Union Schweizerischer Gesellschaften für Experimentelle Biologie

Berichte der 14. Jahresversammlung

Union des Sociétés Suisses de Biologie Expérimentale

Comptes rendus de la 14e Réunion annuelle

Union of Swiss Societies of Experimental Biology

Abstracts of the 14th Annual Meeting

Interlaken, 1./2. April 1982

Unterstützt durch die Schweizerische Naturforschende Gesellschaft
über die Schweizerische Kommission für Experimentelle Biologie

PHYSIOLOGIE - PHYSIOLOGY

Spatial interactions of prolonged potentials in barnacle photoreceptors

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By a suitable choice of wavelengths, the visual pigment of the barnacle photoreceptor can be converted from one stable state R to a 2nd stable state M, or back. Conversion of a large fraction of R to M leads to a prolonged depolarizing afterpotential (PDA). The PDA can be blocked by an 'Anti-PDA' produced by converting the same pigment molecules or their neighbors from M to R (Hillman et al., J. gen. Physiol. *68*, 227, 1976). What is the maximum range of this interaction between PDA and Anti-PDA? The pigment molecules are in the membrane of microvilli which are arranged in groups. In each group all the microvilli are parallel. Polarized light converted most of the molecules in certain groups but not those in other groups. Within 1 min there was little or no interaction between neighboring groups which are on average about 6 μm apart. I conclude that mediators of the interaction must either be confined within the microvillar group, or have a diffusion coefficient $< 10^{-9}$ cm^2/sec.

Cellular actions of hypothalamic factors: inhibition of cytoplasmic proteases?

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To explain the higher ACTH-releasing potency of median eminence extracts (ME) versus AVP, we tested their actions on proteases in a cell-free system. The supernatant of an anterior pituitary saline homogenate (AP) was incubated at 37 °C and pH 7.4 for 20 min. The bioassayable ACTH content of 1 AP (256±31 ng, mean±SEM, n=4–6) decreases exponentially by 69±5%. Only part of this degradation is non-enzymatic since a boiled extract loses only 14±7% of its ACTH content. AVP (10 mU) and skeletal muscle extracts (2 mg) have no effect on the degradation ($p > 0.3$), but the presence of ME and cortex extracts (2 mg) evoke an increase of 51±4% and 37±10%, respectively, of ACTH content over control. In the presence of 10 microdissected ME (total weight equal to 1 ME), increase of ACTH content over control is only 34±19%. The results indicate that brain extracts contain antiproteases, but suggest that these are not a component of corticotropin releasing factor.

Dopamine synthesis in nonneural pancreatic islet cells

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Monoamines are found in pancreatic β-cells. Their function in those cells is unknown. Dopamine (D), noradrenaline (NA) and adrenaline were measured in freshly isolated or cultured islets of rats or *Acomys cahirinus*. Insuline synthesis and release were similar before and after culture. In both species, NA content decreased by ⩾93% after 2 days in culture. The addition of L-DOPA did not restore NA, but caused a large increase in D content (up to 300 times the fresh islet values). The decrease of catecholamines was not due to tyrosine lack. Thus, the 2-days cultured islets have no sympathetic nerve terminals, but can synthesize D from L-DOPA. Ultrastructurally, the endocrine pancreas of *Acomys* – in contrast to rat – has been claimed to lack sympathetic innervation. However, although fresh *Acomys* islets contain 3 times less NA than rat islets, they have nonendocrine NA synthesizing elements. The modification of endogenous D in cultured islets should prove useful in studies of its function in β-cells.

Insulin response to vagal stimulation

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Neural modulation of insulin secretion occurs via the vagus nerves, partly but not exclusively through their coeliac branches, as described by other behavioral data. We have studied the effect of selective vagotomies on the well-described insulin release upon cervical vagal electrical stimulation in the anesthetized rat. Transsection of both subdiaphragmatic vagal trunks abolished the insulin response to right vagal stimulation, as did atropine treatment. Transsection of either the coeliac or gastric trunks significantly reduced the response while hepatic vagotomy induced no change. Left vagal stimulation resulted in a response which could be abolished by double subdiaphragmatic vagotomy or atropine; gastric or coeliac or hepatic vagotomy each significantly decreased the insulin output. The accompanying hyperglycemia was mimicked in control experiments and accounted only partially for the observed hyperinsulinemia.

Effects of chronic hypoxia on maximal aerobic performance of trained individuals

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In the course of a mountaineering expedition to the Himalayas the independent effects of altitude exposure and training on maximal aerobic power ($\dot{V}_{O2}$max) was determined on 7 subjects (25–33 years). $\dot{V}_{O2}$max was assessed by extrapolating to maximal heart rate (HR max) individual HR/$\dot{V}_{O2}$ functions determined at 5 work levels: a) at sea level (s.l. = 350 m) before departure (active untrained, U, with hematocrit Hct = 44.3±1.6%); b) at 3200 and 5200 m (trained, T, with Hct = 50.5±2.9%); c) at 350 m 10 days after return (T, Hct = 48.8±4.3%). HR max was the same (181±13.5 and 181±10) at s.l. before and after training, while it dropped to 0.91 and 0.82 of the control at 3200 and 5200 m, respectively. Average $\dot{V}_{O2}$max at 5200 m was 37.9±3.2 ml · kg^{-1} · min^{-1}, i.e. 0.84 and 0.76 of the s.l. U and T controls, respectively. At 3200 m $\dot{V}_{O2}$max was 0.96 and 0.87 of U and T, respectively. It is concluded that the effect of chronic hypoxia per se on $\dot{V}_{O2}$max of acclimatized individuals is identical with that of acute hypoxia.

Channel gating at denervated rat endplates

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During the development of vertebrate endplates, the gating of the acetylcholine (ACh) activated ion channels mediating neuromuscular transmission becomes more rapid. This change is controlled by the innervating neuron. At rat ectopic junctions, it is reflected by a shortening of the mean channel open time from about 4 to 1 ms. To further examine the neural influence, we have measured the mean channel open time in rat muscles a) at ectopic soleus endplates whose development was interrupted by denervation before channel gating had changed; the soleus nerve was allowed to reinnervate the muscle at the original endplate sites, b) at endplates of the diaphragm denervated for more than 2 weeks. Analysis of ACh-induced membrane current noise showed in both sets of experiments the

appearance and persistence of rapid gating, respectively, which indicates that the neuron does not change gating by direct modification of channel behavior.

Inhibition of T_4-induced metamorphosis by telepaque administration to *Xenopus laevis* tadpoles

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In 1977 we demonstrated by radioimmunoassay an increase in plasma levels of iodothyronines during metamorphosis of *Xenopus laevis*. The increase in T_3, particularly prominent during midclimax, was shown to result essentially from extrathyroidal 5'-monodeiodination of T_4 to T_3. Telepaque (iopanoic acid) considerably reduces this transformation in *Xenopus* tadpoles. Nonmetamorphosing tadpoles treated with perchlorate (0.1% in aquarium water) underwent normal metamorphosis after immersion in a T_4 solution (3×10^{-8} M). Daily i.p. injection of 40 ng telepaque (b.wt approx. 0.6 g) slows down the T_4-induced metamorphosis (8 days of delay). On the other hand telepaque does not inhibit metamorphosis induced by T_3 (2.5×10^{-9} M). Telepaque treated nonmetamorphosing tadpoles show normal T_4 levels in the plasma and in the carcass. On the contrary T_3 is considerably reduced in the carcass. So T_4 must be deiodinated to T_3 in order to exert its metamorphosic actions.

Effect of pH on rabbit intestinal phosphate transport

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A Na-dependent phosphate (P_i) transport system is located in the brush border membrane of rabbit duodenum. Brush border membrane vesicles (BBMV) from rabbit duodenum were prepared by a Mg^{2+}-precipitation method and the effect of pH on the P_i transport system was investigated. P_i uptake in the presence of Na increased when the external pH was decreased. The Na specificity of the P_i transport system was preserved as the uptake of P_i in the absence of Na was only 5–10% of the uptake in its presence at all pHs (K for Na). When inside negative K diffusion potentials were generated in the presence of valinomycin, the P_i uptake was strikingly increased at pH 6, moderately only at pH 7.4 and slightly, if at all, at 8.1. The results appear to suggest that both, the mono and the divalent forms of P_i are transported into BBMV by the Na-dependent P_i transport system and that at acidic pH the Na-P_i-carrier complex bears a net positive charge.

The effect of ischaemia on surface and intracellular pH in rat soleus muscle fibers

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Surface pH (pH_s) and intracellular pH (pH_i) have been simultaneously recorded with single and double-barrelled glass microelectrodes on superficial fibers of the isolated rat soleus muscle superfused in vitro. The superfusate was buffered to pH 7.3–7.4 with 5% CO_2/21 mM HCO_3^- at 37 °C. Under conditions simulating ischaemia, i.e. when the superfusion flow was stopped and the extracellular volume was reduced having the preparation immersed in paraffine oil, pH_s decreased quickly, by $2.36 \pm 0.55 \cdot 10^{-2}$ pH/min ($\bar{x} \pm$SEM, n = 6) while pH_i was slowly reduced, by $0.77 \pm 0.20 \cdot 10^{-2}$ pH/min. A progressive depolarization, $+0.73 \pm 0.17$ mV/min, could be observed. These effects were quickly and fully reversible on reperfusion. Further experiments were performed with 3 metabolic inhibitors (2 mM NaCN, 1 mM Na-iodoacetate, cold). The results illustrate the influence of intracellular production of CO_2 and lactic acid on pH_i and, by subsequent diffusion to the extracellular space, on pH_s.

Protein phosphorylation may regulate the Ca^{2+}-activated K^+ conductance in snail neurones

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Internal perfusion of neurons from the land snail *Helix roseneri* with catalytic subunit (C-subunit) of the cAMP-dependent protein kinase enhances the slow, Ca-dependent K outward current (Ic). The amplitude as well as the rate of activation are increased. Perfusion of neurons with DTNB (5,5'-dithiobis (2-nitrobenzoic acid))-inactivated C-subunit is without effect. Both increase in amplitude and decrease in activation time can be mimicked by perfusing neurons with a solution containing 10^{-5} M Ca^{2+} ions. In contrast, none of those effects appears if the Ic is reduced either by lowering external Ca^{2+}, or by adding EGTA to the perfusate. We conclude that the C-subunit of the cAMP-dependent protein kinase may phosphorylate a regulatory component of the Ca^{2+}-activated K^+ channel such as to increase its affinity to Ca^{2+}.

Effects of hemodilution on the maximal aerobic performance at high altitude (Himalaya)

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The effects of hemodilution induced by mouth administration of 2 l in 45 min of an isotonic solution (Isostar®) on maximal aerobic power (V_{O2}max) was investigated on 4 subjects after 4 weeks exposure to altitudes between 5200 and 6200 m when the mean resting hematocrit (Hct) was $54.6 \pm 1.4\%$. V_{O2}max measured by an indirect method based on heart rate assessment during a continuous step protocol decreased from an average of 38.7 to 35.6 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} (individual ΔV_{O2}max values from -5.1 to -1.2), along with a 2.4% decrease of exercise Hct (individual differences from 0 to -4.7%). For a given maximal work load the drop of V_{O2}max was compensated for by an increased anaerobic energy release as indicated by a higher blood lactic acid accumulation ($\Delta = +2.3$ mM). It is concluded that hemodilution is not advantageous for maximal performance at high altitude, probably due to heart overload following blood volume expansion.

Are there multiple overlayed and partially shifted cochleotopic representations in the Medial Geniculate Body?

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A precise mapping of frequencies has been described in lower auditory centers. However, the pars lateralis of the Medial Geniculate Body has a somewhat blurred tonotopic organization (de Ribaupierre et al., Neurosci. Lett., suppl. 5, S148, 1980). Simultaneous recording of single unit pairs with one microelectrode has demonstrated that the

characteristic frequencies of adjacent cells may be quite different. A possible explanation for this apparent anomaly may be that there exists an overlap of multiple cochleotopic representations in that structure. A model of such an organization is proposed. The predictions of the model fit well with the data obtained from single unit pairs and from 3-dimensional reconstructions of over 80 electrode tracks. Best fits were obtained for up to 5 cochleotopic superimposed representations, each being separated from the dominant representation by an integral number of octaves and having a progessively decreasing importance.

Differential modulation of the hydrosmotic actions of vasopressin (VP) and isoproterenol (IP) in toad skin

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Water flow (Jw) can be stimulated in the skin of *B. marinus* by VP or IP. The effect of IP was very reproducible and increased Jw by a factor of 7.92±0.65 (n=50). Since a similar, but much more variable, effect of VP was seen, we performed experiments in which paired skins were sequentially stimulated with VP and IP, at concentrations inducing a maximal response. There was mutual inhibition between VP and IP when either agent induced a high response. For intermediate responses to VP, IP produced a further increase in Jw but, in 18 paired skins, the combined effects of VP+IP were not different from that of IP alone. There were skins in which VP had no hydrosmotic effect while IP induced a normal response, thus showing an intact cAMP-dependent machinery leading to the increase in Jw. This differential modulation of the responses to VP and IP seen in vitro is likely to be related to osmoregulatory mechanisms present in vivo.

Characterization of angiotensin II (AII) binding and corticotropin-releasing activity in rat anterior pituitary cells

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To further characterize the effect of AII in stimulating ACTH release by rat pituitary cells in vitro, we studied the binding and biological activity of AII on isolated rat anterior pituitary cells under various treatments. Scatchard analysis of AII binding indicated the presence of a single class of receptors with $K_d = 4.1 \times 10^{-9}$ M), comparable to the ED_{50} for AII stimulation of ACTH secretion (2×10^{-9} M). The hepapeptide (2-8)-AII ($K_i = 5.5 \times 10^{-8}$ M) and the antagonists des-Asp^1, des-Arg^2, (Ile^8)-AII ($K_i = 0.96 \times 10^{-6}$ M) and (Sar^1, Ala^8)-AII, all inhibited AII binding in competition experiments. (2-8)-AII stimulated ACTH production with an ED_{50} close to its K_i. Changes in dietary salt as well as dexamethasone treatment (5-20 µg/d) for 10 days did not affect the number or affinity of AII receptors. – These data suggest that AII may play a role in the control of ACTH secretion, but that the pituitary is not closely dependent on AII plasma levels.

Brain receptors to angiotensin II (AII) in spontaneously hypertensive rats

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AII-sensitive neurons in the brain of stroke-prone spontaneously hypertensive rats (SHR-sp) and of normotensive Wistar Kyoto rats (WKY) were investigated for possible differences at receptor sites. AII, the competitive antagonist saralasin and acetylcholine (ACh) were applied microiontophoretically onto neurons of the septal area. AII-evoked neuronal firing which was specifically inhibited by saralasin occurred at significantly lower threshold in SHR-sp (23%) and showed an extended postactivity (342%) as compared to the age-matched WKY controls. In contrast, the activity due to ACh remained similar in both strains. – In conclusion, the decreased threshold of AII on excitation of septal neurons in SHR-sp points to an increased sensitivity of those neurons. This, together with the increased duration of action may reflect changes in the brain renin-angiotensin system probably contributing to the genesis of hypertension in these rats.

Development of spatial response strategies in RHA/Verh and RLA/Verh rats

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Adult rats effectively explore the arms of a multi-arm maze with minimal initial repetition (Olton and Samuelson, J. exp. Psych. *2*, 97, 1976) presumably due to spatial memory. To test the spatial memory capabilities of young rats, we used a maze divided into 6 equal sectors which radiated from the center of the maze. 8 RHA/Verh and 8 RLA/Verh rats were tested at each of the following ages: 16, 20, 24, 28, 38 and 50 days. RHA/Verh rats were more active than RLA/Verh rats. The RLA/Verh rats made fewer repetitive alley entries than the RHA/Verh between the ages of 20 and 38 days, with this performance matching the performance levels of adult rats. Further analysis showed, however, that the RLA/Verh rats utilized a simple strategy in about 25% of the choices, as compared to only 15% of the RLA/Verh rats. This strategy involved visiting the adjacent sectors in a clockwise or counterclockwise direction. It is therefore suggested that the higher performance of the RLA/Verh rats was caused by this strategy.

Behavioral comparisons between individually and group-housed rats during the light/dark cycle

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We have previously described that individually housed rats, in comparison to group-housed controls, show a hyperreactivity when exposed to an openfield but a diminished spontaneous activity. In the present experiments we determined locomotor and rearing activities immediately after having exposed animals to a novel environment, and after a 6-h and a 24-h adaptation period. Additionally, after 24 h of adaptation, animals' responses towards a modification of the test environment were observed. 4 experiments were carried out, each starting at a different time point within the light/dark cycle. Individually housed rats were hyperreactive in novel environments and showed a slower habituation, which is not due to an increased spontaneous activity. Behavioral parameters partly show clearcut day/night variations, but differences between individually and group-housed rats do not seem to be influenced by the time of day.

Individual housing in rats is not producing a 'high-stress state'

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Individual housing in rats results in a number of abnormal behavioral and physiological responses, which lead to the concept of 'isolation syndrome'. However, there are contradictory reports concerning physiological stress parameters in unstressed individually and group-housed rats. Since previous studies mostly neglected ontogenetic aspects of such parameters, we determined several physiological and anatomical parameters, which have been proposed as indicators for stress, after various periods of different housing. Determinations of organ weights (adrenals, spleen) and blood parameters (corticosterones, glucose) after long-term isolation (> 12 weeks) did not indicate isolation to be stressful. Even during ontogeny, no marked signs for any temporary 'high-stress state' in isolated rats could be detected. Therefore, the pronounced behavioral alterations in isolated rats can not be explained as a consequence of chronic stress.

Alactic and lactic energy sources at high altitude (Himalaya)

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Peak blood lactate concentration above resting (ΔLa_b) was determined in 4 subjects acclimatized (30 days) at 5200 m, after 5, 10, 15 and 20 standing high jumps off both feet, carried out without interruption at maximal power. Work (W_j) and power ($\dot{W}_j$) per jump were calculated from cinematographically determined time of flight and duration of knee extension. Average W_j over first 5 jumps (23 W/kg) was not different than at sea level. Up to the 8th jump ($\Sigma W_j = 70$ J/kg) $\Delta La_b = 0$. Thus, as the O_2 consumed during this phase (about 8 sec) is negligible, alactic stores account for (at least) 70 J/kg of mechanical work, as observed at sea level (60–100 J/kg). From the 8th to the 20th jump ΔLa_b increased linearly with time (t_e) of exercise. As the latter is maximal, $\Delta La_b/t_e$ is the maximal rate of La accumulation in blood, i.e. ~0.2 mM/sec, about half that at sea level. Thus, while La production seems to be greatly affected by high altitude, alactic energy sources (mainly PC splitting) are not.

Influence of insulin resistance on glucose induced thermogenesis in man

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Glucose induced thermogenesis (GIT) to a 100 g oral glucose load was measured over 3 h by indirect calorimetry in 42 nondiabetic and diabetic obese subjects. They were divided into 3 groups: group A, normal glucose tolerance (GT); group B, impaired GT and group C, diabetics with hyperinsulinemic response. In a control group (n = 17), GIT was 8.6±0.7% ($\bar{X}$±SEM) of the energy content of the glucose load, whereas GIT was significantly reduced, i.e. 6.6±0.9%, 6.4±0.6% and 3.7±0.7% in the obese groups A, B and C respectively ($0.001 > p < 0.05$). The mean change in plasma insulin concentration (ΔIRI), an index of insulin resistance, was 50±5, 74±11, 123±20 and 131±21 μU/ml in controls and groups A, B and C respectively. There was an inverse correlation between GIT and ΔIRI ($= -0.48$, $p < 0.001$). These results suggest that insulin resistance reduces GIT, which may contribute to an energy sparing effect in the obese.

Differential reactivity of the skin and the bladder of *B. marinus* to vasopressin (VP)

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Under the action of VP the rate-limiting barrier of the skin and of the bladder of *B. marinus* undergoes a similar structural rearrangement that correlates with the change from a low to a high state of water permeability. In this work we compared the hydrosmotic effects induced by supramaximal concentrations of VP in epithelia taken from the same animal. Most often the response of the skin was lower than that of the bladder. Whenever it was high, the bladder also had a similarly high response. Interestingly, there were animals in which the bladder reacted normally to VP whereas the skin was totally unresponsive. These different patterns of the VP effect have interesting implications in the osmoregulation of amphibians and probably reflect the need, in vivo, for local modulation of the action of VP in different target epithelia.

Released proteins of cultured brain cells stimulate cholinergic differentiation

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Reaggregating cells of fetal (15–16 days gestation) rat telencephalon grown under chemically defined conditions release various proteins into the culture medium. We have isolated and fractionated these proteins, and we have tested the resulting fractions for biological activity in the same culture system. One of the partially purified protein fractions greatly stimulates the development of the neuronal enzyme, choline acetyltransferase (CAT), whereas equivalent amounts of other proteins isolated from the culture medium or from newborn rat brain extracts have little or no effect. The time course and extent of CAT stimulation by the active protein fraction is similar to that observed with nerve growth factor (Honegger and Lenoir, Dev. Brain Res. *2*, 1982) but other characteristics differ, suggesting distinct mechanisms involved in the enhancement of the in vitro differentiation of cholinergic telencephalic neurons.

The directional error of O_2 and CO blood contents measurements by the gasometric method

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The analysis of a blood sample for O_2 and CO by conventional gasometric technique according to Van Slyke is known to be inaccurate because during the O_2 absorption by $Na_2S_2O_4$ deoxyhemoglobin capable of reabsorbing the extraced CO is formed. We have quantified this error by measuring, by the gas chromatographic technique of Farhi, the amount of CO reabsorbed during the O_2 absorption phase. The error, ΔC_{CO}, was found to be a function of the CO content of the blood (C_{CO}) and of its hemoglobin concentration but was not correlated with the O_2 content. ΔC_{CO} vol% = 0.02 (Hb) g% + 0.026 C_{CO}. The 2 components of ΔC_{CO} could be related to the 2 physicochemical steps of the O_2 absorption phase. Application of the above formula for correcting the Van Slyke data reduces the error of the method to ±0.1 vol%, i.e. the variability of the method when no CO is present. The proposed correction allows

simultaneous measurements of O_2 and CO_2 contents by conventional Van Slyke technique without directional error.

Dual mechanism for angiotensin excitation of hippocampal pyramidal cells in vitro

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The excitatory action of angiotensins (AII, AIII) in the hippocampus has previously been suggested to depend on a disinhibitory mechanism (Haas et al., Experientia *36*, 1394, 1980). We have now confirmed this action by demonstrating a reduction of a) spontaneous inhibitory postsynaptic potentials and b) the conductance increase caused by stimulation evoked IPSPs. In addition we report a direct depolarization by topically applied AII associated with a conductance increase, which is short lasting and persists in the presence of tetrodotoxin. All effects are reversibly blocked by bath applied saralasin.

The role of intracellular [Ca] in the positive inotropic and toxic effect of cardiac glycosides in canine Purkinje fibers

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We used the Ca-activated photoprotein Aequorin to measure changes in $[Ca]_i$ induced by cardiac glycosides. The positive inotropic effect of ouabain (50–100 nM) or strophanthidin (100–200 nM) on canine Purkinje fibers stimulated at 1 Hz was closely correlated to an increase of the 2nd component (L_2) of the Ca-transient which represents Ca^{2+} released from intracellular stores. The first component (L_1), the source of Ca^{2+} for which is uncertain, was also increased but the drug effects on L_1 were less pronounced and more variable than those on L_2. No elevation of diastolic $[Ca]_i$ was detected. Toxic effects were obtained with higher drug concentrations: Reversal of the initial positive inotropic effect during sustained elevation of the Ca-transient and temporally concomitant oscillations of diastolic membrane potential, tension and $[Ca]_i$ (with oscillatory peaks of 1–1.5 μM). Very similar toxic effects were obtained in the absence of drugs by elevating $[Ca]_o$ from 2.7 mM to 18 mM.

Effect of calcium on phosphate efflux from nonmyelinated nerve fibers

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Phosphate efflux from desheathed rabbit vagus nerves was measured in conditions supposed to modify the intracellular Ca concentration. An increase in extracellular Ca, or application of Ca ionophore A23187, produces an increase of the phosphate efflux. The effect is enhanced in solutions with low Na, presumably by reducing the effectiveness of Na/Ca exchange. On the other hand, D600, a drug which is known to inhibit Ca entry and so to lower the intracellular Ca, produces a decrease of the phosphate efflux. Measurements of the P_i/ATP balance after homogenization and extraction indicate that the observed effects are not due to an alteration of the intracellular metabolism, but rather to a direct effect of Ca on the phosphate efflux mechanism.

Calcium ionophore A23187 stimulates phosphate efflux from nonmyelinated nerve fibers

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Phosphate efflux from desheathed rabbit vagus nerves can be stimulated by increased external Ca, thought to be due to a rise in internal free Ca (Jirounek et al., J. Membrane Biol., in press). Application of ionophore A23187 produces an increased phosphate efflux, up to about twice normal. The increase develops slowly during 1–2 h after application of ionophore; it is dose-dependent (maximal at about 10 μM ionophore). The absolute increase in efflux appears to be independent of external phosphate concentration (range 0–2 mM), but largely dependent on the presence of external Ca. The phosphate efflux becomes sensitive to mM changes in external Ca (saturating at about 3 mM), in contrast to normal perfusion, when a just detectable stimulation is found for 3 mM Ca. Stimulation of efflux by the ionophore occurs in the absence of external Ca – a detectable increase is found in solution with EGTA, but the effect is small and develops slowly (after 2 h).

Stimulation of dorsomotor nucleus of the vagus nerve (DMX) on insulin secretion

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The effect of electrical stimulation of brain stem sites on plasma insulin levels was investigated in anesthetized rats. Unilateral stimulation of DMX was made with monopolar electrodes (50 μA, 30 Hz, 0.2 msec) during infusion of glucose producing a glycemia of 150 mg/100 ml. DMX stimulation produced a rapid rise (within 1 min) in plasma insulin levels (200% increase) that was not correlated with observed small changes in glycemia. Stimulation of the adjacent nucleus solitarius tract (NTS), anatomically connected with DMX, also produced significant increases (50%) in insulinemia. Stimulation of another adjacent structure, the nucleus of the XIIth nerve, was ineffective in promoting insulin secretion. The observed effects were decreased by atropine pretreatment. – As stimulation of nucleus ambiguus (AMB) was previously shown by this Laboratory to promote insulin secretion, it is concluded that AMB, DMX and to a lesser extent NTS are important sources of vagal efferent fibres that facilitate insulin release.

Changes in resting membrane potential and extracellular potassium concentration during acute global ischemia of ventricular myocardium

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Guinea-pig hearts were perfused according to the Langendorff method with a mixture of bovine erythrocytes and Tyrode solution containing dextrane. Intracellular potentials were measured by means of floating microelectrodes. K^+ liquid-ion-exchanger microelectrodes served for determination of extracellular potassium concentration (K^+_o). Global ischemia was induced by interrupting aortic flow. K^+_o increased from an initial value of 4.5 mM (±1.0 SEM; n = 9) to 18.6 mM (±1.5; 9) after 15 min. Within this period the potassium equilibrium potentials (E_K) were calculated from the measured K^+_o levels, assuming an initial internal K^+ value of 136 mM and an intracellular to extracellular K^+ activity ratio of 0.82. Resting membrane potentials

(E_{RM}) after aortic clamping decreased from an initial value of −78.3 mV (±3.1; 8) to −49.8 mV (±7.0; 6) after 15 min of occlusion. The results suggest that E_{RM} is close to E_K during K^+_o accumulation in acute ischemia.

Circadian rhythm and strain differences in behavior

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Spontaneous hypertensive rats (SHR) are a selectively bred strain with known abnormalities in behavior. Circadian aspects of these behaviors were investigated. 1. Under entrained light:dark (L:D 12:12) conditions SHR had a rest activity cycle with early onset and late offset of activity in the light phase and no difference in amplitude. 2. Initial hyperactivity after a bedding change was similar in SHR and WKY, but the rate of habituation showed strain and circadian differences: at $L+4^h$, WKY habituated more rapidly than SHR, at $D+6^h$, both strains were similar. 3. In the open field paradigm, higher ambulation, rearing and less boli occurred in SHR than in WKY. Initial hyperactivity was higher at $D+6^h$ for SHR than at $L+4^h$, and the inverse for WKY. – Thus complex intereactions between vigilance state related to time of day and strain responses to a variety of stimuli preclude a simple explanation for the WKY/SHR differences in behavior.

Mechanical responses of chick embryonic cells to electrical stimulation

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The mechanical activity of the epiblastic cells of the gastrulating chick embryo have been studied using a new technique which allows the recording of the tissue viscosity in situ (Kucera and de Ribaupierre, J. Physiol. *318*, 5P, 1981). In addition to spontaneous and periodic variations of viscosity it has been found that the embryonic cells are able to respond to brief pulses of electrical current. The response is biphasic: an immediate, rapid and transient viscosity increase followed by a slower, long-lasting and sometimes oscillatory activity. The time-lapse microcinematography shows that such responses appear near the electrode tip, do not propagate to distant regions and represent a complex behavior of the cells (changes in tension, displacement, modulation of migratory movements). These results support the idea that the early embryonic pattern formation could be controlled by the electrical currents generated by the epiblast itself (Jaffe and Stern, Science *206*, 569, 1979).

Seasonality in autonomous nervous functions of depressed patients

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Retrospective data concerning vegetative functions in 1040 depressed patients (661 ♀; 379 ♂) collected over three years (1978–80) were analyzed with regard to annual cyclic variations. Seasonal changes were found in thermoregulatory parameters. In female patients, the lowest basal skin temperature and the least efficient thermoregulatory response to cold stress occurred each year in March and November, and a marked main peak in May. In male patients basal skin temperature and response to cold stress appear to follow the gradual change in environmental temperature with peak values in the summer months and low values in winter. There was also a seasonal trend in salivary flow rate with a similar time course in both sexes, whereas none could be detected in heart rate, blood pressure and orthostatic reaction. – These results are of interest in connection with the known seasonality of biochemical and physiological systems thought to be involved in affective illness and the seasonality of the illness itself.

Analysis of several individual Ia-EPSPs in single motoneurons

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Individual excitatory postsynaptic potentials (EPSPs) recorded from medial gastrocnemius motoneurons (MN) elicited by stretch evoked single Ia afferents have been analyzed in the cat to study functional connectivity between Ia terminals and MN. By means of an improved spike triggered averaging technique up to 15 different individual EPSPs could be recorded from the same MN. Cable properties of the MNs were estimated from the decay time course of a voltage transient following a current pulse applied to the soma. Theoretical shape index curves for EPSPs located at different electrotonic locations were calculated for each MN. The location of the synaptic contacts was estimated from the time course of the EPSP. The majority of the EPSPs had time courses fitting the shape index curve very closly suggesting that they were generated by contacts located at about the same electrotonic distance. EPSPs whose rise time was too short for their half-width showed composite decay time courses indicating different location of the active contacts.

A dual time-voltage window discriminator for multiunit spike train analysis

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Extracellular recording from small nerve or spinal root filaments frequently picks up the electrical activity of several axons simultaneously. Provided that the multiunit nerve spike sequences can be decomposed into the component single units a great variety of techniques can be employed to analyze the separated spike trains. Construction and operation of a spike discriminator will be presented which is not only sensitive to spike height but also to spike width. The combination of 2 adjustable time-voltage windows with an AND operation leads to a high yield of reliably discriminated spike trains. We are currently using the spike discriminator to decompose multiunit spike trains recorded from small dorsal root filaments in order to study projection and functional connectivity of several muscle spindle afferents onto single motoneurons in the anesthetized cat by means of spike triggered averaging.

Passive electrical properties of insect salivary gland cells

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Cell pairs from insect salivary glands (*Chironomus nuditarsis*, 4th instar stage larvae) were obtained by mechanical elimination of neighboring cells. Insertion of a current-passing and a voltage-sensing microelectrode into each cell allowed to measure both the input resistances and the transfer resistances (Watanabe and Grundfest, J. gen. Physiol. *45*, 267, 1961). Analysis revealed quantitative information about the resistance of the surface membrane and the gap junctional membrane. Taking into account the visually determined geometry of the cells, the specific surface membrane resistance, R_m, turned out to be 2250

Ωcm^2 (n=8). The resistance of the gap junction, r_g (geometrical data not available), ranged from 0.16 MΩ to 2.45 MΩ. It was found that R_m and r_g show an ohmic behavior over the potential range tested. Using this 2-cell preparation, experiments were performed to study the effect on r_g of interventions known to modify the intracellular concentration of Ca^{2+} or H^+.

