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PHYSIOLOGIE – PHYSIOLOGY

A Direct Effect of Estrogen on the β-Adrenergic Control of Pineal Function

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The pineal of the female rat is known to be a steroid target organ and to concentrate estradiol via specific receptors. It has been reported (Weiss and Crayton, Endocrinology *87*, 527, 1970) that estradiol benzoate (EB) inhibits pineal adenyl cyclase after stimulation with norepinephrine. To date, however, it has not been shown whether estradiol exerts any effect on pineal *N*-acetyltransferase (NAT), the rate-limiting enzyme for the biosynthesis of melatonin. We have investigated three instances in which estradiol plays a major biological role in the rat: (1) the induction of precocious sexual maturation by hypothalamic lesions, (2) the sterilizing effect of EB when injected into four day-old females, (3) the triggering of a surge of luteinizing hormone in ovariectomized females by two injections of EB. In pineals stimulated in vitro with β-agonists we have found, in all cases, increased induction of NAT (as compared with controls not treated with EB), which is followed by increased melatonin synthesis. These results present further evidence for steroid-catecholamine interactions in neuroendocrine tissues.

Responses of Atrial A- and B-Fibres to Atrial Volume Pulsations

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This study examined (a) the rate sensitivity of atrial B-fibres, and (b) the activation mechanism of A-fibres. Firing patterns of single vagal fibres were monitored in response to artificial pulsations (± 1 ml; 1/sec) (Brain Res. *98*, 189–193, 1975) of cat atria. B-firing patterns of 17/20 fibres responded to rate of change and change in atrial volume. Phase advance of impulse frequency, ranging up to 80°, correlated positively with receptor threshold, and inversely with atrial volume. A-firing patterns of 13 fibres, originating mainly from the septum, were unaffected by atrial pulsing, but A-firing could be modified in 5 fibres from the atriovenous junctions. The interatrial septal receptors fired at lower A- and B-impulse frequencies. The results suggest (a) that high and low threshold B-fibres signal predominantly venous return and thoracic blood volume, respectively; and (b) that A- and B-firing is largely determined by the anatomical location of atrial stretch receptors.

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Influence of 1,25-Dihydroxy-vitamin D_3 (1,25-$(OH)_2D_3$) on (TPTX) Rats

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Thyroparathyroidectomy is characterized in the growing rat fed a 0.5 g% Ca diet by a decrease in plasma Ca ([Ca] Pl.), net intestinal Ca absorption (Vna), Ca retention (Vr), bone formation (Vo +) and bone resorption (Vo −). These alterations could be partly due to the reduced formation of 1,25-$(OH)_2D_3$ occurring in TPTX animals. Ca balance and ^{45}Ca kinetics were studied in sham-operated (S-O) and TPTX rats treated with synthetic 1,25-$(OH)_2D_3$ (2×13 pmole/day i.p. for 10 days) or ethanol and pair-fed a 0.5 g% Ca diet. In TPTX rats 1,25-$(OH)_2D_3$ increased 1) [Ca]Pl. (mg% ± SEM) from 6.2 ± 0.2 to 8.9 ± 0.2 (S-O: 10.3 ± 0.2) 2) Vna (mg Ca/day) from 13.4 ± 1.8 to 27.3 ± 1.0 (S-O: 23.7 ± 1.2) 3) Vr (mg Ca/day) from 11.8 ± 1.9 to 22.5 ± 1.1 (S-O: 22.6 ± 1.2) 4) Vo + (mg Ca/day) from 39.5 ± 2.1 to 53.0 ± 1.9 (S-O: 64.2 ± 2.9) and 5) urinary excretion (mg Ca/day) from 1.6 ± 0.3 to 4.8 ± 0.6 (S-O: 1.1 ± 0.1). Thus doses of 1,25-$(OH)_2D_3$ which normalize Vna and Vr and increase calciuria do not completely correct [Ca] Pl. and Vo +. The increased Vna caused by 1,25-$(OH)_2D_3$, in contrast to that caused by raising dietary Ca, was not associated with a decreased Vo-, suggesting that 1,25-$(OH)_2D_3$ influences bone resorption in TPTX rats. This study rationalizes the possible therapeutic use of 1,25-$(OH)_2D_3$ in human hypoparathyroidism, if hypercalciuria does not limit its application.

