expression of axonal proteins in response to the different topographical provenance of the surrounding cells might reflect environmental modulation of axonal functions such as axon growth, fasciculation, pathfinding and synapse formation during the development of the nervous system and in its repair processes after injury.

Synthesis of RuBP carboxylase/oxygenase subunits in *Chlamydomonas* chloroplast mutants that lack the holoenzyme

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Two light-sensitive, acetate-requiring chloroplast mutants of *C. reinhardii* were recently recovered that lack the ribulose-1, 5-bisphosphate carboxylase/oxygenase holoenzyme. Both the nuclearencoded small subunit and chloroplast-encoded large subunit are also absent when total cell proteins are visualized on Coomassieblue-stained SDS-PAGE gels (Spreitzer and Ogren, PNAS *80*, 6293, 1983). We have found that large- and small-subunit mRNAs are present in the mutants by probing Northern blots with large- and small-subunit gene probes. Immunoprecipitation of $^{35}SO_4^{2-}$-pulselabeled cell proteins revealed that small subunits are synthesized, but rapidly degraded during the chase period. SDS-PAGE of total pulse-labeled cell proteins suggests that a truncated large subunit is synthesized and then degraded in one of the mutants. These results provide further evidence that the mutants may be large-subunit structural gene mutants and suggest that small-subunit synthesis is not affected by the specific absence of the large subunit.

EcoA: enzyme structure and DNA recognition sequence

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The *Eco*A restriction enzyme from *E. coli* 15T$^-$ has been isolated. It proves to be an unusual enzyme, clearly related functionally to the classical type I restriction enzymes. The basic enzyme is a two subunit modification methylase. Another protein species which by itself has no enzymatic activities can be purified that converts the modification methylase to an ATP and S-adenosylmethionine dependent restriction endonuclease. The DNA recognition sequence of *Eco*A also resembles previously determined type I sequences. It is:

5′-GAGNNNNNNGTCA-3′,
3′-CTCNNNNNNCAGT-5′.

Modification methylates the A residue in the specific trinucleotide and the A residue in the lower strand of the specific tetranucleotide. The genetic and transcriptional organisation of the *Eco*A genes will be presented.

Characterization of a xenopsin precursor as deduced from its cDNA sequence

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We have cloned and analyzed a cDNA complementary to a mRNA which encodes the protein precursor of xenopsin. Xenopsin is a biologically active octapeptide from *X. laevis* skin, it bears a striking sequence homology and also shares a number of biological activities with the mammalian neurotransmitter neurotensin. The known amino acid composition of xenopsin was used to design and synthesize by the triester method two oligodeoxynucleotide mixtures complementary to different portions of the hypothetical xenopsin mRNA sequence. A cDNA library was screened with both probes and one of the recombinants (pXP) was found to contain a nearly full length copy of a mRNA of 480 nucleotides which encodes a xenosin precursor. The deduced polypeptide consists of 80 amino acids, exhibits a typical leader sequence and contains the mature peptide at the very C-terminal end. Sequence similarities between pXP and other cloned skin mRNAs strongly suggest that the xenopsin precursor is encoded by a gene which is a member of a larger gene family.

DNA polymerase activities in multiplying and arrested mammalian cell-cycle mutants and wild-type cells

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Heat-sensitive (hs) and cold-sensitive (cs) cell-cycle mutants of murine P-815 cells were reversibly arrested at the nonpermissive temperature (39.5 °C for hs, 33 °C for cs), and wild-type (WT) cells were arrested by 2 mM butyrate (Bt) or 2.5% DMSO. In hs cells at 39.5 °C and cs cells at 33 °C, DNA polymerase *a* decreased to 10–15% of the initial value, while the number of DNA-synthesizing cells decreased from approx. 50% more rapidly to less than 2%. In WT cells arrested by Bt

or DMSO (less than 10% DNA-synthesizing cells), polymerase α activity was as high as 32–55% of the initial value. After return of arrested hs and cs cells to the permissive temperatures of 33 °C and 39.5 °C, polymerase α increased with nearly the same time course as the number of DNA-synthesizing cells. For polymerases β and γ, only minor differences between multiplying and arrested cells were observed.

I–V characteristics for carrier transport in bilayer lipid membranes: Explanation for the observed deviation from ideal behavior at higher applied voltages

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Current-voltage characteristics are often used to determine rate constants and barrier geometry for carrier transport in bilayers, but the voltage-dependent membrane geometry (electrostriction) may lead to distortions of the *I–V* curves. We present a procedure to correct for these effects and demonstrate its use with nonactin-K+ transport across asymmetrically shielded phosphatidyl serine bilayers. The voltage-dependence of bilayer thickness and area, the geometry of the potential energy barrier for the charged species, and the intrabilayer diffusion constant are considered specifically. Whereas parameters fitted to the uncorrected curves often take on unrealistic values, the corrected curves yield parameters consistent with both general expectations and different experimental techniques.

Central versus peripheral nerve explants as substrates for axonal growth in vitro

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Rat optic or sciatic nerve explants were offered to dissociated sympathetic neurons (growing in the middle chamber of a 3-compartment culture ring) as 'bridges' to the 2 side-chambers. If given the choice between optic and sciatic nerve, neurites grew almost exclusively into and through the sciatic. The proportion of surviving glial cells was manipulated by treatment with cytosine arabinoside. EM showed that with many glial cells present, axons were predominantly growing on Schwann cells in the sciatic, but seemed to actively avoid the glial cells of the optic nerve. With only a few surviving glial cells, however, axons in the sciatic preferably grew on the Schwann cell-side of the empty endoneurial basement membranes (BM). In the optic nerve the few axons present under these conditions were associated with perivascular collagen and BM. In contrast to Schwann cells and their BM, which are favorable substrates for axonal growth, optic nerve glia seems to inhibit fiber growth inspite of optimal culture conditions.

Membrane-bound and soluble precursors of acid phosphatase from yeast

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Yeast cells were grown in the presence and absence of tunicamycin. Aliquots of crude cell extracts, 75000 g sediments with corresponding supernatants and purified membranes were examined for acid phosphatase protein. In membranes of cells treated with tunicamycin, 3 immunoreactive bands with apparent molecular weights of 60000, 58000 and 56000 were identified. The same banding pattern was obtained for purified acid phosphatase deglycosylated *in vitro*. We conclude that the 60kd, 58kd and 56kd proteins represent *in vivo* synthesized unglycosylated acid phosphatase precursors that accumulate in membranes. Mutant *sec* 59 accumulates the same proteins in membranes. In extracts and membranes of untreated control cells, two bands of an apparent molecular weight of 80000 and 76000 can be detected. Mutant *sec* 18 accumulates these two core glycosylated proteins in a soluble as well as a membrane-bound form.

Cell cycle stage specific function of the *mei-9* and *mei-41* locus in *Drosophila*

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Different ratios of chromatid versus isochromatid breaks were observed by Gatti et al., PNAS, *77* 1980, 1575, in neuroblasts of *mei-9* and *mei-41* mutant larvae at MI of cells irradiated during S-phase. Their interpretation of the data was that chromatid and isochromatid breaks were produced by partially different metabolic pathways. Repair defects in either mutant at different points in time of the cell cycle were not considered to be a likely cause of the locus specific aberration pattern. Applying autoradiography I scored in G_2 irradiated cells (200 rd) at MI, 40.1% chromatid and 8.2% isochromatid breaks in *mei-9*^{a} and 11.1% chromatid and 2.8% isochromatid breaks in *mei-41*^{D5} cells. Conversly, in G_1 irradiated cells I scored 2.4% chromatid and 4.8% chromosome breaks in *mei-9*^{a} but 13.9% chromatid and 29.6% chromosome breaks in *mei-41*^{D5} cells. In order to maintain the chromosome structural integrity, the *mei-9*$^+$ gene function is apparently important particularly during S and G_2 of the cell cycle and that of the *mei-41*$^+$ gene during the S and G_1.

A morphometric approach to the molecular architecture of mitochondria

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Mitochondria were isolated from rat liver according to established procedures. Samples were taken for measuring biochemical activities: respiratory rates with different substrates, ATPase activity, content of cytochromes of the respiratory chain. Aliquots of the same mitochondira were prepared for electron microscopy and their structural data estimated by means of morphometry applying advanced stereological procedures. By relating the morphometric with the biochemical measurements precise estimates of some parameters regarding the molecular buildup of mitochondria could be obtained: the 'flow' of O_2 per mitochondrial volume and surface in different metabolic states, the rates of ATP/ADP exchange per volume and surface area, and the densities and average distances of cytochromes in the inner mitochondrial membrane.

Partial purification of an immunosuppressive factor released by human glioblastoma cells

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Glioblastoma patients show impaired T cell mediated immunity, as evidenced by cutaneous anergy, diminished numbers of T cells and impaired lymphocyte blastogenic responsiveness. Supernatant fluids from cultured human glioblastoma cells contain both an interleukin-1 like activity and a 97.000 m.w. factor which suppresses the lectin response of mouse thymocytes, the generation of cytotoxic T cells in mixed lymphocyte cultures, and the interleukin-2 dependent growth of cloned T cell lines [Fontana et al., J. Immunol., in press]. We aim to purify and characterize this factor because it may be responsible for an escape of glioblastoma cells from T cell mediated antitumor mechanisms. We developed a rapid and quantitative assay which measures suppressor activity of

chromatographic fractions even if they contain salt. In a three-step procedure, the suppressor factor could then be separated from the bulk of the protein and from the interleukin-1 like activity.

Conserved sequences far upstream in the 5′ flanking region of the adult α globin genes of *Xenopus laevis*

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The globin gene family of *Xenopus laevis* is an attractive model of cell and stage specific gene expression. These genes are arranged in two clusters (I and II) each containing larval (L) and adult (A) α and β genes. The corresponding globin genes of each cluster are expressed coordinately and a complete switch from larval to adult globin gene expression occurs during metamorphosis.

To detect putative control regions of globin gene expression we determined the nucleotide sequence within 1 kb 5′ to the α_I^A and α_{II}^A genes. We found a relatively conserved region (83% homology) from the cap site to position − 290 and three boxes of homology (87–95%) further upstream, each separated by more diverged sequences (< 50% homology) or regions representing either deletions or insertions.