In vivo dark regeneration of visual pigment in the honeybee drone

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Photoreceptors of drone contain a pigment with 2 states, rhodopsin (R) and metarhodopsin (M). The possibility that concentration of R and M can change in the dark was investigated by microspectrophotometry. Before dark adaptation, drones were exposed to strong blue light (409 nm, 90 min), so that most of the pigment would be in the M state. In the dark, the relative R concentration increased to a steady value of 85% with exponential kinetics ($\tau = 3.5 \pm 0.5$ h). In complementary experiments the pigment was converted to the R state by green light (543 nm, 90 min). In the dark, the relative concentration again reached the same equilibrium ratio: 85% R, 15% M with $\tau = 3.5 \pm 0.8$ h. Regeneration did not reach 100% due to spontaneous conversion from R to M in darkness. Total pigment concentration did not change. In drones that died shortly after illumination, the total concentration remained constant and no changes of R and M were observed, suggesting that pigment conversion may be linked to the metabolic activity of the photoreceptor cells.

Comparison between Na channels in rat and frog Ranvier nodes

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The conductance γ and number N of Na channels in Ranvier nodes of rat and frog nerve fibres was determined from nonstationary Na current fluctuations (Sigworth, J. Physiol. *307*, 97, 1980). From measurements at depolarizations V between 40 and 80 mV we obtained for rat nodes $\gamma = 14.5$ pS, N = 19,000 (extracellular Na concentration $[Na]_0 = 154$ mM, temperature 20° C, holding potential $V_H = -32$ mV) and for frog nodes $\gamma = 10.9$ pS, N = 70,000 ($[Na]_0 = 112$ mM, 15° C, $V_H = -28$ mV). The lower conductance of frog Na channels is due to the lower extracellular Na concentration and the lower temperature. The higher number of Na channels in frog nodes reflects species-dependent differences between amphibian and mammalian myelinated nerve fibres.

Intracellular Na activity in skeletal muscle – influence of 50% Na_0 and twice normal tonicity

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Solutions of increased tonicity (to inhibit contraction) and decreased sodium content (to reduce upstroke and conduction velocity of the action potential) are often used in muscle studies without information about their effects on internal sodium activity, $a_{Na,i}$. We therefore measured $a_{Na,i}$ in frog muscle fibres during exposure to 50% Na_0 solutions of normal and doubled tonicity using microelectrodes based on a neutral carrier (gift Dr Ammann, ETHZ). In normal Ringer $a_{Na,i}$ was 12.3 ± 0.7 mM and 34.4 ± 1.3 mM in twice hypertonic solutions. The overproportional increase of Na_i seems to be related to the observed reduction in resting potential (from −89 to −81 mV). In normotonic and twice hypertonic solutions both containing 50% of the normal Na (NaCl replaced by sucrose to maintain tonicity) $a_{Na,i}$ is not measurably changed during at least 20 min. This result agrees with a measured reduction of conduction velocity in 50% Na_0 to an extent which indicates stable $a_{Na,i}$.

Effects of maze geometry on spontaneous exploration in 2 lines of rats

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Rats from each of 2 selected lines (RHA/Verh, RLA/Verh) underwent 6 days of testing in a maze with 6 symmetrical alleys leading from the center, followed by an 8-day period with configurations in which the main arm and short blind alley were oriented clockwise (configurations used during habituation) or counterclockwise on alternate days. Locomotion, patrolling efficiency (occurrence of a first repetitive entry) and the number of blind alley entries were calculated. RHA/Verh rats were more active, entered blind alleys more and showed higher patrolling efficiency than RLA/Verh rats. The patrolling efficiency of RLA/Verh rats gradually increased over days, reaching the level of the RHA/Verh rats by the end of the experiment. For both lines of rats, variation of the maze geometry produced higher activity, decreased patrolling efficiency and, for the counterclockwise configurations, a dramatic increase in the number of blind alley entries. These results suggest that both lines developed a maze concept during the first 6 days.

Progressive muscle weakness of myogenic origin in the cat – a feline dystrophy?

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The conductance γ and number N of Na channels in Ranvier nodes of rat and frog nerve fibers were determined all dogs and cats showing muscle weakness of apparent myogenic origin that were brought to our clinic. One case, a male Siamese cat, is described here. Progressive muscle weakness (especially of the limb extensors) causing overknuckling of the forepaws and inability to jump was first seen at 4 months, and by 6 months the cat could not stand unaided. Infection of the muscle and CNS and neurological abnormalities were excluded. Electromyography of affected muscles revealed pathological spontaneous activity, and the histochemical appearance of the muscle biopsies was clearly myopathic, strongly resembling dystrophic muscle. A female littermate with motor deficits was destroyed at $4\frac{1}{2}$ months.

Monovalent cations and cAMP in the regulation of parathyroid hormone (PTH) secretion

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High potassium (60 mM) and isoproterenol (10^{-5} M) stimulated the release of PTH both in the presence and absence of calcium. Choline (55 and 117 mM), or low sodium (20 and 82 mM) were ineffective. Ouabain (10^{-3} M) suppressed the stimulation of PTH by low calcium and raised potassium, but not by isoproterenol, while propranolol (10^{-3} M) specifically blocked the effect

of isoproterenol. The PTH response to isoproterenol was accompanied by a stimulation of cAMP, whereas low calcium and potassium did not affect cAMP levels. – In conclusion, the stimulation of PTH secretion by potassium is similar to its effects in other endocrine systems, but does not require extracellular calcium. More likely release of stored cellular calcium in response to potassium, isoproterenol and possibly low calcium point to a common mechanism of PTH secretion.

T3 modulates its own nuclear receptors in rat pituitary

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The narrow range at which T3 exerts its control on TSH secretion depends on T3 levels and on the number of T3 nuclear receptors. To establish whether T3 regulates its receptors in the pituitary, they were measured in salt solubilized nuclei from adult male rats 1, 2, 8 and 16 weeks after thyroidectomy (-T). Saturation analysis was performed with 10^{-12} to 10^{-9} M ^{125}I-T3 on 20 µg protein solubilized receptors. In normal rats, maximal binding capacity was 598±49 fmoles/mg protein. In rats 2 weeks after -T there was a 60% reduction in thyroid hormones and T3 nuclear receptors concentrations (197±52 fmoles T3/mg protein) which remained so at 8 and 16 weeks after -T. There was a positive correlation between plasma T3 and T3 nuclear receptors ($r = 0.86$, $p < 0.01$). One injection of T3 (1.0 µg T3/100 g b.wt) to 2-week -T rats was able to restore in 24 h a normal level of T3 nuclear receptors in the pituitary. This suggests that in rat pituitary, T3 modulates its own nuclear receptors, providing a further mechanism of increased TSH secretion in thyroid insufficiency.

Alveolar-arterial equilibration in sheep lung

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In order to compare the efficiency of sheep lung with that of dog (Savoy et al., Respir. Physiol. *42*, 43, 1980) gas exchange in hypoxia (F_{IO_2} 0.12–0.13) with and without CO (F_{ICO} 0.001) was measured in 9 sheep under pentothal anesthesia and artificial ventilation. Alveolar-arterial P_{O_2} difference, percent venous admixture and $V_A/\dot{Q}$ inhomogeneity were found to be much larger than in dog. In sheep, the standard deviation of $\dot{V}_A/\dot{Q}$ lognormal distributions was 1.2 and the Q_s/Q_t ratio 7%. – Steady-state diffusing capacity, both for O_2 and for CO, was about 40% smaller than in dog. D_L without inhomogeneity correction was 19 and 13 µmoles · min^{-1} · Torr^{-1} for CO and O_2, respectively. The $D_{L_{O_2}}/D_{L_{CO}}$ ratios were not significantly different from those of dog; thus the presumed beneficial effect of sheep small erythrocytes on O_2 transfer was not confirmed. Because of its low $D_{L_{O_2}}/M_{O_2}$ ratio the sheep appears to be more adequate than the dog as a model for human gas exchange.

Influence of light adaptation and extracellular calcium on the receptor potential of drone retinula cells

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The effects of changes of extracellular calcium on the size and shape of the response to light of drone photoreceptors have been studied and compared with the effects of light adaptation. The light stimuli used were so weak that the amplitude of the response was linearly related to the number of photons absorbed and the effects of voltage-dependent mechanisms were negligible. Decreasing external calcium caused an increase in the peak amplitude and duration of the receptor potential. Increasing external calcium had opposite effects. Changing external calcium did not affect the rate at which cells depolarized following illumination. The reduction in time scale and sensitivity caused by light adaptation could be quantitatively mimicked by an appropriate increase in extracellular calcium. The experimental results can be described quantitatively by a model with the basic assumption that calcium acts in the process of light adaptation by regulating the open time of the light-dependent channels.

Single-site uptake of monosaccharides by guinea-pig small intestine

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To examine whether D-galactose and α- and β-methyl-D-glucoside are transported by a single site in guinea-pig small intestinal rings, large-scale kinetic experiments were performed in which substrate and inhibitor concentrations were both varied and interchanged within the same animal. Uptake data, obtained after 2-min incubations, were not corrected for extracellular space but were fitted by non-linear regression analysis to an equation comprising the sum of a saturable and a diffusive component (the latter including the extracellular uptake). The 2 half-experiments – with one sugar acting as substrate and the other as inhibitor, and vice versa – were analyzed separately and then combined for a pooled estimate under the restriction that the K_m of each sugar was equal to its corresponding K_i. Statistical tests showed that this constraint is permitted. It is concluded that the single site model satisfactorily describes the uptake of these monosaccharides in the guinea-pig intestine.

ECG changes due to altitude and to catecholamines

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The ECG changes occurring during stepwise ascent to high altitude are mainly due to the activity of the sympatho-adrenal system (Experientia *35*, 917, 1979). In order to distinguish the effects of betareceptor stimulation on the ECG at high altitude from other factors, the ECG of 19 volunteers were compared after isoprenaline inhalation at ground level and during exposure to altitude (6000 m) with and without betareceptor blockade (propranolol). The results show that during exposure to high altitude the ECG changes are due not only a) to betareceptor stimulation (increase in heart rate, shortening of P–Q interval, lengthening of Q–T interval, flattening of the ST–T segment), but also b) to vagal withdrawal (increase in heart rate), c) to direct effects of hypoxia on the heart (lengthening of P–Q) and d) to hypoxic increase of pulmonary resistance (deviation of the mean electrical axis and of the T-vector).

Vagal pathways from the lower brain stem to the abdominal viscera

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The left (LCv) or right (RCv) cervical vagal trunk of anesthetized rats was electrically stimulated and the elicited nerve conduction was extracellularly recorded on the anterior (AS) and posterior (PS) subdiaphragmatic, as well as on the anterior (ACl) and posterior (PCl) coeliac trunks. 2 main axis to the coeliac branches have been observed: a) RCv – PS – PCl, b) LCv – AS – ACl. 2 accessory axes

were also seen, pointing to a crossing of fibres in the heart-diaphragm area: c) LCv - PS -PCl (10% of the fibres in the PS), d) RCv - AS - ACl (5% od the fibres in the AS). Conduction speeds (0.8±0.1 m/sec, n = 7 in the PCl or ACl) suggest that C fibres predominate in the PS and AS, and that other fibre types are virtually absent from PCl and ACl. By stimulating the PS and recording the antidromically invaded cell bodies with an extracellular micropipette in the brain stem, the neurons of origin (n = 12) have been localized in the 3rd quarter of the rostrocaudal length of the right dorsal motor nucleus of the vagus.

Variation of membrane potential and flavoprotein redox state in rat brown adipose tissue during nerve stimulation

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The thermogenic response of brown adipose tissue is controlled by the sympathetic nervous system. In order to obtain further information about the postsynaptic events during tissue stimulation, we recorded continuously in vitro membrane potential and flavoprotein redox state (an index of the metabolic activity of the tissue). Electrical nerve stimulation at 4 Hz for 25 sec was followed after a latency of 2 sec by a first depolarization. This reached a maximum at ~10 sec, when flavoprotein reduction became detectable. After ~1 min a 2nd depolarization was observed with its maximum at ~6 min. The maximum of flavoprotein reduction was at ~1 min. The first depolarization was blocked by an α-antagonist, the 2nd by a β-antagonist. The α-antagonist did not block the flavoprotein reduction, the β-antagonist did. The results indicate that 2 temporally distinct depolarizations, which precede and accompany the metabolic activity of the cell, are mediated by different adrenergic receptors.

Comparison of birefringence and calcium signals in frog skeletal muscle

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A cut fibre voltage clamp system was used to activate fibres by depolarizing pulses and to monitor intrinsic birefringence signals (BS) and absorption changes of the calcium indicator antipyralazo III (CaS), introduced into the fibre by diffusion. In both signals, latency and time to peak are reduced, while amplitude and maximal rate of rise increase with increasing pulse amplitude. Along a strength duration curve (just visible movement) the amplitude of both signals stays constant. The twitch potentiator caffeine and the muscle relaxant dantrolene affect both signals in the same way (caffeine: increase; dantrolene: decrease). While both signals begin simultaneously, BS peaks later and declines more slowly than CaS under all conditions. The most consistent interpretation is that CaS reflects free Ca^{++}, while BS indicates a structural change upon Ca^{++}-binding e.g. to the Ca^{++}-ATPase of the SR. An SR potential change cannot be excluded completely.

Characterization of uptake and retrograde axonal transport of noradrenaline in an in vitro system

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In order to study the mechanism of uptake and the compartment involved in retrograde axonal transport of noradrenaline (NA), which has been shown to label CNS aminergic neurons by retrograde transport (Streit, J. comp. Neurol. *191*, 429), dissociated neurons from newborn rat sympathetic ganglia are grown in a culture dish divided into 3 separate chambers by a Teflon ring sealed with silicone grease (Campenot, PNAS *74*, 4516). From the cell bodies in the middle chamber axons grow into the side chambers and form dense plexus of fibres within 3 weeks. If H^3-NA (0.7×10^{-6} M) is added to the axons, the label is rapidly taken up into the varicosities, and a small but significant part is transported retrogradely to the cell bodies. Uptake and transport of labeled NA are blocked by desmethylimipramine, a blocker of the membrane amine pump. The retrograde transport is not affected by reserpine but bocked by colchicine. A reserpine-resistant compartment different from synaptic vesicles is postulated.

Do insulin levels affect the metabolic responses of brown adipose tissue?

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In obese rodents the brown adipose tissue (BAT) response to norepinephrine (NE) and its capacity to utilize fatty acids were found to be reduced. Since obese rodents are hyperinsulinemic, the role of insulin was investigated by using rats chronically infused with insulin or made diabetic by streptozotocin. The BAT metabolic response was measured by monitoring the NAP(P) redox state. In BAT from control rats NE produced an increase of this redox state. This increase was not modified in insulin-infused rats whereas in diabetic rats NE produced a decrease in NAD(P) redox state. The capacity of BAT to β-oxidize and to esterify fatty acids were both significantly increased in insulin-infused rats whereas the opposite was observed in diabetic rats. The results suggest that insulin might play an important role in the control of energy dissipation by BAT.

Regional blood flows (RBF) in aortic coarctation in dogs

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The importance of local versus systemic neurohumoral factors in the control of RBF was examined by constricting the descending thoracic aorta in 7 conscious dogs. Cardiac output (CO, electromagnetic flowmeter), mean arterial pressure (MAP) above (a) and below the constriction (b), and RBF (radioactive microspheres) were measured before, 1 h and 6 h after constriction (40 mm Hg pressure gradient). MAPa increased from 99±5 mm Hg to 113±3 mm Hg after 1 h and remained at this level. CO decreased by about 10%. As a whole, RBF were increased by 50% after 1 h and 6 h in the areas a, in particular in the skin, the bones and the skeletal muscles. Several RBF were decreased in the territories b, notably in the kidneys and the splanchnic area. Vascular conductance increased in several tissues, both a and b. Since local autoregulatory responses would induce vasoconstriction in the upper part of the body, these results suggest that systemic factors predominate over local ones in the early stages of aortic coarctation.

Heterogeneity of rat small intestinal brush border membrane vesicles

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Brush border membrane vesicles from rat small intestine were isolated by a Mg/EGTA precipitation method. Further fractionation either by free flow electrophoresis or by sucrose density gradients leads to 2 different fractions in respect to enzyme enrichments and SDS-PAGE. In one fraction an enrichment of $Na^+ + K^+$-ATPase is found, whereas in the other maltase and alkaline phosphatase are enriched. The 2 vesicle populations have different D-glucose transport properties, measured with radioactively labeled D-glucose or with a membrane potential-sensitive fluorescent dye. These results indicate that the vesicles obtained by the cation precipitation methods are not homogeneous. Recent findings on sodium-independent transport systems in brush border membranes might be a result of the 2 different vesicle populations. One of them might either be a cross-contamination by basal lateral membranes or membranes of not fully differentiated cells.

Calcium-dependent activation of peripheral osmoreceptors in rats

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To investigate the cellular mechanisms in portal vein osmoreception (J. Physiol., Lond. *313*, 217, 1981), we locally applied $CaCl_2$ (10-50 mM), EDTA (5-50 mM), TFP and CP (trifluoperazine, chlorpromazine 0.1-1 mM) and bradykinin (1-10 µM) or NaCl solutions (0.2 ml 1.20 sm/kg) to the portal vein and measured the response of the hypothalamo-neurohypophysial system (HNS) by an electrophysiological method. Bradykinin activated the HNS, but neither bradykinin nor indomethacin (10 µM) modified the HNS response to NaCl, suggesting that NaCl does not act through pain receptors. TFP, CP and EDTA diminished the response to NaCl by 25-40% ($p < 0.025$), and $CaCl_2$ and 1 mM c-AMP evoked a significant HNS response. The results favor the view that osmotic stimulation induces an increase of free calcium concentrations within osmosensitive cells and possibly the activation of a calmodulin-dependent process.

Energetic cost of glucose storage in man during euglycemic insulin clamp

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The euglycemic insulin clamp technique (DeFronzo et al., Am. J. Physiol. *237*, E214, 1979) combined with continuous indirect calorimetry was performed on 24 male volunteers. Insulin was infused at 4 rates in order to achieve steady state insulinemia of 62, 103, 170 and 423 µU/ml. This required glucose infusions (i.e. glucose uptakes) of 0.41, 0.50, 0.66, 0.74 g/min for each group respectively. Glucose storage (i.e. glucose uptake minus glucose oxidation) was 0.25, 0.29, 0.43, 0.49 g/min and energy expenditure increased by 0.08, 0.10, 0.14 and 0.17 kcal/min respectively. There was a significant relationship ($r = 0.93$; $p < 0.001$) between the increment in energy expenditure and glucose storage indicating a cost of 0.36 kcal/g glucose stored, i.e. 9.5% of the energy content of glucose.

Effect of sleep deprivation in rats with suprachiasmatic lesions

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Bilateral lesions of the nucleus suprachiasmaticus (SCN) were performed in enucleated rats. The loss of the circadian rest-activity rhythm in these rats was documented by motor activity recordings over several weeks. Subsequently, the rats were chronically implanted with electrodes and recorded via EEG telemetry. During the 2 baseline days the arhythmic animals showed the typical episodic recurrence of waking bouts. Sleep deprivation for 24 h in a slowly revolving drum had a similar effect on recovery sleep as that observed in intact rats: an increase in the slow wave fraction of nonREM sleep and in REM sleep, and the rhythmic alternation of synchronized and desynchronized EEG. Our results show that sleep regulation is functional also in the absence of the circadian sleep-wake rhythm.

Kinetics of oxygen consumption after a single flash of light in photoreceptors of the drone *(Apis mellifera)*

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The time course of oxygen consumption (QO_2) after a single flash of light has been measured in 300-µm slices of drone retina at 22 °C. Transients of PO_2 were recorded with O_2 microelectrodes simultaneously at 2 sites in the slice and ΔQO_2 was calculated. After a 40-msec flash of intense light, ΔQO_2 reached a peak of 40 µl O_2/g of tissue · min above the base line and then declined exponentially. The time constant of the rise (τ_1) was 2.0 sec; of the fall (τ_2) 5.0 sec. The peak amplitude, (ΔQ_m), increased linearly with the log of the light intensity over 3.5-log units. Replacement of Na^+ by choline, known to decrease greatly the light-induced current, caused a 63% decrease of ΔQ_m. These changes did not alter the kinetics of $\Delta QO_2(t)$. Exposure of the retina to high concentrations of ouabain reduced ΔQ_m by about 80% and increased τ_2. Hence, it seems likely that the greatest part of ΔQO_2 is used for the working of a sodium pump.

Circadian activity in socially-raised and isolated rats as assessed in an open-field device

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Behavior of isolated and yoked socially-raised rats was studied in an open-field at different times during the light- and the dark-phase of the day. - The diurnal rhythmicity of ambulation and rearing, was abolished in isolated rats, and changes in the 24 h means and shifts in the phase-related maxima and minima of some behaviors, if compared to controls, were observed. Isolated rats showed high ambulation and low grooming (both during the light and the dark phase) and a delay in the night maxima of about 4 h. There were no differences in the 24 h means of the rearing between the groups. The maximum defacation in isolated rat was also clearly delayed (ca 20 h). The results of the study indicate that in comparative studies with socially-raised rats, diurnal variations of some behaviors of isolated rats should be taken into account.

BIOCHEMIE - BIOCHIMIE - BIOCHEMISTRY

Influence of thyroid hormones (T3) and starvation on hepatic nuclear globulins in the rat

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Barsano (1980) demonstrated qualitative changes in rat liver nuclear globulins under the influence of T3 and starvation. On the basis of these studies, we intended to quantify these changes and to study their time course. Analysis of SDS-electrophoresis with microdensitometry showed that 2 bands varied significantly: in thyroidectomized (Tx) rats the n-band was lower compared to the one in normal (Eu) rats while the t-band was higher in Tx than in Eu. 18 h after injection of 1 dose of T3 to Tx, the n-band increased significantly compared to Tx controles. The level of the n-band in Eu was not achieved in Tx even after a T3 substitution during 7 days. The t-band in Tx was reduced to Eu-values 24 h after 1 injection of T3. In conclusion: starvation and hypothyroidism induced similar changes in nuclear globulins. However, contrary to the findings of Barsano, none of the bands was only dependent on thyroid hormones.

Functional properties of the subunits of cytochrome c oxidases

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The properties of the subunits in cytochrome c oxidases from bovine heart mitochondria have been studied using the following approaches: 1. Covalent inhibition (dicyclohexylcarbodiimides) of the redox associated H^+-translocation to locate the subunit(s) related to the proton pump. 2. Selective extraction, using covalent chromatography of polypeptide III involved in H^+-pumping and functional studies on the deficient enzyme. 3. Preparation of a cytochrome c oxidase depleted of the low mol.wt subunits (IV–VII) by controlled denaturation and its characterization. 4. Renaturation of denatured subunits and their functional reconstitution. 5. Affinity chromatography purification *(Rps. sphaeroides, B. subtilis, B. caldolyticus)* of bacterial a-type oxidases, possessing a limited number of subunits, and their spectroscopic-functional comparison. By these approaches a more detailed molecular picture of this complex enzyme has already been obtained.

Cytochrome c peroxidase – cytochrome c electron transfer complex: modification of carboxyl groups

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Cytochrome c peroxidase (CcP), a hemoprotein from yeast, catalyzes the reaction: 2 cyt $c_{red} + 2\ H^+ + H_2O_2 \rightarrow 2$ cyt $c_{ox} + 2\ H_2O$. To localize on the peroxidase the cyt c recognition site, which is known to contain negatively charged groups, we use the method of differential chemical modification. CcP either alone or bound to cyt c is modified with a trace amount of a watersoluble carbodiimide (EDC) and [^{3}H]taurine whereby carboxyl groups are amidated according to their reactivity. Thereafter the peroxidase is fully modified with non-radioactive reagents in excess and mixed with fully ^{14}C-labeled CcP. The reactivity of individual aspartate and glutamate residues is obtained from the $^3H/^{14}C$ ratios of isolated peptides. Preliminary results indicate that only a few carboxyl groups and none of the heme propionates are involved in the binding of cytochrome c.

In perfused rat livers insulin inhibition of α-adrenergic responses is more pronounced at low than at high extracellular Ca^{2+}

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Livers from fed rats respond to phenylephrine (0.5 μM) with an activation of glycogenolysis, paralleled by a release of Ca^{2+}. Inhibition of the α-adrenergic effects by insulin was studied at 2 extracellular Ca^{2+} concentrations. Insulin alone (10 nM) exerted no short-term effect on basal rates of glycogenolysis and did not acutely affect the Ca^{2+} distribution. Insulin inhibited α-adrenergic glycogenolysis and Ca^{2+} release by 35% and 25% respectively at 1.27 mM extracellular Ca^{2+}. At 0.01 mM the metabolic response to phenylephrine was depressed by 80%, Ca^{2+} release by 50%. Thus insulin suppressed α-adrenergic effects more drastically at low extracellular Ca^{2+} concentrations. Low Ca^{2+} may produce a partial depletion of the phenylephrine-sensitive Ca^{2+} pools and/or a decreased binding of the α-agonist to its receptor. Low extracellular Ca^{2+} and insulin seem to act synergistically by diminishing the α-adrenergic increase of cytosolic free Ca^{2+} concentrations.

Tryptophan fluorescence in different forms of *Neurospora* tyrosinase

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Upon excitation at 294 nm, *Neurospora* tyrosinase exhibits tryptophan fluorescence with a maximum intensity near 330 nm, indicative of a protein containing buried tryptophans. However, quenching experiments with acrylamide and iodide indicate environmental heterogeneity. In fact, both in apo- and mettyrosinase, about 40% of the total fluorescence can be attributed to solvent exposed tryptophans. In oxytyrosinase, no quenching is observed suggesting that its fluorescence emanates exclusively from buried residues. The comparison of emission spectra obtained for different enzyme derivatives shows that binding of metal and oxygen at the active site leads to large effects on the tryptophan fluorescence. The quantum yield decreases in the order: apo (0.129), met (0.051), Co^{2+} substituted (0.050), desoxy (0.046), oxytyrosinase (0.026). These quenching effects suggest the presence of tryptophan residue(s) at the active site pocket.

Golgi and cell surface localization of galactosyltransferase

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Monospecific antibodies against soluble human milk galactosyltransferase (GT) were used to localize the enzyme in various human tissues. GT was found adjacent to the nucleus as well as on the cell surface. Intracellular staining was compatible with the Golgi area and was most prominent in cells with known secretory function, e.g. pancreatic acinar or gastric chief cells. Ecto-GT was confined to microvilli containing surfaces, e.g. of gastric epithelium, jejunal villus tip and pancreatic ducts. In jejunal crypts only Paneth cells were positive. Staining in the middle portions along the crypt/villus gradient was found in the region of the terminal web, while villus tip cells showed intensely stained brush borders. Surface membrane and 'terminal web' staining are suggestive for a distinct mechanism of luminal membrane biogenesis and renewal.

Isolation of plasmamembranes from the proximal colon of the guinea-pig

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Coloncytes were isolated by incubation in an EDTA containing buffer. The cells were homogenized in an isotonic sucrose buffer and then centrifuged at 370×g for 10 min. Hereby 60% of the activities of Na/K-ATPase and alkaline phosphatase - potential marker enzymes for the basallateral and/or luminal membrane - remained in the supernatant. Centrifugation through a 15–30% sucrose gradient at 100,000×g for 5 min resulted in more than $^2/_3$ of the plasmamembrane enzyme activities in the upper third of the gradient. In isopycnic centrifugation (15–30/37/55% sucrose) Na/K-ATPase and adenyl cyclase activity were enriched 10-fold at the 30/37% interface. For the fraction recovered from the 37/55% interface no enrichment of plasmamembrane or intracellular membrane marker enzymes was found. Surface labeling experiments suggest that the membranes collected at the 30/37% interface are basallateral membranes, whereas the membranes at the 37/55% interface represent luminal membranes.

Measurement of in situ rates and parameters of platelet 3′,5′-cAMP-phosphodiesterase with a new technique, 'free enzyme buffering'

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Using a new technique, in situ 'free enzyme buffering', cAMP-phosphodiesterase (PDE) was shown to change its behavior in intact platelets toward specific inhibitors following hormonal (PGE_1) stimulation of adenylate cyclase. - With PGE_1 (10^{-7} M) the in situ inhibitor constant for papaverine increases about 30 times while that for IBMX (1-methyl,-3-isobutylxanthine) is moderately decreased. In situ Michaelis-Menten parameters are likewise shifted in a characteristic fashion. - The data bear out an earlier proposal (Honegger and Bally, Adv. Cycl. Nucl. Res. *5*, 825, 1975) that following hormonal stimulation of adenylate cyclase in platelets PDE undergoes a calcium-dependent change in situ which is maintained as long as the hormone is present.

Proteolytic processing of cytoplasmically-made mitochondrial proteins

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Most mitochondrial proteins are synthesized as larger precursors in the cytoplasm. These can then be imported into the organelle without further protein synthesis. This import requires an electrochemical potential across the mitochondrial inner membrane and is accompanied by the proteolytic conversion of precursors to their mature forms. - 2 different mitochondrial proteases appear to participate in this process. - We have isolated and characterized a soluble, chelator-sensitive protease from the yeast mitochondrial matrix which cleaves precursors of proteins for transport across the inner membrane. Conversion of these precursors to the corresponding mature polypeptides appears to occur in a single step. However, cytochrome b_2 (a component of the soluble intermembrane space) and cytochrome c_1 (a component located on the outer face of the inner membrane) are converted to their mature forms in *two* steps. The first of these steps is catalyzed by the matrix protease; the 2nd step apparently involves a detergent-sensitive, membrane-bound protease.

Biochemical and structural analyses of microtubules in the pellicular membrane of *Leishmania tropica*

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The pellicular membrane of *L. tropica* contains a major protein of about 50,000 d. It was found that under suitable electrophoretic conditions this protein separated into 2 equimolar components. The components cross-reacted immunologically with the α and β subunits of pig-brain tubulin. The β subunit of pig tubulin and the faster migrating component also generated virtually identical peptides after partial proteolysis. Thus the major polypeptides found in the pellicular membrane of *L. tropica* were the α and β subunits of tubulin. Immunoelectron microscopy indicated that tubulin is located in the microtubule array closely associated with pellicular membrane. Analysis of electron micrographs revealed the classic helical substructure of microtubules and side projections for membrane attachment, implying that the nature and organization of tubulin in mammalian brain or *L. tropica* pellicular membranes microtubules are identical.

Cytochrome c peroxidase-cytochrome c electron transfer complex: experimental support of a hypothetical model

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Cytochrome c and cytochrome c peroxidase are electron transferring hemoproteins of known amino acid sequence and crystal structure. Their mode of interaction is exemplary for other electron transfer reactions involving cytochrome c, notably the interaction with mitochondrial cytochrome c oxidase, since several hemoproteins are now known to recognize the same active site of the cytochrome c molecule. A hypothetical model of the peroxidase-cytochrome c complex based on the crystal structure of the 2 molecules will be presented. The main feature of the model is a ring of aspartate residues surrounding the heme edge of the peroxidase which is juxtaposed to a similar ring of lysine residues around the exposed heme edge of the cytochrome c molecule. The model is corroborated by results from chemical modification of carboxyl groups and by the analysis of a covalent peroxidase-cytochrome c complex.

Do calcium ions inhibit pyruvate carboxylation in rat liver mitochondria?

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Conflicting results exist on the inhibition of pyruvate carboxylation by Ca^{2+} in isolated rat liver mitochondria (Mörikofer-Zwez et al., J. biol. Chem. *248*, 7588; Foldes and Barrit, J. biol. Chem. *252*, 5372). We found that the discrepancy may be related to the nutritional state of the animal since added Ca^{2+} (50 nmoles/mg protein) caused a decrease of pyruvate carboxylation in mitochondria from fasted rats whereas less than 5% inhibition was observed in those from fed rats. In the fasted state, the uptake of Ca^{2+} caused a temporary fall in mitochondrial

ATP/ADP ratio which in turn led to the observed inhibition of pyruvate carboxylation; 5–10 min after Ca^{2+} addition, the ATP/ADP ratio returned to normal and pyruvate carboxylation was no longer inhibited. In the fed state, no change in the ATP/ADP ratio was observed at the earliest measurement at 3 min after Ca^{2+} addition. We conclude that Ca^{2+} has no direct effect on pyruvate carboxylation under steady state conditions.