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Sympathetic Nervous Control on Endocrine Pancreas during in vivo Selective Pancreatic Hyperglycemia in the Dog

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In 12 anesthetized and atropinized male or female mongrel dogs weighing 20–30 kg the cranial pancreaticoduodenal artery was catheterized after its first duodenal branch. The superior pancreaticoduodenal vein was cannulated and an extracorporeal circuit established by reinserting the catheter back into the portal vein. This shunt was used to sample pancreatic blood and measure venous flow. Pancreatic nerves both running along and beside the superior pancreaticoduodenal artery were isolated. Injection of glucose through the arterial catheter increased and maintained glycemia of the pancreatic venous effluent at up to 300 mg % during 30 min with negligible increase in systemic glycemia. Stimulation of the pancreatic nervous supply at 2 Hz (i.e. within the physiological range) stimulated the release of glucagon by about 50% and inhibited that of insulin by 40%; both effects were *independent of the pancreatic glycemia*. It is concluded that sympathetic activity contributes to the control of plasma glucose and that this is not overridden by hyperglycemia.

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Subcellular Distribution of ATPase and Creatine Kinase in Mammalian Unmyelinated Nerve

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The activity of ATPase and creatine kinase (CPK) was measured in homogenates of rabbit vagus nerve at 37°C. They were 293 ± 9.3 and 116.8 ± 9.5 nmole/mg prot./min respectively. In three crude subcellular fractions, containing mainly either mitochondria, microsomes and membrane fragments, or cytoplasme, the ATPase activity was 200 ± 26, 1131 ± 30 and 405 ± 25, while that of CPK was 159 ± 18, 56 ± 8 and 247 ± 17 respectively (means of 4–5 experiments ± S.E.). Thus the highest activity of ATPase was found in the fraction containing membranes and microsomes and the CPK in the soluble fraction. The distribution pattern of these two enzymes in the vagus nerve was similar to that in other nerve tissues studied so far, however the ATPase activity was found to be high and that of CPK low compared with corresponding values in other nerve tissues. In the mitochondrial fraction both activities were lower in the vagus nerve than in the brain (Sullivan et al., J. Neurochem. *15*, 115, 1968) probably because the rabbit vagus nerve is composed essentially of axons and contains few mitochondria.

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Nicotine Induced Changes in the Regulation of Body Weight in Growing Rats

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10 male Roman Low Avoidance rats, $3^1/_2$ months old, received twice daily 0.4 mg/kg nicotine subcutaneously. Food and water intake was measured once daily. During the 45 days treatment periode they initially lost weight. From day 10 to day 25 the rats stabilized their weights at a level 10% below the weight of the saline control group. From day 25 on the nicotine rats gained weight at the same rate as the control rats did. Food intake first decreased to about 80% of the control and recovered slowly until it reached control values after a month. Water intake paralleled food intake but changes were less pronounced. Upon discontinuation of the nicotine treatment the experimental rats ate about 10% more than the saline rats. However, after about 3 weeks food intake decreased again and reached control levels after $1^1/_2$ months. 3 months after the cessation of the treatment the weight deficit amounted still to about 10 g with identical body lenghts for both groups, suggesting therefore a shift of the set-point for weight regulation.