We also compared the 5′ flanking sequence of the α_I^A gene from erythrocytes of an adult frog where the gene is expressed with the corresponding sequence from tailbud embryos where the gene is not yet expressed. Within 640 nucleotides 5′ to the cap site the sequences were identical exept for 10 base changes presumably due to polymorphism. This suggest that no sequence rearrangement occurs within the putative 5′ control region of the α_I^A gene between the embryonic and the adult stage.

A unique host cell protein is involved in the interferon type specific antiviral response of mouse cells towards influenza viruses

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Interferons (IFNs) induce in responsive cells the synthesis of severel proteins and the establishment of an antiviral state. The relative roles of these proteins in inhibiting particular viruses is largely unknown. To analyse this, we have used mouse cells that differ at a gene locus responsible for the antiviral action of IFN-α/β against influenza viruses (alleles Mx^+ and Mx^-). IFN-α/β (but not IFN-γ) rendered Mx^+ cells (but not Mx^- cells) resistant against influenza virus replication and induced in Mx^+ cells beside the usual set of proteins an additional unique protein of Mr 78,000. By immunizing Mx^- mice with extracts of IFN-α/β treated cells from congenic Mx^+ mice, we obtained antibodies specific for the Mr 78,000 protein. A 2.5 kb mRNA was identified that was able to direct the *in vitro* synthesis of this protein. We are now in the process of cloning the relevant cDNA.

Establishment of epithelial cell cultures from human small intestine

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A method is described for the establishment of primary cultures of human small intestinal epithelial cells. Starting material is foetal small intestinal mucosa or mucosal biopsies. The method initially involves collagenase digestion followed by culture at 37°C in an atmosphere of 5% CO_2 in air. The medium routinely used is Dulbecco's MEM, 4.5 g/l glucose, 4 mM glutamine, 10% NU-serum, 50 U/ml penicillin/streptomycin, 5 µg/ml amphotericin B.

The isolation of the epitheloid cells may be achieved by use of cloning cylinders or film-lined dishes. Alternatively the proliferation of fibroblasts may be supressed by the use of horse serum (10%) and/or D-Valine Dulbecco's MEM.

Intestinal epitheloid cells have been maintained in culture for up to 6 months. They form uniform sheets of polygonal cells. Preliminary results have indicated that the cultured cells contain at least one brush-border enzyme (aminopeptidase N).

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Complete primary structures of the four light-harvesting polypeptides from *Rhodopseudomonas sphaeroides* R-26.1

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Four low molecular weight polypeptides have been isolated and purified from chromatophore membranes of *Rp. sphaeroides* R-26.1 by a combination of gel filtration and ion-exchange chromatography in organic solvents. On dodecyl sulfate polyacrylamide gels, the purified polypeptides comigrate with bands LH-1, LH-2 and LH-3 known to be related to the antenna pigment protein complexes. The complete primary structures have been elucidated by automated Edman degradation of the intact polypeptides and of overlapping C-terminal fragments obtained after chemical cleavage at tryptophan and methionine residues. The sequences, when used together with data obtained from other species of purple non-sulfur photosynthetic bacteria, illustrate a common structural principle of this class of integral membrane proteins (see Abstracts by Brunisholz et al. and Zuber et al.).

Activation of the vitellogenin locus in oocytes by injection of purified estrogen receptor protein from *xenopus* liver

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Partly purified estradiol receptor protein from Xenopus liver nuclei was injected into Xenopus oocytes. It activates transcription at the silent vitellogenin locus which is under estradiol control in the liver. Albumin genes, whose expression in liver is suppressed by high concentrations of estradiol are not activated. We have analyzed the expression of the vitellogenin B2 gene: transcription starts within the 5′end region and probably continues to the 3′-third of the gene. The transcripts accumulate to high levels after 3–4 days; however, most of them seem to be inaccurately processed. Correspondingly, new proteins, the largest of these running close to vitellogenin on SDS gels, are made and secreted by the injected oocytes.

Mouse complement C4 gene duplications correlate with the testosterone-independent expression of the sex-limited C4 isotype S1p

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Sex-limited protein (S1p) is biochemically and genetically closely related to the fourth component of murine complement (C4). The expression of S1p is inducible (testosterone-depen-

dent) in several strains and constitutive (testosterone-independent) in others. Both S1p and C4 are encoded in the S region of the murine major histocompatibility complex. We have examined the number of gene copies and the restriction site polymorphism in this genetic region using C4 clones obtained from a 28S liver cDNA library. The murine C4 gene family consists of two copies per haploid genome in the common mouse strains, including strains with the S1p-negative phenotype. Three strains in which S1p expression is constitutive all have multiple gene duplications within the S region. Thus, the S1p genes cloned from these mutants in testosterone control provide a powerful tool for studying the molecular aspects of androgen regulation.

Calcitonin gene-related peptide (CGRP) in the human thyroid, pituitary and brain

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Alternative splicing of calcitonin (CT) RNA resulting in the production of CGRP in neural tissue and of CT in the thyroid has been proposed by Rosenfeld et al. (Nature *304*, 129, 1983) in rats. We have analyzed CGRP and CT with non-crossreacting and specific RIAs in man. The CGRP content of the periventricular mesencephalic region and of the thyroid was comparable (mean ± SE, 1.84 ± 0.12 and 6.85 ± 1.25 ngeq/g wet tissue, respectively), whereas pituitary glands and medullary thyroid carcinomas contained higher levels (20.0 and 7660 ± 5420 ngeq/g, respectively). CT concentrations of the same tissues were 0.26 ± 0.09, 146 ± 26, 2.4, and 6.8 ± 3.7 × 10^5 ngeq/ml, respectively. The predominant CGRP component had a mol wt of ~ 4000 on gel filtration analysis and a different retention time from previously characterized CT-gene products on HPLC. In conclusion, the identification of CGRP in the thyroid, pituitary and brain suggests that the production of CGRP is not tissue specific in man.

A highly conserved amino acid residue in human interferon α 2 can be mutationally replaced without loss of antiviral activity

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The human IFN-α gene family consists of at least 12 non-allelic members. Although the amino acid sequences they encode differ from each other up to about 20%, there are several positions in which all human α-IFNs, and even the distantly related human IFN-β, are identical, for example Phe^{48}. It is commonly believed that strict conservation of a particular amino acid implies that the latter is important for the function of the protein. Using synthetic mismatch-containing primers we converted the Phe^{48} codon of the human IFN-α2 gene to a Tyr, Cys or Ser codon. We found that E. coli transformed with wild type or any of the mutant IFN-α2 genes produced the same level of antiviral activity. We conclude that Phe^{48} is not required for antiviral activity and that the evolutionary conservation of the Phe^{48} codon is not due to selection for this function of the protein.

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

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Four distinct chloroplast DNA segments from *Chlamydomonas reinhardii* of 400, 415, 730 and 2300 bp which promote autonomous replication in yeast have been mapped on the chloroplast genome. Plasmids carrying these chloroplast DNA fragments are unstable in yeast when the cells are grown under non selective conditions. Sequence analysis of three of these chloroplast ARS regions (autonomously replicating sequences in yeast) reveals a high AT content, numerous short direct and inverted repeats and the presence of at least one element in each region that is related to the yeast ARS consensus sequence A/T TTTATPuTTT A/T. These three chloroplast regions share in addition two common elements of 10 and 11 bp which may play a role in promoting autonomous replication.

Creatine kinase (CK) isoenzymes in spermatozoa from chicken and man

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Washed chicken spermatozoa retain CK-activity (1.5 EU/mg protein), some of which can be extracted by Triton X-100. Two CK-isoforms, brain-type (BB-CK) and mitochondrial-bound (MiMi-CK) were identified by cellulose polyacetate electrophoresis and immunoblots using specific anti B-CK and anti MiMi-CK antibodies. ON SDS-PAGE MiMi-CK migrates between B-CK (M_r 42000) and M-CK (40000). BB-CK was localized by immunofluorescence within the spermatozoan tail excluding the head. Seminal plasma from man contains in contrast to chicken considerable BB-CK activity. BB-CK is also found in human spermatozoa freed of seminal plasma by percoll (Arcidiacono et al 1983, Int J Andrology, (in press)) centrifugation. The two CK-isoforms present in spermatozoa are an indication for a similar CP-shuttle as described in muscle (Wallimann and Eppenberger 1984, Muscle and Cell Motility Vol 7, Plenum Press, (in press)). BB-CK may be functionally coupled to the dynein ATPase, MiMi-CK to the ATP/ADP translocase. Sperm mobility seems to depend on presence of CK and CP.

Culture and morphology of human testicular tumor cells in semi-solid agar medium*

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Fragments of a human testicular tumor (embryonal carcinoma) grown in nude mice (NMRI-strain) were dispersed and cultivated as single cells in semi-solid medium. Small colonies (< 1 m) remained round, larger ones flattened out. Cell suspensions and fragments were prepared from single clones and replated in petri dishes with fresh medium. New tumor colonies grew during three months over five generations. Light- and electron microscopy of colonies showed structures similar to the tumors grown in nude mice and in the surgical specimen. The basic structure is epithelial, with cells displaying nuclei varying in shape, numerous mitoses, microvilli and partly differentiated junctional complexes. In the cytoplasm annulate lamellae were detected. No evidence of a basal lamina was found in colonies but was present in the tumor grown in nude mice. * Supported by the Krebsliga des Kantons Zürich.

An SV40 'enhancer trap' incorporates exogenous enhancers or generates enhancers from its own sequences

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We have transfected monkey CV-1 cells with non-infectious, linear SV40 DNA, lacking the 72 bp repeated enhancer region. Infectious virus was recovered from this 'enhancer trap' upon co-transfection with enhancer DNA segments from various viruses such as polyoma virus (from which a truncated en-

hancer was integrated as a dimer), cytomegalovirus and Rous sarcoma virus. In some transfection experiments without added viral enhancer DNA, SV40 variants were generated which have a segment of their flanking 'late' DNA duplicated to substitute for the deleted 72 bp repeat. The duplication creates a strong enhancer from this non-enhancing DNA region. Both the polyoma enhancer fragment and the spontaneously created enhancers lack the structural features which have been associated with enhancer elements, namely the 'GTGG(A/T)-box', the 'CACA-box', or stretches of alternating purines-pyrimidines.