Primary structure analyses of the light harvesting polypeptides from various photosynthetic bacteria

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Recently, the complete amino-acid sequence of the single light harvesting polypeptide (LHP) from *R. rubrum* G-9$^+$ was reported (Brunisholz et al., FEBS Lett. *129*, 150, 1981). Similar isolation techniques were applied to prepare the homologous LHPs from *Rps. viridis* and *Rps. gelatinosa.* Gel filtration on LH-60 of a C/M extract from *Rps. viridis* reveals at least 3 low mol.wt components. Separation of the LHP homologous constituent from an acidic polypeptide of unknown function was achieved on a DE-32 column in C/M/NH_4OAc. In the case of *Rps. gelatinosa* the LHP was sufficiently purified on the LH-60 column. Complete amino-acid sequence determination of all of these LHPs exhibit a single His within a hydrophobic stretch which might be involved in the pigment-protein interaction. The knowledge of the primary structure of the LHPs offers a helpful tool to establish their localization and aggregational state within the photosynthetic membrane.

Antibodies against human liver alcohol dehydrogenase (ADH). Localization of the enzyme in human tissue and cultured cells

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Antibodies against human liver ADH, which was purified by affinity chromatography, were elicited in rabbits. Their specificity was tested by double immunodiffusion, adsorption of anti-ADH antibodies to immobilized ADH, ELISA and immunoprecipitation of ADH-activity. Protein-A peroxidase with diaminobenzidine as substrate was used to detect anti-ADH binding in human-liver sections, cultured fibroblasts from human skin and lung and Hela cells. In human liver, ADH is localized in the cytoplasm of hepatocytes. The hepatocytes do not stain uniformely, indicating an uneven distribution of ADH. Strongly stained hepatocytes are localized mainly around the central veins. Human skin and lung fibroblasts as well as Hela cells all exhibit positive staining for ADH. The staining intensity is much weaker, reflecting a lower ADH-content.

Activation of rabbit muscle phosphorylase kinase requires the binding of 3 calcium ions on its δ subunit

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Rabbit skeletal muscle phosphorylase kinase (PhK) has the subunit structure $(\alpha\beta\gamma\delta)_4$ and requires micromolar [Ca^{2+}] for its activity. The δ subunit has been shown to be identical with calmodulin and claimed to be the Ca^{2+}-binding subunit of the enzyme. Our purpose was to correlate Ca^{2+}-binding to the Ca^{2+} dependence of the enzyme activity. Ca^{2+}-binding to PhK was carried out by equilibrium gel filtration in the same ionic conditions as the Ca^{2+} activation of the enzyme. The data show that binding of 3 calcium ions per δ subunit is required for activity. Interestingly, free calmodulin also requires the binding of at least 3 Ca^{2+} to stimulate the activities of phosphodiesterase (Cox, J. biol. Chem. *256*, 3218) or adenylate cyclase (Malnoë, Biochim. biophys. Acta *714*, in press). Thus, even when calmodulin is an intrinsic subunit as in PhK, the binding of 3 Ca^{2+} is required to activate the enzyme.

Isolation of distinct lysosomal populations from mouse brain

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Brain lysosomes difficult to obtain in good quality by conventional techniques can be purified using a novel method. From 17-day mouse brains 25% homogenates (w/v) were prepared in 0.25 M sucrose and 1 mM EDTA. A crude postnuclear fraction (1000 × g SN) was centrifuged for 10 min at 25,000 × g to give a pellet (P_2). The P_2 material was resuspended in 0.25 M sucrose and centrifuged on a continuous Percoll density gradient system self-generated by colloidal silica-gel particles. The overlayered isotonic Percoll (1.07 g/ml) was centrifuged for 1 h at 20,000 × g. The lower third of the gradient contained an essentially pure lysosomal population. The upper $^2/_3$ of the gradient were repelleted (1 h/105,000 × g), resuspended and recentrifuged on a 2nd Percoll system (1.05 g/ml) for 2 h at 20,000 × g. 2 more distinct lysosomal populations could be isolated one being associated with synaptosomes. All subcellular fractions were characterized by biochemical markers and by electron microscopy.

Modulation of the leukotriene (LT) generation of eosinophils by different supplements

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Pure (> 98%) horse eosinophils (2.5×10^7/ml) incubated with 10 µg/ml Ca-Ionophore A 23187 for 15 min at 37 °C generated 60±34 units (= 100%) guinea-pig ileum contracting activity, which could be resolved by HPLC into 2 LTB_4 peaks and 1 LTC_4 peak. Preincubation for 5 min of the eosinophils with 100 µg/ml vitamin C raised the contracting activity to 174±51% and on HPLC the LTC_4 peak was also significantly increased. Vitamine E (100 µg/ml) reduced the contracting activity to 41±17% and on HPLC the LTB_4 peaks were decreased, whilst the LTC_4 peak was increased. Vitamin A (100 µg/ml) reduced the contracting activity to 5±3% and on HPLC the LT peaks disappeared. NaF and the scavengers histidine, mannitol, 2,5-dimethylfuran and uric acid can also modulate the LT generation.

Regulation of human red blood cell (Ca-Mg) ATPase by calmodulin and calcium

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A quantitative analysis of the effect of Ca^{2+} and calmodulin on the human erythrocyte (Ca-Mg)ATPase activity has been carried out. 3 major conclusions can be drawn: 1. The biochemically active species are $CaM \cdot Ca_3 + CaM \cdot Ca_4$. 2. These species bind to the enzyme with a K_{diss} of 6×10^{-10} M or 1.1×10^{-8} M, depending on whether Ca^{2+} saturates the substrate binding site of the enzyme or not. 3.

The binding of $CaM \cdot Ca_3 + CaM \cdot Ca_4$ to the enzyme decreases the K_{diss} of the enzyme for Ca^{2+} at the substrate binding site from 51.5 to 2.8 μM. Contrary to general belief calmodulin does not induce pronounced positive cooperativity in Ca-binding: an apparent cooperativity is seen exclusively when only part of the enzyme binds CaM, but disappears in the presence of saturating concentrations of $CaM \cdot Ca_3 + CaM \cdot Ca_4$. In healthy erythrocytes, the Ca pump keeps free [Ca^{2+}] low (ca. 0.25 μM) with a minimal dissipation of energy; a small increase in intracellular Ca^{2+} results in a strong amplification of the pumping activity.

Phase transition behavior of trypanocide-containing liposomes

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Liposomes of dipalmitoyllecithin were prepared in the presence of 15% of ethidium bromide, salicylhydroxamic acid, and pentamidine mesylate. The inclusion of the drugs lowered the main transition temperature as shown by scanning differential calorimetry. With ethidium bromide and salicylhydroxamic acid the pretransition has totally vanished. The phase transition determined by the efflux rates, as a function of temperature, of trapped carboxyfluorescein shows similar transition shifts, indicating an alteration of the physical properties of the lipid bilayer by the drugs.

Does troponin C cross-react biologically with calmodulin in the case of the erythrocyte (Ca-Mg) ATPase system?

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Calmodulin substitutes for troponin C in the regulation of actomyosin ATPase; does biological cross-reactivity of these 2 proteins also exist in calmodulin-regulated processes? A negative answer was obtained in the instance of phosphodiesterase and myosin light chain kinase, whereas Larsen and Vincenzi gave a positive answer for erythrocyte (Ca-Mg)ATPase (Science *204*, 306). We report here that the classical troponin C preparations activate (Ca-Mg)ATPase, but at concentrations 300-fold higher than those of calmodulin. Upon sephadex G-100 chromatography in a Ca-free medium the endogenous activating factor elutes distinctly beyond the peak of troponin C. The K_{av} value of this factor corresponds to that of bovine brain calmodulin. Thus the activation of (Ca-Mg)ATPase, attributed to troponin C, is really due to contamination by calmodulin.

Peptide inhibitors of the reactivation of dissociated lactate dehydrogenase. Reversion of the inhibition by NADH and NAD^+

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The catalytic activity of the LDH-isoenzymes depends on their tetrameric structure. Low pH or other denaturants lead to dissociation into monomers and to the loss of the specific activity. After removal of the denaturing conditions reassociation and reactivation occur spontaneously. Neither NADH *nor* NAD^+ show a significant effect on the reactivation. We have isolated 2 different peptides which isoenzyme-specifically inhibit the reactivation of dissociated LDH. Inhibition was abolished by treating with proteases. Additionally, NAD and NADH were found to be antagonists of the inhibitors. The heart-type enzyme inhibitor system is especially susceptible for NADH whereas NAD^+ affects the inhibition only slightly. The muscle-type system shows the opposite behavior, e.g. the completely inhibited system can be fully reactivated by NAD^+ but not by NADH. These findings suggest a possible specific regulatory function of these peptides.

Reductive studies of the iron-sulphur cluster of bovine heart aconitase: multiple forms of the reduced Fe–S cluster

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It is now well established that the long-known citric cycle enzyme aconitase contains an Fe–S cluster, although the enzyme is not involved in any kind of electron-transfer reaction. The Fe–S cluster in aconitase seems to be of a novel type, namely a [3Fe–3S] cluster and is paramagnetic (i.e. EPR-detectable) in the oxydized state. Reductive studies reveal various EPR-detectable species, depending upon the experimental conditions, besides the classical reduced (EPR-undetectable) form of the [3Fe–3S] cluster. 3 different EPR-detectable species of the reduced cluster could be generated, depending upon the pH of reduction and/or the reductants used. The EPR-parameters of 2 such signals present striking similarities with those of the EPR-detectable Fe–S centers of Photosystem I, including center X. Whether the generation of such signals is artefactual either in aconitase or particularly in center X of photosystem I, is discussed here.

Modifications of crystalline hemoglobins

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We have studied oxygen affinity of hemoglobin (Hb) in 2 model systems: tetrameric human and monomeric liver fluke *(Dicrocoelium dendriticum)* Hb. Certain benzoates are allosteric effectors of hemoglobin (Laver et al., J. appl. Physiol. *43*, 632, 1977). Orthorhombic human deoxy-Hb crystals have been grown from PEG-solutions in an O_2-poor ($\leqslant 10$ ppm) atmosphere: space group $P2_12_12$, a = 96.6 Å, b = 98.0 Å, c = 65.9 Å, Z = 4. Co-crystallization with o-iodobenzoate yields isomorphous crystals with changed intensities. Binding site(s) of this allosteric effector are currently being determined crystallographically. We have previously crystallized *D. dendriticum* hemoglobin in CN-met-form at acid pH (Smit and Winterhalter, J. molec. Biol. *146*, 641, 1981). The structure determination by isomorphous replacement is in progress. Spectral evidence is presented that this crystalline Hb can be converted to other liganded states.

Improvement of detection of proteins and peptides in polyacrylamide gels by formaldehyde fixation followed by silver staining

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Nanogram amounts of protein can be detected after fixation of proteins and peptides by covalent binding to polyacrylamide with formaldehyde and subsequent staining with silver. Silver staining was made reliable by defining the ammonia concentration in the silver nitrate reagent,

by extending differentiation in water until free silver was undetectable and by introducing a methylamine 'stopper' to terminate development (Bürk et al., Meth. Enzymol., in press, 1982). Reproducible results were obtained with native gels, SDS (sodium dodecylsulphate) and urea gels, and with several acrylamide concentrations. A variant of the method was also applicable for staining proteins after isoelectric focussing.

Mannan synthesis in the osmophilic yeast *Saccharomyces rouxii*

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The osmophilic yeast *S. rouxii* which is involved in food fermentations can grow readily in NaCl concentrations up to 3.0 M, which can lead to wall thickening. As mannan is an important component of the cell wall of *S. rouxii* (mannan to glucan ratio of 1.0:1.2) it was decided to investigate mannan synthesis in this organism. Mannan synthetase activity has been achieved using spheroplast lysates and membrane preparations. The enzyme has a pH optimum of pH 7.0–7.2 and requires the presence of 7.5 mM $MnCl_2$ for maximum activity. Enzymic activity was examined by following the incorporation of (^{14}C) mannose from guanosine 5′-diphosphate (^{14}C) mannose into mannan polymer. The effect of growth in the absence and in the presence of different concentrations of NaCl on mannan synthetase has been examined.

Multi-aggregation states of the cardiac fatty acid-binding protein; its implications in energy production in the heart

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Self-aggregation of the cytoplasmic fatty acid-binding and carrier protein purified from the heart is described for the first time. Experimental evidence is provided by the variations of O_{225} nm vs. the protein concentration as observed in CD spectra, by segregation into 4 electrophoretic bands in the PAGE system and by the appearance of sigmoidal components after deconvolution of the fatty acid-binding capacity curve obtained by ESR analysis. At pH 7.2, corresponding to the physiological pH of the cardiac cell, 4 structural species of this protein are coexisting in equilibrium. A mathematical model is proposed that calculates the concentration of each of the 4 species. The possible interactions of this protein with fatty acid or acylCoA-dependent enzymes bound to membranes, is also analyzed. Strong modulation of the enzyme activity is predicted when, selectively, 1 protein species only interacts with the membrane enzymes.

Transcobalamin II synthesis by human skin fibroblasts

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Studies concerning the site of synthesis of human transcobalamin II (TC II) have shown that bone marrow can participate, but that other cellular sources must exist as well (Blood *56*, 560, 1980). A report describing intracellular TC II in fibroblasts led us to investigate media of cultured fibroblasts using a solid-phase immunoassay (RIA) and polyacrylamide gel-electrophoresis (PAGE) to quantitate and identify the secretory protein. Results: 1. After confluence, TC II production (RIA) increased linearly up to 30 days. 2. Cycloheximid inhibited TC II synthesis in a reversible fashion. 3. TC II variants (PAGE) corresponded to the serum phenotype. 4. 2M ammoniumsulfate precipitated TC II. 5. Gelfiltration (G-150) separated a vitamin B_{12} binding fraction corresponding to TC II. 6. Antihuman TC II antiserum removed the TC II-like activity from the medium. Conclusion: fibroblasts are capable of secreting and synthesizing TC II.

Inhibition of fibrin polymerization by synthetic peptides

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Human fibrinogen was treated with batroxobin, *Ankistrodon contortrix* thrombin-like enzyme or thrombin; the resulting fibrin monomers were devoid of fibrinopeptide A, fibrinopeptide B, or both, respectively. The synthetic peptide Gly-Pro-Arg (1 mM), an analogue of the aminoterminal sequence of fibrin α-chain, dramatically inhibited polymerization of desA- as well as of desB-fibrin monomers. Inhibition was strongly accentuated at low calcium concentration (< 1 mM). Gly-His-Arg (5 mM), the amino-terminal tripeptide of the fibrin β-chain, had no effect upon polymerization of desA- or desAB-fibrin monomers at physiological calcium concentration but enhanced their aggregation in the absence of calcium ions. However, polymerization of desB-fibrin monomers was only slightly inhibited by Gly-His-Arg. Our results indicate that competitive binding of Gly-Pro-Arg and Gly-His-Arg to the respective binding sites in the carboxy-terminal domain of fibrin monomer cannot explain the observed inhibition.

Lymphocyte membrane proteins and their activation by phytohemagglutinin

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The surface proteins of human peripheral blood lymphocytes were studied during phytohemagglutinin (pha) activation. The cells were incubated with pha for up to 16 h, transferred to mitogen-free medium and surface iodinated after various time intervals. The partitioning of labeled proteins in Triton X-100-soluble and insoluble fractions, and in surface membrane fractions was studied by autoradiography of 2-dimensional gels. – Of the surface-labeled species 2 microheterogeneic proteins partition quantitatively into the Triton-insoluble fraction. They are membrane proteins, because they are enriched in one of various membrane fractions isolated without detergent and are sensitive to neuraminidase treatment of intact cells. Known intracellular proteins are not labeled. These 2 proteins can be separated from the Triton-soluble and the nuclear pellet fractions on sucrose density gradients. There are changes in the pattern of surface-labeled proteins during pha activation of cells. Pha persists for up to 72 h at the cell surface.

Murine monoclonal IgE against bovine β-lactoglobulin

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A monoclonal IgE hybridoma was produced by the fusion of NS2 myeloma cells and spleen cells from Balb/c mice immunized with β-lactoglobulin adsorbed on alum. Affinity chromatography was used to isolate the pure IgE from

ascitic fluid. Production of rabbit anti murine IgE, rendered specific for the ε-chain has allowed us to develop a radioimmunoassay for the quantitation of murine IgE, and to study the specificity of the hybridoma product. Furthermore, we are at present investigating the morphology and localisation of mast cells from Balb/c mice bearing the hybridoma and fed with cow's milk or β-lactoglobulin.

Structure of phosphoribosylanthranilate isomerase: Indoleglycerolphosphate synthase (PRAI:IGPS) at 5 Å resolution

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PRAI:IGPS from *Escherichia coli* is one of the few simple representatives of multifunctional enzymes. It is a monomer which catalyzes the 2 metabolic steps of tryptophan biosynthesis preceding the tryptophan synthase reaction. Biochemical and genetic studies showed that the N-terminal portion of the polypeptide chain is responsible for IGP-synthase activity while the C-terminal part comprises the active site of PRA-isomerase. - The enzyme crystallizes in space group $P4_1$ with $a=b=105$ Å and $c=67.9$ Å. 3 heavy atom derivatives were found, their respective Patterson maps interpreted and heavy atom parameters refined. A 5 Å electron density map was calculated. In the map one can distinguish separate molecules of size $70\times50\times40$ Å in which the 2 domains can be recognized. Crystal data of the enzyme in the presence of a substrate analogue for both reactions should reveal the locations of the 2 active sites.

In vitro effects of citrate on calcium handling by isolated rat liver mitochondria

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Energized mitochondria respond to small amounts of EDTA or $CaCl_2$ to the suspension medium by a net release resp. uptake of Ca^{2+}, so as to maintain the Ca^{2+} concentration of the extramitochondrial fluid at a steady value (set point). As measured with a pCa electrode, the set point established by mitochondria incubated in a KCl-medium with succinate + rotenone (pH 7.4, 25 °C) was decreased from 1.18 ($\pm$0.05) to 0.75 ($\pm$0.02) ng-ion Ca^{2+}/ml by admixing 1.2 mM citrate to the medium ($n=4$). With increasing amounts of added $CaCl_2$, on the other hand, the capacity of mitochondria to accumulate and retain Ca^{2+}, which is limited by the occurrence of Ca^{2+}-induced uncoupling with collapse of the Ca^{2+} gradient across the inner membrane, was markedly increased by citrate. Strong effects of citrate were also observed with Pi present (1-2 mM). Citrate effects on both the set point and the Ca^{2+}-retention capacity resemble known effects of ATP.

The mechanism of action of cholera toxin: inhibition by blockers of protein synthesis

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Pretreatment of macrophages with cycloheximide blocked the intracellular accumulation of cAMP after exposure to cholera toxin (CT) in a time- and dose-dependent manner which paralleled the inhibition of protein synthesis. The response to isoproterenol, on the other hand, was not affected, nor did the number of receptors for CT decrease. In broken cells, however, adenylate cyclase could be stimulated by the A_1 fragment of CT regardless of pretreatment. - Presumably, the A protomer of CT has to enter the cell in order to be reduced and release the active A_1 fragment. Our experiments showed that the generation of A_1 after incubation of cells with ^{125}I-CT was inhibited in pretreated cells. Furthermore, ^{125}I-CT bound to treated cells was not degraded. - Since both the receptor for CT and the adenylate cyclase were intact, we concluded that the translocation of the A subunit of CT through the cell membrane is inhibited by pretreatment with blockers of protein synthesis and that a cell membrane protein is necessary for the translocation to take place.

Ultrastructural localization of Kunitz trypsin inhibitor in soybeans using gold granules labeled with protein A

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The Kunitz trypsin inhibitor (SBTI) is one of the best characterized inhibitor of serine proteases but its cellular location is unknown and its physiological role in soybeans is obscure. SBTI has now been localized at the ultrastructural level on thin sections of *Glycine max* (Soybean) var. Maple Arrow by the gold method (Horisberger and Rosset, J. Histochem. Cytochem. *25*, 295, 1977) using protein A. Thin sections were incubated with an anti-SBTI immunoglobulin fraction and then exposed to gold granules (12 nm in size) labeled with protein A. SBTI was found localized in the cell wall and in most of the protein bodies but not in the cytoplasm. However, in the embryonic axis, marking was also associated with the cytoplasm. Cotyledons after 4 days germination were also examined. The results were similar. These observations were corroborated by immunofluorescence staining. Numerous controls indicated that the methods were specific.

Spatial structures of snake venom toxins by NMR: Sequential ^{1}H NMR assignments in cardiotoxin $V^{II}2$ from *Naja mossambica mossambica*

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Cardiotoxins from snake venoms produce a variety of toxic effects, e.g. depolarization of membranes, hemolysis and synergistic action with phospholipase A_2. Relatively little is known about the mechanisms of action of cardiotoxins and additional insight might come from a knowledge of the spatial structures. We have started work on the determination of the spatial structure of cardiotoxin $V^{II}2$ from *N. moss. moss.* (obtained from Prof. M. Lazdunski), following a strategy based on the use of 2-dimensional ^{1}H NMR at 500 MHz as was recently described in detail (Wüthrich, Wider, Wagner and Braun, J. molec. Biol., in press). Nearly complete ^{1}H NMR assignments will be presented and the gross features of the conformation of toxin $V^{II}2$ will be discussed.

Replicases appear to be highly conserved enzymes

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DNA polymerases responsible for chromosomal DNA replication of the bacterium *Escherichia coli*, the fungus *Ustilago maydis*, the fly *Drosophila melanogaster*, the chicken and

the mammals including human all possess a high M_r (125,000–160,000) catalytic polypeptide. Not only crude extracts from these cells and organisms possess such high M_r activities but also highly purified or even homogeneous enzymes. When limited endogeneous proteolysis is allowed to occur a defined and remarkably similar pattern of 4–5 intermediate M_r (74,000–110,000) catalytically active fragments is created in prokaryotes as well as in eukaryotes (Hübscher, Spanos, Albert, Grummt and Banks, Proc. nat. Acad. Sci. USA *78*, 6771, 1981). Protease inhibitors can retard the formation of some of these catalytically active proteolytic fragments. The high M_r polypeptide for DNA-chain elongation in prokaryotes and in eukaryotes appears to possess a conserved 3-dimensional structure.

Are microtubules the tracks for the intracellular traffic of coated vesicles?

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It has been shown that coated vesicles are involved in the intracellular transport of newly synthesized and of endocytosed proteins. The question arises whether these coated vesicles are transported by random diffusion or whether they are specifically carried along cytoskeletal tracks. – We have found that preparations of microtubules contain a component (~ 10% of total proteins) with the mol.wt of 180 kd. Immunological data indicate that this material is clathrin, the main component of coated vesicles. Direct immunoprecipitation of coated vesicles from bovine brain showed, in addition to the coat proteins, a microtubular component with a mol.wt of 185 kd, which might represent the linker between the vesicles and the tubules. Analysis of negatively stained preparations of microtubules in the electron microscope indicates that, in fact, there is a connection between microtubules and coated vesicles.

Monoclonal antibodies against Torpedo nicotinic acetylcholine receptor

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Monoclonal antibodies (mAbs) raised against nicotinic acetylcholine receptor (nAcChR) were classified into 3 groups, based on inhibition with cholinergic ligands. Group A, comprising 2, probably identical mAbs, binds at the cholinergic ligand-binding site. These mAbs are inhibited from binding to nAcChR by a range (8) of cholinergic ligands, including α-bungarotoxin (α-Bgt), in a dose-dependent manner. Group B, comprising 2 mAbs, binds close to, but not at, the cholinergic ligand-binding site. They are inhibited from binding by α-Bgt (mol.wt 8000) but not by cholinergic ligands of smaller mol.wts. Group C, over 20 mAbs, binds outside the ligand-binding site of nAcChR. The majority bind to a region of nAcChR exposed at the cell surface. Mutual inhibition studies with 7 mAbs from this group suggest that they bind within a limited area of the receptor. This area is distinct from that occupied by mAbs from group A.

Complement activation by monoclonal antibodies

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Complement (C) binding and activation by monoclonal anti-sheep red blood cell (SRBC) and anti-trinitrophenyl (TNP) antibodies were studied. Increasing amounts (2×10^5 to 2.5×10^6 molecules per cell) of TNP were covalently linked to SRBC using ^{3}H-labeled trinitrobenzene sulfonate. Complement activation was maximal at 10^6 TNP molecules per SRBC and declined sharply at higher densities. Over this same range (10^6 to 2.5×10^6 TNP per SRBC) of derivitization, binding of ^{125}I-labeled anti-TNP monoclonal γ_2b antibodies remained constant, suggesting that optimal binding and activation occurs at a well-defined angle of the 2 Fab arms of the Ig molecule. Monoclonal γ_2a and γ_2b-antibodies were comparably efficient for Clq binding and complement activation, whereas γ_3 was 5–10 times less active and γ_1 inactive. Dilutions of TNP- and Forssman-specific γ_2b monoclonal antibodies, each producing 1–2% lysis of target cells, gave upon mixing a synergistic effect corresponding to a lysis 5–10 times stronger than the expected theoretically value.

Monoclonal antibodies against insulin-like growth factor I (IGF I)

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IGF I (Mr 7500) has been isolated from human serum. The hybridoma technique was used to raise monoclonal antibodies against this polypeptide. Mouse F0 myeloma cells were fused with spleen cells of 4 immunized animals (3 BALB/c mice and 1 Wistar rat). Hybrid supernatants were tested for the presence of antibodies to IGF I in a solid and in a liquid phase radioimmunoassay (RIA). 4 clones secreting anti-IGF I antibodies of the IgG_1-type were isolated. 1 antibody had a suitable affinity (10^9 M^{-1}) for IGF I serum level determinations by RIA (normal subjects: 160 ng/ml, acromegalic patients: 850 ng/ml). In the RIA, IGF II cross-reacts slightly (3%) with the anti-IGF I antibody but porc insulin does not. Crude IGF (0.03% purity) was purified 400-fold by chromatography on sepharose-linked antibody. Both IGF I and II were absorbed by the affinity column.

Oxotremorine binding induces a change in apparent molecular size of muscarinic cholinergic receptor

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Muscarinic cholinergic receptor from frog heart membranes was solubilized by a digitonin and gitonin detergent system. High specific binding of radiolabeled ligands was recovered in the solubilized preparation. The molecular characteristics of the receptor were studied by gel-filtration chromatography on Bio-Gel A-0.5 m column. The receptor can be identified by [^{3}H]-oxotremorine, an agonist, and [^{3}H]-quinuclidinyl-benzylate (QNB), an antagonist. Exposure of the receptors to [^{3}H]-oxotremorine prior to solubilization resulted in an increase in apparent receptor size, which was not found in the antagonist binding experiments. Binding of [^{3}H]-QNB to the same column eluates demonstrated a shift of the peak to a lower apparent molecular size. The cause of such a change in apparent receptor size remains unknown. It is speculated that either agonist-induced receptor aggregation of interaction of the receptor with a guanine nucleotide regulatory protein may be responsible for this change.

Effect of EGTA on actin filament self-association in the presence of calcium

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The presence of EGTA strongly affects the calcium dependence of actin filament self-association. In the absence of

EGTA, calcium ions below pCa = 3.5 enhanced the actin network formation, as seen by low-shear, falling-ball viscometry. Gel point (infinite falling time) was obtained at pCa below 2.5 for an actin concentration of 0.27 mg/ml. In the presence of millimolar concentrations of EGTA, the gel point was shifted to lower $[Ca^{2+}]$ ($pCa_{free} \sim 4.0$). This effect was observed provided that the total concentration of Ca^{2+} was at least in the mM range. Under such conditions, no change in high-shear Ostwald viscometry was observed, i.e. the number and the average length of actin filaments remained unchanged. These results suggest that, in addition to the effect of calcium per se on actin filament self-association, calcium may cross-link F actin via EGTA associated with actin. Thus particular caution should be exercised whenever an EGTA buffer system is used in studies on Ca^{2+}-sensitive actin gelation factors.

Fluorescence studies on the Ca^{++}-dependent adenosine triphosphatase from sarcoplasmic reticulum labeled with N-(3-pyrene)maleimide

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The microviscosity of sarcoplasmic reticulum vesicles from skeletal muscle was determined using 1,6-diphenyl-1,3,5-hexatriene (Shinitzky and Barenholz, J. biol. Chem. *249*, 2652, 1974). The viscosity continuously decreased from 3 P at 5 °C to 0.9 P at 40 °C. The vesicles were labeled to different extents with N-(3-pyrene)maleimide (NPM) at pH 7.0 (0.01–5 moles NPM per mole ATPase). The calcium-dependent dinitrophenylphosphatase activity was unaffected after the labeling. As expected from the viscosity measurements and from the mol.wt of the monomeric protein, the fluorescence polarization of NPM did not change between 5 °C and 40 °C, whereas the lifetime slightly decreased. From the concentration dependence of the polarization it is concluded that the NPM-fluorescence polarization did not monitor the rotation of the whole ATPase molecule within the membrane. The occurrence of a temperature-dependent fluorescence polarization required the solubilization of the membrane.

The structural alterations on erythrocytes (RBC) which allow a naturally occurring autoantibody to distinguish between old and young RBC

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An IgG autoantibody binds preferentially to old RBC and thereby initiates a selective erythrophagocytosis. This preferential binding to old RBC is due to dimerization of a small fraction of preexisting antigen monomers. This change in valency doubles the change in free energy of autoantibody binding and yields ≤ 200 firmly bound autoantibodies on old RBC. Antigen monomers are present on both young and old RBD and constitute a subpopulation of band 3 protein. Dimerization of antigen monomers is likely to occur by a local detachment of the cytoskeleton from the membrane, because ATP-depletion, known to detach the cytoskeleton from the membrane, also results in increased autoantibody binding to ATP-depleted RBC and to spectrin-free vesicles. Hence, exposure of a cell-age-specific antigen is not due to a chemical modification, but to changes within the cell that affect the lateral restriction of proteins.

Adenylate kinase regulates transient kinetics of mitochondrial ATP production

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The transient response of mitochondrial ATP production towards perturbations was studied by analyzing the trajectories leading from arbitrary initial conditions to steady state. Trajectories were calculated from differential equations based on linear relations between flows and thermodynamic forces in the adenylate kinase system (AKS). The motion of the system along the trajectories consists of 2 phases: 1. A rapid phase leading from initial states to a common attractor along loci of constant adenylic energy charge. Analytical calculations revealed that this charge is a constant of motion of the AKS. 2. A slow phase of motion along the attractor leading to the final steady state. The attractor corresponds to states close to equilibrium of the AKS. Thus AKS not only optimizes the efficiency of oxidative phosphorylation through thermodynamic buffering but also deeply influences the transient kinetics of the whole system. Incubations with isolated mitochondria were in excellent agreement with the theoretical predictions.

Controlled expression of a human interferon-α gene introduced into mouse L cells

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A cloned chromosomal human interferon-α1 (IFN-α1) gene linked to a thymidine kinase gene as a selectable marker was introduced into mouse L cells. Of 13 transformed cell lines, 8 produced correctly initiated IFN-α1 mRNA after infection with Newcastle disease virus, but not under normal growth conditions. The IFN-α1 mRNA was polyadenylated and had the same 5′- and 3′-ends as IFN RNA from IFN-producing human leukocytes. Uninduced cells contained some human IFN RNA initiating upstream from the cap site. Since the incorrect transcripts were about as stable in noninduced as in induced mouse cells, we conclude that the effect of induction is not inhibition of IFN RNA degradation, but rather new production of correct transcripts. Since the levels of human IFN-α1 RNA follow the same kinetics as those of mouse IFN mRNA, the foreign gene is likely subject to the same control mechanisms of the host cell as are the resident mouse IFN genes.

Limited proteolytic cleavage of aspartate aminotransferase isoenzymes (AAT)

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Treatment of both the cytosolic (c) and mitochondrial (m) AAT from chicken and pig in their native state with trypsin coupled to sepharose results in the selective cleavage of 1 or 2 peptide bonds: after Arg 26 and Lys 31 in mAAT from chicken (Sandmeier and Christen, JBC *255*, 10284, 1980) and pig; after Arg 25 and Arg 31 in cAAT from chicken and only after Arg 25 in cAAT from pig. In all 4 cases the N-terminal peptides are lost on gel filtration. The enzymic activity is decreased to a few percent although none of the residues in the N-terminal segment that bridges the active site cleft between the 2 subunits is part of the active site. Quantitative immunological characterization of the shortened enzyme derivatives indicates their conformational integrity. In both isoenzymes the tryptic cleavage is substantially retarded in the presence of a transaminating

substrate pair suggesting that the bridging segment participates in the propagation of syncatalytic conformational changes.