Analysis of K^+ and Na^+ in the Adrenal Cortex of Rats by Electron Microprobe

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Intracellular zona glomerulosa K^+ could be the final trigger of aldosterone secretion. In order to verify this hypothesis K^+ and Na^+ were measured in the adrenal cortex of rats by electron microprobe. Four groups of rats were given the following one week regimen: (a) standard diet, (b) low Na^+ diet, (c) high Na^+ diet, (d) high K^+ diet. The animals were then anesthesized with pentobarbital, their adrenals removed and frozen in liquid nitrogen. Lyophilized adrenal sections (8 μ) were processed for electron microprobe analysis. K^+ and Na^+ contents through the cortex were graphically recorded and their mean value in each zone calculated from digital data. The Si^{4+} signal from the quartz slide was monitored to reveal cracks in the sections. Albumin K^+ and Na^+ standards and liver sections were used as controls. Plasma aldosterone and corticosterone were determined. – Results: Plasma aldosterone varied with each regimen as expected while no change of corticosterone was observed. For every regimen, a constant K^+ concentration through zona glomerulosa and zona fasciculata and a statistically significant higher Na^+ content in zona glomerulosa were observed. – In conclusion, no correlation was found between plasma aldosterone and K^+ content of zona glomerulosa in situations of hyper- and hypoaldosteronism in rat.

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Recurrent Excitation of 'Vasopressin-Neurones' in the Rat

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Spontaneously active supraoptic neurones either discharge continuously at a low average rate (random neurones) or are intermittently active (phasic neurones). There is evidence that phasic firing may be confined to vasopressin-secreting cells (Harris et al., Nature *258*, 80, 1975). In urethane-anaesthetized rats, supraoptic neurones were identified by antidromic invasion following stimulation of the supraoptico-neurohypophysial tract. In many phasic neurones, 2–5 stimuli applied to the tract during the silent phase of the cycle elicited a long-lasting burst of action potentials. This was not seen in random neurones. To produce an antidromically-evoked burst in phasic neurones, the stimuli had to excite the axon of the perikaryon from which records were obtained. The existence of recurrent collateral excitation, which might contribute in part to the tendency of some magnocellular endocrine neurones to fire in phases, may be yet another characteristic of vasopressin-neurones.

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Efflux of Inorganic Phosphate and Intracellular Phosphate Turnover in Nerve

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In desheathed rabbit vagus, loaded during 150 min by exposure to ^{32}P labelled phosphate Locke, the efflux of radiophosphate was measured during washing with inactive Locke. In these experiments a gradual decrease of the efflux of radiophosphate was found. Calculation of the fraction of radioactivity lost per min showed that the rate constant of the efflux apparently decreased during the washing. Extracts of the nerves (see Anner

et al., J. Physiol. *247*, 759, 1975), prepared at various times during the washout, showed a continuous lowering of the amounts of labelled ATP, ADP, CrP and P_i. In spite of the gradual decrease of the specific activity of these compounds, the labelling of a water insoluble fraction of the extracts increased slowly during the washout: for instance from 4% of the total radioactivity (or 0.04 mmole/kg w.w.) at the beginning of the efflux, to 21% (or 0.14 mmole/kg) after 120 min at 0.2 m*M* external phosphate. The rate of labelling of these phospholipids and phosphoproteins is necessary to know for calculating the true rate constants of the phosphate efflux.

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Lack of Inhibition of the Pancreatic Glucagon Production during in vivo Selective Pancreatic Hyperglycemia in the Dog

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It has been shown by Campfield, Seydoux and Girardier (this meeting) that injection of glucose through a catheter inserted in the cranial pancreatic duodenal artery increased glucose concentration in the pancreatic venous effluent up to 300 mg % with negligible increase of systemic glycemia. It was observed that, whereas pancreatic insulin output was invariably increased, glucagon output was not affected. However, it is a well established fact that glucose, infused at the periphery, induces an inhibition of glucagon output. We were thus led to compare the effects of glucose infusion in both pancreatic artery and femoral vein *of the same dog*. Injection of glucose in pancreatic artery failed to inhibit glucagon output whereas glucose injection at the periphery, matching the previous concentration in the pancreatic venous effluent, inhibited pancreatic glucagon output by 30%. This effect was not modified by section of the mixed nerve alongside the pancreaticoduodenal artery. Thus, we are led to the conclusion that glucagon release by pancreatic A cells is not directly sensitive to hyperglycemia.