Effect of an acidic environment on the biological activities of influenza virus

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Influenza virus requires an acidic environement for the infectious entry. To study the effect of low pH on infectivity, hemolysis and hemagglutination we treated influenza virus PR/8/34 at pH5 for increasing periods of time from 0°C to 40°C. Infectivity and hemolytic activity are rapidly reduced within 5 min at pH5 above 33°C. Hemagglutination is not affected under the same conditions for up to 60 min of incubation over the whole temperature range. These effects concide with a change in antigenicity of the hemagglutinin (HA) glycoprotein which is characterized by monoclonal antibodies. With an antibody recognizing HA after acid treatment the entry of the virus into the endosomal compartment, where the virus is uncoated by fusion with a membrane, was visualized by immunofluorescence microscopy. Thus the acid activation of influenza virus for fusion and hemolysis appears as a process with characteristic temperature dependence and kinetics, which can be correlated with the molecular conformation of the HA molecule.

Localization and dynamics of the membrane-bound α-MSH receptor

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Recent investigations reveal the importance of α-MSH in man. However, α-MSH receptors are still poorly characterized. Ternary conjugates of tobacco mosaic virus (TMV) with rhodamine and α-MSH interact specifically and almost irreversibly with receptors on cultured mouse melanoma cells (1981, 1983). They are potential reagents for studying the localization and internalization of MSH receptors. In these experiments, new conjugates, in which the α-MSH is linked to TMV with a disulfide bond, were used. They showed enhanced pharmacological potency in the tyrosinase assay and enhanced receptor binding. Reductive cleavage of the disulfide bond abolishes the receptor interaction. Our recent studies indicate an allosteric regulation of the receptor affinity for α-MSH via disulfide and thiol groups of the receptor. Furthermore, it was clearly shown that tyrosinase activation is independent of adenylate cyclase activation (cAMP production). Results will be discussed according to classical transduction concepts and in terms of our melanotropin-receptor regulation model.

Transcription signals of *Xenopus laevis* U snRNA genes

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Genes coding for snRNAs from *X. laevis* have been cloned and sequenced. By microinjection into oocytes it was shown that all the sequences necessary for faithful transcription of U1 and U2 snRNA are contained within 343 bp (U1) or 306 bp (U2). By Ba131-deletion studies of these snRNA genes the regions in the 5′ and 3′ flanking sequences important for faithful transcription have been delineated. Within the 82 base pairs of flanking sequence necessary for transcription of the U2 gene, i.e. for promoter activity, the *X. laevis* U1 and U2 snRNA genes show extensive homology. A sequence homologous to that reported by J. Dahlberg and E. Lund to be necessary for faithful human U1 snRNA transcription, is also located within this region in both genes. In the 3′ flanking sequence at a distance of about 10 bp from the end of the RNA coding sequence there is a region of homology conserved in all U snRNA genes sofar sequenced. By deletion analysis it was shown that removal of this sequence reduces the efficiency of generation of correct 3′ ends of the RNA transcribed from the injected U snRNA gene.

PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

Effect of diet and an oral caffeine dose on caffeine absorption and gastric emptying in the rat

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Pharmacokinetics studies showed that caffeine administered orally was rapidly and completely absorbed in fasted rats. The effect of diet has never been studied, while at doses as high as 100 mg/kg, Latini et al. (Toxicol. Letters, 1978, 2, 267) reported that intestinal absorption seemed to be affected.

Rats fed ad libitum different diets received orally a dose of 5, 20, 40 and 80 mg/kg labeled caffeine. Radioactivity in the stomach, small intestine, blood and expired CO_2 was measured.

The results showed that intestinal absorption did not seem to be impaired. The amount of radioactivity present in the stomach increased with the dose. For 5 and 80 mg/kg, 19 and 37%, 7 and 36%, 3 and 21%, 0.1 and 10% of the dose was recovered in the stomach after 2, 4, 8 and 24 h respectively. Kinetics of radioactivity of blood and CO_2 expired can be explained by the delayed gastric emptying observed. Dose dependent kinetics may not be due to limited capacity for caffeine metabolism but to an absorption dose effect.

Biochemical aspects of cardiotoxicity of methyl-2-tetradecylglycidate, a new hypoglycemic agent, in rats

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Based on the hypothesis of Randle's glucose-fatty acid cycle, methyl-2-tetradecylglycidate was introduced as a new type of hypoglycemic agent, acting by inhibiting fatty acid oxidation. 2 × 10 and 2 × 25 mg/kg in corn oil were given to female rats

by gavage for 4 weeks. Food and water were provided ad lib. Despite enlargement and severe fatty change of liver and kidneys, their mitochondria showed only slight deterioration of functions. Cardiomegaly and loss of muscle tonus were conspicuous and dose dependent. Heart mitochondria showed progressive uncoupling of oxidative phosphorylation and changes in oxygen consumption. Simultaneously their creatine-phosphate kinase was inhibited, and their creatine content increased, indicating a loss in membrane semipermeability. Concomitantly phosphilipid and triglyceride concentrations of heart tissue and mitochondria increased. Electronmicroscopic studies of myocardial tissue showed marked increase in lipid droplets, thinness of myofibrillar bundles, occasionally with dissociation of Z-bands.

Impaired elimination of triazolam in patients with cirrhosis of the liver

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Since administration of benzodiazepines (BZD) to cirrhotics (CI) may be followed by exaggerated pharmacological responses, ultrashort acting BZD might be clinically advantageous. The pharmacokinetics of triazolam were therefore investigated in 8 CI (56±SD 10 years) and as controls in 8 patients with minimal liver disease (MLD, 42±11). After 0.25 mg triazolam p.o. plasma concentrations were assessed for 8 h by capillary GLC with ECD and on-column injection. A Grob-type persilanized glass capillary column (15 m × 0.3 mm) coated with 0.4% OV-1701 was used together with a 1.5 m fused silica retention gap. Because of condensed by-products of the sample, the first 20 cm of the fused silica were cut off every 10 injections. The method was suitable for triazolam concentrations of 0.5 to 5 ng/ml. Half-lives were 11±SD 7 and 4±2 h and apparent oral clearances 155±62 and 322±198 ml/min in CI and MLD, respectively. It is concluded that in CI elimination of triazolam is impaired, and pharmacological effects may be prolonged.

Stimulation of adrenocortical and pigment cells by α-MSH

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α-MSH stimulates pigment dispersion in melanophores of cold-blooded vertebrates, pigment production in melanoma cells and steroidogenesis in rat adrenocortical cells. The EC_{50}'s of α-MSH for pigment cells are 2.5×10^{-11} M (Rana pipiens), 2×10^{-10} M (Anolis carolinensis), 5×10^{-10} M (Xenopus laevis) and 2.5×10^{-9} M (Cloudman S-91 mouse melanoma) as compared to 5×10^{-8} M for rat adrenocortical cells. The long-lasting and potent pigment-dispersing analogue [Nle^4, D-Phe^7]-α-MSH is inactive in the adrenocortical cell assay in concentrations up to 2.5×10^{-6} M. Cyclic[Cys^4, Cys^{10}]-α-MSH which has been reported to be superpotent in the Rana assay (Sawyer et al., Proc. Natl. Acad. Sci. USA *79:* 1751, 1982), was slightly less active than α-MSH in the Anolis and the mouse melanoma assay, and 100 times less potent in the adrenocortical assay. These and additional experiments suggest that α-MSH acts through ACTH-type receptors in adrenocortical cells, and that the MSH-receptors on different pigment cells are not identical.

Early changes of the rat adrenal medulla induced by nicotine

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A model is presented to assess quantitatively early biochemical, hyperplastic and hypertrophic alterations in rat adrenals. *S.c.* administration of nicotine (4 mg/kg/day) first caused a twofold increase in dopamine-β-hydroxylase (DBH) activity after 1 week, followed by a marked increase of endogenous catecholamine (CA) levels (107% increase in norepinephrine, 18% increase in epinephrine) and DBH activity (by 116%) after 6 weeks. At that time hyperplasia and hypertrophy were demonstrated by stereological determination of total chromaffin cell number (increase by 21%) and total cell volume (increase by 55%), whereas the numerical density of chromaffin cells per reference volume was decreased (by 22%). Thus, stimulation of the adrenal medulla by nicotine leads to an increased CA biosynthesis, followed by proliferative activity of chromaffin cells.

Antibody against rat metallothionein

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Metallothioneins are small MW proteins which physiologically bind zinc or copper. The rate of synthesis of metallothioneins (Mt) is enhanced by heavy metals such as cadmium. Mt may play a role in the transport of Cd from liver to kidney. In order to investigate the renal glomerular and tubular handling of Cd-Mt, a very sensitive method for quantitative measurement of the protein is needed. We therefore undertook to set up a radioimmunoassay for this protein. Liver Mt was obtained from rats exposed to Cd (500 μg/kg · day, 30 days), and purified by high speed centrifugation (110000 g), G-75SF Sephadex gel filtration, and ion exchange chromatography. Two isometallothioneins containing respectively, 102 and 280 μg/mg protein were obtained. These proteins were injected into rabbits, and sera were tested 2 months later for detection of antibodies, by immunoblotting. The antisera contained antibody recognizing very specifically metallothioneins. This antibody was absent from preimmune serum. The antisera obtained are currently used for the development of a Mt RIA.

Ontogeny of beta-adrenergic binding sites in rat brain

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The development of beta-adrenergic receptor sites was studied in fetal and postnatal rat brain by biochemical techniques and by *in vitro* autoradiography. In both cases the beta-adrenergic antagonist ^{3}H-dihydroalprenolol (^{3}H-DHA) was used as a ligand. Non-specific binding was defined as binding in the presence of 330 nM (±)oxprenolol. Stereospecificity of binding to forebrain homogenates was demonstrated with isoproterenol and propranolol. Scatchard plots of ^{3}H-DHA binding in immature rats yielded a single straight line, with a dissociation constant (Kd) of 3.39 nM. Binding data of ^{3}H-DHA in fetal rats (gestational day (GD) 19 3/4) indicate that the Kd does not change during development.

Beta-adrenergic binding sites were detected in forebrain homogenates first at GD 15 3/4. With the autoradiografic technique, significant specific binding to some regions could already been detected at GD 15. The time of appearance of this binding site coincides with the developmental stage when catecholaminergic fibers innervate forebrain target areas.