Cytochrome P-450 content and substrate-induced difference spectra in capsular adrenal of K^+ depleted and K^+ repleted rats

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The mineralocorticoid aldosterone is synthesized in the capsular portion of rat adrenal cortex. K^+ ions have a direct stimulatory effect on the late steps of aldosterone biosynthesis (corticosterone 18 methyl oxydation I and II). We investigated the influence of a K^+ loading on the concentration of total cytochrome P-450 and substrate-induced difference spectra in mitochondria and microsomes of capsular adrenal. This K^+ treatment had no influence on total cytochrome P-450 concentration, cholesterol- and deoxycorticosterone-induced difference spectra in mitochondria and the progesterone-induced difference spectrum in microsomes. In contrast, the corticosterone-induced difference spectrum was increased by K^+ repletion of K^+ depleted rats, indicating increased corticosterone 18 methyl oxidation. This effect might be another example for the induction of a specific cytochrome P-450 mixed function oxidase.

Effect of trifluoperazine on the release of spectrin-free vesicles from ATP-depleted human red blood cells

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The release of spectrin-free vesicles from ATP-depleted human red blood cells can be inhibited by high EDTA concentrations (Müller, Schmidt and Lutz, BBA, in press). The apparent inhibition by EDTA suggests the involvement of bound divalent cations, possibly of the Ca^{2+}-calmodulin complex, in the mechanism of vesiculation. Trifluoperazine, a calmodulin-blocking agent, inhibits vesicle release completely at very low concentration (30 µM) when present during ATP-depletion of cells. Trifluoperazine also prevents a complete shape change to spheroechinocytes and facilitates a reversal of the shape change to discocytes. Dephosphorylation of cytoskeletal proteins bound to IOV is clearly reduced in ATP-depleted cells when trifluoperazine is present. This suggests that calmodulin could be involved in the control of cytoskeletal interactions with the membrane necessary to maintain discoid shape.

The subunits of the rat alpha-macroglobulins

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Rats produce 2 α-macroglobulin (αM) proteinase inhibitors, the α_1M, normally found in the plasma, and the α_2M, an acute phase protein. On the basis of M_w, pI, AA composition and proteinase-binding characteristics both are considered to be functional and (by implication) structural homologues of human α_2M. Comparison of the purified proteins on SDS PAGE following mild reduction (1% SDS, 1%-mercaptoethanol, 37 °C, 45 min) reveals a 185,000 M_w species for rat and human α_2M and a 167,000 plus a 38,000 M_w species for rat α_1M. Heat treatment (pH 11, 37 °C for 45 min) prior to reduction results in the appearance of 125,000 M_w and 60,000 M_w components from rat α_2M analogous to the pattern of human α_2M. In contrast α_1M showed, in addition to the 125,000 M_w and the 38,000 M_w bands, 2 bands of approx. 25,000 M_w. Incubation with trypsin (~1 mole/mole αM) prior to reduction causes formation of ~90,000 M_w components from both rat inhibitors. – These data suggest that only rat α_2M but not α_1M is structurally homologous to human α_2M.

Cell concentration dependency of corticosterone production in vitro

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The effect of cell concentration on corticosterone (B) production by rat adrenal fasciculata cells under ACTH stimulation (3×10^{-8} M) was studied. After a 2-h incubation period, intra- and extracellular B and total cAMP were measured. When the number of cells/incubate was raised, the total B production/cell varied in a biphasic way: it increased from 10^2 to 10^4 cells/ml, and decreased from 10^4 to 2×10^5 cells/ml (by up to 80%), as did total cAMP, while the intracellular B content kept increasing. Moreover the dose-response curves of B production under AVTH stimulation (10^{-12} to 10^{-7} M) were progressively shifted to the right. The supernatant of cells (2×10^5 cells/ml) incubated for 2 h was stripped of steroids and supplemented with exogenous B (200 ng/ml). This supernatant inhibited the maximal ACTH stimulation in freshly dispersed cells (10^4 cells/ml) by up to 50%. These results suggest that accumulation of steroids affects steroidogenesis at the level of both cAMP production and steroid release.

Proteins of spinach chloroplast envelopes

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Proteins of purified chloroplast envelopes, isolated according to Joyard and Douce (Physiol. Vég. *14*, 31, 1976), were separated by electrophoresis in a linear acrylamide gel gradient (SDS-PAGE) and by isoelectric focusing (PAGIF). SDS-PAGE resolved 37 polypeptides. The 2 major polypeptides (i.e. 54,000–56,000 and 15,000–16,000) of the envelope fraction were compared with the corresponding ones of the stroma and thylakoid fractions and identified as the subunits of the RuBP carboxylase or of the coupling factor by peptide mapping after limited proteolysis or by PAGIF in a 2nd dimension. Solubilization conditions of the envelope membranes for the PAGIF separation were studied in the presence of nonionic and ionic detergents. An adaptation of the method of Ames and Nikaido (Biochemistry *15*, 616, 1976), using solubilization in SDS and PAGIF in the presence of high concentration of Nonidet P40, gave the best separation and permitted to resolve the envelope fraction into 23–25 proteins.

Effect of (+) and (−)catechin [cyanidanol-(3)] on glycogen metabolism in isolated rat hepatocytes

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0.5 mM of either (+) or (−)catechin (cat.) showed opposite effects on glycogen metabolism when added to isolated rat liver cells incubated for 60 min in Krebs-Ringer bicarbonate buffer containing albumin. In liver cells from fasted rats, net glycogen production was stimulated by (+)cat. by 108% and inhibited by (−)cat. by 92% (substrate: 20 mM glucose). Inhibition was also observed when glycogen synthesis was stimulated by potassium, insulin, lithium or

amino acids. (DL)cat. inhibited glycogen deposition to the same extent as (−)cat. The stimulatory effect of (+)cat. was not mediated by cyclic AMP since (+)cat. antagonized the inhibitory action of glucagon on glycogen production without changing cyclic AMP levels. In livers from fed rats, addition of (+)cat. inhibited glycogenolysis by 34% whereas (−)cat. stimulated it by 37%. The mechanism of these effects and whether there is a correlation of the known hepatoprotective action of (+)cat. (catergen®) is under investigation.

Conformation of laminin fragments

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Elastase, chymotrypsin, trypsin, subtilisin and *Staphylococcus aureus* protease produced similar fragment patterns upon prolonged laminin digestion. 4 laminin fragments could be purified and they differed in size, amino-acid composition, spectral properties and antigenicity. Fragment 1 ($M_r \approx 280{,}000$) showed a circular dichroism spectrum indicative of aperiodic structure. Fragment 3 ($M_r \approx 50{,}000$) possessed β-structure. Fragments 2 ($M_r \approx 50{,}000$) and 4 ($M_r \approx 75{,}000$) exhibited mainly aperiodic structure. - Electron microscopy showed that fragment 1 consists of 3 rod-like elements connected to each other at one end. Fragment 3 appeared globular, fragment 2 as a short rod and fragment 4 as a globule connected to fragment 2.

An active monomeric form of human erythrocyte membrane acetylcholinesterase (AChE)

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After treatment of detergent-solubilized AChE with mercaptoethanol and iodoacetic acid, analysis by sucrose density gradient centrifugation revealed a conversion of the dimeric 6.3 S form to a monomeric 4.1 S form. More than 70% of the enzymatic activity were recovered after monomerization. Similar treatments of erythrocyte membrane preparations yielded only 4.1 S AChE and no loss of activity could be detected. On the other hand, predominantly 6.3 S AChE was solubilized from untreated membranes. This same result was obtained when red blood cells were washed and hemolyzed in the presence of Ellman's reagent which was used to block free SH-groups. These results indicate that AChEs exist predominantly as dimeric species in the erythrocyte membrane. The dimeric form should not be considered as an artifact of the solubilization procedure, due to oxidation of free SH groups, despite the fact that the enzyme can be monomerized in vitro.

Acetylcholinesterase from human erythrocyte membranes: dimers as functional units

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The mol. wt of purified, detergent-solubilized human erythrocyte acetylcholinesterase was determined by a sedimentation equilibrium ultracentrifugation procedure in presence of the nonionic detergent octyl-tetraoxyethylene. Because of its density of 0.9914 g/cm^3, the buoyancy term of the detergent bound to the protein could be neglected in the mol. wt calculation. The value obtained for acetylcholinesterase was 151,000±8000. Sedimentation velocity experiments yielded an $s_{20,w}$-value of 5.7 S. The enzyme showed no tendency to aggregate. Determination of the subunit mol. wt by sodium dodecyl sulfate polyacrylamide gel electrophoresis gave an apparent subunit mol. wt of 73,000. Thus, acetylcholinesterase exists as dimer in nonionic detergent solutions.

Alcohol dehydrogenase (ADH) in the gastrointestinal tract: an immunohistochemical study

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Antibodies against human liver ADH were used to localize ADH in human gastrointestinal tissue. ADH is localized in the mucosa of all parts of the gastrointestinal tract. There is a gradual decrease in staining intensity from stomach to rectum, indicating decreasing amounts of ADH. In the gut a similar gradient can be observed from strongly stained cells exposed to the lumen to unstained cells at the bottom of the crypts. Goblet cells and Brunner's glands as well as the mucoid glands of the antrum are all negative. Mucus producing cells of the stomach corpus stain very strongly. Chief and parietal cells stain weaker but the staining intensity is comparable to the gut villus cells. In conclusion, ADH is an intrinsic component of the gastrointestinal mucosa cells and cannot be attributed exclusively to the microbial flora.

Transient opening of rat kidney brush border vesicles

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Brush border membranes as prepared by the Ca^{++}- or Mg/EGTA precipitation methods are obtained as closed and right side out orientated vesicles. Experiments on membrane protein phosphorylation by γ-^{32}P-ATP indicate a transient opening of the vesicles under osmotic shock conditions. Under isoosmotic conditions alkaline phosphatase is the only prominent phosphoprotein, whereas under hypotonic conditions (1:16 dilution) approx. 18 phosphoproteins can be identified. The phosphorylation pattern obtained under osmotic shock conditions is identical to that obtained under isotonic conditions in the presence of 0.1% saponine. Osmotic shock does not alter the integrity of the membrane: a) When the vesicles were phosphorylated after hypotonic treatment, only alkaline phosphatase was phosphorylated by γ-^{32}P-ATP, indicating a similar membrane orientation after transient opening. b) Transports of D-glucose and of phosphate are identical in vesicles which have been osmotically shocked as compared to control vesicles.

TRH accelerates metabolism of phosphatidylinositol in clonal pituitary cells: a possible mechanism in stimulus-secretion coupling

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Thyrotropin releasing hormone (TRH) affects metabolism, secretory activity and morphology of clonal pituitary cells (GH cells) in culture. TRH at low concentrations ($K_a = 0.8$ nM) stimulates metabolism of phosphatidylinositol (PI) in GH_3 cells. A significant increase in ^{32}P-phosphate incorporation into PI can be observed as early as 10 min after stimulation by TRH. Neither increasing the cAMP levels

with isobutylmethylxanthin (IBMX) nor provoking an entry of Ca^{2+} by depolarization of the plasma membrane with high concentrations of K^+ cause an increased PI metabolism. Therefore if the PI response was implicated in stimulus-secretion coupling it would precede these manifestations of TRH action. We postulate that an increased metabolism of PI reflects an early event following TRH-receptor interaction, probably involved in the mechanisms that change Ca^{2+} distribution in GH cells following TRH action.

Physiological significance of M-line bound creatine kinase (CK)

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After 10 washings (50 v/w) 0.8 EU of CK activity remains bound to 1 mg chicken pectoralis myofibrils freed of mitochondria and SR representing 7% of the total CK present in the muscle. The bound CK is located at the M-line contributing to the electrondensity of this structure (Wallimann, PNAS *75*, 4292). By measuring the combined actin-activated MgATPase and CK reactions of myofibrils in a pH-stat assay it was shown that the M-line bound CK was active. The amount was sufficient to rephosphorylate the ATP hydrolyzed in vitro by the myofibrillar ATPase at maximal velocity and in steady state to support an ATP turnover rate of 6 sec^{-1} per myosin head corresponding to 30–60% of the ATPase turnover rate measured in vivo. Specific blocking of CK activity or extraction of M-line bound CK abolished the ATP-regeneration potential. The experiments provide evidence for a physiological significant role of M-line bound CK as an ATP-regenerating system at the myofibrillar end of the phosphocreatine shuttle.

Do spinach chloroplasts contain ferralterin?

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The activities of several chloroplast enzymes are light-regulated via the ferredoxin (Fd)/thioredoxin (Th) system. Its key enzyme is Fd/Th-reductase, taking electrons from photochemically reduced Fd to reduce Th. Recently another protein, ferralterin, was described (Lara et al., BBRC *94*, 1337, 1980) mediating the light regulation without the need for Fd nor Th. Ferralterin isolated from *Nostoc muscorum* shows striking molecular similarities (visible spectrum, relative molecular mass, subunit structure) with our Fd/Th-reductase purified from spinach. In no instance however did our preparation exhibit any ferralterin activity. To exclude possible damage to the spinach protein during lengthy purification procedures we carefully searched for ferralterin activity in freshly isolated chloroplasts. But already rapid elimination of Fd and Th from the chloroplast extract by gel filtration on sephadex G-50 abolishes completely any light activation of enzymes. This and other results indicate that ferralterin is absent from spinach chloroplasts.

Reevaluation of protein-protein interactions on human red blood cells

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Glycosylated proteins were labeled with ^{14}C-amines on intact red blood cells or spectrin-free vesicles following aldehyde generation. The use of either ^{14}C-aniline or ^{14}C-aryl-alkyl-diamine provided a tool to differentiate between exo- and cytoplasmic cross-links and to evaluate whether inaccessibility of aminogroups could explain a lack of cross-linking by amino-group specific reagents. Glycophorins and band 3 proteins are cross-linked to less than 1% on intact RBC, whereas on spectrin-free vesicles glycophorins are cross-linked to 7.5% and band 3 to 11% within 8 min. Amino-group supplementation enhances these values in RBC and vesicles by the same factor (~ 2). Thus the low extent of cross-linking observed on intact RBC is not due to inaccessible amino groups but to a restricted lateral mobility of membrane proteins which is eliminated in spectrin-free vesicles. The data imply that band 3 protein exists as a monomer in intact RBC. Cross-linking of band 3 on isolated membranes under the same conditions completely oligomerizes band 3.

Effect of chloramphenicol on the NADH dehydrogenases of *Neurospora* mitochondria

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When *Neurospora crassa* is grown in the presence of chloramphenicol (CAP), the respiration via the cytochrome chain is reduced and an alternative oxidase, sensitive to hydroxamic acids, is derepressed. The rates of oxidation of exogenous NADH and NADPH were also strongly enhanced and were mostly insensitive to cyanide. This would increase the flux through glycolytic pathways and favor cytosolic ATP formation, thus compensate for the deficiency of oxidative phosphorylation. Furthermore, the oxidation of NAD-linked substrates became largely insensitive to rotenone, suggesting that CAP also hindered the biogenesis of a functional NADH-ubiquinone oxidoreductase and that at least one subunit of complex I could be synthetized on mitochondrial ribosomes. The rotenone-resistant bypass was able to give electrons to both cyanide-sensitive and -resistant oxidases, indicating the operation of a branched system rather than of parallel respiratory chains.

Heme iron coordination geometry and functional properties of cytochromes c

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In cytochromes c 2 different modes of attachment of the axial methionine to the heme iron, which correspond to different chirality, R or S, at the sulfur atom have been determined by high resolution ^1H NMR and CD spectroscopy (Senn, Keller and Wüthrich, Biochem. biophys. Res. Commun. *92*, 1362, 1980). Comparison of 14 bacterial and eukaryotic cytochromes c then revealed that the chirality of methionine binding is correlated with the unpaired electron density distribution in the heme of the oxidized protein. Evidence will be discussed that functional properties of cytochromes c, such as the physiological activity and the redox potential, are controlled in part by oxidation state-dependent conformational changes in the ligand sphere of the heme iron.

Structural studies on the light harvesting system of cyanobacteria: amino acid sequence of C-phycoerythrin from *Fremyella diplosiphon*

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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-phycocaynin (CPC) and allophycocyanin (APC) to the reaction centres in the thylakoid membrane. C-Phycoerythrin was separated into 2 subunits ($\alpha+\beta$, 17,200 and 18,000 mol.wt, linear tetrapyrroles [phycoerythrobilin] covalently bound to cysteine residues). Large fragments of the chains were prepared by cleavage with chemical methods and separated by gel filtration. The amino-acid sequence was determined by sequenator analysis with polybrene as carrier. The primary structure was compared with CPC and APC. It was found that the 2nd chromophore of the β-chains are inserted together with a peptide of 10 amino-acid residues at position 150 of all the chains compared.

Cellular uptake of lipophilic β-adrenergic ligands, an explanation for apparently low agonist affinity?

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The hydrophilic ligand [^{3}H]CGP-12177 can be displaced by low concentrations of isoproterenol from β-adrenergic receptors on intact cells in contrast to lipophilic ligands (Porzig et al., Experientia *37*, 72, 1981). Using C_6 glioma cells we have found that the discrepancy between hydrophilic and lipophilic ligands is only present in intact cells but not in membranes. Since lipophilic ligands show high unspecific binding, we have studied the nature of the unspecific binding of a lipophilic ligand, ([^{3}H-]DHA) to intact cells. We conclude that [^{3}H]DHA is actually taken up by cells, since this unspecific binding is released much more slowly from intact cells than from membranes but is released immediately upon sonication. As an explanation for the apparently different agonist affinities with hydrophilic and lipophilic ligands, we propose that β-adrenergic receptors undergo an agonist-mediated endocytosis. The internalized receptors are accessible to lipophilic ligands but not to hydrophilic agonists or ligands.

Direct peptide mapping of protein bands from polyacrylamide gel electrophoresis by chemical cleavage in gel pieces and re-electrophoresis

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A convenient method has been developed for peptide mapping of protein bands obtained by polyacrylamide gel electrophoresis in sodium dodecylsulphate or under nondenaturing conditions. The procedure is based on selective acid hydrolysis of aspartyl-prolyl bonds. The gel piece containing the protein band to be analyzed is cut out and soaked with 75% formic acid. After equilibration, the gel piece is immersed in liquid paraffin and incubated at 37 °C for 24 h. Formic acid is removed by lyophilization and the gel piece is rehydrated in buffer. It is placed into the sample well of a 2nd polyacrylamide gel for electrophoretic separation of the generated peptides. An essential advantage of the chemical cleavage is its independence from the amount of substrate. Large protein bands in the µg range give the same peptide pattern as bands in the pg range detectable only by their radioactivity.

Possible correlation between the stimulation of glycogen synthesis by some amino acids and the synthesis of purines in hepatocytes from starved rats

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As known from the literature, amino acids such as glutamine, asparagine, alanine, etc. strongly stimulate glycogen deposition from glucose and lactate/pyruvate in hepatocytes, whereas leucine, arginine and lysine have no or only small effects. Since the stimulatory amino acids are also direct or indirect precursors for purine synthesis, it was tested whether inhibitors of purine synthesis can prevent this stimulation of glycogen synthesis. Purine synthesis inhibitors such as amethopterin, azaserine and 6-mercaptopurine were shown to suppress stimulated glycogen synthesis by 75, 78 and 52% respectively. Furthermore, some purines were tested for their effectiveness to stimulate glycogen synthesis. Among these, 3'-deoxyadenosine (cordycepin) showed a significant stimulation whereas adenosine was inactive. It is concluded that the stimulation of glycogen synthesis by amino acids may be mediated by a purine derivative.

The cytochrome c oxidase of *Paracoccus denitrificans*, pumps protons in a reconstituted system

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The purified 2-subunit cytochrome c oxidase of *P. denitrificans* was reconstituted into phospholipid vesicles having a high internal buffering capacity and exhibiting a respiratory control index greater than 6.6. With these proteoliposomes, pH-changes of the suspending medium were monitored in response to reductant-pulses in the presence of valinomycin and potassium. When reduced cytochrome c was added to allow for a limited number of turnovers (2–12), a net acidification of the extravesicular space could be observed. This apparent proton ejection by the vesicles was sensitive to azide and uncouplers. We conclude that cytochrome c oxidase of *P. denitrificans* is a proton pump. An apparent stoichiometry of 0.6 protons/electron was obtained by extrapolation to zero turnovers. The effect of dicyclohexyl carbodiimide on this system was investigated.

2 classes of acetylcholinesterase in human brain

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2 classes of acetylcholinesterase (AChE) E.C. 3.1.1.7 occur in human brain caudate nucleus. 20% of the total activity belongs to class A AChE (soluble in buffer of high ionic strength, s=5 S and 12 S). The enzyme does not interact with detergents and it is not a hydrophobic protein. 80% of the total enzyme activity is soluble only in the presence of detergent (s=10 S). This enzyme activity is designated as class D AChE as is possesses a hydrophobic segment which binds detergents and as its catalytic activity depends on the presence of amphiphilic molecules. Studies on the immunochemical cross-reactivity showed that the D enzyme is related to class D enzyme from human erythrocytes. The salt extractable forms of AChE (class A) appear to be only distantly related to class D AChE.

Azo dye labeling of bacteriorhodopsin

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Bacteriorhodopsin (BR) is modified by the hydrophobic azo dye 4-N,N-dimethylaminoazobenzene-4′-isothiocyanate (DABITC). The reagent is directed towards the hydrophobic domains of the membrane protein, interacting with nucleophilic groups. Covalent binding is ascertained by resistance to organic solvent extraction and SDS-gel electrophoresis. Upon chymotrypsin digestion of modified purple membranes followed by chromatography on sephadex LH-60, the label is recovered almost exclusively within the N-terminal fragment. CNBr treatment and subsequent fractionation by HPLC yields a peptide fraction which contains the covalently bound azo dye. The sequence of this fraction corresponds to amino acids 33-56 of BR. – The covalent modification of BR does not affect the structure of the purple membrane. Proton pumping activity is fully retained. The specific binding of DABITC to BR and the functional integrity of the modified protein provide the necessary requirements for structural and functional investigations with heterobifunctional azo cross-linking reagents.

Gluconeogenesis in vitro: Formation of glucose 6-phosphate from malate by a cell-free rat liver system consisting of cytosol and mitochondria

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Gluconeogenesis is one of the few metabolic pathways for which no cell-free system has been described with the exception of pigeon liver homogenate. A cell-free system has now been prepared from rat liver which forms glucose 6-phosphate at physiological rates (1.1 $\mu moles \cdot min^{-1} \cdot g^{-1}$ liver, 30 °C) from malate + 3-phosphoglycerate as substrates. At least 70% of glucose 6-phosphate formed was derived from malate based on experiments with [^{14}C]malate. The system contained cytosol and mitochondria as well as a number of cofactors at near physiological concentrations. A comparison between incubations with or without mitochondria revealed that mitochondria decreased the lactate/pyruvate ratio and increased the ratio of (ATP + ITP)/(ADP + IDP). It was shown that glucose 6-phosphate formation was closely linked to this nucleotide ratio whereas changing redox ratios had little influence on the gluconeogenic rate.

Glyceollin, a soybean phytoalexin

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Glyceollin production in hypocotyls of soybean (*Glycine max* (L.) Merr.) seedlings was studied following induction by abiotic elicitors and was compared with the production after inoculation of seedlings with zoospores of *Phytophthora megasperma* f.sp. *glycinea*. Silver nitrate was the most effective abiotic elicitor, inducing 0.65% hypocotyl dry weight glyceollin. There was considerably less glyceollin following fungal infection. With the exception of triton X-100, glyceollin accumulation depended on the concentration of abiotic elicitor. Production of glyceollin depended also on seedling age, light conditions and nutrient amendment during seedling growth, and the method of elicitor application. Glyceollin, purified by TLC and gel filtration on sephadex LH-20, was used to test its biological activity.

Inactivation of nitrate reductase and glutamine synthetase in extracts of wheat and bean leaves

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Extracts of young leaves were mixed with extraction buffer (pH 7.5) or with extracts of senescing leaves and incubated at 2 °C or at 30 °C. The inactivation of nitrate reductase (NR) and of glutamine synthetase (GS) was accelerated when extracts of senescing leaves were added. The inactivating factor extracted from senescing leaves was a) excluded by sephadex G-25, b) precipitated by ammonium sulfate (60% saturation) and was c) heat-sensitive. These properties support the hypothesis that the inactivation could be due to endopeptidase activity. NADH (3 mM) improved the stability of NR and of GS and inhibited endopeptidase activity against azocasein, while NAD had no effect. NR was better protected by NADH than GS. Our results suggest that, besides energy charge, the concentration of NAD(P)H or NAD(P) could affect proteolysis by interaction with the substrate proteins (NR, GS) or with the peptide hydrolase(s).

Maturation studies on galactosyltransferase in HeLa cells

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Biosynthesis and intracellular processing of galactosyltransferase (GT) was investigated in HeLa cells using a pulse/chase protocol with ^{35}S-methionine as label. GT was identified by immunoprecipitation followed by SDS-PAGE/fluorography. Specificity of the antibody used was ascertained by the appearance of a single band of 54 kD on SDS-PAGE. In addition, previous immunocytochemical studies revealed this antigen in trans Golgi cisternae (Roth and Berger, Biochem. Soc. Trans. *1981*, 147P, abstr.). After 10 min pulse, 2 forms of GT with 45 and 47 kD were identified, both sensitive to Endo H treatment. After 20 min chase, an Endo H resistant form of 54 kD was detected. GT was thereafter released into the supernatant as a 52-kD form. Average half-life of GT along this pathway was 15 h. Thus, GT follows a similar maturation pathway as membrane and secretory glycoproteins but appears to be retarded at the level of the trans-Golgi cisternae.

Lipid-protein interaction: deuterium-, phosphorus- and nitrogen-14-NMR studies on reconstituted cytochrome c oxidase

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Lipid-depleted bovine-heart cytochrome c oxidase has been reconstituted with a number of different selectively deuterated phospholipid molecules. ^{2}H-, ^{31}P- and ^{14}N-NMR spectra of these membranes do not indicate any 'boundary'-lipid component. The lineshape analysis of these spectra rather reveals a similar (or slightly reduced) degree of segmental order. At the extremely high protein concentrations used (up to 75 w/w) the presence of rather slow motions (10^{3}–10^{5} Hz) are manifested in the lineshapes of all recorded spectra (head group and side chains) as well as in the measured ^{2}H-T_2-relaxation rates. The correlation times for the reorientation of the various phospholipid segments have been determined from ^{2}H- and ^{31}P-T_1-relaxation

measurements. They are in the ns range for the phosphate group and the 0.1-ns range for the methylene segments. The motions are slowed down by 10–30% in the presence of cytochrome c oxidase.

Metal-thiolate clusters in metallothionein

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Mammalian metallothioneins contain 20 Cys in a total of 61 amino-acid residues and bind 7 bivalent metal ions. Since each metal ion is coordinated tetrahedrally to 4 thiolate ligands and since all Cys participate in metal binding, it follows from considerations of stoichiometry that the metals must be joined to form metal-thiolate clusters. We have now confirmed the presence of such oligonuclear complexes in metallothionein both by standard EPR and magnetic susceptibility measurements of Co(II)-metallothionein and by X-ray photoelectron spectroscopy of Cd(II)-metallothionein. The results document spin-spin interaction between the bound metal ions and prove the existence of both bridging and non-bridging thiolate ligands postulated for such structures. The data are consistent with either 2 or 3 metal-thiolate clusters built up from distorted tetrahedral units. Perturbed angular correlation of γ-ray spectroscopy (PAC) of excited ^{111}Cd-metallothionein indicates 2 different populations of distorted sites.

Inhibition of calf intestinal alkaline phosphatase by phosphonic and arsenic acids

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Phenyl-, carboxymethyl-, and 2-aminoethylphosphonate as well as 3-chloropropyl-, 3-aminopropyl-, and N-benzoyl-3-aminopropylarsonate were examined as possible inhibitors of alk. phosphatase. Their inhibition was compared with the inhibitory effect of phosphate, phosphite, and arsenate. Phenylphosphonate, phosphate and arsenate were found to be potent inhibitors. In contrast phosphite, alkylphosphonate- and alkylarsonate derivatives were only weak inhibitors. In order to analyze the kinetic data we have developed a useful BASIC-program, which permits the estimation of the kinetic paramters V_{max}, K_m and K_i on the basis of different methods: 4 conventional diagrams as primary and secondary plots an a direct curve-fitting method by multiple linear regression analysis of the general form: $y = a_0 + a_1x_1 + a_2x_2 + \ldots + a_nx_n$ (e.g. competitive inhibition: $n = 2$, $y = 1/v$, $x_1 = 1/[S]$, and $x_2 = [I]/[S]$. Diagrams are plotted and the results are printed out.

Cytochrome c peroxidase-cytochrome c electron transfer complex: cross-linking by a carbodiimide

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Cross-linking of cytochrome c peroxidase with cytochrome c by the carbodiimide EDC lead to a new compound of apparent Mr = 46,000. The compound was partially purified by sephadex chromatography. It exhibited a typical heme spectrum (Soret-maximum at 408 nm) and had 16% residual peroxidase activity. For a preliminary characterization of the linkage sites the complex was cleaved with CNBr and fragments were analyzed by polyacrylamide gel electrophoresis: 3 fragments composed of a peroxidase and a cytochrome c part were obtained. From the size of the 3 fragments and by comparison with the CNBr-fragments from free peroxidase and cytochrome c we conclude that cross-linking had occurred at carboxyl groups of the N-terminal fragment 1–119 and of fragment 172–229. The results agree with a hypothetical model of the complex based on the known crystal structures of the 2 hemoproteins (Poulos and Kraut, J. biol. Chem. *255*, 10322, 1980).

A hydrophilic form of human erythrocyte membrane acetylcholinesterase

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A monospecific rabbit antibody to purified acetylcholinesterase (AChE) of human erythrocyte membranes was raised. AChE shows its amphiphilic nature in charge-shift crossed immunoelectrophoresis (CS-CIE) as well as in hydrophobic interaction electrophoresis on phenyl-sepharose (Ph-CIE). Limited proteolytic digestion of AChE results in 2 precipitates in Ph-CIE. Both precipitates show esterase activity. One corresponds to the undigested control that appears with zero migration. The other precipitate – appearing with higher mobility – represents a less hydrophobic form. The form with zero migration in Ph-CIE binds detergents like the undigested enzyme. The form derived by proteolysis is a hydrophilic form as it does not bind detergents. The amphiphilic and the hydrophilic forms are immunochemically identical, indicating that limited proteolysis cleaves off a nonantigenic, hydrophobic part that presumably serves as the anchor of AChE to the lipid bilayer of the erythrocyte.

Human aldehyde and aldose reductase show isocorticosteroid reductase activity

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Isocorticosteroids (21-oxo-20-hydroxysteroids) are formed in an alternative pathway of the corticosteroid catabolism. We show that human aldehyde and aldose reductase catalyze the reduction of these intermediates to the corresponding glycol metabolites. The α- and β-isomers of corticosterone and cortisol and their 11-deoxyderivatives were synthesized. With aldehyde reductase the K_m values for the (11-deoxy-)isocorticosterones were 3–5 µM for the α- and 14–17 µM for the β-isomers. K_m for the (11-deoxy-)isocortisols were between 65 and 130 µM. V for the corticosterone and cortisol derivatives was 120–160 and 16–35%, respectively, of the rate with D-glucuronate. Aldose reductase reduced all steroids at the same rate, equal to that of dl-glyceraldehyde. K_m values were below 1 µM. Our results suggest that in vivo aldose reductase catalyzes the reduction of the isocorticosteroids.

Altered circadian rhythms in neurotransmitter receptors and plasma hormones in spontaneous hypertensive (SHR) rats

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Abnormal central catecholaminergic mechanisms, as well as inversed circadian rhythms in hypothalamic noradrenaline content have been found in SHR rats. We found

abnormal circadian rhythms of plasma hormones in SHR rats: corticosterone was elevated throughout the light phase, and prolactin was phase-advanced. Circadian rhythms in binding to α_1-, α_2-, β-adrenergic and muscarinic cholinergic receptors in brain regions from WKY rats showed complex differences in waveform, phase, amplitude and 24-h mean in SHR rats, with a predominance of increased receptor binding in the 2nd half of the dark phase. It will be interesting to see whether this 'supersensitive' period at the end of wake is related to functional hypertension. These findings indicate that the disturbances in adrenergic receptor functions in SHR are more complex than previously recognized.