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Electrophoretic Studies on Presynaptic Inhibition in the Mammalian Spinal Cord

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GABA has been postulated to be the transmitter mediating presynaptic inhibition in the mammalian spinal cord (review: R. F. Schmidt, Rev. Physiol. *63*, 20–101, 1971). The experiments, performed on 20 cats anaesthetized by Nembutal and immobilized with Flaxedil, involved laminectomy from L_1 to L_7 and transsection at L_1. The nerves peroneus superficialis (SP) and suralis (SU) of the left leg were exposed. Excitability testing of single SU-fibres was used for measuring the primary afferent depolarization (PAD) with a platinum-wire-stimulation microelectrode in the dorsal horn. This electrode was combined with a three-channel electrophoresis-pipette for releasing GABA and the antagonist bicuculline-methochloride (BIC) near the terminal regions of the nerve fibres. It was demonstrated, that GABA had nearly the same depolarizing action (threshold depression $\Delta T = 22\%$) as was induced by producing PAD ($\Delta T = 28\%$) through stimulating the SP. On the other hand with BIC the PAD could be blocked reversibly ($\Delta T < 2\%$). The results give very good evidence, that GABA is the transmitter mediating presynaptic inhibition.

Impulse Conduction in a Myelinated Giant Nerve Fibre

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In tenches (*Tinca tinca* L., 150–250 g) the cable constants of the Mauthner axon were determined using conventional methods (two independent microelectrodes inserted at various distances from each other). In order to analyze the impulse conduction, two rectangular currents of equal duration were simultaneously passed through both microelectrodes. The current-strengths were varied independently, until the combined effect reached excitation-threshold. The paired current-strength values thus obtained allowed the calculation of the effected, passive potential distribution inside the axon. The intersection of these resultant curves was always situated at midpoint between the electrodes, even after a displacement of both electrodes along the axon, provided the interelectrode distance did not exceed 5 mm. These results are consistent with the assumption that Mauthner axon excitation is initiated by simultaneous activation of the collaterals distributed over a minimal axonal length, and not by discrete, individually excitable parts of the axon such as e.g. the nodes of Ranvier.

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Free-Water Reabsorption in Chloride-Depleted Rabbits

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The relationship between osmolal clearance (C-osm) and the reabsorption of solute free-water (TcH_2O) was examined during sequential hypertonic saline and hypertonic sodium bicarbonate diuresis in both normal and chloride-depleted rabbits. In normal rabbits during hypertonic saline diuresis TcH_2O increases with C-osm in a curvilinear fashion. This increment in TcH_2O during hypertonic saline diuresis appears to be due to the ability of Henle's loop to respond to increasing sodium delivery by increased sodium transport. During hypertonic bicarbonate diuresis, there is an initial curvilinear increase in TcH_2O with increasing C-osm. Eventually TcH_2O reaches a maximum at osmolal clearances close to 2.5 ml/min. At C-osm approximating to 3 ml/min the mean values for TcH_2O during hypertonic saline and bicarbonate diuresis were 1.07 ml/min and 0.7 ml/min, respectively. This difference was highly significant. In a second group of rabbits, chloride depletion was induced by giving the animals furosemide for 2 days, and artificial chloride-free diet for 10 days. In chloride-depleted animals, free-water reabsorption was depressed during both hypertonic saline and hypertonic bicarbonate

diuresis. At osmolal clearances approximating to 3.0 ml/min, TcH_2O was 0.08 ml/min during hypertonic saline diuresis. During hypertonic bicarbonate diuresis, a negative mean value for TcH_2O was actually observed. These observations suggest that sodium chloride, but not sodium bicarbonate is transported in the ascending limb of Henle's loop. They are in agreement with the in vitro demonstration that sodium reabsorption in this segment is the passive consequence of active chloride transport.