Stimulation of DNA synthesis in rat liver by carcinogens with different modes of action

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In order to investigate the role of the stimulation of cell division for the initiation (and possibly promotion) of liver tumors by chemical carcinogens, the incorporation of radiolabelled thymidine into liver DNA was determined in male rats. Single doses of various levels of aflatoxin B_1, benzidine and carbon tetrachloride (all known to be genotoxic via DNA binding), and of gamma-hexachloro-cyclohexane (HCH), did not affect cell division, whereas several hepatocarcinogens known *not* to bind to DNA (alpha-HCH, clofibrate, phenobarbital and 2,3,7,8-tetrachlorodibenzo-p-dioxin) gave rise to a dose-dependent stimulation of liver DNA synthesis within 24 h. An equation combining the influences of mitotic stimulation (expressed as dose required to double the control level of DNA synthesis) and DNA binding potency (expressed as the Covalent Binding Index) correlated well with the carcinogenic potency for both classes of hepatocarcinogens.

Sex-linked genotoxicity and metabolism of senecionine in the rat

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Senecionine, a common member of the structurally diversed pyrrolizidine alkaloids, has been found in the medicinal herbs *Petasites hybridus* L. and *Tussilago farfara* L. Retronecine-labelled (^{3}H)-senecionine with a high specific radioactivity was prepared biosynthetically with seedlings of *Senecio vulgaris* L. using (2,3-^{3}H)-putrescine as precursor. Covalent binding of (^{3}H)-senecionine to liver DNA of male and female Sprague-Dawley rats was determined 6 hours and 4 days after oral administration. The effectiveness of covalent binding, expressed in the units of a 'Covalent Binding Index' (CBI), was found to be 49 and 49, respectively, for male, and 210 and 196, respectively, for female rats. Female rats excreted significantly more of the metabolite 19-hydroxy-senecionine in the urine than male rats. These results give further evidence that senecionine is a genotoxic carcinogen and support results of a study, in which female SD rats showed a higher sensitivity to long-term feeding of a senecionine-containing alkaloidal plant extract.

Genetically determined oxidation of β-adrenoceptor blocking drugs. Stereoselective metoprolol metabolism and concentration-effect relationship

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The oxidation polymorphism (debrisoquine type) is a major determinant of the oxidation of β-adrenoceptor blocking drugs (Lancet 1, 509, 1982). The influence of different phenotypes on stereoselectivity of metoprolol metabolism and on its effect was investigated in selected extensive (6) and poor (4) metabolizers. Direct measurements of (−) and (+) isomers in plasma were achieved using ion pair HPLC and correlated with racemic mixture measurements ($r = 0.995$) and with a radioreceptor assay ($r = 0.954$) using soluble guinea pig lung receptors. Genetically determined metabolism is stereoselective: the (−)/(+) ratios 3 hrs after an oral dose are between 1.01 and 2.27 and correlate with debrisoquine (rho = −0.79) or bufuralol (rho = −0.78) metabolic ratios. However, determination of the active (−) isomer or use of the radioreceptor assay do not improve the overall correlation between concentration and effect.

Abnormal novelty-oriented responses in spontaneous hypertensive (SHR) and normotensive control (WKY) rats

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Novelty-oriented responses such as latency to approach novel object (white-colored cube) in unfamiliar open-field, duration of object exploration, ambulation, rearing, grooming and defecation were investigated in spontaneous hypertensive rats (SHR), their normotensive controls (WKY) and standard laboratory Wistar rats (Tif: RAIf). Remarkable differences in behavior of all three strains were observed. By comparison to RAIf rats, SHR showed less neophobia in the presence of novelty. WKY rats distinguished themselves from SHR and RAIf by high level of anxiety leading to almost total supression of all behavioral responses in the presence of novel object. This behavioral supression was antagonized by 7.5 mg/kg i.p. of chlordiazepoxide. The results of this study indicate that, by comparison to standard laboratory rats, both SHR and WKY show, possibly genetically determined, alterd behaviors which are diametrically opposite to each other and perhaps due to impaired central noradrenergic functioning.

Postsynaptic densities (PSDs) contain N-methyl-D-aspartate (NMDA)- and quisqualate-sensitive L-glutamate binding sites

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Receptors for the excitatory amino acid neurotransmitters have been classified into 4 types which are selective for (1) NMDA, (2) quisqualate, (3) kainate and (4) L-2-amino-4-phosphonobutyrate (APB). Studies using isolated synaptic membranes (SPM) have revealed 2 populations of L-[^{3}H]-glutamate binding sites, the major one dependent on Cl^- and Ca^{2+} and resembling the L-APB receptor class, and a smaller component independent of Cl^- and Ca^{2+}. In PSDs (prepared from SPMs by Triton X-100 treatment) the Cl^-/Ca^{2+}-dependent, APB-sensitive class of binding sites is absent. However, L-glutamate binding sites in PSDs can be separated into 2 types based on their sensitivity to (1) NMDA ($K_i = 12$ μM) and (2) quisqualate ($K_i = 1$ μM). Other agonists overlap into both types, in agreement with their mixed actions as determined electrophysiologically. This is the first demonstration of distinct NMDA and quisqualate receptor-like recognition sites using an L-glutamate receptor binding technique.

Effect of triazolam on flicker sensitivity at different frequencies

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Psychophysical methods allow accurate assessment of various functions in the peripheral visual system, which may reflect changes in transmitter concentrations. Thus, they could serve as models for changes induced by psychoactive drugs. In a double blind, placebo controlled study with volunteers ($n = 13$), effects of the benzodiazepine triazolam, 0.25 mg, p.o. were assessed on the threshold for perception of flickering of a sinusoidally modulated white light and on critical flicker fusion (CFF). Measurements were made before and 1.5 h after drug intake. After triazolam the threshold rose from a modulation of 7.2 to 10.2% at 40 Hz, from 2.3 to 3.4% at 15 Hz, from 2.8 to 4.1% at 10 Hz and from 5.6 to 8.5% at 5 Hz. When change for the placebo and drug group were compared, $p < 0.05$ re-

sulted for 5 and 30, but not 15 and 10 Hz. CFF was not systematically influenced. It is suggested, that triazolam may have frequency-specific effects on visual perception.

Effects of anesthetics on the baroreceptor reflex of the rat

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We have investigated the effects of the anesthetics urethane (U), pentobarbital (P), Dial (urethane plus allobarbital, D) and chloralose (C) on the reflex bradycardia following pressor responses to i.v. phenylephrine (PE) in adult male normotensive, Wistar rats. Basal diastolic arterial pressure (DAP, mmHg, femoral artery cannula) and heart rate (HR, bpm) values were U 62 ± 23, 413 ± 75, P 90 ± 17, 399 ± 36, D 74 ± 19, 334 ± 29, and C 113 ± 15 362 ± 49 (n = 4/group). Doses of PE required to induce a 50 mmHg increase in DAP were U 6+(−2, P 0.2 ± 0.1, D 3 ± 2, and C 6 ± 4, moles × 10−8/kg. Decrease in HR following a 50 mmHg increase in DAP were U 46 ± 6, P 65 ± 10, D 26 ± 7 and C 56 ± 10.
Our results show that U and D attenuate the baroreceptor flex when compared to P.

Vascular reactivity in hypertensive and normotensive rats

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It has previously been reported that the spontaneously hypertensive rat (SHR) presents an increased vascular reactivity to various vasoconstrictor agents. We have investigated whether such increased reactivity is linked to the genetic origin or the hypertension of the SHR. We compared the vasoconstriction produced by electrical field stimulation, noradrenaline or potassium in isolated perfused/superfused tail arteries from SHR, inbred Wistar-Kyoto (WKY), outbred Wistar (Ico: WI) and outbred Sprague Dawley (Ico: OFA.SD) male rats of 3 months of age. Maximal responses to field stimulation were as follows SHR > WI = SD = WKY, noradrenaline SHR = SD > WKY = WI, and potassium SHR = SD = WKY > WI. ED50s for electrical stimulation were as follows SHR = WKY = WI = SD, noradrenaline SHR = SD = WKY > WI and potassium SHR = SD = WKY > WI.
We conclude that the increased vascular reactivity of the SHR, when estimated on the basis of maximal vasoconstriction only, is linked to high blood pressure.

Genotoxicity of *Symphytum* pyrrolizidine alkaloids in somatic cells of *Drosophila*

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Pyrrolizidine alkaloids (PAs) are natural plant products and several are known to be mutagenic in *Drosophila*. The diastereomeric alkaloids of *Symphytum officinale*, 7-acetylintermedine (AIN) and 7-acetyllycopsamine (ALY) were separated by preparative HPLC and studied for their genotoxic effects in mitotically active cells of *Drosophila melanogaster*. Senkirkine (SNK) and seneciphylline (SNP) were used as reference PAs. The PAs (conc. 0.05 mM) were fed to 72 h old *Drosophila* larvae. These were trans-heterozygous for the 2 recessive wing cell marker mutations *mwh* (3-0.0) and *flr* (3-39.0). After metamorphosis, mutational events induced in larval cells were recognizable on the wings on the flies as marked single clones (*mwh* or *flr*) or as twin spots (*mwh*//*flr*). All 4 PAs were significantly genotoxic ($P < 0.01$), with the following rates of clone induction per mitotic cycle: SNK 146×10^{-5}, SNP 54×10^{-5}, AIN 39×10^{-5}, ALY 8×10^{-5} (values corrected for the spontaneous rate of 4×10^{-5}).

Effects of adenosine on calcium movements in the depolarized taenia of the guinea-pig caecum

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Adenosine (AD) receptor activation seems to reduce the availability of calcium ions (T.W. Stone, 1982, Bioscience Reports *2*:77). The effects of AD on the calcium contraction of high K^+ (low Na^+) depolarized preparations, as well as on the uptake and release of ^{45}Ca, were studied in the guinea-pig taenia coli. AD (10^{-4}–10^{-3} M) inhibited the calcium contraction of high K^+-depolarized preparations, but did not change the high K^+-stimulated calcium influx measured by 3–6 min exposures to ^{45}Ca. In efflux experiments, ^{45}Ca-loaded tissues were first washed for 60 min in non-labelled Krebs, then transferred to high K^+-Krebs with or without calcium. After 50 min, AD increased the ^{45}Ca fractional rate of efflux by 24±4% (mean±SEM, n = 6) in the absence of external calcium, and by 6±1% (n = 4) in the presence of calcium. It is concluded that, in the taenia, stimulation of calcium extrusion, but not inhibition of calcium influx, may be part of the mechanism of adenosine-induced relaxation.