Purification of an inhibitor protein of erythrocyte membrane ($Ca^{2+} + Mg^{2+}$)-ATPase from hemolysate

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An endogenous inhibitor of erythrocyte ($Ca^{2+} + Mg^{2+}$)-ATPase has first been observed by Au (Int. J. Biochem. *9*, 477, 1978). We have now purified a small protein (apparent mol.wt $\approx$ 19,000) from the cytosol of human red cells to homogeneity by a combination of ion-exchange chromatography, ultrafiltration, $(NH_4)_2SO_4$- and heat precipitation. This protein inhibits the Ca pump in nM concentrations by decreasing the Ca affinity of the system. The dose-response curve at a fixed [Ca^{2+}] shows marked sigmoidity (Hill coefficient $\approx$ 4). With calmodulin (CaM), which shifts the Ca^{2+}-affinity in the opposite direction, the inhibition cannot be overcome. It can therefore be excluded that the inhibitor interacts directly with CaM or competes with CaM for the same site. Membrane ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase are not affected by the inhibitor.

Glucose transport in hearts of genetically obese *(fa/fa)* rats

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As 2-D-deoxyglucose (2DG) is transported through plasma membranes *and* phosphorylated, it cannot be used for studying glucose transport per se. Therefore the nonmetabolizable analog of D-glucose, 3-0-methyl-D-glucose (3-0-MG) was used to study glucose transport by perfused hearts, using L-glucose as extracellular marker. 3-0-MG transport was completely abolished by the presence of cytochalasin B, a drug that specifically binds to glucose transport units, thereby assessing the reliability of the system. It was observed that basal 3-0-MG transport by heart of obese rats was 4 times lower than that of lean ones, a defect that could not be compensated for by the addition of even supramaximal concentrations of insulin, indicating decreased insulin responsiveness. The latter abnormality was related to altered V_{max} without change in apparent K_m. This is the first demonstration of a defectual glucose transport system per se in the heart muscle of obese rodents.

Arachidonic acid metabolism and peroxidase release from horse eosinophils

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Pure (> 98%) horse eosinophils (2.5×10^7/ml) incubated with 10 µg/ml Ca-ionophore A 23187 for 15 min at 37 °C released more than 40% of the granular peroxidase (EPO) into the medium and less than 5% of the LDH. Preincubation of the cells for 5 min with the cyclooxygenase inhibitor indomethacine (140 µM) reduced the ionophore stimulated release (= 100%) to 63±15%, with the lipoxygenase inhibitor NDGA (10 µM) to 32±14% and with ETYA (10 µM) an inhibitor of cyclooxygenase and lipoxygenase to 55±20%. The phospholipase A2 inhibitor bromophenacylbromide (10 µM) reduced the EPO liberation to 19±7% and the Ca/Mg ATPase inhibitor ethacrinic acid (50 µM) blocked the releaee almost completely (11±7%). The esterase inhibitor Pms-F (50 µM) enhanced the ionophore-induced release (128±22%). Our data suggest that for the EPO release, the lipoxygenase-, the cyclooxygenase products and may be lysolipids and a Ca/Mg ATPase play an important role.

ZELL- UND MOLEKULARBIOLOGIE

BIOLOGIE CELLULAIRE ET MOLÉCULAIRE

CELL AND MOLECULAR BIOLOGY

Permeability of *Escherichia coli* outer membranes: pores and pumps

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Transport of small solutes ($\leq$ 650 d) across *E. coli* outer membranes occurs by facilitated diffusion through channels formed by proteins called porins. In vitro, pores exist in either an open or closed state, the equilibrium between the 2 being voltage-dependent. In vivo, pores appear mostly closed. We have studied the state of pores in isolated, unextracted outer-membrane vesicles and compared their properties with vesicles derived from plasma membranes. Although separation of the 2 populations was excellent by several criteria, enzyme activities (dehydrogenases) and active transport (permease-mediated proline and phosphotransferase-dependent sugar) were equipartitioned, as were the 2 components of the electrochemical gradient (ΔpH and $\Delta\varphi$). These puzzling observations are unlikely due to cross-contamination, but concentration of active proteins around dynamic fusion points may explain them. As to porin channels, pores are closed also in outer-membrane vesicles.

Sequence comparison of a larval and an adult α-globin gene of *Xenopus laevis*

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The larval α_1- and the adult α_1-globin genes of *Xenopus* as well as the 2 corresponding globin cDNA clones were investigated by sequence analysis. – Comparison of the adult α_1-globin gene and its cDNA revealed complete homology of the coding sequences. The splicing points of the 2 introns are at the same positions as in all other vertebrate α-globin genes, namely between the codons for amino acids 31 and 32, and amino acids 99 and 100, respectively. – The sequences of the larval α_1-globin gene and its cDNA differ from other α-globin genes in the following: Both, the gene and the cDNA have an extraordinarily long noncoding region at the 3′-end (260 bp instead of 90–130 bp). This extra long 3′-noncoding region could be due to an altered polyadenylation signal (AACAAA instead of AATAAA) at the expected position about 100 bp from the termination codon.

Organization of the rDNA repeating units of *Ascaris lumbricoides*

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The rDNA cistron of *A. lumbricoides* exists in at least 2 different molecular forms, an 8.9 kb and an 8.5 kb long repeat. The latter lacks a 450 bp long DNA fragment in the nontranscribed spacer region. Each form is present in a tandem array and in a quantitative ratio of roughly 15:1. By comparing the restriction patterns of cloned 8.9 kb and 8.5 kb repeats, several differences are observed at the 5′-end of the 28 S coding region. Since no insertion sequences have been detected, the different restriction patterns must reflect true sequence heterologies. The S_1-mapping technique of hybrids between hnRNAs from different origins and adequate, end-labeled restriction fragments produces a different banding pattern in both the external and the internal transcribed spacer regions. From these results the question arises whether or not both forms of rDNA repeats are transcribed.

cDNA clones derived from genomic measles virus RNA

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50 S measles virus RNA was isolated from nucleocapsid and polyadenylated in vitro. Double-stranded cDNA was prepared, joined to pBR322 by dC-dG-tailing, and cloned in *Escherichia coli*. Plasmids with inserts ranging from 500 to 1000 nucleotides were nick-translated and hybridized to Northern blots of cytoplasmic RNA from infected and noninfected cells. From the hybridization to RNAs of different sizes (supposedly viral mRNAs) 5 classes of clones could be distinguished; all hybridized in addition to 50 S RNA. One class was positive in filter dot hybridization using 3′ terminally labeled 50 S genomic RNA as a probe. DNA sequencing revealed that these plasmids share insert sequences with one derived from nucleocapsid mRNA (Gorecki and Rozenblatt, PNAS *77*, 3686, 1980), indicating that the region coding for nucleocapsid is located close to the 3′-end of the genome, as in VSV.

Isolation of mouse basophil/mast cell lines

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A number of permanent cell lines have been isolated from long-term mouse bone marrow cultures. According to several biochemical and histochemical tests, these cells appear to represent pure populations of basophil/mast cells. They are dependent for their continued growth upon a factor(s) present in medium conditioned by a myelomonocytic leukemia cell line (WEHI-3) or by pokeweed-mitogen-stimulated spleen cells. The lines have a population doubling time of 70 to 80 h and have been maintained in suspension culture for 12 months. These basophil/mast cell lines should be useful for studies of the mechanisms of allergic reaction and inflammation, as well as the differentiation pathways involving this subset of hemopoietic cells. Furthermore, these cells also be employed in transformation studies.

Analysis of the transcriptional 'enhancer' effect

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In vivo transcription of a cloned rabbit β-globin gene is enhanced by 2 orders of magnitude when the gene is linked to a small segment of SV40 DNA (Banerji, Rusconi and Schaffner, Cell *27*, 299, 1981) or polyoma virus DNA (de Villiers and Schaffner, Nucl. Acids Res. *9*, 6251, 1981). This effect is being subjected to a detailed analysis by the following experiments: a) Mutagenesis of the 72-bp enhancer element of SV40, b) linkage of the SV40 enhancer to other genes, including sea-urchin histone genes, c) replacement of the SV40 enhancer by the enhancer segment of other viruses, and d) characterization of mammalian cell DNA sequences with enhancer activity.

Transcription of simian virus 40 chromosomes in an extract of HeLa cells

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Simian virus 40 (SV40) chromosomes were incubated with a concentrated extract of HeLa cells containing RNA polymerase II and other factors involved in transcription. SV40-specific RNA was synthesized. In the absence of HeLa cell extract the synthesis of labeled RNA by endogenous RNA polymerase in the chromosome preparations amounted to les than $1/10$ of that when the HeLa cell extract was present. When restriction endonucleases able to cut SV40 DNA were added to transcription reactions containing SV40 chromosomes and the HeLa cell extract, RNAs of discrete length were produced. The RNAs were identified as correctly initiated run-off transcripts of the early and late genes. Most of the RNA synthesized by the HeLa extract from SV40 chromosomes was from the region of the late genes, whereas transcription of purified SV40 DNA was from both the early and the late regions, with the early transcripts predominating.

Studies on the expression of β-glucuronidase in mouse hepatocytes

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Hepatocytes of C57 Bl/6 were cultured at 37 °C/95% O_2/5% CO_2 in William's medium E, supplemented with insulin and, during the attachment period of 18 h, 15% fetal calf serum (FCS). Cells could be kept in culture for more than 8 days with a cell detachment ranging from 2 to 10% per day. β-Glucuronidase expression was gauged during the culture period at different cell densities and in the presence of varying concentrations of FCS and compared with cytoplasmic lactate dehydrogenase, protein content and synthesis rate. β-Glucuronidase activity remained constant during the first 6 days of culture and then decreased slightly under all the conditions tested. The other parameters showed essentially the same changes. On day 4, the proportional distribution of the lysosomal and the microsomal forms of β-glucuronidase was similar to that in total liver (by electrophoresis). The relative rate of synthesis was $51.4 \cdot 10^{-6}$, in the same range as that measured in vivo. These findings allow us to study the regulation of the processing of β-glucuronidase in cultures of hepatocytes.

Parvalbumin, a neuronal marker protein in rat brain. Microisolation and comparison with the muscle protein

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Parvalbumins, highly specific Ca^{2+}-binding proteins, have so far been isolated from muscle tissues. Recently, we demonstrated parvalbumin immunoreactivity in a subpopulation of both short and long axon neurons in all regions of the central nervous system of the rat (Nature *293*, 300, 1981). We have now isolated this neuronal parvalbumin from rat brain. For this purpose a new HPLC-method was applied, allowing isolation of parvalbumin from heat-treated extracts. After passage of the sample through 4 columns under different conditions, parvalbumin, well separated from S-100, troponin-C and calmodulin, was eluted as a single peak. The direct comparison of the brain and muscle parvalbumin revealed identities in their migration on 2D-gels, HPLC-elution profiles, amino-acid compositions and immunological properties.

Blocking of the stimulation of cytotoxic lymphocytes by tumor cell membranes and soluble membrane proteins

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Cancer cells may differ from their normal counterparts in having structures which are recognized by the autochthonous or syngeneic host as foreign, i.e. tumors may be antigenic. Tumor antigens are presumably located on the cell surface. Protection against a subsequent challenge with viable tumor cells can only be achieved by pretreatment of the tumor hosts with intact tumor cells and not with cell membranes. - In vivo priming with viable tumor cells resulted in weak and variable cytotoxicity in the syngeneic P-815/DBA/2 model. After in vitro restimulation with intact nonproliferating tumor cells highly cytotoxic lymphocytes could be obtained. Isolated tumor cell membranes or soluble membrane (glyco-)proteins were not only incapable of stimulating cytotoxicity but inhibited the stimulation by intact tumor cells. The blocking capacity could be shown to reside in the lymphocyte population.

Sequence requirements for the formation of authentic 3-ends of a H2A histone gene

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In order to define the sequences important for the production of correct 3′-ends of the sea urchin *Psammechinus miliaris* H2A gene, deletions of various parts of the H2A coding, trailing or spacer sequences were constructed in vitro. The effects of these deletions were tested by injection of the manipulated DNAs into centrifuged oocytes of *Xenopus laevis*, and by the analysis of the resulting transcripts. A highly conserved sequence element found at the 3′-end of histone genes, which includes a dyad symmetry element, was demonstrated to be essential for the formation of authentic 3′-ends. However, the presence of this dyad symmetry element alone does not provide all the necessary information. The additional important sequences are not situated in the structural gene, as was shown by the analysis of deletions in this region, but can be traced to spacer sequences downstream of the 3′-terminus of the H2A mRNA.

Stimulation and inhibition of DNA synthesis by cell extracts from mammalian cell cycle mutants

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A series of heat- and cold-sensitive cell-cycle mutant clones of the P-815 line (Zimmermann et al., Somat. Cell Genet. *7*, 591, 1981) was used for the preparation of cell extracts. These were tested for their effects on DNA synthesis in a subcellular system consisting of P-815 cells partially lysed with Brij-58 (Reinhard et al., Biochim. biophys. Acta *564*, 141, 1979). Extracts prepared from the mutant clones at 4 days after shift to the nonpermissive temperature inhibited ^{3}H-dTTP incorporation by partially lysed cells, whereas extracts from mutant cells maintained at the permissive temperature, similar to those from wild-type cells, stimulated DNA synthesis. Both stimulating and inhibiting factors are nondialyzable and sensitive to heating.

Initiation site for transcription of the ribosomal genes of *Physarum polycephalum*

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The ribosomal genes of *P. polycephalum* are situated on an extrachromosomal palindrome of 60 kb length, repeated 100 times per nucleolus. The mature 19 S, 5.8 S and 26 S rRNA molecules are first transcribed from this rDNA as a large precursor of 13.3 kb which is then processed. - As a first step, we tentatively localized the initiation site of ribosomal transcription by Sl-mapping. - The surrounding DNA was then sequenced by the method of Maxam and Gilbert. Subsequently the precise position (within 3 nucleotides) of the 5′-end of the primary transcript was determind by electrophoresing the Sl-resistent DNA on sequencing gels and direct comparison with the sequencing pattern. - Partial sequence homology was found with the transcriptional initiation site of ribosomal genes from other species.

Characterization of *Paramecium primaurelia* surface proteins by IgG conjugated WGA labeling on nitrocellulose blots

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The ciliate *P. primaurelia* can transform its surface coat proteins in response to alterations of its culture medium (e.g. temperature changes). This transformation has been studied by immunological techniques (*Paramecium* serotypes; Capdeville, J. Cell Physiol. *99*, 383, 1979). We present evidence that the alternative states can also be characterized by their reactivity to wheat-germ agglutinin, but not to the other lectins tested. Paramecia of the 2 serotypes investigated had different sensitivities to the lectin, in vivo. The extracted surface coat proteins were subjected to electrophoresis, then blotted on nitrocellulose paper. A sensitive labeling was achieved by means of IgG conjugated WGA. The labeling pattern was markedly different for the 2 surface coats.

Proliferation and differentiation of oligodendrocytes in embryonic and neonatal mouse brain cell cultures

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Oligodendrocytes (O) differentiation was monitored with antisera against galactocerebroside (GC) and myelin basic protein (MBP). Proliferation was checked with ^{3}H-thymidine autoradiography. In embryonic cultures, O express both GC and MBP from the beginning and they are able of proliferation. In neonatal cultures, GC is expressed earlier than MBP, only a small part of GC^+ are also MBP^+ and only the cells which are GC^+ and MBP^- are able of proliferation. The $\gamma\gamma$ enolase staining revealed the presence of many neurons in embryonic, but not in neonatal cultures. This suggests that in embryonic cultures in comparison to neonatal cultures the differentiation of O is accelerated and highly differentiated O (GC^+ MBP^+) are able of proliferation. These facts may be due to the influence of neurons in embryonic cultures.

Investigation of the viral DNA region responsible for the hormonal stimulation of transcription of mouse mammary tumor virus (MMTV)

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We have recently shown that cloned MMTV DNA is biologically active in transfected cells and that its expression is stimulated by glucocorticoid hormones, suggesting that the hormone-sensitive sequences are located on the viral DNA. In order to locate these more precisely, we have now studied MMTV RNA synthesis in cell lines transfected with subgenomic DNA fragments or with aberrant recombinant DNA containing incomplete MMTV genomes. Virus-specific RNA synthesis was stimulated by dexamethasone in a cell line transfected with the 1.45-kb Pst I fragment which includes almost the complete terminal repeat (LTR) plus 120 nucleotides of unique sequence DNA. The hormonal effect was also present in cells transfected with an aberrant recombinant DNA lacking U5 and part of adjacent regulatory U3 sequences.

A cytoplasmic factor modulates the binding of agonists to alpha-adrenergic receptor in human platelets

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The effect of guanine nucleotides on agonist binding and adenylate cyclase activity in the alpha-adrenergic system of human platelets is a well-known phenomenon. As shown previously agonist competition curves are shallow in washed plasma membranes, indicating high and low affinity agonist binding sites. In the presence of guanine nucleotides these curves become steeper and shift to the right, representing only the low affinity agonist state. We now present evidence that a soluble component in the lysate of human platelets modulates the guanine nucleotide-sensitive (−)-epinephrine binding in a concentration-dependent manner, as measured by competition with [^{3}H]-yohimbine. In the presence of the lysate the high affinity agonist binding site is prevented and the effect of guanine nucleotide is weak. Ultrafiltration and gel-chromatography experiments show that this endogenous component is a soluble protein (mol.wt 20,000–30,000), heat-stable and not identical with calmodulin.

Actin and vimentin filaments in young and senescent human diploid fibroblasts

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Changes of actin and vimentin filaments in young and senescent cultures of human embryo lung fibroblasts have been investigated using: 1. Antiactin immunofluorescent staining with and without pretreatment with an actin depolymerizing factor (ADF). 2. Antivimentin immunofluorescent staining. 3. Planimetric analysis of SDS-PAGE scans of total extracts and after G/F actin separation by ultracentrifugation. 4–12 h after seeding, actin stress lines are more sensitive ADF in young cultures than in senescent, whereas at confluence both cultures are similarly resistant. On the other hand, old cultures contain more F-actin (4 h after seeding and at confluence), and the amount of vimentin is generally higher in all senescent cells. Our results indicate that the differences in the organization of microfilaments and of 10-nm filaments may be associated to the decline of the growth potential that characterizes cell aging in vitro.

Precursor cell cycle in mouse marrow

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A method based on vital staining using Hoechst 33342 bisbenzimidazole dye, followed by fluorescent activated cell sorter (FACS) separation of cells according to individual DNA content, and final bioassay of sorted cells for clonogenicity in vitro and in vivo, has been worked out. This method preserves cell viability, allowing valid evaluation of cell cycle parameters in precursor-enriched cell populations. – Balb/c strain murine bone marrow progenitor cells have thus been sorted in 2 steps, the first based on cell physical characteristics, the 2nd on DNA-content. Final Go/Gl fractions, when compared to S/G_2 cells, contained BFU-E and CFU-C in a proportion corresponding to 25-fold enrichment. A similar enrichment was obtained with other mouse strains, DBA, CBH and C3H, whereas it was only 4-fold with C57B1/6 mice.

The role of viral protein p19 in controlling the functions of Rous sarcoma virus RNA

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Cells infected with Rous sarcoma virus (RSV) synthesize viral RNA by transcription of the integrated proviral DNA and the RNA is involved in several different biological processes: translation, reverse transcription, RNA splicing and virion assembly. We show here that the viral protein p19 may exert a negative control on RNA translation and splicing and a positive control on virion assembly. P19 molecules bind strongly to specific regions of RSV RNA located: a) within the dimer linkage structure, b) at the 3′-end of p19 and p15 coding sequences, c) at the 3′-end of the *pol* gene and d) in the *env-src* gene junction. In addition all the p19 binding sites are located adjacent to or within stable structured regions of the RNA itself that can interact with each other. On the basis of these data we propose and discuss a model structure for the association between p19 and RSV RNA which takes into account RNA-RNA, RNA-protein and protein-protein interactions.

Polyoma virus DNA mediates cell type-specific differences in the expression of a linked β-globin gene

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The expression of a cloned rabbit β-globin gene, after transfection of mouse 3T3, mouse 3T6, human Hela and monkey CV-1 cells, is greatly enhanced when it is linked to the genome of SV40 or to a 244 bp 'enhancer' fragment from the closely related DNA tumor virus polyoma (Banerji, Rusconi and Schaffner, Cell *27*, 299, 1981; de Villiers and Schaffner, Nucl. Acids Res. *9*, 6251, 1981). In contrast, when the β-globin gene is linked to the entire polyoma virus genome the expression of the globin gene is cell-type specific: No globin gene transcripts are detectable in Hela and CV-1 cells whereas the murine cells produce β-globin mRNA. This host range is not explained by a differential replication of the polyoma virus DNA in mouse and primate cells. Experiments to investigate this phenomenon will be presented.

Characterization of complexes between recA protein and duplex DNA by electron microscopy

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Stable complexes were formed between the recA protein of *Escherichia coli* and duplex DNA in the presence of adenosin 5′-γ-thio triphosphate. From the known number of bp of the plasmid used, from the appearance of the complexes in the electron microscope after platinum shadowing and negative staining and from in-situ mass determinations by scanning transmission electron microscopy, we deduce a structure in which 18.6 bp and 6.4 recA monomers contribute to one turn of a right-handed helix with a pitch of 9.7 nm and a width of 11 nm. The results suggest an intercalative mode of binding which partially unwinds the DNA.

Analysis of the rabbit β-globin promoter by DNA restructuring and site-directed mutagenesis

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Promoter sequences which influence the transcription of the rabbit β-globin gene were identified by analyzing the transient expression of modified β-globin genes in mouse 3T6 cells. 3 regions required for efficient transcription have been identified: the region containing the ATA box sequence (−31)CATAAAA(−25), the region containing the CCAAT box sequence (−77)GGCCAATCT(−69), and a region located between positions −82 and −109. Deletion of any of these regions resulted in at least an 8-fold decrease in the level of transcription. The region containing the ATA box sequence also constitutes the major determinant in directing the site of initiation of transcription. The importance of the ATA box and CCAAT box canonical sequences was verified by analysis of point mutants. Single or double point mutations at positions −26 to −28 and −73 to −75 reduced transcription 2- to 5-fold and 4- to 8-fold, respectively.

Structure and expression of the gene encoding mouse C3, the third component of the complement system

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Complement is a system of serum glycoproteins that play a major role in the defense of mammals against infections by microorganisms. C3 is the key element of the system. We have prepared cDNA-clones that represent 4700 nucleotides of the mouse liver C3-mRNA which is 5100±200 nucleotides long. By direct DNA sequencing it was found that a) the beta-subunit of the protein is located in the amino-terminal portion of the precursor, preproC3, b) the amino-acid sequence of mouse C3-beta is identical in 12/15 residues with that of guinea pig and in 9/10 with that of human C3-beta. - A gene-bank was prepared from mouse DNA in phage lambda. Using cloned cDNA probes, 4 C3-genomic clones were isolated, 2 of which comprise the entire C3-gene (24 kb). At the 5′-end of the gene a TATAAA-box was found and an initiation codon for translation. DNA-sequence from this region predicts that C3 is initially synthesized as a preproC3 molecule containing at its amino end a signal peptide as yet undetected at the protein level.

Sequence of the chloroplast DNA region containing the gene of the large subunit of the ribulose bisphosphate carboxylase of *Chlamydomonas*

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The sequences of the gene of ribulose bisphosphate carboxylase (LS) of *Chlamydomonas reinhardii* and its neighboring regions has been determined by the chemical cleavage method of Maxam and Gilbert. The LS gene shows 80% sequence homology with the corresponding genes of maize and spinach; the active sites of the protein are conserved in the 3 species. The LS mRNA untranslated region comprises 50 and 84±3 bases at the 5′- and 3′-ends, respectively. The first AUG codon is preceded by a UAA termination codon. There is therefore no precursor of LS in *C. reinhardii*. The sequence upstream of the LS gene displays prokaryotic features: a Shine-Dalgarno sequence, a sequence related to

the Pribnow box 10 bp upstream from the LS mRNA start and a sequence TTTACA which is related to the prokaryotic '−35' box.

The long terminal repeat of mouse mammary tumor virus (MMTV) DNA includes a long coding sequence and signals for hormonally regulated transcription

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Long terminal repeats (LTR) of MMTV DNA might play an important role in the regulation of viral gene expression. The complete sequence of a MMTV LTR was determined. In addition to the expected signals for initiation of viral transcription and polyadenylation, the 3'-end of the MMTV genome contains an open reading frame large enough to code for a 36-K protein. On the basis of the amino-acid sequence B. Gutte (University of Zürich) synthesized a peptide of 23 amino acids by solid phase. This synthetic peptide was injected into rabbits in order to produce antibodies which will be used to search for this unidentified protein in mammary tumor cells. Furthermore the MMTV LTR seems to contain all of the necessary sequences for the response to glucocorticoid hormones. To localize the relevant sequences more precisely we are analyzing aberrant MMTV fragments which also induce the hormonal response upon transfection.

Intracellular location of the precursor of mitochondrial aspartate aminotransferase and its in vitro import into mitochondria

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An equal amount of [^{35}S]methionine was incorporated into the higher MW precursor and the mature form of mitochondrial aspartate aminotransferase by pulsing chicken embryo fibroblasts for 20 min and adding the uncoupler CCCP 5 min after the onset of the pulse. After centrifugation of the cell homogenate, the mature enzyme was quantitatively recovered in the pellet whereas > 90% of the precursor was in the membrane-free supernatant. In contrast to the mature enzyme and most other cell proteins, the precursor was completely digested after mild trypsinization of the homogenate. Its exceptional susceptibility to proteolysis indicates that its conformation differs from that of the mature enzyme. Attempts to convert the precursor in fibroblast homogenates to the mature form by adding mitochondria were not successful. However, precursor synthesized in vitro was processed by exogenous mitochondria.

Strand-specific DNA probes of high specific activity

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A general method to obtain DNA probes of high specific activity (2×10^{10} dpm/µg) is described. The probes are integrated into the single-stranded bacteriophage M13mp8 or M13mp9 and hence are strand-specific. Some applications demonstrating the advantages of this method are shown.

Crystallization and preliminary crystallographic studies of porin, an integral membrane protein from *Escherichia coli*

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Tetragonal and hexagonal crystals of porin have been grown from detergent solutions. They diffract to 3.2 Å and 3.8 Å, respectively. On cooling, the tetragonal crystals change space group from $P4_2$ to $P4_222$ and become ordered to at least 2.9 Å resolution. Collection of 3-d data has been started at −18 °C using synchroton radiation and initial evaluation shows good agreement of equivalents (R = 7.15% on 1917 pairs of intensities from crystal 1). The hexagonal form confirms the 3-fold axis of the porin trimer and exhibits a packing arrangement very similar to that observed in 2-d crystalline sheets both with regard to cell dimensions and symmetry. Diffuse scattering from this form is characteristic of β-sheet with the chains colinear with the c axis and thus perpendicular to the membrane.

Cloning of a rabbit Ig λ-light chain gene

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Rabbits usually synthesize immunoglobulins (Ig) with light (L) chains of k-type. However, the variant strain BASILEA (BAS) mainly uses L-chains of λ-type to build up its Ig. To clone the λ-chain gene, mRNA was isolated from spleen cells of hyperimmunized BAS rabbits and transcribed into cDNA. Double-stranded DNA produced from this cDNA was integrated into plasmid vector pBr 322. The recombinant clones were hybridized with radioactively labeled cDNA obtained by reverse transcription of 'L-chain-sized' mRNA isolated from spleen or liver cells. Clones hybridizing to cDNA from spleen cells only (i.e. cells competent for the production of Ig), were selected. The plasmid of one selected clone was subjected to DNA sequence analysis. A nucleotide sequence corresponding to residues 77–99 of the BAS λ-chain C region amino-acid sequence (Garcia and Jaton, Biochem. J. *197*, 177, 1981) was found. Therefore, the cloned DNA contained at least part of the BAS rabbit Cλ-chain gene.

The biogenesis of outer mitochondrial membrane

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The outer mitochondrial membrane of yeast mitochondria is characterized by 3 major proteins (mol.wt = 29 k, 45 k, 68 kdaltons). In contrast to many proteins of the inner mitochondrial membrane, none of these is synthesized as a larger precursor in vitro (as determined by gel electrophoresis). After incubation of isolated mitochondria with the radiolabeled products of in-vitro translation, these proteins reisolate with the mitochondria; moreover, the 29 k- and the 45 k-polypeptide become resistant to externally added protease. Unlike the import of inner mitochondrial membrane components, this uptake a) occurs in the absence of an electrochemical gradient across the inner membrane, and b) is unaffected by a light trypsin treatment of isolated mitochondria prior to in-vitro import. We have recently probed the orientation of in vitro-inserted proteins of the outer mitochondrial membrane with limited proteolytic digestion and monoclonal antibodies. The mechanisms for protein import into the outer and inner mitochondrial membranes are different.

No change in the methylation pattern of the *Xenopus laevis* vitellogenin genes upon estrogen treatment

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To study the methylation pattern of the A_1 and A_2 vitellogenin genes, DNA from vitellogenic and nonvitellogenic tissues was probed with the restriction enzymes HhaI and HpaII. Hybridization of southern blots of such DNA with vitellogenin gene fragments showed that these sites are fully methylated and that there is no difference in the methylation pattern between DNA from cells actively expressing these genes (estrogen-stimulated hepatocytes) and from cells which do not express these genes (erythrocytes or unstimulated hepatocytes). In contrast, within the β_1-globin gene an unmethylated site is found in erythrocyte DNA where this gene is active and this same site is fully methylated in hepatocyte DNA where globin genes are inactive. These results show that the estrogen-dependent expression of the vitellogenin genes cannot be correlated to undermethylation, in contrast to other genes, suggesting that demethylation of specific gene loci is not a prerequisite for gene activation.

Dynamic and morphological aspects of contacts between lymphocytes and mononuclear macrophages in human effusions of tumoral origin

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The in-vivo observations have been made on the cells of liquid centrifugation pellets, either smeared for optical microscopy, or fixed and embedded for electron microscopy. The in-vitro studies have been carried out on living cultivated cells by phase contrast microscopy. The lymphocytes adhere to the macrophage surface, forming some kind of rosette. In the zone of contact the electron microscope reveals, between the intact cell membranes, a dense, often striated material. A 6-h treatment with hyaluronidase does not provoke the withdrawal of the lymphocytes. Phase contrast observations have shown that the lymphocyte draws near to the macrophage and adheres to it by means of a dense uropode; it may detach itself after a while, or else it may head for the nuclear region of the macrophage, come to rest, then withdraw and most commonly leave the cell. Rarely, the lymphocyte remains as imprisoned in a recess of the macrophage cytoplasm, where it shows signs of nuclear pycnosis and dies.

Monoclonal antibodies against myomesin used to study myofibrillogenesis

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Hybridomas were produced by fusion of spleen cells from BALB/c mice immunized with purified chicken myomesin with NS-1 myeloma cells. Supernatants were screened by ELISA and 10 clones out of 109 from one fusion were found to produce antimyomesins, most of them being IgG_1. Seven of these mAb bound to the M-line of myofibrils. Molecular specificity of interaction was shown by immunoreplicas of blotted proteins. The complex binding patterns revealed the same bands (some of which may be degradation products) that reacted with polyclonal antibodies. Of the 5 mAb which were positive in the blots only 2 bound to the M-line. The 2 groups could also be distinguished by their blotting pattern. mAb B-4 was shown to bind to myofibrils of 5 different vertebrate classes, mAb B-2 bound only to chicken and frog myofibrils. Another group of mAb bound only to chicken organelles, thus these mAB represent at least 4 different specificities.

Tissue-specific promotors regulate the expression of the mouse α-amylase gene *Amy*-1^a in the parotid gland and the liver

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Amy-1^a is a single copy gene and specifies the α-amylase mRNAs with tissue-specific leader sequences in the parotid gland and the liver. - S_1 nuclease analysis of nuclear transcripts as well as the localization and quantitation of RNA polymerase molecules engaged in *Amy*-1^a transcription demonstrate that: a) transcription initiation occurs at the tissue-specific cap sites in the parotid gland and the liver, b) the concentration of RNA polymerase on *Amy*-1^a is only 10-fold higher in the parotid gland than in the liver. Thus transcriptional and posttranscriptional modulation might be required for the 100-fold difference in accumulation of α-amylase mRNA in these 2 tissues. c) a low amount of RNA polymerase molecules starting at an unknown distance upstream of the various cap sites and running through *Amy*-1^a were detected in the parotid gland, the liver and the brain.