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Angiographic Estimation of Right Ventricular Volume

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Left ventricular volume in man is routinely determined by single-plane cineangiography according to the area-length-method of Sandler and Dodge. Some attempts to estimate right ventricular (RV) volume have been made, a generally accepted method is however still lacking. – In the present study, the RV volume of 8 human and 12 canine casts was assessed by angiographically in the right and left anterior oblique projection by 5 different methods: method 1 was based on the geometric model of a pyramid, method 2 on a cone, method 3 on a cylinder, method 4 on the assumption that the product of the two areas at right angles is proportional to the true volume and method 5 on Simpson's rule. The true volume was obtained by water displacement. – All 5 methods gave high correlation coefficients between 0.955 and 0.988. The standard error of estimate (SEE) of the linear regression between calculated and true volume, expressed as per cent of the mean value of the true volume, was 11% in method 5, 13% in 3, 14% in 1, 14% in 2 and 25% in 4. Method 5 showed not only the smallest SEE, but also the highest correlation coefficient. It is concluded, that Simpson's rule is the most accurate method for quantitating RV volume, but the methods based on a simple geometric model seem to be particularly attractive for routine assessment of RV function.

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Effect of K^+ on the CO_2/HCO_3^--Induced Depolarization in Frog Skeletal Muscle

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We have shown previously (Experientia *31*, 711, 1975) that the CO_2/HCO_3^- system depolarizes frog skeletal muscle under a variety of experimental conditions. This effect is quantitatively explained if one assumes the cell membrane to be weakly permeable to the HCO_3^- ion, P_{HCO_3} being about $0.1\times$ resting PK^+. If this interpretation is correct, it follows from the constant field theory that the depolarizing effect of the CO_2/HCO_3^- system in Cl^--free solutions should be a characteristic function of the K^+ concentration. This prediction has been tested by studying the depolarizing effect of Cl^--free solutions having a PCO_2 of 97 mm Hg and a $(HCO_3^-) = 5$ mM/l and containing variable (K^+) in the range of 1–80 mM/l. The depolarization caused initially by the introduction of the CO_2/HCO_3^- system was +48 mV at a $(K^+) = 1.0$, +11 mV at a $(K^+) = 2.5$ and nearly zero at $(K^+) = 10$ and 80 mM/l. These results agree quantitatively with the prediction and thus strengthen the hypothesis of a weak membrane permeability to HCO_3^-.

Facilitation of Avoidance Learning by Post-Trial Reinforcing Hypothalamic Stimulation

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Since post-trial food reinforcement was shown to facilitate learning of a passive avoidance task in mice (Huston et al., Experientia *30*, 1038–1040), presumably by a direct influence on memory processes, we tested the possibility that post-trial reinforcing brain stimulation in rats could similarly improve learning of an avoidance task. 17 rats in whom electrical stimulation of the lateral hypothalamus was reinforcing, were given daily training in a one-way step-through avoidance task. After one week of training all animals ran to the safe goal compartment within 10 sec to avoid electrical shock in the start-box. They were then subjected to a reversal learning task: as soon as the animals reached the safe compartment they received there a 2-sec footshock and were immediately removed from the experimental situation. After 30 sec the experimental group received reinforcing brain stimulation for 30 sec (0.2 sec on, 0.8 sec off); the control group did not receive stimulation. When tested 24 h later the control group showed a mean step-through latency of 9 sec (no learning), whereas the experimental animals showed a mean latency of 59 sec ($p < 0.05$, Wilcoxon Test). Thus, post-trial hypothalamic stimulation facilitated learning of an avoidance task, providing further evidence for direct control of memory processes by reinforcement.