Pitrazepin, a novel $GABA_A$-antagonist

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Pitrazepin is a potent new antagonist which differs from bicuculline in its chemical structure and its interactions with both ^3H-muscimol and ^3H-flunitrazepam binding sites, whereby the potency of pitrazepin in displacing ^3H-muscimol exceeds that of bicuculline by at least a factor of 10. In physiological experiments, blockade of synaptically released GABA by pitrazepin was shown to reduce inhibitory postsynaptic potentials and resulted in the onset of bursting activity which persisted for hours following drug application. The effect of pitrazepin was not tissue-specific since it induced a bursting discharge pattern in cultures derived from hippocampus and hypothalamus. Bursting activity was abolished by GABA, baclofen, and nembutal, but only weakly reduced by midazolam. Pitrazepin also antagonized the action of exogenous GABA, but failed to influence the action of baclofen.

^3H-Imipramine and ^3H-cyano-imipramine binding in rat brain tissue: effect of chronic antidepressant administration

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1) High affinity binding of ^3H-imipramine (^3H-IMI) and ^3H-cyano-imipramine (^3H-CN-IMI) was compared in rat brain tissues. 2) IMI and desimipramine (DMI) were administrated (21 days; 10 mg/kg/day) to groups of rats and then, possible drug effect on ^3H-IMI, ^3H-CN-IMI and ^{125}I-cyano-pindolol binding was determined in rat whole-brain-homogenates. Whereas the regional distribution was similar, a significant difference in KD values, but not in Bmax values, was found, depending on the ligand used to label 5HT-uptake sites. When compared to control rats, chronic treatment with IMI or DMI did not induce any change in Bmax values for ^3H-IMI and ^3H-CN-IMI, but, transient increases in apparent Kd-values were determined. Down-regulation of β-adrenergic receptors after chronic IMI confirmed the propriety of drug adminis-

tration. The present findings are discussed in respect to previous reports, which have been highly contraversial. (Supported by «Schweiz. Nationalfonds»; grant 3.911.0.82).

Effects of muscarinic agonists and pirenzepine in the hippocampal slice preparation

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In coronal hippocampal slices of rat brains, we have tested the action of oxotremorine, acetylcholine, muscarine and RS 86 applied in the bath. The population spike amplitude in the CA_1 layer induced by stimulating the statum radiatum near the CA_3 cell layer, and the extracellular potentials (spikes) were measured. In small doses (e.g. starting with 0.5 µM for muscarine) the population spike amplitude increased and decreased distinct under the control amplitude applying higher doses (e.g. 3 µM for muscarine). The pD_2-values of the dose-response curves obtained from the spike rate measurements were: oxotremorine 7.70±0.06 (4), acetylcholine (in the presence of 1 µM eserine) 5.72±0.23 (4), muscarine 5.55±0.19 (10) and RS 86 5.76±0.24 (4). When pirenzepine 1 µM was applied in the bath the population spike amplitude did not increase when the muscarinic agonists were applied in lower doses and did not decrease at higher doses.
The increase in the spikerate induced by the four muscarinic agonists tested was blocked by pirenzepine.

Noradrenaline desensitizes the isolated rat tail artery (TA) to nerve stimulation

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Exposure to agonists can induce desensitization of the end organ involved; we have tested this hypothesis in the TA preparation. TA's were removed from normotensive, Sprague-Dawley rats and perfused/superfused with Krebs solution containing cocaine, dl propranolol, indomethacin and ascorbic acid. They were either field stimulated for 15 sec (supramaximal voltage, 0.3 msec, 1 to 30 Hz) or exposed to various concentrations of alpha adrenoceptor agonists (AA: noradrenaline and phenylephrine) for 2 minutes. Alpha 1 AA and field stimulation induced dose-related contractions. Alpha 1 AA – at concentrations above the ED 50 of 10^{-7}M – blocked field stimulation by up to 80% for 30 minutes. Thereafter responses recovered. The alpha 2 AA, guanfacine, had no effect on the TA (up to 3×10^{-6}M).
We conclude that short exposure to alpha 1 AA desensitizes the TA to nerve stimulation.

Inhibitory effects of Zy 15028 (Clobenoside) on inflammation and generalized edemas in rats

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Zy 15028 (Clobenoside 1-O-ethyl-3-O-propyl-5,6-di-O-p-chlorobenzyl-D-glucofuranose) is a congener of a known anti-inflammatory agent, tribenoside (Glyvenol®). This compound was used to evaluate it for its anti-edema and anti-inflammatory effects in a variety of experimental test models with different pathogenesis. Zy 15028 inhibited D-galactosamine and dextran induced edemas, when administered either by i.p. (300 mg/kg) or by oral (1500 mg/kg) route in rats. It produced dose dependent inhibitory effects in carrageenin edema at a relatively lower dose range (240 to 750 mg/kg p.o.). However, it failed to inhibit the genesis of ovalbumin edema. Anti-edema mechanisms of Zy 15028 appeared to be similar to that of tribenoside, either by inhibiting the release and/or antagonizing the actions of histamine and/or kinins. Zy 15028 appeared to be relatively more potent than tribenoside.

Characterization of the human hepatic cytochrome P450 isozyme which causes polymorphic debrisoquine (De) and bufuralol (Bu) metabolism

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In microsomes from human liver biopsies hydroxylation of De and Bu corresponded to the *in vivo* hydroxylation phenotype. The cytochrome P450 mediated formation of 4-OH-De and 1'-OH-Bu was 1.33±0.82 $nmol \cdot mg^{-1} \cdot 15\ min^{-1}$ (n = 9) and 4.2±1.0 $nmol \cdot mg^{-1} \cdot 60\ min^{-1}$ (n = 32), respectively, and was markedly reduced in two poor metabolizers (Meier P.J. et al., Gastroenterology 85, 682, 1983; Minder E. et al., in press). Several isozymes of cytochrome P450 were purified from human kidney donor livers and bufuralol hydroxylation was measured after reconstitution with NADPH cytochrome P450 reductase and phospholipids. Isozymes with high activity are being further characterized to define the molecular genetic defect causing altered metabolism of this class of drugs.

In vitro evidence for two types of vascular α-adrenoceptor (α-R)

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While in vivo experiments have yielded convincing evidence for the existence of α_1- and α_2-R in arterial blood vessels, the demonstration of the two α-R subtypes under in vitro conditions was met with variable success. Concentration-response curves were obained in strips of rabbit main pulmonary artery exposed to the following α-agonists: methoxamine (α_1-selective), the azepine derivative B-HT 920 (α_2-selective), the two imidazoline derivatives clonidine (preference for α_2-R) and St 587 (α_1-selective). Contractions to B-HT 920 or clonidine, but not to methoxamine or St 587, were highly susceptible to the inhibitory effect of the Ca antagonist verapamil or to Ca deprivation. Since contractions in response to the structurally related imidazolines clonidine and St 587 differed in their dependence on extracellular Ca, the involvement of two α-R subtypes rather than that of a unitary α-R with two recognition sites (phenylethylamines, imidazolines) is suggested. Results of experiments with α-R antagonists support this conclusion.

Interactions of excitatory amino acids and their antagonists with membrane potentials of cat caudate neurons

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Membrane potentials were recorded in halothane anaesthetised cats during iontophoretic application of drugs and stimulation of the cortico-caudate pathway. Agonists elicited three types of responses (Herrling et al., J. Physiol. 339: 207, 1983): type I, depolarizations with regular firing, was induced by glutamate (GLU), quisqualate (QUIS) and kainate (KA); type II, where action potentials occurred on depolarizations of ca. 30 mV with abrupt onset and end, was induced by N-methyl-D-aspartate (NMDA) and quinolinic acid (QUIN); the last type was intermediate, type I components predominating and was induced by aspartate (ASP) and cysteine sulfinic acid (CSA). The antagonists AP-5 and AP-7 reversibly inhibited the effects of NMDA and QUIN but not those of other agonists or the cortically evoked EPSP. γ-glutamyl-glycine inhibited

NMDA > KA only. Kynurenic acid inhibited NMDA > KA = QUIS and the cortically evoked EPSP reversibly and specifically.

Assay for somatic point mutations resulting in metallothionein (MT) protein variants isolated from the liver of rats treated with the carcinogen diethylnitrosamine (DENA)

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As a further development of an *in vivo* assay for somatic point mutations induced by genotoxic carcinogens resulting in altered amino acid composition [Jauch and Lutz, Chem.-Biol. Interact. *46* (1983) 139] a more sensitive system on the basis of MT was investigated. MT contains no aromatic amino acids but 20 CYS residues coded for by DNA triplets able to be mutated to codons for aromatic amino acids. Male rats were treated with the ethylating hepatocarcinogen DENA; controls received no carcinogen. All animals were then given p.o. 10 μCi/kg [^{14}C]CYS, and 15 mCi/kg each of [^{3}H]PHE and [^{3}H]TYR. After 6 h, MT was isolated from the liver and extensively purified. After acid hydrolysis and collection of CYS, PHE and TYR from an HPLC system, the [^{3}H]/[^{14}C] ratio was determined for each animal. In accordance with model calculations, the carcinogen-treated rats indeed hat incorporated more erroneous aromatic amino acids.

Effects of phosphate and sodium on calcium influx in mammalian non-myelinated nerve fibers

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The movements of ^{45}Ca in rabbit vagus nerve were studied by a new method which allows continuous monitoring of the radioactivity of the preparation (Jirounek et al,, J. Physiol., *334*, 117P, 1982). The results show a large increase of calcium uptake when sodium was replaced by choline, indicating that in this preparation the Na-Ca exchange mechanism plays an important role in the regulation of the cellular calcium.
In presence of extracellular sodium, an increase in calcium influx is also observed after addition of phosphate (0.1–1 mM), whereas in the absence of Na this effect of phosphate is almost completely abolished. As it is known that the influx of phosphate depends on the extracellular Na, the observed increase in calcium influx can be explain by a modification of the internal phosphate pool, rather than by a direct effect of the extracellular phosphate concentration.