Cyclic AMP-binding proteins and hormone receptors in normal and neoplastic human breast tissue

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There is evidence that the expression of cAMP-dependent protein kinases are linked to cell growth and differentiation by showing alterations in the ratio of type I to type II isoenzymes between control and malignant tissues. Since differences between the 2 isozyme types are due to different cAMP-binding proteins (regulatory subunits R-I and R-II), we investigated the distribution of R-proteins in normal (M-I) and neoplastic (CA) human breast tissue specimens by fotoaffinity labeling with 8-N_3-[^{32}P] cAMP and SDS gel electrophoresis. It was found that the ratio of R-I/R-II in cytosols of CA samples is not significantly different from that of M-I samples, but that proteolytic fragments of R-proteins are much more abundant in CA than in N-I. Furthermore a significant inverse relationship between R-proteins and estrogen- and/or progesterone receptors is observed in the respective neoplastic tissues.

The study of the function of modified nucleosides in tRNA of *Schizosaccharomyces pombe*

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Recently accurate high performance liquid chromatography has been developed for the analysis of the nature and quantity of modified nucleosides in small amounts of tRNA. We screened various antisuppressor mutants (which abolish the function of tRNA nonsense suppressors in vivo) for alterations in modified nucleosides of their unlabelled or C^3H_3 and ^{35}S labeled tRNAs. The labeled tRNAs were also characterized by gel electrophoresis. Several mutants show altered patterns. Most interesting is *sin3:* Its tRNA is

devoid of 5-methoxycarbonylmethyl-2-thiouridine, a nucleoside present in the wobble position of the anticodon of several tRNAs. Our working hypothesis is that the mutation affects the transferase that introduces sulfur to position 2 of uridine. We plan to study the effect of the missing modification on tRNA function in the decoding process.

Production and characterization of antibodies against the brain-lipid sulfatide

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One of the enriched lipids in the myelin-sheath is sulfatide, a galacto-sphingolipid. The sulfate group of this molecule is attached to the 3'-C of the galactose moiety. Our aim was the development of a radioimmunoassay (RIA) for the detection of antisera against this lipid. BALB/c-mice were immunized according to M.-J. Coulon-Morelec (Ann. Inst. Pasteur *123*, 619, 1972). For the detection of mouse antisulfatide antibodies, a solid-phase microtube RIA was used. ^{125}I labeled rabbit antimouse Ig antibodies did not distinguish between mouse antisulfatide antiserum and control sera from animals immunized with different antigens. No reaction was observed with sera from untreated mice. By using protein A, which binds only to the immunoglobulins of the Ig G-classes, we found an exclusive reaction with antisulfatide antibodies. This method may provide a tool for detection and characterization of mouse antibodies against lipids.

Structure and arrangement of *Xenopus laevis* globin genes

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2 libraries of cloned genomic DNA of *X. laevis* were screened using cloned globin cDNA sequences as probes. Out of 52 clones carrying globin genes 27 partly overlapping clones contained a contiguous stretch of 66 kilobases of *Xenopus* DNA. On this DNA 5 globin genes arranged in the order of 5'-larval α_1-larval α_1-adult α_1-adult β_1-larval β_2-3' were localized. 25 other clones carrying further globin genes could not be linked to the 27 clones described above. As *X. laevis* is likely to have undergone a genome duplication, these clones might contain DNA of another, separate globin gene cluster. - The internal gene organization was analyzed by electron microscopy. R-loops formed with globin mRNA demonstrated that all coding sequences are interrupted by 2 introns of variable sizes. In general, these introns are larger in *Xenopus* than in birds or mammals and also larger in larval genes as compared to the adult ones.

The chromatin repeat lengths of cortex and cerebellar neurons change differentially during development

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Chromatin repeat lengths were determined in the rat by micrococcal nuclease digestion followed by gel electrophoresis. The repeat length of cortex neurons decreased from 200 bp in fetuses to 170 bp at 14 days and all subsequent stages. Administration of [^{3}H]-thymidine to pregnant rats during the period of fetal neurogenesis allowed the neurons of individual cortex layers to be labeled differentially. This revealed that the shortening of the chromatin repeat length affected only neurons of layers IV-VI. In contrast, cerebellar neurons (granule cells) underwent lengthening of the repeat length from 165 bp at fetal and early postnatal stages (up to day 5) to 218 bp at 30 days. Thus, in both cortex and cerebellar neurons the changes occurred temporally coincident with major developmental processes. No changes were detected in liver nuclei during the same period. Non-astrocytic glia cells of the adult cortex had 200 bp repeats.

Rapid degradation of the precursor of mitochondrial aspartate aminotransferase in uncoupler-treated chicken embryo fibroblasts

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Mitochondrial aspartate aminotransferase is synthesized as a higher MW precursor on free polysomes in the cytosol (Sonderegger et al., J. biol. Chem., in press, 1982). Treatment of chicken embryo fibroblasts with the uncoupler CCCP completely blocks the uptake of the precursor into mitochondria and its conversion into the mature enzyme. The resulting accumulation of the precursor in the cytosol is limited by rapid proteolytic degradation ($t_{1/2} \sim 5$ min). This degradation is specific and does not attack proteins destined for location in the cytosol. The cytosolic isoenzyme of aspartate aminotransferase which is homologous to its mitochondrial counterpart and has an almost identical chain folding is not subject to this degradation. On treatment of the cells with cysteamine, an antagonist of CCCP, the precursor escapes degradation and is processed to its mature form.

Topology of reaction center protein in the photosynthetic bacteria *Rhodopseudomonas viridis*

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In order to understand the structure-function relationship of photosynthetic proteins studies on the orientation and localization of the principle components reaction center (RC) and light harvesting proteins (LHP) have been initiated. - Proteins were isolated for antibody production by means of several techniques: using detergents, urea or organic solvents. Antibody specificity was primarily determined by the immunoblotting technique and visualized as ferritin conjugates in transmission electron microscopy. - Labeling studies, both chemical and antibody-mediated, carried out on membranes and derived inside-out vesicles suggest the cytoplasmic location of several RC components.

Sequence analysis of the V_k24 multigene family of immunoglobulin germ-line genes

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We have used a cDNA plasmid derived from the kappa chain mRNA of M167 to clone several related V_k germ-line genes from a BALB/c sperm DNA library. We showed that no germ-line gene for the M167 and the related M511 kappa chain is present in BALB/c mice and concluded that the sequences of M167 and M511 were derived by somatic diversification. The germ-line gene V_k24 which gives rise to the somatic variants expressed in M167 and M511 is followed in its 3'-flanking sequence by a stretch of repeated DNA. Sequence analysis revealed that the tetrameric unit AAAT is tandemly repeated 8 times. This AT cluster is followed by a palindromic sequence of 14 bp. DNA

sequence analysis of several members of this V_k24 multigene family confirmes our previous knowledge that flanking sequences of immunoglobulin genes are conserved and will allow us to study the evolution of genes of a multigene family.

Mammalian 'heat-shock' proteins are also induced by viral infections

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Infection of mouse and monkey cells with polyoma virus and SV40 leads to increased synthesis of most cellular proteins, but particularly of 2 proteins with M_r of 92,000 and 72,000. Thermal treatment of mouse and monkey cells ('heat-shock') induces strong stimulation of proteins with M_r of 92,000, 72,000 and 70,000. We compared by 2-dimensional gel electrophoresis and by partial proteolytic digestion the virus-induced and the heat-shock-induced proteins. Our results show that a) the 92,000 and the 72,000 proteins induced under both conditions are the same, b) the 92,000 proteins, as well as the 72,000 proteins of mouse and monkey cells are closely similar, c) the major heat-shock protein (70,000) of mouse and monkey cells which is barely stimulated by virus infection is related to the 72,000 protein. Since increased synthesis of similar proteins has been observed in cells exposed to various chemicals and in virus-transformed cells, these well-conserved proteins may be important for cell survival.

Electron microscopy and image analysis of a crystalline bacterial photosynthetic membrane

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The thylakoid membrane of the photosynthetic bacterium *Rhodopseudomonas viridis* contains extensive 2-dimensional crystalline arrays of a photosynthetic unit. The units are arranged on a hexagonal lattice with a centre to centre distance of about 130 Å. Studies on freeze-dried heavy metal shadowed membranes have suggested a rosette-like structure for the photosynthetic unit. - Sheets consisting of 2 apposed membranes can be isolated by fracturing the bacterial cells. Treatment of thylakoids with mild detergents produces single membranes. We have studied negatively stained double and single membranes by electron microscopy and image analysis. Computer processing of electron micrographs revealed the structure of the membrane in projection.

Diet-induced accumulation of vascular cholesterol (CH) is localized in macrophages

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Several authors have reported that macrophages participate in the formation of arterial lesions in animals on an atherogenic diet. We have investigated to what extent monocytes/macrophages participate in the formation of vascular lesions in rabbits on a CH-rich diet for up to 10 weeks. Adhesion of monocytes to aortic endothelium was increased from day 4 onwards. On day 16 subendothelial foam cells (macrophages) were present and subsequently increased steadily. Highly significant correlations were found between the number of aortic foam cells counted in cross-sections and the aortic CH ($r=0.91$) and between the number of aortic foam cells and the cross-sectional area of the neointima ($r=0.92$). The smooth muscle cells remained free of recognizible fat inclusions and we found no evidence for smooth muscle cell migration. We conclude that aortic DH is predominantly stored in macrophages which fully account for the neointima formation in CH fed rabbits.

Involvement of higher order chromatin structures in metaphase chromosome organization

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We have prepared both interphase nucleoids and metaphase chromosomes from CHO-cells by a modified Hancock procedure, dissociated them in O, 0.3 and 0.5 M NaCl and studied their salt-dependent appearance by electron microscopy. There is no difference in the salt-dependent condensation of the chromatin fibres between interphase and metaphase chromatin. We found that dissociation of chromosomes in up to 0.3 M NaCl does not affect their overall structure and their condensation with increasing ionic strength. However, chromosomes dissociated in 0.5 M NaCl have totally lost their shape and are unable to condense into typical chromosome structures with increasing ionic strength. Since concomitantly the chromatin fibres of such chromosomes no longer form higher order structures, we suggest that an intact higher order structure of the chromatin fibre is a prerequisite for the formation of the metaphase chromosome structure. Our results support a 'folded-fibre' model for chromosomes.

Proteins from thylakoids of *Chlamydomonas* studied with antisera

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The use of antibodies is a helpful tool in the investigation of membrane polypeptides. Sera directed against CF_1 and CP-complexes of the thylakoid membranes of *C. reinhardii* were raised in rabbits. These antisera have been used to characterize and to identify polypeptides synthesized in vivo as well as in vitro. With an antiserum against LHCP it has been shown that in a chlorophyll b-deficient mutant of *Chlamydomonas* the LHC-apoproteins have different electrophoretic properties as compared to the wild type. Antisera against chloroplast made and cytoplasmically synthesized polypeptides, i.e. CF_1 and LHCP, resp., have been used to study the products of in-vitro protein synthesis, directed by chloroplast RNA-containing fractions isolated from chloroplasts of *Chlamydomonas*.

2 steps in the activation of an ecdysone-sensitive chromosome region

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In 1963, Karlson postulated that ecdysone (Ec) directly activates chromosome region I-18C in the larval salivary glands of *Chironomus* whereas Kroeger hypothesized that it does so by changing intracellular ion concentrations. Pong's group (Bochum) localized Ec in I-18C while Wuhrmann (our lab) measured an increased intracellular K^+ activity (a_{Ki}) in glands of prepupae (high Ec). We now postulate 2 necessary steps in the activation of I-18C: 1. Chromatin decondensation mediated by ions. 2. Induction of transcription by Ec. This view is based on the following morphometric, autoradiographic and electrophysiological findings: during development decondensation precedes transcription

in I-18C, decondensation is paralleled by an increase in a_{Ki}, Ec induces transcription only in glands with decondensed I-18C regions, ouabain which causes a decrease in a_{Ki} and I-18C decondensation prevents Ec from inducing transcription in this region while leaving other regions unaffected.

Molecular cloning of HLA-DR antigen cDNA fragments

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HLA-DR antigens are the human equivalent of the murine Ia antigens. These antigens, present at the surface of B lymphocytes, play a crucial role in the regulation of the immune response. Their mechanism of action, as well as the basis for their high degree of polymorphism, is not yet understood. Molecular cloning of their genes will help to resolve these questions. We have demonstrated that translation and assembly of the 3 DR polypeptide chains take place in *Xenopus* oocytes injected with mRNA from a human B-cell line. This reconstituted DR antigen is immunoprecipitated with specific monoclonal antibodies. cDNA clones were made from a mRNA fraction that had been enriched by gel electrophoresis. mRNA selected by hybridization to specific cDNA fragments was analyzed by injection in oocytes and immunoprecipitation with anti-DR antibodies. Using this screening procedure, we have isolated cDNA molecules encoding HLA-DR antigens.

Membrane characteristics of peripheral blood mononuclear cells (PBMC) from patients with solid tumors

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PBMC of patients with solid tumors were analyzed for surface thymic antigens (Leu 1, Leu 2, Leu 3), immunoglobulins (SIg), α-naphthylacetate esterase (ANAE), plasma membrane enzymes 5′-nucletidase (5′-AMPase) and γ-glutamyltranspeptidase (GLUPAase). When compared to the values obtained with normal PBMC, i.e. Leu 1, 78.7%±4.9; GLUPAase, 13.3±3.4 nmoles/h 10^6 cells; 5′-AMPase, 28.9±15.9 nmoles/h 10^6 cells, the variations were: breast carcinomas: Leu 1, 52.2%±14.9; GLUPAase, 42.5±21.8; 5′-AMPase, 14.9±8.3 nmoles/h 10^6 cells. Metastasis of breast carcinomas: Leu 1, 52.7%±7.1; GLUPAase, 43.0±14.4; 5′-AMPase, 2.3±1.7 nmoles/h 10^6 cells. Other primary neoplasias: Leu 1, 47.3%±17.6; GLUPAase, 59.9±34.8 nmole/hr/10^6 cells. Benign proliferative diseases: Leu 1, 56.0%±6.2; GLUPAase, 35.6±15.8; 5′-AMPase, 18.3±11.2 nmoles/h 10^6 cells. These variations may reflect a PBMC ability in detecting proliferation of malignant and benign solid tumors as well as altered immune properties.

Influence of pO_2 on primary cultures of cells derived from granulation tissue of rats

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In most tissues, pO_2 is considerably lower than in standard cell cultures. Therefore the influence of pO_2 on cells obtained from enzymically dissociated granulation tissues of rats was investigated. Cells were incubated in air with 8% CO_2 in which O_2 was reduced by N_2 either to 1% or 5% or enhanced by O_2 to 31%. Under hypoxic conditions, cell cycles determined by differential staining of sister chromatids were shorter, cloning efficiencies nearly doubled, and mutation frequencies at the $HGPRT^-$ locus enhanced compared to 21% and 31% O_2. These effects were maintained in cells subcloned for several passages. The results suggest that low pO_2 favors maintenance of cells in the proliferative state.

Characterization of *Herpes suis* virus, labeled radioactively

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Neurons recognize, internalize and transport within themselves specific molecules. Neurotropic viruses may serve as a model to study these mechanisms. To this end we have labeled a strain of *Herpes suis* virus, grown on PK15 (pork kidney) cells, with either ^{25}S-Met or a cocktail of 4 ^{14}C-amino-acids (Leu, Val, Lys, Arg). After isolation and purification of the virus by gel chromatography, centrifugations and fractionation on a self-generated Percoll gradient we have characterized the viral proteins on SDS-PAGE and 2-D gels by PAGE-blue staining and autoradiography. The differences between the 2 labeling patterns are discussed.

Transcription termination of eukaryotic tRNA genes

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Transcription termination of cloned and sequenced tRNA genes from *Xenopus laevis* has been studied by gel and sequence analysis of the transcripts made in a homologous cell-free system. Transcription of a $tRNA^{Phe}$ gene mostly terminates at the first dT tract following the gene, but some read-through transcription also occurs, resulting in a series of longer precursors. In contrast, transcription terminates very efficiently at the first dT tract following a $tRNA^{Lys}$ gene. In addition, it also terminates within the gene itself at a dT_4 tract corresponding to part of the anticodon loop. Exchange of the 3′-flanking sequences of these 2 genes shows that termination efficiency is determined by these 3′-flanking sequences and is independent of the tRNA portion of the precursor. When the $tRNA^{Phe}$ gene is microinjected into *Xenopus* oocytes, transcription terminates exclusively at the first dT cluster. This suggests that the cell-free extract has lost the factor which leads to termination of transcription of the $tRNA^{Phe}$ gene.

Intracellular nucleoprotein structure of the DNA of minute virus of mice (MVM)

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We studied the nucleoprotein structure of the DNA of MVM in infected mouse L-cells. To test for nucleosome-like structures infected cell nuclei were digested with micrococcal nuclease and the purified DNA fragments electrophoresed then transferred to nitrocellulose filters. The filters were hybridized with radioactive cloned viral DNA, or with L-cell DNA, and autoradiographed. DNA fragments with lengths characteristic of nucleosomes were seen with the cell DNA probe but not with the viral probe. Viral nucleoprotein complexes were extracted from infected cell nuclei. On sucrose gradients these sedimented in 2 peaks at 50 S and 110 S. Their protein and DNA content were analyzed by gel electrophoresis. Both peaks contained replicative form DNA and virus capsid proteins A and B. Histones were not seen. The complexes were examined by

electron microscopy. The 110-S fraction contained DNA attached to what seem to be viral capsids, while in the 50-S peak only DNA was seen. No beaded nucleosome-like structures were observed.

The promoter for the regulated acid phosphatase in yeast

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The structural genes for repressible *(pho5)* and constitutive *(pho3)* acid phosphatase from yeast *(S. cerevisiae)* have been cloned by complementation of a *pho5; pho3* yeast double mutant using a cosmid vector. Subcloning and functional in-vivo analysis confirmed genetic data that the 2 structural genes are tightly linked in the order of (5') *pho5–pho3*. – Sequence analysis of the 5'-nontranslated region of the *pho5* structural gene revealed a promoter-like structure with a TATAA sequence at position −99. The first ATG after the major transcription start (at position −40) leads to an open reading frame that extends into the *pho5* gene as far as sequence data are available (more than 600 bp). The mRNA codes for a stretch of 20 hydrophobic N-terminal amino acids framed by hydrophilic lysine residues. Such a signal peptide was also found for other secreted yeast proteins (e.g. glucose repressible invertase). The phosphatase promoter is being used for the expression of foreign genes in yeast.

Organogenesis during metamorphosis of marine sponge larvae

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The major organ of a sponge is the flagellated chamber, which takes in food and pumps water through the canal system. It is thought that the cells forming the chambers (choanocytes) are derived from the superficial flagellated larvae cells which have migrated into the interior of the larvae, reflecting some form of gastrulation. To investigate this repeated gastrulation in *Microciona prolifera*, we have used 3 different approaches: a) following the fate of labeled flagellated epithelial cells of the larvae during organ-formation, b) detection of de novo cell formation via cell division, c) time lapse cinematography. The results of each of the approaches led to conclude that the choanocytes that eventually build this sponge organ do not arise from the flagellated epithelial cells but are formed de novo from stem cells. Thus sponges may not display gastrulation and should be reclassified into their own subkingdom.

Do tumor promotors cause a transition of Go cells to the Gl state?

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Nuclear fluorescent patterns from cultured cells stained with quinacrine-dihydrochloride allow the discrimination of cell cycle position within the Gl phase (Moser et al., J. Cell Physiol. *106*, 293, 1981). The use of this method in vivo was investigated in nuclei of single cells from dissociated tissue, in smears and in histological sections. The cell cycle related fluorescent nuclear patterns were confirmed by autoradiography in liver cells, granulation tissue and fibrosarcomas of rats. The method was applied to examine whether tumor promotors push Go cells to a Gl state. Liver smears taken from fine needle biopsies of phenobarbital treated rats (50 mg/kg per day for 9 weeks in drinking water) contained cells with a much lower degree of fluorescence intensity in their nuclei as compared to those of untreated rats. This indicates that phenobarbital shifts liver cells from Go arrest to the late Gl phase.

Calmodulin stimulates Ca^{2+} uptake and ATPase activity of human platelet microsomes

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Ca^{2+} uptake was measured in microsomes from washed human platelets as a function of time. Addition of bovine brain calmodulin stimulated the net uptake of Ca^{2+}, e.g. from 1.6 to 2.2 nmoles/mg protein in 30 min. Subsequent addition of 10^{-5} M Ca-ionophore A23187 strongly reversed the Ca^{2+} uptake. With 5 mM oxalate Ca^{2+} transport kinetics were linear. The latter 2 findings are evidence for the accumulation of free Ca^{2+} inside the vesicles and against Ca^{2+} being sequestered by microsomal membranes. Calmodulin also stimulated microsomal ATPase activity from 2.0 to 2.2 µmoles P_i/mg protein in 30 min. Stimulation of the Ca^{2+} transport and ATPase activity by calmodulin could be abolished by the calmodulin antagonist R24571 (10^{-5} M). Thus, it is likely that – similar to other cells – calmodulin plays an important role in the Ca^{2+} homeostasis in platelets.

Molecular cloning and characterization of DNA sequences isolated from the DNA eliminating nematode *Ascaris lumbricoides*

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A. lumbricoides contains a DNA satellite which is mostly eliminated during the process of chromatin diminution. Several satellite DNA containing fragments about 5–10 kb long were isolated and cloned in pBR 327/*E. coli* HB 101. These DNA sequences were then digested with Rsa I, the resulting DNA fragments separated by agarose gel-electrophoresis, transferred to nitrocellulose filters and hybridized with a labeled probe of *Ascaris* satellite DNA. Clones which contained satellite as well as nonsatellite DNA sequences were selected. The nonsatellite DNA fraction of such clones were hybridized to southern blots of germ line and somatic DNA digested with various restriction enzymes. It is hoped that this technique permits to fish out border sequences between eliminated and retained DNA. Molecular analysis of these border sequences should give us some clues about the mechanism of chromatin diminution at the molecular level.

Surface component at focal contacts of fibroblasts defined by a monoclonal antibody

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Balb/c mice were immunized with chicken embryo fibroblasts (CEF) and their sera were found to prevent attachment of CEF to artificial substrates. Spleen cells of such mice were fused with F0-myeloma cells and the supernatants of the resulting hybridomas were tested for their ability to prevent attachment of fibroblasts. The supernatant of a particular clone (C-1) in fact showed such an antiattachment activity and, additionally, stained the focal contacts in the immunofluorescence. These 2 activities were

not separated by limited dilution of the C-1 hybridoma population and the active antibody was identified as an IgG_1. Furthermore, the action of the antibody was blocked by cholate extracts of CEF, while urea, low and high salt extracts were ineffective. By immunoprecipitation the corresponding antigen was identified as a 60-kd protein. We thus have identified a new membrane protein at focal contacts presumably facing the cell exterior.

Mouse mammary tumor virus DNA clones transfected into nonmurine cells exhibit glucocorticoid-dependent expression

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2 cloned MMTV genes, a 9-kb clone of the unintegrated exogenous viral genome and a 14-kb clone containing the integrated endogenous genome along with cellular flanking sequences, have been transfected into mink lung cells which do not contain endogenous MMTV sequences. By cotransfection with SV-40 DNA, cell clones containing MMTV could be isolated using SV-40 transformation as a selective marker. Cell clones transfected with either the endogenous or exogenous MMTV DNA express normal MMTV RNA species in response to glucocorticoid hormones. Expression of low levels of viral proteins is also hormone-dependent. Since recombination with endogenous viral sequences is not possible in these cells, we conclude that the MMTV DNA clones contain all the information necessary for synthesis of normal viral RNA and proteins. These results also suggest that the DNA sequences involved in the hormone responsiveness of MMTV expression are contained within the viral genome.

Transcription of a cloned mouse immunoglobulin gene in various cell lines

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An immunoglobulin λ-light chain gene (Bernard, Hozumi and Tonegawa, Cell *15*, 1133, 1978) was linked to SV40 DNA containing a transcriptional 'enhancer' element and transfected by the calcium phosphate method into human Hela cells. In a transient expression assay, up to 10,000 λ-gene transcripts per cell were detected which were correctly spliced and polyadenylated. However, several λ-gene-SV40 recombinants gave transcripts with defined but incorrect 5'-termini and these transcripts were not translated into detectable amounts of protein. Since monkey CV-1 and mouse 3T6 cells were also unable to properly recognize the λ-gene promoter, we have attempted to transfect mouse myeloma cells. In our hands, these cells were refractory to calcium phosphate transfection. Using a modification of the protoplast fusion technique (Schaffner, Proc. nat. Acad. Sci. USA *77*, 2163, 1980) about 1% of the myeloma cells could be transfected. The expression of the λ-chain gene is under investigation.

Ontogenetic changes in rabbit myelinating optic nerves

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In rabbit optic nerves, at 5 days of age, a few axons are already myelinated. The peaks of maximal activity for 2 myelin lipid synthesizing enzymes, ceramide galactosyltransferase and cerebroside sulfo-transferase were found between the 10th and 20th day of age, the latter preceding the former. This period slightly precedes the active phase of myelination as shown by the increase in the number of myelinated axons and the thickness of the myelin sheaths. During this time, the concentration of myelin basic protein (MBP) increased dramatically. 2 enzymes associated with myelin membranes: 2',3'-cyclic nucleotide 3'-phosphodiesterase and carbonic anhydrase (CA) showed developmental patterns similar to MBP but the rapid increase occurred earlier. CA activities decreased after the early myelination phase confirming that it is preferentially localized in oligodendroglial membranes rather than in compacted myelin. No difference was observed in the ontogenetic program between the left and right optic nerves.

Characterization and sequence analysis of interspersed repetitive DNA sequences transcribed in *Xenopus laevis* embryos

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In order to investigate the possible involvement of repetitive sequences in the process of gene expression, clones containing repetitive as well as transcribed sequences were selected from a genomic *X. laevis* library. A detailed examination of one of these clones demonstrated that it consists of a cluster of different repetitive elements adjacent to a stretch of unique DNA. Transcripts complementary both to the unique and repetitive regions of the clone have been detected in nuclear and polyribosomal RNA of stage 40 embryos. The repetitive sequences are largely found on polyadenylated molecules. Moreover a number of cDNA clones obtained from polyribosomal RNA habe been shown to contain the repeats. Whether or not mRNA molecules carry these repetitive sequences is a question now being investigated by sequence analysis of the cDNA clones and by means of hybridization selection and in-vitro translation experiments.

A repetitive sequence in genomic DNA clones hybridizing with cDNA from various developmental stages of *Xenopus laevis*

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Genomic DNA clones (111) containing sequences homologous to polysomal RNA from stage 45 embryos were classified according to their hybridization with cDNA prepared from cytoplasmic RNA of different tissues. 56 of these clones hybridized with cDNA from all tissues analyzed, whereas the others showed a certain pattern of 'tissue specificity'. 6 out of these 56 clones contain a repetitive sequence homologous to the tandem repeat described by Spohr et al. (JMB *151*, 573, 1981). Sequence analysis indicates that this repetitive sequence is highly conserved. Heteroduplex analysis of 2 clones, containing a 3 and 10 kb *X. laevis* insert respectively, shows a region of homology limited to 300 bases. Northern analysis revealed repetitive transcripts present in RNA molecules cosedimenting with polyribosomes in sucrose gradients. We are now studying whether repetitive sequence transcripts are present in mRNA molecules.

Cytochemistry of nucleic acids in *Dictyostelium discoideum*

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With the DNA-specific fluorescent dyes DAPI and mithramycin, chromatin in interphase cells fluoresced uniformly, except for a brighter heterochromatic patch near the tapered end of the nucleus. The nucleolus did not fluoresce. Chromosomes in mitotic cells were individually distinguishable. A faint, but distinct fluorescent line was visible at the nuclear envelope in interphase and mitotic cells. Mitochondria fluoresced faintly and the fluorescence of ingested bacteria varied. With ethidium bromide and acridine orange, the nucleolus fluoresced brightly and chromatin moderately. Except for the spindle area, mitotic nuclei fluoresced uniformly, chromosomes being invisible. None of the 4 dyes stained the nucleus-associated body (NAB) of interphase cells or the spindle pole bodies of mitotic cells. Regressive staining of ultrathin sections left open the question whether the core and the matrix of the NAB contain DNA and RNA, respectively, but the reaction with osmium ammines revealed no DNA in the NAB.

Abundance and expression of M-CK genes

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A library of clones containing muscle-specific cDNA sequences was constructed by generating recombinant plasmids pBR322 with inserted DNA sequences complementary to polyA muscle RNA. Colonies hybridizing to radioactive cDNA from muscle RNA but not to cDNA from gizzard were selected. Identification was achieved by hybrid-selected translation and analysis of the translation products by immuno-precipitation and by 2d-gelelectrophoresis. The clone pMCK hybridized to RNA that was translated into the muscle-specific form of creatine kinase M-CK. Northern blots of muscle RNA indicated that pMCK sequences hybridized to a RNA species in the size range of 15 S. The cloned sequence has been further analyzed by restriction mapping. Genomic abundance and arrangement of M-CK genes are being studied by southern transfers of restricted genomic chicken DNA and hybridization to nicktranslated pMCK sequences.

Localization of vitamin D-dependent calcium binding protein (CaBP)

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The immunocytochemical localization of CaBP which is involved in vitamin D regulated Ca^{2+} metabolism is reported. CaBP is found in chick intestinal absorptive cells and in principal but not dark cells of distal convoluted tubules, connecting segments, and the early part of collecting ducts of chick and mammalian kidney. Subcellularly, CaBP is present in the cytosol and euchromatin, but is not preferentially associated with membranes. Therefore, CaBP may function in intracellular regulation of Ca^{2+} level. – By mapping CaBP in chick and rat central nervous system, it is found in certain neurons in all areas and in ependymal cells (only rat). In the chick pancreas, CaBP is present only in B-(insulin)cells.

Temperature controlled transcription of a *Drosophila* heat shock gene in *Xenopus* oocytes

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The *Drosophila* gene coding for the 70,000 d heat shock protein (hsp 70 gene) has been injected into *Xenopus* oocyte nuclei. This gene is not specifically transcribed at normal temperatures, but is activated when oocytes are heat treated (35 °C). Transcription then gives rise to RNA of correct size and sequence content. These observations show that the DNA sequences controlling the expression of the *Drosophila* hsp 70 genes are recognized by the *Xenopus* heat shock control mechanism. Mutant hsp 70 genes are used to map the DNA region involved in heat shock gene activation.

Vimentin-containing smooth muscle cells are responsible for localized thickening of the aortic intima after endothelial injury

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Changes in the aortic wall, as they occur during formation of atheromatous plaques, have been studied in rat by removing mechanically the endothelial layer and inducing intimal smooth muscle accumulation (intimal thickening). Tunica media of normal aorta consists of smooth muscle cells containing filaments composed of vimentin and desmin. Areas of intimal thickening, however, are formed by cells containing practically only vimentin filaments. This is demonstrated by double immunofluorescent staining using guinea-pig antibodies against vimentin and rabbit antibodies against desmin, and by densitometric quantitation of these 2 proteins on SDS-PAGE. It appears that vimentin-rich cells only contribute to the experimentally induced intimal accumulation of smooth muscle cells. Similar studies on experimentally induced hypertension will be presented.

Gene transfer into whole organisms

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Our earlier studies demonstrated that rabbit β-globin gene DNA injected into fertilized frog eggs persists through development (Rusconi and Schaffner, Proc. nat. Acad. Sci. USA *78*, 5051, 1981). Closer analysis of DNA from transformed frogs shows that the persisting foreign genes are unevenly distributed among tissues of the same individual, in amounts varying from 0.1 to 50 copies per cell, without any apparent tissue preference. Furthermore, none of the persisting rabbit β-globin genes were found to be transcribed at a detectable level, in contrast to the previously reported activity at early stages of frog development. – Recently we have applied similar experimental approaches to the mouse system.

Transfection of *Xenopus* embryos with a cloned vitellogenin gene

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The A2 vitellogenin gene of *Xenopus* cloned in Charon 4A was injected into *Xenopus* eggs fertilized in vitro. 1 day after injection, at the early tailbud stage, on the average 5

copies of the injected gene were still present and low amounts of transcripts could be detected, whereas in larvae the injected DNA was usually no more detectable and in all frogs analyzed no persisting DNA was found. Using a minigene consisting of the 5′- and 3′-fragments of the A2 gene cloned into pBR 322, the injected DNA was detectable frequently in tadpoles and in frogs. Low levels of transcripts of the injected minigene were detected 1 day after injection when about 20 copies of the injected gene were found per cell, whereas no transcripts were found at later stages, although the minigene was still present. Analysis of the RNA in tissues of frogs containing minigenes will reveal whether the injected minigene persisting larval development can be activated by estrogen in a tissue specific manner.