The Atropine-Sensitive Bronchomotor Response in the Histamine-Induced Asthma Attack

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The role of the vagal bronchoconstrictor fibres in the histamine-induced asthma attack has been studied in the anaesthetized guinea-pig by recording the bronchial resistance combined with body-plethysmography and respiratory air mass spectrometry. – The results indicate that the increase in bronchial resistance at onset of the asthma attack has an atropine-independent as well as an atropine-sensitive component: The first (atropine-independent) is due to the local effects of histamine on the airways, resulting in uneven ventilation responsible for the lung deflation reflex. The second (atropine-sensitive) component of the increase in bronchial resistance takes part in the short-lasting, vagally mediated vicious circle in which changes in pulmonary mechanics (bronchoconstriction, uneven distribution) and respiratory reactions (tachypnoea, increase in lung volume) follow one another. – It is assumed that the vagal bronchoconstrictor reflex is mediated by vagal lung deflation/irritant fibres.

Hippocampal Influences on the Auditory Cortex

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The influence of the dorsal hippocampal output (driven by trains of 5 stimuli: 7/sec, 0.2 msec, 5–10 V) on unit responses of the auditory cortex to clicks, beeps and complex auditory stimuli was studied in unanaesthetized, gallamine paralyzed and artificially ventilated cats. Extracellular recordings (tungsten microelectrodes) were made by means of the closed chamber technique the day after the surgical preparation of the animal was carried out under sodium pentobarbital anaesthesia. The hippocampal output exerted excitatory and inhibitory influences that resulted in the following changes of unit responses to auditory stimulation: (1) increase in the stability and the number of spikes of the unit response, (2) random firing depression bringing into relief the unit response, (3) unit response modification evident for many minutes after the end of hippocampal stimulation. The observed effects suggest that the hippocampal output influences the information processing in the auditory cortex.

Ag^+-Induced Changes in Na and Water Permeability in Amphibian Skins

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Effects of Ag^+ (10^{-4} *M*) on electrical conductance (k), short-circuit current (SCC) and osmotic water flow (J_{H_2O}) were studied in amphibian skins exposed to Na_2SO_4 Ringer solutions. SCC and k were monitored with a voltage clamp holding trans-epithelial potential alternately at 0 and 5 mV; J_{H_2O} was continuously monitored by an optical technique. Addition of Ag^+ to the external side of frog skin resulted in: (a) an increase in k and J_{H_2O} and a rise followed by a decrease in SCC; (b) abolition of oxytocin-induced stimulation of Na and H_2O transport; (c) decreased sensitivity to amiloride; (d) reappearance of an ouabain-sensitive SCC in amiloride-blocked skins. In contrast, Ag^+ added to the internal side resulted in no change in k but a rise in SCC sensitive to amiloride. This effect on SCC was completely absent in skins pre-treated with amiloride or ouabain, but still present in skins maximally stimulated by oxytocin. Being a sulfhydryl reagent, Ag^+ is an interesting tool for relating transport properties to specific groups of membrane structure.

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Zeitabhängige Systemeigenschaften der motorischen Innervation des Zwerchfelles

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An vagotomierten, beatmeten Kaninchen wurden vagale A-Beta-Fasern (25–40 m/sec) afferent gereizt (100/sec) und die Veränderungen im Phrenicogramm in Abhängigkeit von der zeitlichen Beziehung zwischen Reiz- und Atmungsphase analysiert. Der Beginn der Reizphase wurde gegenüber dem Inspirations- bzw. Exspirationsbeginn zunehmend verzögert und die Reizphase durch den Beginn der nächstfolgenden Atmungsphase beendet. Resultate: 1. Vorzeitiger Abbruch der Inspiration erfolgte bei Koinzidenz der Reizphase mit der Inspirationsphase, Verlängerung derselben, wenn die Reizphase unmittelbar vor dem zu erwartenden Inspirationsende gestoppt wurde. 2. Verlängerung der Exspiration fand sich bei frühexspiratorischem Reizbeginn, eine Verkürzung bei Reizbeginn im letzten Drittel der Exspiration. Diese zeitabhängigen Eigenschaften der motorischen Zwerchfellinnervation lassen sich an einem mathematischen Modell demonstrieren, welches auf einem von Lotka und Volterra eingeführten, nichtlinearen Differentialgleichungssystem erster Ordnung beruht.