Choline acetyltransferase and acetylcholinesterase in organotypic cultures of rat septum and hippocampus

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Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activity in the hippocampus originates to a great extent in axons from cholinergic neurons located in the medial septum. In the rat, the development of ChAT and AChE in the hippocampus takes place during the first few weeks after birth. We show that organotypic cultures of fetal rat septum develop ChAT and AChE during the first 4 weeks *in vitro*. Thus it appears that cultured septal cholinergic neurons differentiate even without making any contact with their target cells. Explants of postnatal rat hippocampus cultured together with septal explants become innervated by cholinergic fibers coming from the septum, as shown by AChE histochemistry. The innervation of the hippocampus seems to be specific, since the cholinergic axons from the septum do not grow into cerebellar explants.

Hypothalamic α_2- and β-adrenoceptor rhythms regulate circadian feeding: evidence from chronic methamphetamine treatment and withdrawal

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The circadian regulation of food intake in rats is mediated through a bimodal rhythm of β-adrenoceptor binding in the lateral hypothalamus and a unimodal rhythm of α_2-adrenoceptor binding in the medial hypothalamus. Chronic methamphetamine treatment provides evidence for this functional connection: β-adrenoceptor binding in the lateral hypothalamus is down-regulated at dusk, together with reduction of food intake; α_2-adrenoceptor binding in the medial hypothalamus is up-regulated at dawn, together with persistent food intake. Long-term changes in these two adrenergic systems regulate homeostasis of food intake: 24-hour mean β-adrenoceptor binding is reduced and α_2-adrenoceptor binding is increased upon methamphetamine withdrawal, when rebound feeding occurs. Corticosterone, although normally coupled to adrenergic control of feeding, is phase delayed after chronic methamphetamine treatment.

Metabolic activation of aldrin in extrahepatic cells

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Aldrin is a selective substrate for cytochrome P-450 dependent monooxygenases. In extrahepatic cells, alternatively or in addition, aldrin might be converted to dieldrin by a prostaglandin endoperoxide synthetase (PES)-dependent cooxydation. Therefore the influence of indomethacin (IM) and arachidonic acid (AA) on the aldrin epoxidation was determined. In freshly isolated intact mesenchymal rat cells derived from a subcutaneous granulation tissue and kept in culture for 4 days, dieldrin formation was determined by capillary gas chromatography. IM (100 μM) incubated together with aldrin decreased the dieldrin formation by 90 percent. AA (10 μM) increased the activity 2.5 fold. These results indicate that in extrahepatic rat cells, the oxidative conversion of xenobiotics can be linked with the PES activity.

Direct determination of R- and S-metoprolol in biological fluids by ion-pair-HPLC

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Metoprolol is a beta-blocking agent sold as a racemate. The S-isomer is the active moiety. Its main elimination pathway is through oxidative metabolism, which has been shown to be genetically determined. We have developed an ion-pair-HPLC assay for the direct determination of metoprolol isomers in biological fluids.
Dichloromethane is used as the organic mobile phase, with propanol as a modifier and (+)-10-camphor-sulfonic acid as a chiral counter ion. The separation is performed on a Lichrosorb-Diol (5 μm) packing and a fluorescence detector is used to monitor the effluent at an excitation wavelength of 227 nm. Alprenolol is used as an internal standard.
The standard curve is linear over the range of 5–500 ng/ml for each isomer. The limit of measurement is 5 ng/ml for one ml samples. For concentrations above this limit the coefficient of variation is less than 10%. In various pharmacokinetic studies correlation coefficients between this assay and racemate determinations with classical HPLC were above 0.99.

VIP acts synergistically with norepinephrine (NE), histamine and certain ergot derivatives to increase cAMP levels in mouse cerebral cortical slices

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We have recently observed that VIP and NE act synergistically to increase cAMP in mouse cerebral cortex (Soc. Neurosci. Abstr. *9*, 715, 1983). The mechanism of this synergism is the potentiation by NE, via alpha-adrenergic receptors, of the stimulatory effect of VIP on cAMP levels, since the synergism was antagonized by phentolamine but not propranolol and mimicked by phenylephrine but not isoproterenol. The EC_{50} of NE for this effect is approximately 5 μM. We have observed that histamine and ergotamine, an ergot derivative acting at alpha-adrenergic receptors, also act synergistically with VIP to increase cAMP in mouse cerebral cortex. We are currently investigating the identity of the histamine receptor subtype and the pharmacological profile of the ergot derivatives that may interact synergistically with VIP to stimulate cAMP formation.

Chronic caffeine attenuates the effect of pargyline in rat brain

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Caffeine is one of the most widely consumed drugs, ingested in beverages or in OTC drug formulae. Nevertheless, its mechanism of action in the CNS is still unclear. It has been reported that serotonin may play a role in its pharmacology and marked interactions have been shown between caffeine and monoamine oxidase inhibitors (MAOI).

We have studied the interaction of chronic caffeine administration (0.1% in the diet for 8 days) with the MAOI pargyline. On the 8th day, rats received a 75 mg/kg dose of pargyline i.p. 45 min. before sacrifice. The levels of 5-HT, 5-HIAA, DA, NA, DOPAC and HVA were determined in the locus coeruleus (LC), raphé dorsalis (RD) and the VMT-A10 area by a HPLC method.

Caffeine alone provoked no effect in the LC, and increase of 5-HT and 5-HIAA in the RD and a decrease of 5-HT and 5-HIAA an A10. As expected, in the 3 nuclei, pargyline alone markedly increased the levels of 5-HT, DA and NA and depleted completely DOPAC and HVA but 5-HIAA by 50% only. The effects of pargyline were decreased in rats fed the caffeine containing diet as compared to pargyline treated controls, as if caffeine partially antagonized pargyline.

The phosphodiesterase inhibitor Ro 15-2041 inhibits the increase and accelerates the decrease of cytosolic Ca^{2+} in thrombin stimulated platelets

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Stimulation of platelets leads to an increase of cytosolic Ca^{2+}. These changes can be monitored by the fluorescent Ca^{2+}-indicator Quin-2. Adenylate cyclase stimulators, which elevate cAMP and inhibit platelet function, have been shown to inhibit the increase and to accelerate the decrease of Ca^{2+}. We investigated if this holds also true for the phosphodiesterase inhibitor Ro 15-2041, which elevates cAMP and is a potent, broad-spectrum platelet function inhibitor. We demonstrated in gelfiltered human platelets 1) a dose-dependent increase of cytosolic Ca^{2+} after thrombin stimulation (0.03–1.0 U/ml), 2) a strong and dose-dependent inhibition of this increase by Ro 15-2041 (0.04–5 μM) added 1 min *before* thrombin and 3) a dose-dependent acceleration of the decrease of Ca^{2+} by Ro 15-2041 (0.2–5 μM) added 1 min *after* thrombin (0.3 U/ml). *Conclusion:* Ro 15-2041 amplifies the capacity of platelets to maintain Ca^{2+} homeostasis.

Decrease of β-adrenergic responsiveness during in vitro maturation of rat reticulocytes

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Rat reticulocytes but not mature red cells formed cAMP at a rate of 111 pmoles/10^7cells/10 min if maximally stimulated by the β-adrenergic agonist isoprenaline. Within a 3 h period of in vitro culture, receptor-stimulated cAMP formation dropped by 40–50%. This rapid loss was followed by a slow phase resulting in a further reduction of cAMP formation to 23±9% of the initial value after 5 days in culture. Under identical conditions, the decrease of β-adrenoceptor density in intact cells from 16.8±2.5 fmol/10^7 cells to 9.0±1.0 fmol/10^7 cells followed a similar biphasic time course. Stimulating cAMP formation with forskolin, a receptor-independent activator of adenylate cyclase did not change the kinetics of the decay of enzyme activity. These results suggest that loss of receptors or decoupling between receptor occupation and cyclase activation do not represent initial steps in the loss of hormone responsiveness in maturating reticulocytes.

Acute venous stasis edema in rats: pharmacodynamic studies with HR (O-β-hydroxyethyl-rutoside)

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An increase in venous pressure in the rat tail is known to result in acute edema (H. Nordmann and O.P. Gulati, Meth. and Find. Exptl. Clin. Pharmacol. 5 (6), 347–355, 1983). Acute venous stasis edema of the rat tail was induced by applying a force-controlled banding of standard tension (200 g) proximally for a period of 6 to 12 h. Lowering of tail circulation to 40% to the control is followed by prolonged vascular disorder characterized by genesis of reversible edema, increased total blood flow to the tail and decreased local cutaneous blood flow, without affecting the general hemodynamic. It is shown here that HR (0.5 g/kg b.w. i.v. before edema induction) has a beneficial effect on a 12 h edema ($p < 0.05$); it decreases the total tail blood flow 3 h after the removal of a 7 h ligature ($p < 0.02$); it increases the local cutaneous blood flow from the 1st to the 7th min after the removal of a 8 h ligature ($p < 0.05$). The general hemodynamic is not affected by the compound.

Pharmacological factors affecting dopamine synthesis, storage, metabolism and release in rabbit retina in vitro: an LC-EC study

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We have initially investigated by liquid chromatography coupled with electrochemical detection (LC-EC) the catecholamine content in freshly isolated rabbit retina and found that dopamine (DA) was the principal catecholamine. We have subsequently attempted to influence DA content and metabolism in intact retina incubated for 40 min at 35° in oxygenated Krebs-Ringer containing various factors. It was found that: a) exogenous L-tyrosine (100 μM) in the presence of pteridine cofactor and exogenous L-dopa (10 to 100 μM) increased tissue levels of L-dopa and DA, respectively; b) reserpine (100 μM) largely depleted DA stores; c) MAO inhibitors of A- (pargyline) and B-type (deprenyl) differentially affected DA

and DOPAC levels. These studies confirm the usefulness of isolated rabbit retina for investigations in vitro related to dopaminergic mechanism(s) and DA-receptors.

Evaluation of different test conditions and cell strains for cytotoxicity testing of chemicals in vitro

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Test conditions for the assessment of the cytotoxic potential of new chemicals were optimized and standardized. A quantifiable and reliable assay using routine cell culture techniques for BHK 21-C13 cells (Reinhardt et al., Toxicology *25*, 47, 1982) and human skin fibroblasts was developed. So far more than 40 chemicals of different classes (metal salts, solvents, detergents, reagents, drugs) were tested for their effect on the parameters 'cell detachment', 'growth' and 'cloning efficiency'. 'Cell detachment', the least sensitive parameter, allows morphological control of the cells. 'Growth' could be most reliably measured after 2 days subconfluent culture. 'Cloning efficiency' proved to be the most sensitive but least reliable parameter. The human fibroblasts were often less sensitive in the 'cell detachment' assay, possibly due to their stronger adherence to the culture dish.