Comparison of the cytochrome P450 containing microsomal monooxygenases originating from 2 different yeasts

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Cytochrome P450 occurs in different yeast species. In *Candida tropicalis* it is functionally related to the degradation of n-alkanes. In *Saccharomyces uvarum* it is formed when the cells are grown at quasi-anaerobic conditions but its physiological role is not fully understood. From both yeasts a cytochrome P450 containing microsomal fraction was isolated. The 2 fractions were compared with respect to the absorption maximum in the CO-difference spectrum and the in vitro aliphatic hydroxylation. Cytochrome P450 from *C. tropicalis* showed its absorption maximum at 450 nm, whereas that of *S. uvarum* had its maximum at 448 nm. Hydroxylation activity was high with the microsomal fraction from *C. tropicalis* and negligible with that from *S. uvarum*. The results indicate that the 2 yeasts yield 2 different classes of cytochrome P450.

Both spliced and unspliced mRNAs mediate *Escherichia coli* galactokinase gene expression in mammalian cells

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E. coli galactokinase gene (*gal*K) expression controlled by SV40 signals for transcription initiation, polyadenylation and RNA splicing was obtained in mammalian cell lines transfected by a plasmid vector (Schümperli et al., Proc. nat. Acad. Sci. USA, in press). Removal of the splicing signals did not abolish *gal*K expression, although analogous deletions of the same vector carrying a rabbit β-globin cDNA gene had resulted in complete loss of β-globin production (Howard et al., J. molec. appl. Genet., in press). Analysis of *gal*K-specific RNA by Northern blot hybridization and mapping of the 5′- and 3′-untranslated regions by mung bean nuclease protection indicated that *gal*K transcripts were being spliced in the wild-type plasmid but not in the deletion mutant. Determination of *gal*K enzyme levels normalized for gene copy number by virtue of an internal standard allowed us to compare *gal*K expression efficiencies for spliced vs. unspliced mRNAs.

Formation of defined monolayers from natural and artificial membrane vesicles

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Natural and artificial vesicles were shown to form spontaneously a monolayer at the air-water interface. In this way lipid-protein monolayers with similar compositions to those of original membranes are generated without the need for extraction of components. A characterization of such monolayers requires a complete separation of the monolayer from vesicles. This is achieved by applying hydrodynamic shear forces to vesicles adhered to the monolayer. The velocity of monolayer formation from vesicles strongly depends on the salt concentration, which was examined for univalent and divalent cations. Vesicles made of natural membrane lipid extracts or whole membranes generally exhibit high spreading velocities and yields, whereas small velocities are found for liposomes made of purified lipids. This velocity difference could not be explained by differences in type and structure of phospholipids. Nonphospholipid components, especially proteolipids, seem to govern the spreading velocity.

Separation of splenic T and B lymphocytes of *Xenopus laevis*

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The separation of 2 functionally distinct populations of *Xenopus* splenic lymphocytes was achieved by using the method of anti-Ig antiserum coated petri dish. The recovered nonadherent cells (NAC) were enriched in sIg^- (90% vs 70%) and the adherent cells (AC) were enriched in sIg^+ cells (75% vs 30%). The NAC proliferate actively and enlarge into lymphoblasts upon stimulation with PHA, Con A and PWM, but they respond poorly to anti-Ig antisera. These cells are also MLR-reactive. The AC respond vigorously to anti-Ig and PWM but they do not react to allogeneic cells. Less than 5% of PWM-induced lymphoblasts in the NAC and more than 45% of lymphoblasts in the AC populations differentiate into Ig-producing plasmablasts. These results indicate that the sIg^- lymphocytes in the NAC population are thymus-derived, while the sIg^+ lymphocytes in the AC population belong to the B-cell lineage and can differentiate into Ig-producing lymphoblasts.

Orthophosphate dependent and independent regulation of acid phosphatase in yeast

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The cell surface glycoprotein acid phosphatase (a.Pase) of yeast exists in an enzymatically active and inactive form. The inactive form is membrane-bound. Orthophosphate-dependent and -independent increase of a.Pase activity involves mechanisms that increase the specific activity of the active enzyme and shift the ratio between inactive and active a.Pase towards the side of the active form. Both types of regulation affect glycosylation of a.Pase. We have recently shown for the fission yeast *Schizosaccharomyces pombe* that a.Pase is involved in the control of growth and cell-cell contact (Schweingruber et al., Proc. 9th Congr. int. Soc. devl Biol., 1982, in press). The shift of membrane-bound inactive acid phosphatase to the active cell-wall associated form and differential glycosylation may be part of the cell surface modulation mechanisms regulating cell growth and cell-cell contact.

Characterization of 2 nonsense suppressor tRNAs from *Schizosaccharomyces pombe*

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The opal (UGA) suppressor tRNA from *S. pombe* strain *sup9-e* and the ochre (UAA) suppressor tRNA from *S. pombe* strain *sup3-i* have been purified by several steps of column chromatography and 2-dimensional gel electrophoresis. Suppression was assayed by in vitro translation in a wheat-germ extract programmed with rabbit globin mRNA. Addition of opal suppressor tRNA elongates β-globin, addition of ochre suppressor tRNA elongates α-globin. The pure suppressor tRNAs from both strains can be aminoacylated with serine. Thus they insert serine in response to the termination codons UGA and UAA respectively. We are studying the primary structure of these tRNAs by nucleotide sequencing and by the analysis of the modified nucleosides by HPLC methods (see abstract Heyer et al.). This will provide the basis for our research on the function of modified nucleosides in tRNA.

Persistence of Semliki Forest Virus in *Aedes albopictus* cells

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Infection of the *A. albopictus* cell clone C6/36 with wild type (wt) Semliki Forest Virus (SFV) leads to high virus production, to inhibition of cell functions and to pronounced cytolysis. Surviving cells become persistently infected (C6/36PI) and produce low virus titers. 100 days after infection virus released from C6/36PI cells form small plaques on CEF at 28 °C and do not multiply at 37 °C (SPts SFV). Infection of C6/36 cells with SPts SFV leads to high SPts SFV production at 28 °C and to cytolysis of most of the cells. In contrast, superinfection of C6/36PI cells with wt SFV does not inhibit cell functions nor stimulate virus production or intracellular viral RNA synthesis. By blot hybridization with cloned SFV cDNA we detected 49 S and 26 S virus specific RNA in infected C6/36 and C6/36PI cells. Both wt and SPts virions contained 49 S RNA. In particular, we did not detect defective interfering RNA. Our results suggest a coevolution of virus and cells in a persistently infected culture of C6/36 cells.

A physical map of *Glycine max* plastid DNA

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DNA was isolated from purified *Glycine max* (soybean) chloroplasts. All restriction sites for PstI, SalI and SmaI were located on the circular genome which has a total length of about 150,000 bp. The genome contains 2 inverted repeats each carrying 1 rDNA segment. The structural gene for a M_r 32,000 protein (photogene) was located close to one of the inverted repeats. From a phylogenetic standpoint it is noteworthy that chloroplast DNA from *Glycine max* has about the same length as chloroplast DNA from *Phaseolus mungo* (Palmer and Thompson, PNAS *78*, 5533, 1981). Both genomes contain inverted repeats of about 23,000 bp; however, 2 other leguminous plants, i.e. *Vicia faba* and *Pisum sativum*, have chloroplast genomes of only 120,000 bp, a single rDNA segment and no inverted repeats. It seems that during evolution loss or gain of a large DNA segment has occurred.

Effect of membrane modulators on the catecholamine release in the Pheochromocytoma cell line PC 12

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The Pheochromocytoma cell line PC 12 has been used as a model system to study the role of membrane structure during release of catecholamines. Maximal release (70% of the ^{3}H-noradrenaline taken up during a preincubation period) can be achieved when cells are incubated with 55 mM K^+ for 15 min. High concentrations (up to 300 µg/ml) of 5 different lectins, including Con A, neither stimulate nor inhibit the release of catecholamines. 1 cationic and 2 nonionic detergents, as well as tetracaine and n-octanol have a strong inhibitory effect upon release at low concentrations (at least 10-fold lower than critical micellar concentration). Cell viability was not affected. At high concentrations of most agents the expected promotion of release could be observed together with incipient damage. The anionic detergent SDS does not evoke a similar effect.

Contributions of the domains of histone H1 to chromatin structure

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By comparing the salt-dependent condensation of H1-depleted chromatin with chromatin containing H1 (native or reconstituted) in the electron microscope, we have shown that H1 organizes the chromatin fibres (Thoma and Koller, J. molec. Biol. *149*, 709, 1981). To test which part of H1 was involved in the formation of the fibres, we have added fragments of H1 to H1-depleted chromatin. All the fragments induced condensation and finally precipitation. The formation of fibres similar to those of chromatin containing H1 was obtained when fragments containing the globular domain were used. The fibres were distorted when the pH was shifted from 7 to 10. Binding studies in 100 mM NaCl demonstrated that H1 and fragments containing the basic C-terminal end remained bound at pH 7 and 10, whereas the globular fragment of H1 was released by the shift from pH 7 to 10. The results indicate that the globular domain of H1 is essential for the formation of higher order chromatin structures.

Mouse strain-specific expression of pancreatic α-amylase genes

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Pancreatic α-amylase isoenzymes are encoded at the genetic locus *Amy*-2 on mouse chromosome 3 (Eicher, Mouse News Lett. *60*, 50, 1973). While in most inbred mouse strains including strain A/J only one α-amylase species is found in the pancreas, some inbred strains express multiple pancreatic α-amylase isoenzymes. This suggests that several distinct α-amylase genes exist at the *Amy*-2 locus, and that their expression is regulated in a strain-specific fashion. Therefore, we have begun to examine the expression of the *Amy*-2 locus in mice of strain CE in which 4 distinct pancreatic α-amylase proteins were detected (Bloor, Meisler and Nielsen, J. biol. Chem. *256*, 373, 1981). – Using restriction endonuclease and sequence analysis of cDNA clones we have established that at least 2 distinct pancreatic α-amylase mRNAs are expressed in strain CE mice. The 2 mRNAs for which we have deduced a partial sequence differ by approximately 1% of their nucleotides. Both mRNA species are distinct from the α-amylase mRNA which is found in the pancreas of strain A/J.

Butyrate antagonizes aldosterone-dependent Na^+ transport

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Aldosterone (A) stimulates transepithelial sodium transport (TST) in the urinary bladder of the toad *Bufo marinus* by regulation of gene expression. Sodium butyrate (B) can modulate gene activity. This action correlates well with inhibition of histone deacetylation induced by B. We have therefore studied the effect of B (0.3–10 mM) on a) base-line TST and aldosterone-dependent TST and b) the degree of core histone acetylation after incorporation of ^{3}H-acetate (60 min pulse) followed by a chase in absence or presence of B (0.3–10 mM). B did not change base-line TST but antagonized the late response to A (80 nM) in a dose-dependent manner ($Ki \simeq 0.7$ mM). This effect correlated well with the degree of histone hyperacetylation induced by B ($Ki \simeq 0.8$ mM). The biological activities of 2 analogs (propionate and isobutyrate 1- and 12-fold respectively, less potent than B) were similar both in the physiological and the biochemical assay. We conclude that acetylation of histone (and/or of other unidentified nuclear proteins) antagonize the late response to A.

Penetration of differently metastasizing B16 melanoma cells through Nuclepore filter holes

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Clones of different metastasizing potential as assessed by various assays were seeded on chemically modified filters of different pore sizes (1–12 µm). Clones D, G and H passed with 34, 38 and 122 cells/cm^2 through 3-µm filters in 24 h. Incidence of metastasis in C57bl mice was 35, 56 and 66%, respectively. For the F1 parent line and a clone from F1 we found 101 and 16 passing with 55 and 30% metastasis. Penetrating cells were selected through 2-µm filters 9 times, with 10, 94 and 84 cells penetrating respectively. Colonies picked from the 3rd step showed high variability at assay. 2 extreme clones passing at 299 and 544 (on 3-µm filters) showed 89 and 30% metastasis, an observation supporting the multistep concept of metastasis. It is unlikely and actually unexpected that a single assay monitoring a single property of metastasizing cells will correlate very closely with metastasis as a whole.

Parallel stimulation by dicoumarol of mitochondrial H^+-ATPase and rate of germ tube outgrowth from conidia of *Neurospora*

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The site of germ tube emergence on conidia of *N. crassa* is acidified, presumably by protons vectorially dissipated from the back-positioned mitochondria (Turian, Ber. schweiz. Bot. Ges. *90*, 202, 1980) as suggested by their increased ATP-hydrolyzing and H^+-generating activity, compared with that of mitochondria isolated from semidormant conidia. Such an evocation of latent mit-H^+-ATPase activity (Lehninger, Biochemistry, Worth 1975) was further increased in the presence of an uncoupler of oxidative phosphorylation, 2,4-dinitrophenol (10^{-5} M) with proportional stimulation of the rate of germ tube outgrowth (Turian and Michéa-Hamzehpour, Abstr. IX. Cong. Int. Soc. Devl Biol. Basel, 1981). Even more effective parallel stimulation of both mit-H^+-ATPase and rate of germ tube outgrowth has now been obtained with another uncoupler, dicoumarol (5×10^{-7} M), increasing both processes by 50% after 2.5 h germination in liquid Vogel medium at 25 °C.

Characterization of the transcription initiation site of the A1 and A2 vitellogenin genes of *Xenopus laevis*

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The 5′-ends of the 2 closely related, estrogen-controlled A1 and A2 vitellogenin genes have been analyzed and compared. DNA restriction fragments from the region where the putative 5′-ends had been mapped by electron microscopy were transcribed in an in-vitro transcription system (Hela cell extract). The localization of the transcription initiation sites of the 2 genes was determined from the length of run-off transcripts obtained from DNA fragments truncated with different restriction enzymes. The DNA primary structure at these sites has the characteristic features of RNA polymerase II initiation sites. S1 nuclease mapping experiments were performed to compare the position of the 5′-end of vitellogenin RNA synthesized in vitro and in vivo, i.e. in the hepatocytes of estrogen-stimulated animals.

Tumor-host relationship of an undifferentiated human germ cell tumor xenografted into nude mice

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Fragments of a testicular tumor (embryonal carcinoma, human) were injected into 6-week-old male animals of the thymus-dysgeneic nude-mouse NMRI-strain. 10 weeks after injection, rapid tumor growth was noted. In parallel, the testes of these animals showed prominent swelling. Light- and electron microscopical investigations revealed structural differences between surgical and implanted specimens. The Leydig cells in the testes of the tumor-bearing mice were stimulated and hyperplastic. In addition, the electrophoretical behavior of the lactate dehydrogenase isozymes in the mouse serum displayed variations in comparison to control sera, to homogenated tumor cells, to the serum of the patient, and to mixtures of the various sera. The data suggest that this human tumor and its nude-mouse host represent a sensitive system to observe changes of biochemical and of structural parameters.

Control of RNA synthesis by the circadian clock

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The marine dinoflagellate *Gonyaulax polyedra* shows circadian rhythms in photosynthesis, bioluminescence and cell division which appear to be controlled by a single circadian clock. Using the fluorescence of acridine orange the RNA content was measured in free-running (constant light and temperature), non-dividing cells. The RNA content per cell was found to oscillate with a period of about 24 h with a maximum at circadian time (CT) 18 and a minimum at CT 02. At CT 18 30–40% more RNA could be detected per cell than at CT 02. The phase of this rhythm can be shifted by a short-light pulse. The banding pattern of total RNA on gels (under denaturing conditions) shows significant qualitative and quantitative variations. Various bands in the size range

of messenger RNA appear only at CT 18 (physiological midnight). Our results demonstrate that transcription is under the control of the circadian clock.

The light-harvesting chlorophyll a/b complex: isolation, crystallization and structural characterization

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The light-harvesting chlorophyll a/b complex (LHC) accounts for 50% of the total protein and chlorophyll mass in thylakoid membranes of plants. It collects solar energy and transfers it to the reaction centers. The triton X-100 solubilized complex has the unique property of forming 2-dimensional crystals upon addition of cations (Mullet and Arntzen, Biochim. biophys. Acta *589*, 100, 1980). In order to obtain highly ordered 2-dimensional crystals we purified the complex and tested several parameters to optimize crystallization conditions. Similar crystalline preparations have been obtained by fusion of liposomes with thylacoid membranes (Siegel et al., J. Cell Biol. *91*, 113, 1981). Both types of LHC-crystals have been studied by electron microscopy and digital image processing. The LHC's form hexagonal units containing a central core which are embedded within the membrane and arrange into a hexagonal lattice with 120 A lattice distance.

Expression of globin mRNA sequences in *Xenopus laevis* analyzed at the molecular and cellular level

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Analysis of cloned cDNAs showed that 4 sequences (2α, 2β) are preferentially expressed in either anemic larvae or adults of *X. laevis*. - R_0t-analysis revealed a similar abundance of the stage-specific sequences in the 9 S globin mRNAs of larvae and adults. On northern blots we found that 5–20% of the mRNA from larvae represented adult sequences, whereas only about 0.2% of the mRNA from adults specifically reacted to larval clones. - Northern blots of total erythroid cell RNA showed that all sequences are also expressed in the absence of induced anemia. Whereas anemia had no visible effect on the expression of larval sequences, we found that in the adult mRNA the α_2 and β_2 sequences become more abundant. - By in-situ hybridization of ^{3}H-nick-translated cDNA clones to blood smears of anemic larvae, we could demonstrate the presence of 2 cell populations expressing either larval (L-cells) or adult globin genes (A-cells).

Splicing of rabbit β-globin gene transcripts studied by reversed genetics

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Cloned rabbit β-globin genes, modified by restructuring or site-directed mutagenesis, were introduced into cultured cells and the β-globin transcripts analyzed. 1. Deleting the greater part of the large intron had no effect on splicing accuracy or mRNA level. 2. Deleting *either* the large *or* the small intron had no significant effect whereas deletion of both introns reduced the level of mRNA to about $1/10$ of wild type. 3. Deletion of the 5′ splice site (ss) of the large intron led to normal levels of shortened mRNAs, in which the normal 3′ ss was spliced to a 'cryptic ss' at position +360 in exon 2. 4. Of 5 purine transitions, at positions flanking the large intron 5′ ss only the GT → AT transition (position 495, breaking the 'Chambon rule') affected splicing. 3 products were found: in one, the 3′ ss was joined to a cryptic site just downstream, and in 2 others, to sites upstream of the altered 5′ ss.

RNA transport in microinjected frog oocytes

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When total p^{32}-labeled RNA from Hela cells is injected into the *cytoplasm* of *Xenopus* oocytes, it is found that the RNAs of nuclear origin (U1, U2) migrate back into the cell nucleus, where they become about 50-fold more concentrated than in the cytoplasm. 5S RNA is an intermediate case which only partly reenters the nucleus. While U1 and U2 become distributed uniformly within the oocyte nucleus, 5S RNA migrates to the nucleolus, as determined by autoradiography of sectioned oocytes. The Hela snRNAs become associated with oocyte proteins forming ribonucleoprotein particles; at least 5 of the 7 snRNA-binding proteins are stock-piled in the oocyte cytoplasm in a complex that does not contain RNA. It is known that proteins that are normally of nuclear origin have the ability to migrate and accumulate in the cell nucleus after microinjection into the cytoplasm. It is conceivable that some RNA-binding proteins or the complex of RNA plus protein may also have this nuclear-migrating property.

PHARMAKOLOGIE - PHARMACOLOGIE - PHARMACOLOGY

Caffeine-induced lipolysis in normal-weight human subjects

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We present evidence that oral glucose (OG) consumed with caffeine (C) annuls the lipolytic effect of C. Following a 12-h overnight fast and 16-h abstention from C-containing foods, each of 3 male and 3 female subjects (31.7±3.7 yr) was submitted to 3 separate tests, 2–3 days apart: C (4 mg/kg b.wt) + 250 ml H_2O; C + 100 g OG (in 250 ml H_2O); placebo + 100 g OG. Sera from venous blood at −20, 0, 20, 40, 60, 90, 120, 150 and 180′ were analyzed for glucose (G), nonesterified fatty acids (NEFA) and C. C + H_2O raised NEFA to over 160% of 0′ value at 40–60′ and high values persisted till the end of test; no change in G occurred throughout. C + OG lowered NEFA to 40% at 40–60′ when G rose from 4.4 to 8.3 mmoles/l; placebo + C gave similar values. A lipolytic effect of C was manifest only without OG. Absorption of C was slower after C + OG compared to C + H_2O.

Postnatal establishment of a blood-brain barrier for theobromine (Tb) in the rat

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[8-^{14}C] Tb was synthesized, purified and administered orally to newborn and to 5-, 10-, 14-, 19-, 25-, 30-, 40-, 60-day-old rats (15 rats/group). The labeled metabolites excreted in the urine and present in the liver, the brain and blood were identified and quantified 1, 6 and 24 h after the administration. The ratio of brain/blood Tb concentrations decreased continuously from 0.96±0.02 at birth to 0.60±0.02 in 30-day-old rats, while the ratio for liver remained constant: 1.18±0.05. The kinetics of the establishment of a blood-brain barrier for Tb and theophylline (Tp) are similar (Arnaud and Mariotte, 8th Int. Cong. Pharmac., Tokyo, 1981) but a ratio of 0.49±0.05 was reached for Tp. These results are not in agreement with those reported by Snyder et al. (Proc. nat. Acad. Sci. USA *78*, 3260, 1980) where a 2 times higher Tp brain concentration was measured compared to Tb. In the urine of the newborn rats, Tb, 6-amino-5[N-formylmethylamino]1-methyluracil, 3,7-dimethyluric acid, 3-methyl and 7-methylxanthine were identified.

Chronic choline treatment does not alter blood pressure

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A chronic increase in the plasmatic concentration of choline, produced by increased choline intake, may be expected to elevate blood pressure via stimulation of cerebral or peripheral ganglionic cholinergic receptors leading to increased adrenal medulla-sympathetic nervous activity. We tested this hypothesis in normotensive, developing renovascular and spontaneously hypertensive rats. Animals received 7–17 nmoles choline · kg^{-1} per 24 h for 3–7 days. Blood pressure (and heart rate) were measured by tail plethysmography or intraaortic cannulation. We found no change in blood pressure, heart rate (and plasmatic or renal renin levels). Such 'doses' of choline do, however, increase adrenal medulla tyrosine hydroxylase activity and urinary catecholamine excretion. Thus increased sympathetic nervous system activity is, paradoxically, not accompanied by an increase in blood pressure.

Platelet noradrenaline concentration as an index of long-term peripheral sympathetic activity

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Blood platelets take up noradrenaline (NA) from plasma, in which the NA concentrations depends on NA release from peripheral sympathetic nerves. Platelet and plasma NA concentrations have been measured radioenzymatically in chronically catheterized Sprague-Dawley rats before and during treatments that either increase or decrease sympathetic activity. During swimming stress (1 h), rapid, marked and lasting elevation of plasma NA was followed by a slow NA increase in platelets (~150% of basal values at 1 h). After ganglionic blockade with chlorisondamine (10 mg/kg, 3 times at 6-h intervals) plasma NA declined rapidly, whereas platelet NA was lowered gradually to ~50% of basal values at 24 h. Therefore, platelet NA concentrations reflect changes in plasma NA and may serve as an index of long-term peripheral sympathetic activity.

Benzodiazepine hypnotics: prolonged residual effects after a single dose

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Flunitrazepam (FN, 2 mg), flurazepam (FR, 30 mg) and triazolam (TR, 0.5 mg) were investigated in 8 healthy young volunteers. Drugs and placebo were administered at 1-week intervals in a double-blind crossover design, 30 min before bedtime. All-night EEG spectral analysis was performed on drug nights and first post-drug nights. All 3 drugs impaired performance in a psychomotor test in the morning following drug intake and caused a significant reduction of EEG slow wave activity in the drug night and post-drug night. Self-rated residual effects on vigilance and mood were present for the compounds with a long elimination half-life (FN, FR).

Canalicular paracellular bioelectric resistance to passive anionic movement from blood to bile in the rat

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Evaluation of differences between simultaneous biliary clearances of size-matched, charged and uncharged solutes that neither enter cells nor are metabolized; e.g. ferrocyanide and sucrose or carboxyl and methoxy inulins, has been helpful in estimating the negative charge that retards anion entry into bile. Since secretin does not affect these clearances in dogs they appear to relate only to canalicular flows, presumably across the tight junctions. Over a wide range of clearance patterns produced in rats by estrogen (n=10), hypothyroidism (n=12), and puromycin aminonucleoside (n=20), the barrier to carboxyl-inulin entry (C) was found to range from 17 to 75 mV and that to ferrocyanide (F) from 7 to 23 mV (control: 29 and 10 mV, resp.) with little change in C/F ratio (2.9–3.0; control 2.9). This constancy suggests that the steric determinants of the high normal ratio are not affected.

Pharmacokinetics in man of Jumex, selective MAO-B inhibitor, antiparkinsonian drug

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The pharmacokinetics of ^{14}C-Jumex® (JU), (−)Deprenyl, [−]N-methyl-N-propynyl{[2-phenyl-1-methyl]-ethyl}amine, HCl (Chinoin, Budapest, Hungary; Labatec Pharma SA, Geneva) was studied in man, after oral administration of 1 tablet 5 mg. JU was rapidly absorbed ($t^1/_2 k_a = 0.4\ h^{-1}$), widely distributed into large central compartment (V_1 = 137 h). Peak plasma level, 1 h, 32 ng/ml; clearance, 1.7 ml · min^{-1} · kg^{-1}; biological half-life $t^1/_2\ \beta = 39\ h^{-1}$; in vitro plasma protein binding rate, 94%, peak erythrocyte level, 90 ng/ml. JU was eliminated to a greater extent in urine (59% at 72 h) than in feces (15% at 72 h). In plasma and urine, 5 and 7 metabolites respectively as well as parent drug were detected and quantified. Proposed metabolic pathways included amphetamine, methamphetamine and N-demethyl derivate.

The use of the rat ECG in drug safety evaluations

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Pharmacological methods have rarely been applied to toxicological studies. The ECG, one of the most important diagnostic tools in clinical cardiology, is particularly useful in acute pharmacology and toxicology. Compounds showing a selective pharmacodynamic action in rats, should be evaluated for safety the same species in order to estimate a therapeutic ratio. Recent developments in instrumentation have made it easier to conduct cardiovascular function studies in small laboratory animals, particularly the rat. The development of CGP 12673, an antidiabetic vinamidine, had to be discontinued due to serious cardiodepressive effects in toxicity studies. The cardiovascular analysis of CGP 12673 in rats has shown that there exists a good correlation between toxicological studies and pharmacological observations. The rat ECG is a suitable tool for characterization of pharmacodynamic and toxic cardiac drug activity. The chance of unpleasent effects in (rat) toxicity studies can be reduced.

Biochemical characterization of testosterone binding sites present in the rat uterus

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Adult rats, ovariectomized and adrenalectomized for at least 3 days, were used. Uterine homogenates were divided in a 100,000 × g cytosol and a 700 × g pellet. Both tissue fractions contained at least 1 ^{3}H-testosterone binding site of high affinity. The rate constants of association $k_a(\mu M^{-1} min^{-1})$ and of dissociation k_d (min^{-1}), and the dissociation constant K_D (nM) were determined at 0 °C. The values measured in the cytosol were 1.80, 0.0026 and 1.5, respectively. In the pellet we found these numbers to be 0.76, 0.0100 and 14.5, respectively. The hormone specificity of the sites in the 2 fractions was estimated. The inhibition of the testosterone binding caused by estradiol was greater in the cytosol than in the pellet. Progesterone had an opposite effect. Thus, the binding sites in the cytosol and in the pellet are different. The former site resembles the classical testosterone receptor, whereas the latter site is a secondary binding site of unknown physiological function.

Cholesterol-5α,6α-epoxide (ChE) does not interact covalently with DNA in vivo

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The vast majority of strong chemical carcinogens act by covalent interaction with DNA of the target cell. In many instances, the reactive metabolite is an epoxide intermediate. This study based upon the idea that some endogenous compounds can also be degraded via an epoxidation reaction and could contribute to an unavoidable DNA damage possibly involved in the induction of 'spontaneous' tumors. As a first model compound, ChE, a naturally occurring epoxide, was synthesized in tritiated form and administered to rats to test for DNA binding. DNA isolated from liver, stomach and small and large intestine did not exhibit any radioactivity on a limit of detection that was about 30 times below the level of liver DNA binding of the weak hepatocarcinogen 4-dimethylaminoazobenzene. Low protein binding was, however, detectable. ChE therefore seems not to represent an endogenous genotoxic risk factor, and other mechanisms will have to be discussed.

Determination of β-adrenergic receptor in intact cultured human fibroblasts and its use for drug research

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Experimental evidence indicates that the regulation of β-adrenergic transmission depends on the metabolism of the whole cell. We developed a method of high reproducibility for measuring the density and affinity of β-adrenergic sites in intact cultured human skin fibroblasts. We compared β-adrenergic antagonists of different physical/chemical properties in our system. ^{3}H-CGP 12177 (Simmons and Staehelin, Experientia *36*, 714, 1980) turned out to be an excellent radiolabeled ligand. The Scatchard transformation of the binding data with this ligand exhibits linearity, a high affinity (0.17 nM) and a b_{max} of 6–7 fmoles/mg cell protein. The results imply that this type of cell would be suitable for testing the effects of drugs on the regulation of β-receptors. A preliminary indication of this is that chronic administration of desipramine leads to a dose-dependent decrease in the density of β-adrenergic receptors.

Stabilization of enkephalins by substitution with nonbiological amino acids and by covalent attachment to tobacco mosaic virus (TMV)

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Enkephalin analogues containing, besides D-alanine in position 2, nonbiological amino acids like carboranylalanine (Car), adamantylalanine (Ada), t-butylglycine (Bug) and neopentylglycine (Neo) in position 4 and 5 show increased resistance against soluble proteases such as thermolysine, neutral protease and chymotrypsin in vitro. The covalent attachment to TMV also strongly inhibited the degradation of enkephalin peptides as shown by using reversed-phase HPLC as the analytical method. The effect was marked with soluble proteases as well as with membrane-bound enkephalinases. The observed resistance correlates well with the enhanced affinity and potency of both the free and TMV bound hormone analogues.

Phenylbutazone alters comparative susceptibility to gastric ulceration in food-deprived RHA/Verh and RLA/Verh rats

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It has been previously reported that RHA/Verh rats which were food-deprived (FD) for 4–5 days (water ad lib.) were more susceptible to the formation of gastric lesions than were FD RLA/Verh rats (Experientia *37*, 612, 1981). In the present study, fed and FD subjects were injected i.p. with 100 mg/kg phenylbutazone 24 h before sacrifice. It was found, under these conditions, that susceptibility to gastric ulceration was reversed, i.e. the treated FD RLA/Verh rats had significantly higher lesion scores than did the treated FD RHA/Verh rats. Furthermore, in addition to the pyloric lesions normally seen in FD rats of both selected lines, some of the treated FD RLA/Verh rats also had lesions in the cardia portion of the stomach. Previous experiments have shown RLA/Verh rats to be more sensitive to the toxic effects of various substances, such as pentobarbital and oxotremorine. The present study has shown that RLA/Verh rats are also more sensitive to the ulcer-inducing effects of phenylbutazone than are RHA/Verh rats.

Cardiovascular effects of the calcium antagonist tiapamil in combination with β-adrenoceptor blocking agents. Studies in anesthetized open-chest dogs

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β-Blockers and calcium antagonists are used for the treatment of cardiac arrhythmias, coronary heart disease and hypertension. Both types of drugs are likely to be employed in combination. Therefore, the interaction of tiapamil (T), 1 mg/kg i.v., with propranolol (Pr), 3 mg/kg i.v. or pindolol (Pi), 0.1 mg/kg i.v. was studied in open-chest dogs. Measurements included blood pressure (BP), heart rate (HR), coronary blood flow (CBF), myocardial contractility (MC) and myocardial oxygen consumption (MVO_2). While Pi (high ISA) did not affect cardiovascular function, Pr (without ISA) significantly reduced MC, HR and CBF. Subsequent injection of T reversed Pr-induced decrease in CBF, but augmented the cardiodepressant effects of Pr. Injected after Pi, T caused similar effects as alone and in spite of combined use did not affect MC. It is concluded, that β-blockers with high ISA (such as Pi) are best suited for combination with T, if a reduction in MC must be avoided.