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Protein Composition of CNS Myelin after in situ Autolysis

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The protein composition of myelin isolated from brains which were either frozen or kept at +4 °C or +19 °C, for 24 h was studied. In 25-day-old mice, myelin basic proteins (BP) showed a 35% decrease at +19 °C, while proteolipid protein, DM-20 and 2′,3′-cyclic nucleotide 3′-phosphohydrolase did not show any alteration. (^{3}H)-Fucose-labeled myelin glycoproteins (mGP) from brain kept at +19 °C had the same electrophoretic mobility as (^{14}C)fucose-mGP from brain kept at −70 °C. However, mGP double-labeled with (^{3}H)fucose and (^{35}S) Na_2SO_4, isolated from brain kept at +19 °C showed that the (^{35}S)mGP had a smaller molecular weight than the (^{3}H)-fucose-mGP. This suggests that the major mGP peak observed by electrophoresis contains at least two groups of mGP, one which seems resistant and the other which is susceptible to degradation. Myelin from adult animals (bovine and mouse CNS) at +19 °C did not show any increased degradation of myelin proteins. In mature CNS, compact myelin could present a higher resistance to the invasion by endogenous proteolytic enzymes.

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Effect of Calcium on the Stability of Neurophysin-Vasopressin Complex

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Calcium influences the interaction of lysine vasopressin (LVP) with bovine neurophysin extracted from acetone dried pituitaries or prepared from neurosecretory granules ('native' NP, Pliška and Meyer-Grass, Ann. N.Y. Acad. Sci. *248*, 235) in quite different ways. Whereas the former interaction is not influenced (cf. Pliška and Sachs, Eur. J. Biochem. *41*, 229), the latter showes a clear cut biphasic Ca-dependence. The dialysis of 'native' NP I against EDTA does not abolish binding, but addition of Ca^{2+} up to 10^{-6} *M* enhances the ratio of bound-to-free concentrations for LVP (b/f) in an equilibrium dialysis experiment at pH 5.8 by a factor of 1.5. Between 10^{-6} and 10^{-4}*M* Ca^{2+} a slow fall in b/f was observed, followed by a much steeper decrease at higher Ca-concs. There was practically

no binding at 10^{-3} *M* Ca^{2+}. In view on the process of neurosecretion one can tentatively conclude: (1) LVP-'native' NP I complex reaches maximal stability at Ca-concs. similar to those in axoplasm; (2) At Ca-concs. similar to that found in the periaxonal space the complex is almost fully dissociated.

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Effects of Posttrial Punishment and ECS on Memory Processes

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A total of 258 mice were trained in a passive avoidance step-down task and split into 4 groups, that received posttrial punishment (10 sec in ice water) at various delays after the step-down task (< 5, 30, 60 and 90 sec). When tested 24 h later the 60 sec delay group showed significantly poorer learning (shorter step-down latencies) than the 30 and 90 sec groups ($p < 0.05$, Wilcoxon Test). Hence, posttrial punishment may have a direct influence on memory processes, as posttrial reinforcement was shown to have (Huston et al., Experientia *30*, 1038–1040). To test the possibility that part of the amnestic properties of electroconvulsive shock (ECS) could be accounted for by direct punishment of memory processes, the above experiment was replicated with 234 mice, except that they received posttrial ECS instead of ice-water punishment. As above, the 60 sec delay ECS group exhibited shorter step-down latencies (inferior learning) than the 30 and 90 sec delay groups; but significantly different ($p < 0.05$, Wilcoxon Test) only from the 90 sec group.