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Clorgyline (a MAO type a inhibitor) modifies retinal light responses

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Rat retinal photoreceptor disk-shedding and autophagic degradation exhibit an endogenous circadian rhythm. A single light pulse stimulates these metabolic parameters, continuous light dampens the rhythms. Chronic clorgyline treatment dampens both the rhythm and the response to a light pulse, without any phase shift. Retinal dopamine synthesis also undergoes circadian variations, and is rapidly stimulated by a light pulse. In the light phase, DA synthesis is sixfold higher in clorgyline-treated rats. DA levels are increased, as is rhythm amplitude, without any phase shift. Thus clorgyline may mimic the parametric effect of light on retinal metabolism and dopaminergic neurotransmission, but not its effect on circadian phase.

Role of hormones on the renal handling of indomethacin

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The influence of hormones on the excretory function of the kidney is scarcely known. We thought of interest to study the renal handling of Indomethacin (Ind) in male and female rats. Adult Wistar rats (♂11, ♀17) were anesthetized and tracheotomized. Carotid artery and jugular vein were cannulated for blood sampling and for administration of drugs respectively. A single bolus of Ind was given (10 mg/kg) and plasma half-lives for elimination (t½el) were estimated. The effect of probenecid, an inhibitor of the renal secretion of Ind, was tested as well as the effect of testosterone given to female rats.

Female eliminated Ind at lower rate than male rats (t½el 50.6±4.8 vs 31.1±2.6 min $p < .01$). Probenecid increased t½el in both male and female rats to the same extent (55.9 vs 55.5%). Female rats testosterone-treated had t½el similar to male rats (38.2±2.9 and 35.2±1.7 min). Our results are suggestive of a major role of the sexual hormones on the renal secretory mechanisms.

^{3}H-GABA uptake by oligodendrocytes in primary culture

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Glial cells have been suggested to be involved in the metabolism of amino acid transmitters following their release. To differentiate between neuronal and glial uptake of GABA and to investigate the role of oligodendrocytes in its metabolism we studied ^{3}H-GABA uptake into dissociated cultures by autoradiography. Major cell types were identified and quantified using antisera against specific markers. At 12DIV GABA uptake was predominantly into neurons and oligodendrocytes. At 28DIV only oligodendrocytes were heavily labelled. There was a moderate labelling of all flat cells with heavily labelled astrocytes only rarely seen. Oligodendroglial uptake was confirmed by antibody mediated cytotoxicity against cerebroside positive cells. Oligodendrocytes accumulated GABA more rapidly than astrocytes but slower than neurons. Uptake was inhibited by DABA but not by β-alanine and was thus similar to neuronal uptake. It was Na^+ dependent and ouabain sensitive but only slightly enhanced by AOAA. In contrast glutamate and aspartate were only accumulated by astrocytes.

Monoamine metabolites in fetal and postnatal rat brain: effect of nicotine

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In order to assess the functional state of central monoamine systems in pre- and postnatal rat brain, we determined monoamine metabolites by HPLC-EC and catecholamines by radioenzymatic assay. The normal developmental pattern of DOPAC, HVA, MOPEC, 5 HIAA was studied in Sprague Dawley and Long Evans rats during late gestation and early postnatal life; it was essentially similar in male and female animals. All four compounds were detected in forebrain at the earliest stage studied, i.e., gestational day 17. A single s.c. injection of nicotine (1 mg/kg) caused an increase in DOPAC and MOPEG in forebrain of postnatal Sprague Dawley rats; preliminary data point to a similar effect in late gestation. The effect of chronic drug administration is presently being investigated. Our results indicate that noradrenergic and dopaminergic systems of the fetal and early postnatal rat brain are responsive to nicotine.

Specific influences of nifedipine and verapamil on the time course of the action potential of automatic cardiac Purkinje-cells

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Detailed analysis of the time course of the transmembrane potential under control conditions showed that some of the variables maximum diastolic potential, rapid upstroke and amplitude and duration of the plateau potential are related to each other which allowed to reconstruct action potentials. An early and a late part of diastolic depolarization were discriminated. At 10 µmol/l verapamil and nifedipine exerted quite similar effects, whereas considerable differences of effects were noted at 1 µmol/l. When considering the above mentioned relations, results suggest that effects on the ionic membrane currents depend on concentration. The qualitatively different mode of action of these two compounds at 1 µmol/l underlines their specificity in therapeutical use. The effects of verapamil at 1 µmol/l could partly explain the antiarrhythmic properties of this compound.

Receptor-induced phosphoinositide-breakdown is not altered by Ca channel blockers

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Hormones and neurotransmitters that are known to elevate cytoplasmic Ca^{2+} concentrations also cause a rapid hydrolysis of phosphoinositides (PI). The rat parotid gland was used to study PI-responses of α-adrenergic, substance P and muscarinic cholinergic receptors present in this preparation. Stimulated PI-hydrolysis was blocked competitively by a variety of receptor antagonists.

A population of neurons from rat brain grown in culture showed PI-hydrolysis when stimulated with muscarinic agonists. Ca channel antagonists or agonists did not alter PI-metabolism in these systems. This suggests that the product of PI-hydrolysis is causing changes in cytoplasmic Ca^{2+} by a mechanism independant of Ca^{2+} fluxes accross the plasma membrane.

Long-term motor activity recording of dogs in different environments

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A recently developed wrist worn activity monitoring device has been applied in human subjects (Borbély et al., Schweiz. med. Wschr. *111*, 730–735, 1981). Our objective was to test the feasibility of its application in long-term measures of motor activity of dogs and to evaluate the effects of enclosures differing in size, structure and possibility of interaction with other dogs. Motor activity of 4 well adapted dogs was measured for 6 weeks with the monitor worn on a collar around the neck. On Monday of each week the dogs were placed in a different enclosure for 7 days. Mean total activity count per 24 h over 7 days of individual treatment was calculated. Each dog was characterized by an individual daily motor activity pattern. This pattern varied according to the day of the week, depending on general activity in the laboratory. Total weekly activity and daily pattern depended on enclosure conditions.

An alternative in vitro method for the assessmet of drug induced aberrant embryonic development

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Mammalian embryos can be grown in vitro during the early stages of organogenesis (New Biol. Rev., *53*, 81, 1978). 18 drugs of diverse chemical characteristics were used to investigate the response of rat embryos in vitro to xenobiotics known to affect embryonic development in vivo. An S_9-mix from adult rat livers was used as the activating source (Schmid et al., Toxicol. *25*, 53, 1982). All substances tested induced specific malformation patterns, which occurred at concentrations well below those affecting embryonic growth and differentiation, yolk-sac growth and blood circulation. The results indicate a good correlation between in vitro and in vivo findings. The system can thus be used as a relevant alternative method to current whole-animal testing, offering considerable savings in terms of numbers of animals, time and costs.

Possibility of accumulation of dextromethorphan in genetically poor metabolizer phenotypes

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In view of the previously established genetically determined correlation between rates of debrisoquine and dextromethorphan (DEM) metabolism the main metabolites of DEM were examined in 13 poor (PM) and 13 extensive (EM) DEM metabolizer phenotypes (similar in age and sex). DEM and metabolites were measured in 8-hour urine specimens after an oral dose of 25 mg (71 µmol) using GLC (XE-60 column) with a PN-detector. Output of DEM per 8 hours was 0.16±SD 0.11 and 2.1±1.9 µmol in EM and PM respectively. The corresponding outputs of metabolites were: O-desmethyl-DEM (dextrorphan) 34±9 and 0.85±0.91 µmol, O- and N-desmethyl-DEM (hydroxymorphinan) 10.7±5.4 and < 4 µmol in EM and PM respectively. The sums accounted for 64±18 and 4.3±3.4% of the dose. It is concluded that t.i.d. administration of DEM exposes PM patients to the possibility of drug accumulation.

Inhibition by LTB_4, LTC_4 and LTD_4 of the effect of bradykinin on the pain reflex in the isolated rabbit ear

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The lipoxygenase-products LTB_4, C_4 and D_4 (LT) dose-dependently (≥ 25 ng/ml) reduce the nociceptive effect of intra-arterial injection of 400 ng bradykinin (BK) in the perfused isolated rabbit ear.

This desensitizing effect of the LTs on the perivascular pain receptors was observed even in the presence of 40 ng/ml PGE_2 or 200 ng/ml 5-HT. The leukotriene-antagonist FPL 55 712 was able to block the antinociceptive effect of the LTs with a concentration of 2 µg/ml.

The results imply that in contrast to PGs which sensitize pain receptors for BK, lipoxygenase products like LTB_4, C_4 and D_4 antagonize the algesic action of BK.

Presynaptic adrenoceptors ($\alpha 2$) modulation of neurotransmission in a desert mouse vas deferens

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Neurotransmission was studied on the prostatic half of the vas deferens of the desert mouse *(Meriones ungiculatus)* by imposing a double field stimulation (10 Hz for 1 sec) at 0.5 sec intervals every minute. This stimulation gave two contractions; the first being larger than the second. A catecholamine component was present in the response since after a reserpine pretreatment the second peak was larger than the first. Prazosin, a postsynaptic adrenoceptor antagonist, did not modify the contractions, whereas with the presynaptic antagonist, yohimbine, the second contraction became larger than the first. Clonidine, a presynaptic adrenoceptor agonist, strongly depressed the responses, especially the first peak. The presynaptic adrenoceptors on the vas deferens of the desert mouse can be divided into two components; one is acting continuously (i.e. tonically) and the other acts phasically and is expressed with the stimulation of the nerves.

Effects of the tricyclic antidepressant desipramine (DMI) on growing and stationary fibroblast cultures

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Chronic treatment of cultured human fibroblasts with 10 µM DMI induced a significant increase of DNA content in confluent cultures. In addition a remarkable increase of the total phospholipids (PL)/mg protein was noted, suggesting drug induced PL storage. 3 and 6 days exposure of proliferating and confluent fibroblast cultures with 5, 10, and 20 µM DMI resulted in a lowered protein and DNA content in proliferating cultures, but in a remarkably increased one in confluent cultures. PL accumulation was found in young and in old cul-

tures. 3H-Thymidine (THYM) incorporation into DNA of normal and drug treated non proliferating fibroblasts showed a dose dependent depression of the incorporation after a 24 h period. 3H-THYM preincubation of the cultures increased at 1.5 h but decreased at 24 h the 3H-THYM incorporation depending on the DMI dose. The findings suggest that DMI induces DNA synthesis on stationary cell cultures possibly by induction of the de novo synthesis of thymine.