ATP and adenosine hyperpolarize the guinea-pig taenia caeci through different mechanisms

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The sucrose-gap technique was used to measure ATP- and adenosine (AD)-induced hyperpolarizations in various ionic conditions at room temperature. In Krebs solution, ATP (10^{-3} M) hyperpolarized the taenia by 5.6±0.4 mV and AD (10^{-2} M) by 4.1±0.5 mV (mean±SEM, n = 15). When all but 5 mM chloride was replaced by isethionate, the response to ATP was increased by 18±11% (n = 11, $p < 0.05$), but that to AD was decreased by 53±11% (n = 11, $p < 0.02$). Lowering the K^+ from 4.8 to 2 mM in low chloride solution further increased the effect of ATP but did not change the AD hyperpolarization. Finally, in Ca-free medium (EGTA 0.5 mM + Mg^{2+} 10 mM added), the ATP response was reduced by 90±5%, whereas the AD response was 41±6% of control, even after repeated applications; this difference was significant at the level $p < 0.02$ (t-test, paired data). It is concluded that ATP and AD act through different mechanisms, which confirms the presence of separate purinoceptors in this tissue.

Cytochrome P-450 dependent monooxygenase activities in extrahepatic cells

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Aldrin epoxidase belongs to the phenobarbital-inducible cytochrome P-450 dependent monooxygenases which play a crucial role in the activation of xenobiotics to cytotoxic, mutagenic or carcinogenic metabolites. Capillar gas chromatography was used which reduced the detectable levels of product formation (dieldrin) to less than 0.5 ppb. Aldrin epoxidase activity was determined in freshly isolated, intact spleen lymphocytes and granulation tissue cells of rats. The response of the enzyme to pretreatment with phenobarbital, aldrin and dexamethasone was measured and compared with that in liver. Phenobarbital and aldrin increased the activity in liver and extrahepatic cells 3- and 2-fold respectively whereas dexamethasone decreased the enzyme activity in all cell types. The results demonstrate the presence of inducible P-450 dependent monooxygenases in extrahepatic cells.

Modulatory effect of substance P on norepinephrine-induced vasoconstrictions in the rat mesentery

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Substance P (SP) is a potent hypotensive compound and in vitro it shows spasmogenic effect in many blood vessels. Its possible role in the adrenergic transmission has been studied in the isolated perfused rat mesenteric vasculature. Intraarterial perfusion of SP (10^{-9}, 10^{-8} and 10^{-7} M) caused a dose-dependent potentiation of norepinephrine (NE) responses and a shift of the log dose response curve to the left. Saralasin (S), a specific antagonist of angiotensin II, when perfused at concentrations of 10^{-9} M did not change the NE response. However, the potentiating effect of SP could not be demonstrated in the presence of S (10^{-9} M). Indomethacin, a prostaglandin synthetase inhibitor, in concentrations of 10^{-5} M markedly attenuated the responses to NE which could not be normalized by the addition of SP. It appears that SP's potentiating effect simulates angiotensin II and not prostaglandins for the modulation in the adrenergic transmission.

Contractile and electrical responses of vascular smooth muscle to α-adrenoceptor stimulation

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Full concentration-response curves (CRC) for the contractile and depolarizing effects of noradrenaline (NA) and the α_1-adrenoceptor agonist methoxamine (ME) were obtained in strips of rabbit main pulmonary artery. Both agonists were of similar efficacy. The potency of NA was 3 times higher than that of ME. A concentration-dependent decrease in membrane potential (Vm), as measured with intracellular glass microelectrodes, was recorded over the lower half of the CRC ob both agonists. At higher concentrations contraction increased further in spite of the absence of changes in Vm. Membrane resistance, as determined by measurement of the space constant of the vascular strips, decreased over the entire course of the CRC. Blockade of potassium channels by TEA unmasked α-agonist-induced depolarization in the upper half of the CRC. It appears that α-adrenoceptor mediated elevation of intracellular Ca^{++} progressively increases potassium permeability. The latter process counteracts depolarization in the upper range of α-agonist concentrations.

Serotonin stimulates adenylate cyclase activity in growth-inhibited cultures of rat fibroblasts

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Adenylate cyclase activity in growing, primary cultures of rat embryo fibroblasts can be stimulated by isoproterenol (ISO) and dopamine (DA), but not by serotonin (5-HT). After growth inhibition with 5-fluorodeoxyuridine, sensitivity to 5-HT appears. The amount of cAMP produced in response to 5-HT increases up to 40-fold with time after growth inhibition (2–9 days). The cAMP production in response to ISO and DA changes only 2–4-fold during the same time period. The activity of 5-HT can be blocked by specific 5-HT antagonists and by phenoxybenzamine, but not by morphine, and so seems to be similar to the D-type

receptor found in guinea-pig ileum (Gaddum and Picarelli, Br. J. Pharmac. *12,* 323, 1957). Since adenylate cyclase activity is present at all stages of culture growth, the appearance of sensitivity to 5-HT after growth inhibition may result from the development of 5-HT receptors, an altered coupling between receptor and the catalytic unit, or both.

Agonist-induced reversible reduction of β-adrenergic receptors

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Using the hydrophilic ligand CGP-12177 a reduction of β-adrenergic receptors in C_6 cells is demonstrated after short-time incubation with an agonist. This reduction of binding sites is reversed by incubation at 37 °C, while it is stable for 24 h at 4 °C. A reduction of binding sites can only be demonstrated with the hydrophilic ligand in intact cells. No change is observed when cell homogenates are assayed with a lipophilic ligand such as ^{3}H-dihydroalprenolol. Therefore, an agonist-induced movement of the receptor into a region inaccessible for the hydrophilic ligand is suggested. The formation of cytoplasmic vesicles resulting from agonist-induced endocytosis, as suggested by Harden et al. (Science *210,* 441, 1980), offers a possible explanation.

Characterization of ^{3}H-nifedipine binding sites to rabbit cardiac membranes

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The calcium antagonist ^{3}H-nifedipine (^{3}H-NIF) was found to bind with high affinity ($K_D = 2$ nM) to a site in rabbit cardiac membranes. Scatchard analysis indicated the presence of a single population of specific ^{3}H-NIF binding sites with a B_{max} from 20 to 250 fmoles/mg protein, depending upon the type of membrane preparation. ^{3}H-NIF bound rapidly ($t_{1/2} \cong 4$ min) and reversibly ($t_{1/2} \cong 8$ min) to cardiac membranes. The K_D (1.2 nM) determined from the association and dissociation rate constants confirmed the K_D value obtained from the concentration-saturation curve. ^{3}H-NIF binding was inhibited in a concentration-dependent manner by the following calcium antagonists, in the potency order: nifedipine $\gg$ D-600 = verapamil > tiapamil. The potency order of these drugs in displacing ^{3}H-NIF was similar to their ability to antagonize contractions of the isolated rabbit papillary muscle. Diltiazem did non inhibit ^{3}H-NIF binding. It is possible that the ^{3}H-NIF binding site is related to the calcium channel in cardiac muscle.

Methylation of DNA by N-methylnitrosamine formed in vivo from methylamine and nitrite

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The formation of nitrosamines from *secondary* amines and nitrite under acidic conditions might represent an important source for the in-vivo generation of carcinogens requiring *metabolic* activation. The nitrosation of *primary* amines leads to unstable products which decay *spontaneously* to alkylating agents. Alkylation of DNA is known to be an important step in tumorigenesis. The aim of this study was to determine whether the half-life of N-methylnitrosamine formed from the primary amine methylamine in the stomach is long enough to allow for a reaction with DNA of the lining cells. Rats were given orally [^{14}C]methylamine and a 50-fold molar excess of potassium nitrite. DNA isolated from the GI-tract was found to be methylated. The extent of DNA methylations was about 50 times lower than was reached with naked DNA under similar conditions in vitro. The role of primary amines in the induction of GI-tumors in man will thus have to be more carefully assessed.

Muscarinic receptors in mouse brain: different influence by agonists and antagonists in vivo and in vitro

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Muscarinic cholinergic receptors in mouse brain were labeled in vitro and in vivo with the muscarinic antagonist ^{3}H-3-quinuclidinyl benzilate (QNB). Muscarinic agonists (oxotremorine, arecoline) and antagonists (atropine, scopolamine, clozapine, thioridazine) were applied prior to i.v. or s.c. administration of a trace dose of QNB. Total and membrane-bound radioactivity was assayed in several brain regions. In-vitro studies were performed according to the methods of Yamamura et al. (PNAS *71,* 1725, 1974). - The dose-dependent inhibition of QNB binding by agonists, both in vivo and in vitro, extended over a wider range of concentration than that of antagonists. In contrast to the in vitro results, oxotremorine was found to be more potent than atropine inhibiting QNB binding in vivo. However, complete inhibition by agonists could not be achieved. These results indicate that muscarinic drugs may act via different mechanisms in vivo and in vitro.

Are female rats more sensitive to dopamine (DA) agonists than male rats?

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Recent studies have shown that sexual dimorphism of the nigrostriatal DA system exists and that estrogens influence behavioral changes induced by DA-agonists. We observed that in intact rats the postural asymmetry induced by apomorphine in females was more pronounced than in males. The naturally existing asymmetry of striatal DA was increased only in apomorphine treated females. In rats with unilateral 6-hydroxy-dopamine lesion of the nigrostriatal pathway, apomorphine or bromocriptine induced contralateral turning. The females responded with a higher intensity than the males although there were no significant sex-dependent differences in terms of the lesion-induced asymmetry in striatal DA and HVA levels. Moreover, the sex differences in behavioral response to DA agonists became apparent 6 months after the lesion. These results show that female rats are more sensitive to DA-agonists.

Role of the renin-angiotensin system in morphine-induced drinking

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Morphine (1-10 mg · kg^{-1} s.c.) stimulated water intake in the water-satiated rat during the light, but not during the dark period (12 h/12 h). Bilateral nephrectomy, carried out 1 day prior to administration of morphine, completely abolished morphine-induced water intake. In renin-depleted rats (renal artery clipping with subsequent removal of clipped kidney) with subnormal plasma and renal renin levels, morphine-induced water intake was linearly related to preinjection basal plasma renin level;

such a significant relationship was not found in normal renin rats. The angiotensin converting enzyme inhibitor captopril (1 or 10 mg · kg^{-1} s.c.), however, increased morphine-induced water intake, and an equimolar dose of the competitive angiotensin antagonist, saralasin was without effect on morphine-induced drinking. Our results point to a permissive interaction between the dipsogenic effect of morphine and circulating angiotensins or renin.

On the origin of 'C_{26} bile alcohol' in man

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The major urinary bile alcohol 27-nor-5β-cholestane3α,7α,12α,24,25-pentol ('C_{26} pentol') occurs in healthy human beings and in increased amounts in patients with liver disease (Karlaganis et al., J. Steroid Biochem. *14*, 341, 1981). In order to determine the precursor, (4-^{14}C)-cholesterol and β-(4-^{14}C)-sitosterol were administered to patients with primary biliary cirrhosis. Urine was extracted with SEP-PAK C_{18} cartridges, and sterol glucuronides and bile acid conjugates were isolated on Lipidex-DEAP. Following hydrolysis the C_{26} bile alcohol and 2 bile acids were isolated by HPLC. 7 days after administration of (4-^{14}C)-cholesterol, the specific activity was found to be 111 dpm/μmole for the C_{26} pentol, 119 for cholic and 64 for chenodeoxycholic acid. The corresponding values after administration of β-(4-^{14}C)-sitosterol were 5, 14 and 7 dpm/μmole, respectively. It is concluded that cholesterol is the precursor of the C_{26} pentol.

Effects of cyclazocine on open-field and residential-maze behavior of rats, a comparative study

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Cyclazocine (C) is a synthetic opiate with mixed agonistic-antagonistic activities that produces a syndrome of subjective disturbances like drunkenness and hallucinations in man. - The present study compares the effects of 6 different doses (0.1-3.0 mg/kg s.c.) of C on the spontaneous locomotor behavior of rats, which was measured by 2 different methods: the open-field (OF) and the automated analysis of locomotor patterns in a residential-maze (RM). In the OF significant effects i.e. decreased locomotion in the central squares were observed only with the 2 highest doses. In the RM all except the lowest dose caused behavioral changes. In general locomotion was increased with the highest dose and reduced with the lower doses. Behavior varied during the 23-h test and with respect to different maze locations. The reproducibility of the behavioral changes was considerably better in the RN situation. This technique also was more sensitive and gave more varied information than the OF.

A novel in vivo assay for somatic point mutations induced by genotoxic carcinogens measures the incorporation of [^{35}S]methionine into trypsinized rat liver cytochrome b_5 normally lacking sulphur-containing amino acids

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The trypsin fragments of rat microsomal cytochrome b_5 (Tb_5) lack both methionine (met) and cystein (cys), i.e. the sulphur-containing amino acids. Tb_5 should therefore contain no [^{35}S]radioactivity after isolation from animals treated with [^{35}S]met or [^{35}S]cys. If, however, the gene for this polypeptide has been damaged by a mutagenic carcinogen, a point mutation could lead to a coding for met or cys so that Tb_5 could now incorporate [^{35}S]radioactivity. The results of 2 experiments are given, the first one where a toxic regimen of N-nitrosomorpholine to rats resulted in a detectable increase of [^{35}S]radioactivity in the Tb_5 of liver microsomes, and a 2nd experiment with a nontoxic regimen of N,N-diethylnitrosamine, where the current methodology for the assay did not allow the detection of the respective mutational events.

Differential oxotremorine effects in 2 psychogenetically-selected lines of rats

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The behavior of RHA/Verh and RLA/Verh rats was differentially affected by muscarinic agonists (Martin et al., Psychopharmacology *72*, 135, 1981) despite similar numbers and affinity of muscarinic receptors in the 2 rat lines for several brain regions (Overstreet et al., Psychopharmacology *72*, 143, 1981). The present experiment evaluated tremors, chromodacryorrhea (CHR), salivation and rectal temperature for 1 h after IP oxotremorine sesquifumarate (OXO; 0,5, 1.0 or 2.0 mg/kg) or saline vehicle in RHA/Verh and RLA/Verh rats. RLA/Verh rats exhibited stronger tremor, CHR and hypothermia responses and a comparable salivary response to that of RHA/Verh rats after OXO. Pretreatment with scopolamine completely blocked OXO-induced tremors, CHR and salivation and reduced the hypothermic response. Pretreatment with methscopolamine produced minor decreases in tremor and hypothermia and blocked CHR and salivation produced by OXO.

Evidence for 2 bradykinin receptors in the rat portal vein

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Bradykinin (BK) has 2 effects on the spontaneous contractile activity of the rat portal vein in vitro: an initial inhibition is followed by a dose-dependent increase in myogenic activity. The actions of BK on the stimulatory phase exhibits the following properties: 1. A biphasic dose-response curve. 2. A lack of an effect of the B1-antagonist, [Leu^8] des-Arg^9-BK (10^{-6} M), on BK at concentrations from 10^{-9} to 10^{-7} M. 3. An inhibition of BK at concentrations greater than 10^{-7} M by this antagonist. 4. An increased responsiveness of the vein to both BK and the specific B1-receptor agonist, des-Arg^9-BK, during the course of the experiment. These observations suggest the presence of the 2 receptors B1 and B2. The blockade by indomethacin (10^{-6} M) of BK stimulatory action indicates that activation of both receptors increases the synthesis of prostaglandins. The mechanism responsible for the inhibitory action of BK has not yet been identified.

Simultaneous measurement of tryptophan, 5-hydroxytryptamine and 5-hydroxyindole-acetic acid by HPLC with electrochemical detection

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LC techniques for measurement of serotonin (5-HT) are time-consuming and do not permit simultaneous determination of tryptophan (TRP) and 5-hydroxy-indole-acetic acid (5-HIAA). This was achieved, in whole brains and brain regions, by a simple method. Samples were homogenized in 5 vol. 0.1 M $HClO_4$, centrifuged at 3000 rpm for 10 min, filtered through Millex 0.45-µm filters and diluted 4 times before injection onto a C_{18}-µm Bondapack column maintained at 25 °C and protected by an RP-18 10 µm precolumn. The mobile phase, 0.74 M NH_4COOH, pH 4.7, contained 7.4% methanol and was pumped at 1.5 ml/min. The detector (Bioanalytical Systems, USA) was set at +0.85 V. Retention times (R_T) for 5-HT, TRP and 5-HIAA were 9.0, 10.0 and 12.0 min, respectively. The method also allows quantification of dihydroxyphenylacetic acid (R_T, 6 min) and homovanillic acid (R_T, 13 min), the 2 principal metabolites of dopamine.

Changes in the β-adrenoceptor adenylate cyclase system of rat reticulocytes during in vitro maturation

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A homogeneous population of reticulocytes was isolated from the blood of acetic acid phenylhydrazide-treated rats. The cells were incubated under sterile conditions for 5–7 days in RPMI 1640 medium supplemented with 10 mM inosine and 10% newborn calf serum. The reticulocyte count decreased exponentially from 80% (day 1) to 15% (day 4) and 5% (day 7). β-Adrenoceptor densities on the intact cell increased by 30–100% within the first 18 h of incubation but did not change further during the following 4 days. Isoprenaline (ISO)-stimulated adenylate cyclase activity barely changed between day 1 and day 3, but then decreased by 80% till day 5. Chronic β-adrenergic stimulation by ISO (0.1 µM) and incubation in the presence of 8-Br-cAMP (50 µM) significantly delayed the decay of cyclase activity. In conclusion, the activity of the β-adrenergic system in circulating reticulocytes may be subject to 'tonic' adrenergic control but is not directly coupled to morphologic cell maturation.

The locus coeruleus as a target for the activating action of vincamine, nicotine and caffeine

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In the awake rat, the neuronal firing rate of the noradrenergic neurons of the locus coeruleus has previously been shown to correlate with the level of vigilance (Aston-Jones and Bloom, J. Neurosci., in press). In the present investigation performed on anesthetized rats, i.p. administered vincamine, nicotine and caffeine were found to increase the spontaneous firing rate of these neurons in a dose-dependent fashion. It is hypothesized that this nucleus is involved in the mediation of some of these drugs' activating, behavioral effects.

Absolute bioavailability of oral isosorbid-dinitrate (ISDN) in a slow release (SR) preparation

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For a long time bioavailability of oral ISDN has been assumed to be negligible due to a high first pass effect in the liver. Hemodynamic measurements in patients with congestive heart failure clearly established the systemic availability of oral ISDN. We administered ISDN to 5 healthy volunteers either as an i.v. bolus of 3 mg (n=3) or as a continuous infusion with 18 mg in 3 h (n=2) and as 100 mg SR preparation. Plasma levels of ISDN were determined by a gas-liquid chromatographic assay. After i.v. doses ISDN in plasma showed a biexponential decline with a terminal half life of 71 min (68–74) (mean, range). Plasma clearance of ISDN was 1.6 l/min (1.2–2.1). After the SR-form plasma levels were sustained for up to 24 h. Bioavailability of ISDN (AUC p.o./AUC i.v., AUC=area under the plasma-concentration-time curve) was 22% (15–31). We conclude that SR-ISDN has a higher availability than expected and that sustained levels can be obtained.

Bioflavonoid-mediated stabilization of collagen in normal and arthritic rats

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The effect of bioflavonoids on the metabolism and cross-linking of collagen in normal and adjuvant-induced arthritic rats was studied after pulsing with ^3H-proline. In arthritis, collagen turnover was increased due to impaired maturation of collagen and hence increased degradation of newly formed collagen. Administration of 2 different bioflavonoids [(+)-catechin and O-(β-hydroxyethyl) rutosides] to arthritic rats partially stabilized collagen as evidenced by a decrease in a) the relative proportions of ^3H-hydroxyproline in soluble and insoluble dermal collagens, b) urinary hydroxyproline, c) solubility of collagen to denaturing agents and pronase and in d) the α/β-chain ratio. The bioflavonoids also promoted collagen cross-linking in control rats but to a lesser degree. Our results suggest that these nontoxic bioflavonoids may be beneficial in the treatment of certain inflammatory diseases affecting connective tissue.

Nuclear size and DNA content in liver of rats, treated with N-nitrosomorpholine (NNM) followed by phenobarbital (Pb)

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Serial biopsies were obtained from livers of rats treated for 4 weeks with NNM at 2 and 10 mg/kg. Distribution of size and DNA content of nuclei were measured repeatedly with an electronic particle counter and a pulse cytophotometer. After NNM treatment a dose-dependent increase of octoploid (8N) nuclei and an increase in the 2N:4N ratio were observed. During subsequent treatment with Pb the 8N nuclear fraction continued to increase and the shift in 2N:4N ratio decreased slightly. In rats receiving NNM only the 8N fraction remained unchanged and the 2N:4N ratio decreased to control levels. Rats receiving NNM followed by Pb showed a larger number of gamma-glutamyltranspeptidase positive islands than animals treated with NNM only. It is concluded that treatment with Pb can enhance and maintain various cellular changes caused by short-term administration of a hepatocarcinogen.

Effects of lysosomotropic agents on the rat yolk-sac membrane and embryonic development

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Rat embryos within their yolk sacs (YS) were cultured for 24 or 48 h in the presence or absence of 1.5×10^{-5} M cyproheptadine (CPH), on day 10.5 of gestation. After 24 h some of the treated embryos were kept in fresh untreated medium for another 24 h. Thereafter, the embryonic growth and differentiation were checked, and the YS examined electronmicroscopically. The embryos treated for 24 and 48 h were retarded in growth and development. Extensive lysosomal proliferation was observed after a 24-h CPH exposure; it was almost completely reversible after 24 recovery h in untreated medium. After 48 h, multilamellated inclusions indicated intralysosomal storage of polar lipids. It is suggested that these changes were responsible for the effects observed in the embryos. The findings in the YS were identical with those produced in vivo in other cell types. Therefore, the culture technique described may be of considerable value in the assessment of lysosomotropic agents within very short time periods.

Urinary malic dehydrogenase as an indicator for subacute nephrotoxicity

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Urinary excretion of enzymes has been studied as indicator for nephrotoxicity. Most enzymes show excretion peaks 1-3 days after the renal lesion. Malic dehydrogenase (MDH), a mitochondrial enzyme located in the epithelial cells of the kidney, shows a different excretion pattern. Male rats received daily i.p. doses of $HgCl_2$ (0.5 mg/kg) or gentamycin (80 mg/kg). Urine was collected after 24 h and 7 days for the $HgCl_2$ group. Excretion values were not altered after 24 h, but significantly increased on the 7th day from 0.464 ± 0.098 U/17 h to 3.658 ± 1.96 U/17 h. In the gentamycin group urine was collected after 24 h (no alterations) and 13 days. On this day MDH was significantly increased from 0.523 ± 0.121 U/17 h to 2.021 ± 0.638 U/17 h. It is concluded that MDH is particularly suited for the detection of subacute kidney lesions.

GENETIK - GÉNÉTIQUES - GENETICS

Genotoxicity of pyrrolizidine alkaloids

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Seneciphylline and senkirkine, 2 pyrrolizidine alkaloids occurring in feedingstuffs and medical herbs, respectively, have been tested for their ability to produce sex-linked recessive lethals (SLRL) in males of *Drosophila melanogaster* using the *Basc* test (3-day feeding method). Seneciphylline was found to be mutagenic at concentrations of 10^{-5} M 10^{-4} M and 10^{-3} M/ 3.8% SLRL (983 chromosomes tested), 9.0% (708) and 15.3% (327), respectively. Senkirkine produced 4.4% SLRL (2541) at a concentration of 10^{-5} M. Control: 0.17% (9081). The brood pattern analysis with senkirkine showed maximum sensitivity in the late spermatid-stage of spermatogenesis. This is in agreement with the fact that pyrrolizidine alkaloids act as indirect mutagens. Flies fed with milk of seneciphylline-treated lactating rats (25 mg/kg b.wt) produced 1.2% SLRL (1477). Control: 0.3% (1533). In contrast to the salmonella/microsome assay the SLRL assay is a convenient and sensitive mutagenicity test for pyrrolizidine alkaloids.

Inducible cytolytic activity in rat × mouse hybrids

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A mouse near-diploid cytolytic T-cell line dependent on Interleukin 2 (IL-2) for growth has been fused with a rat thymoma line. Hybrids were selected in normal medium or in medium supplemented with IL-2-containing supernatant from Con A stimulated rat spleen cells (CS). In CS-medium we obtained strongly cytolytic hybrids which were cloned and maintained activity through 200 divisions. Some of these hybrids depend on CS for growth. - Without CS in the selective medium we obtained only noncytolytic hybrids in which, however, cytolytic activity could be induced by 24-48 h culture in CS. We do not know whether induction is due to IL-2 or other factors in CS. CS had no significant effect on the growth rate of these hybrids. Induction was fully reversible. 2 out of 2 CS-independent hybrid clones analyzed contained at least 1 copy of all murine chromosomes except chromosome 11. Together with our previous results (Nabholz et al., Nature *287*, 437, 1980) this suggests that mouse genes which render a cell CS-dependent may be located on chromosome 11.

Stability of the activation system of the Salmonella/microsome assay

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One of the most important steps of microbiological mutagenicity assays, as the Ames test, is the addition of liver homogenate (S-9) as a substitute for the mammalian metabolism. The S-9 contains monooxygenases among many other enzymes. During incubation at 37 °C their activity will be partially lost due to thermal inactivation. Such a loss influences the sensitivity of the test. We therefore compared the activity of the S-9 after different times of storage at 20 and 37 °C. The activity was determined as mutagenic effect of benzo-a-pyrene (BaP), dimethylbenzanthracene (DMBA) and 2-aminoanthracene (2-AA) with strain TA 100 (all solved in DMSO). After storage at 20 and 37 °C on minimal medium plates as well as in suspension, the ability of the S-9 to activate BaP and DMBA decreased between 30 min and 7 h, showing an almost complete loss of activity after 7 h. The activation of 2-AA was not influenced by storage of S-9 for up to 22 h.

Molecular cloning of the *white* locus region of *Drosophila melanogaster* using a large transposable element

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We report the molecular cloning of a chromosome segment including the *white* locus of *D. melanogaster*. This region was isolated using a deficiency extending from the previously cloned 87A7 heat shock puff sequences to a large transposable element containing the loci *white* and *roughest*. FB-NOF, a 6.5-kb element carrying inverted repeat sequences, is found at the deficiency breakpoint and is followed by DNA originating from the *white* locus region. Sequences totaling about 60 kb surrounding this initial entry point were obtained by the cloning of successively overlapping fragments from a wildtype strain. The *white* proximal region, thought to be involved in gene regulation, and the distal region have been mapped relative to the cloned DNA. Insertion of the dispersed repetitive element *copia* into the *white* locus is observed in strains carrying the *white-apricot* allele.

rad2-44: a UV-sensitive mutant of *Schizosaccharomyces pombe* that enhances mitotic recombination

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Intragenic recombination is a rare event in a mitotically dividing cell population of *S. pombe*. However, gene conversion events are highly coincidental. In a diploid heteroallelic at 2 genes, recombination frequencies for the 2nd gene among recombinants of the first gene are much higher than in the total cell population. This is interpreted to be due to a subpopulation of cells competent to undergo recombination (Minet et al., Current Genet. *2*, 53, 1980). The weakly UV-sensitive mutant *rad2-44:* enhances mitotic recombination rates (meiosis as well as meiotic recombination is normal). 5 intervals located on the 3 chromosomes have been tested for intragenic mitotic recombination and all of them showed 4–10-fold increased rates. Since the increase in the rate of double recombinants is of the same order of magnitude (7-fold), we conclude that the fraction of cells competent to recombine is enhanced in a *rad2-44* background.

Test for chromosome loss with repair-defective females in *Drosophila melanogaster*

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The registration of ring-X chromosome losses in *D. melanogaster* using repair-defective females is an easily performed 1-generation mutagenicity test. Males carrying a ring-X chromosome are treated with a mutagen and then crossed either to wildtype or to repair-defective females. The chromosome losses are scored in the F_1 progeny as apparent XO-males. The use of excision-repair-defective *(mei-9)* females leads to greatly enhanced frequencies of chromosome losses compared to wildtype females in the case of monofunctional alkylating agents. This potentiating effect is also seen with indirectly acting mutagens needing metabolic activation (cyclophosphamide, N-nitroso-dimethylamine) but not with polyfunctional alkylating agents. Frameshift mutagens are very weak inducers of chromosome losses. The hydrazine derivative hydralazine is tested in a brood-pattern experiment using an additional repair-defective mutant *(mus302)*.

MMS-sensitive mutants of *Aspergillus* and their effects on recombination

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Many *uvs* mutants have been isolated in *A. nidulans* but few have been assigned to specific genes or been tested for effects on mutation and recombination. All clearly UV-sensitive ones were found to be also sensitive to MMS (methyl methane sulfonate). This property facilitated complementation tests between pairs of mutants and 3 new genes, *uvs*F, H and J, have been mapped. Since mutants in several genes are practically sterile in homozygous crosses, their effect on mitotic crossing over in homozygous or heteroallelic diploids was analyzed and compared. In such diploids, which were heterozygous for 2 color markers in repulsion on the same chromosome arm, *hyperrec* mutants produced high frequencies of colored twin sectors. On the other hand, *rec*$^-$ diploids showed no twin sectors, even after UV-treatments which cause pronounced increases in controls. Any rec$^-$ mutants are being tested for reduced activity of the fungal endo-exonuclease which may be analogous to the recBC enzyme of *E. coli*.

Recombination between members of a family of dispersed serine tRNA genes in *Schizosaccharomyces pombe*

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We are studying the transfer of genetic information between identical or similar DNA sequences located on different chromosomes. The 3 genes investigated code in their wildtype form for $tRNA^{Ser}$ reading UCA *(sup3*$^+$, sup9$^+$) and UCG *(sup12*$^+$). The basic experiment consists in the comparision of the frequency of active suppressors found in control crosses (diploid parents homozygous for a UGA mutation and *sup3*$^+$) vs crosses homozygous for an inactivated UGA suppressor *(sup3-e,rX)*. The 40-fold enhancement of active suppressors in the experimental cross must be due to recombination (conversion) rather than mutation. Starting from *sup3-e,rX* 3 types of active suppressors were found: *sup3-e, sup9-e* and *sup12-e*. While *sup3-e* and sup9-e isolates correspond to well-known suppressors, the new haplo-lethal suppressor *sup12-e* could be correlated with the $tRNA^{Ser}_{UCG}$ gene by restriction analysis: The EcoRI site spanning the anticodon of *sup12*$^+$ is eliminated upon its change to *sup12-e*.

Unscheduled DNA synthesis (UDS) assessed in male mice germ cells as an indicator of DNA damage

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DNA damage represents a key step in mutagenesis and carcinogenesis. Excision repair has been described as a major pathway in restoring DNA damage. Assaying DNA repair may thus elucidate mechanisms of mutagenesis and carcinogenesesis. – Experimentally, DNA repair was assessed by demonstration of UDS in meiotic and postmeiotic sperm cells of CD-1 mice. For this purpose, ^{3}H-labeled thymidine was administered intratesticularly 1 h after i.p.

injection of the test compound. Subsequently, spermatozoa were collected from the cauda epididymidis 14, 16, 18, 21 and 31 days after treatment, and their radioactivity measured by LSC. - The test was validated with several well-known mutagens (e.g. methylmethane sulfonate, cyclophosphamide, azathioprine), a nonmutagen (cyclosporine A) and 1 suspected genotoxic chemical (diethylstilbestrol). - Significant radioactivity indicating UDS was measured only in sperms of mice treated with methylmethane sulfonate, cyclophosphamide and azathioprine.

Genetic instability of *sup3* alleles in the fission yeast *Schizosaccharomyces pombe*

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Sup3 is a structural gene for $tRNA^{Ser}_{UCA}$. *Sup3* alleles producing an inactive gene product are meiotically unstable and revert with a rate of up to one in 10^5 meioses. This unstability is ascribed to heterologous recombination between *sup3* and 2 unlinked genes, *sup9* and *sup12* belonging to the same gene family but genetically unlinked to *sup3*. The properties of mutations altering this unstability will be discussed.

Variations of the expression of dynein between germ cells and somatic cells in a ciliary mutant in man

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A ciliary mutant in man induces specific effects in cilia and sperm and is described as the immotile cilia syndrome. By the genetically caused lack of the ATPase dynein, cilia and sperm, normally equipped with their 9+2 tubules-dynein mechanism, are commonly immotile. Men carrying this syndrome are sterile, Chronic bronchitis is common, cilia dysfunction leading to loss of clearance effect. In one case, however, our ultrastructural investigation demonstrated differences in the expression of dynein. In the sperm flagella dynein was absent while in cilia of the nasal mucosa dynein was present. These variations suggest that in cases of the immotile cilia syndrome, the Keimbahn and the soma are capable of different expression.