Contribution of the variants of acid alphal-glycoprotein (AAG) to the binding of antidepressive drugs

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Antidepressive drugs like amitriptyline (At) and nortriptyline (Nt) are bound to AAG in plasma. This glycoprotein has two types of genetic polymorphism: 1. the polymorphic forms show four different patterns with 5, 6, 7 or 8 bands – 2. the variants show three different patterns with two bands: FF, SS, FS.

In 28 depressive patients treated with 150 mg At daily, free and total At and Nt, and also AAG were determined in plasma. The polymorphic forms and variants of AAG were assayed by IEF.

The results show that the free fraction of At and Nt correlates negatively ($p < 0.02$) with the concentration of the S form, but not of the F form of the variants. It is suggested that the binding site of these tricyclics on AAG is located in a region of the peptide which determines the S form of the variants.

Chronic methamphetamine induces phase dispersion in circadian rhythms of imipramine binding

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Specific binding of imipramine is considered to be a parameter linked to presynaptic serotonin uptake mechanisms. Circadian rhythms of imipramine binding occur in many rat brain regions (e.g. the suprachiasmatic nuclei, frontal and occipital cortex, lateral hypothalamus, preoptic area, caudate putamen, raphé nuclei, brainstem). Although slightly differing in wave form, they share a common phase minimum at dusk and a broad maximum at dawn. Chronic methamphetamine treatment has no effect on 24-hour mean imipramine binding in any brain region, but induces a dispersion of phase such that minima and maxima throughout the brain occur at different times of day. Thus imipramine binding exhibits a spatio-temporal coherence throughout the brain that may be important for serotonergic function, and this coherence is disrupted by a psychoactive drug.

GENETIK – GÉNÉTIQUES – GENETICS

The influence of roasting-procedures on the formation of genotoxic compounds in coffee

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Recently, mutagenic activity in several strains of *Salmonella typhimurium* has been found in many heat-processed foodstuffs. We investigated the mutagenic activities of 4 different coffee varieties (different species, different origins: Coffea arabica Santos, Coffea arabica Columbia, Coffea robusta Indonesia, Coffea robusta Camerun). The mutagenic activity of all samples was within the same range (20 mg freeze-dried powder prepared from aqueous coffee extracts induced a number of revertants which was between 6–10 times the value of the negative controls). Green coffee beans showed no mutagenic activity. Mutagenicity increased with roasting time (Probat roasting procedure; 220 °C) up to 4 minutes and then remained constant. No further increase in mutagenicity was observed at the normal roasting time of 8 minutes. These data indicate that genotoxic compounds are quickly formed at 220 °C. Mutagenic activity was independent of roasting procedure (Jetson vs Probat).

Chromosomal localization of the transcobalamin II gene and linkage with the hemoglobin α-chain locus in mice

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Transcobalmin II, the vitamin B12 binding protein in mouse serum, is a secretory protein in cell culture. TCN-2 expresses a genetic polymorphism, which is determined by two common alleles s and f. TCN-2 typing by PAGE in recombinant inbred strains (CXS) and in 'spent' medium of mouse-chinese hamster somatic cell hybrids, provisionally placed the *Tcn-2* gene on chromosome 11 (Mouse News Letter 69: 56, 1983). Subsequent investigations in six selected backcross generations confirmed the preliminary findings and established linkage of *Tcn-2* with two loci on chromosome 11: the hemoglobin α-chain locus *(Hba)* and waved *(wa-2)*, both proximal to the centromere, have recombination rates of 14% and 7% with *Tcn-2*. The fact that the two adjacent loci, *Tcn-2* and *Hba*, also have functional similarities (both bind to cofactors with a porphyrin-like structure) may have evolutionary significance.

Mutagenicity of hydrazine derivatives in somatic cells of *Drosophila melanogaster*

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Hydrazine (HZ) and some of its derivatives have been tested in the somatic mutation and recombination test in wings of *Drosophila melanogaster*. 72-h-old larvae trans-heterozygous for the wing hair mutations *multiple wing hairs* (*mwh*, 3–0.0) and *flare* (*flr*, 3–39.0) were fed with the chemicals for 6 h or for 48 h until pupariation. Induced mutational events can lead to the following clones on the wings: *mwh* single spots, *flr* single spots, and twin spots with a *mwh* and a *flr* area.

The chemicals tested are: HZ, 1,1-Dimethyl-HZ, 1,2-Dimethyl-HZ, Phenyl-HZ, Carbamyl-HZ; the mushroom toxin Gyromitrin with its metabolites Methylformyl-HZ and Methyl-HZ; the drugs Hydralazine, Procarbazine and Isoniazid. Most of them show genotoxic activity in our assay; however they are not or onyl weakly mutagenic in bacterial test systems.

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'In vivo' decoding properties of *Drosophila* $tRNA^{His}$ and codon usage in highly and low expressed mRNAs

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The tRNAs specific for asp, asn, his, and tyr of most organisms contain either an unmodified guanosine (G) or the hypermodified nucleoside queuosine (Q) in the wobble position of the anticodon. We have previously shown that the presence or absence of Q can influence the decoding properties of $tRNA^{Tyr}$ in a special situation. These results suggested that the Q modification might affect the normal decoding properties of the four Q-base containing tRNAs. In the present communication we show that in a *Xenopus* oocyte assay, under competing

in vivo conditions, the *Drosophila* $tRNA^{His}$ with the anticodon GUG clearly prefers the codon CAC, whereas the $tRNA^{His}$ with the anticodon QUG slightly prefers the codon CAU. We discuss these findings in terms of optimal codon-anticodon interaction and codon usage in highly and low expressed mRNAs.

Molecular cloning of homeotic genes from *Drosophila* using DNA sequence cross-homology

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Homeotic genes are involved in the control of developmental pathways and the specification of body segments. At least 30 such genes have been mapped and a few have recently been cloned. We have found that three of these cloned genes, *Antennapedia, Ultrabithorax* and *fushi-tarazu,* share a weakly cross-hybridizing DNA sequence. The sequence of the cross-hybridizing region shows it to be a conserved protein-coding domain. We have used the cross-homology to isolate other clones from a *Drosophila* genomic library. The cytogenetic map positions and spatial patterns of expression of genomic sequences homologous to two of the newly isolated clones suggests that they represent other homeotic genes. Our results support the notion that some of the homeotic genes of *Drosophila* form a highly diverged gene family.

Bacterial plasmid-based test system for in vitro studies of mutagen-DNA interactions

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Induced DNA-lesions and mutation fixation were studied in a plasmid-based test system. Plasmid pUC8 was exposed in vitro to the mutagens hydroxylamine, ethyl-nitrosourea, dimethylsulfate or cisplatinum-II-diammine-dichloride. Biochemical analysis showed mutagen-characteristic electrophoretic mobility shifts and differential reactivities to DNA-interacting enzymes (nuclease S1, topoisomerase II and restriction enzymes); biological analysis showed characteristic transformation patterns (loss of plasmid-coded amp® and $lacZ^+$ phenotypes) in E. coli. A 'forced mutant cloning scheme' was used to measure mutation frequencies at restriction sites in the plasmid target genes. Premutational lesions were correlated with mutations fixed after passage through a biological system by using sequence analysis of individual progeny plasmids as well as restriction patterns of in vitro treated plasmid DNA's. The system proved ideal to study mechanistic aspects of both, mutation induction and mutation fixation.

Osteogenesis imperfecta (OI): increased posttranslational modification of abnormal type I collagen

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OI comprises a heterogeneous group of heritable disorders characterized by bone fragility and other generalized connective tissue abnormalities. We report results of studies in 3 patients with OI whose skin fibroblasts synthesized 2 species of α1(I) chains of type I collagen. On SDS-PAGE, one α1(I) chain was normal; the other chain had lower mobility and was designated α1(I)'. All peptides produced by human collagenase or CNBr from the α1(I)' chain had decreased electrophoretic mobility. However, in the presence of the iron chelator, α,α'-dipyridyl, the cells synthesized only normal α1(I) chains. Thus, the studies demonstrate that the abnormal α1(I)' chains in some patients with OI is due to increased posttranslational modification probably as a result of disturbed/delayed helix formation.

Abnormal α2(I) chain in type I collagen from a patient with Ehlers-Danlos syndrome (EDS) type VII

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Collagen extracted from skin or produced by skin fibroblasts from a patient with EDS type VII contained regular and abnormal α2(I) chains in a 1:1 ratio. The abnormal chains migrated more slowly on SDS-PAGE. Cleavage of pepsinized collagen with human collagenase and/or CNBr suggested that the additional peptidyl material was located close to the N-terminus, probably in the α2(I) CB4 peptide. Since both parents were healthy and produced only normal collagen, the structural mutation in this sporadic case is caused by a new mutation similar to that reported (Steinmann et al., J. biol. Chem. *255:* 8887–8893, 1980).

Relationship between number of vibrissal follicles (VFs) and number of their myelinated sensory fibers (SFs). A study in three new strains of mice: NOR, MCP and M/M

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In NOR, bred for normal whiskerpads (WPs), each WP has 33 VFs and a mean of 3318 SFs (SD 62). In MCP, bred for supernumerary whiskers (SWs) at particular loci in a restricted part of the WP, these values are 39–41 and 4058 (SD 60); in M/M, bred for maximum nrs of SWs, 40–45 and 4494 (SD 143). Total nr of SFs innervating the standard set of VFs in MCP is 3670 (SD 99); in M/M, 3977 (SD 101). Within a strain, these values are independent from the nr of SWs, whereas the total nr of SFs innervating the extra VFs is linearly related to the nr of SWs. Wherever tested, the innervation of the extra VFs was expressed as an enlarged cortical representation (the barrelfield) each extra VF having its own barrel. The greater innervation of the standard VFs in the enriched WPs was not reflected in a larger total area of the standard barrels. Total nr of WPs analyzed: 39. Support: Swiss NSF 3.238.