Tubular Localization of the Renal Adaptation to Dietary Inorganic Phosphate (P_i) in Rats

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The renal tubule of rats adapts its transport capacity for Pi in response to change in Pi intake. Tubular localization of this adaptation was studied in rats pair-fed for 10 days low, 0.2 g %, (LPD) or high, 1.8 %, (HPD) phosphorus diet, both groups being acutely infused with Pi (400 µmole/h) during free-flow micropunctures. Inulin (In) and Pi in plasma, urine and tubular fluid were determined chemically. Clearance of In (ml/kidney min) during micropuncture was (mean ± SEM): LPD 1.09 ± 0.05 ($n = 32$), HPD 1.14 ± 0.06 ($n = 33$). Filtered Pi (µmole/kidney min) was: LPD 6.07 ± 0.29, HPD 5.34 ± 0.24. Fractions of filtered Pi found at puncture site [(TF/P)Pi/(TF/P)In] was (1) early proximal tubule [(TF/P)In < 1.5]: LPD 0.80 ± 0.10 ($n = 14$), HPD 1.10 ± 0.12 ($n = 21$) $p < 0.01$; (2) from (TF/P)In 1.5 to 2.5: LPD 0.40 ± 0.03 ($n = 31$), HPD 0.63 ± 0.07 ($n = 29$) $p < 0.01$; (3) distal tubule [(TF/P)In > 3.5]: LPD 0.21 ± 0.03 ($n = 17$), HPD 0.39 ± 0.06 ($n = 14$) $p < 0.01$. Fractional excretion of Pi (FEPi) for the whole kidney was: LPD 0.26 ± 0.02 ($n = 32$), HPD 0.67 ± 0.02 ($n = 33$) $p < 0.001$. With HPD, FEPi was significantly higher ($p < 0.001$) than the fraction found in distal tubule. The results analyzed in terms of segmental tubular Pi transport indicate that adaptation is mainly localized (1) in the proximal tubule, but only in the first convolutions; (2) in the terminal nephron where an apparent net secretion occurs in rats fed HPD.

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Microspectrophotometry of Rhabdomes in the Honeybee Drone

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Visual pigments of the honeybee drone have been examined by microspectrophotometry in the spectral range 250–650 nm. Individual rhabdomes (2 µm × 6 µm) in a slice of retina about 250 µm thick were illuminated end on. Difference spectra showed photopigments with λ_{max} at 512 nm (S.E. of mean = 4 nm) and 334 ± 6 nm. These converted reversibly to photoproducts with λ_{max} at 415 ± 4 nm and 420 ± 8 nm respectively. The photoproducts were stable in the dark (less than 5% decay in 6 h). – The rate at which the 512 nm pigment was converted to photoproduct by illumination was measured. If each photopigment molecule that absorbed a photon were converted to photoproduct then calculation would suggest that the microvilli of each rhabdome in the 250 µm thick slice contained $(4.0 \pm 0.8) \times 10^8$ molecules of photopigment. If the molecules were located only on the microvillar surface membrane this would give a mean separation between them of 20 ± 7 nm.

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Effects of Aspartate and Glutamate on the Function of the Cat Retina

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Aspartate and glutamate have been shown previously to isolate the receptor potential (P_{III}-component of the electroretinogram, ERG) in the retinae of several lower vertebrates and of the rabbit, and also to depolarize horizontal cells in the turtle and in the skate. Application of these amino-acids to the retina of the cat was carried out by injection into an arterial perfusion system of isolated eyes in vitro (Niemeyer, Documenta Ophthal. *39*, 53–116, 1975). Stabile ERGs and recordings of action potentials from the optic nerve monitored the responsiveness of the entire retina to light over 4 to 8 h. These signals were strongly affected by applications of aspartate and of glutamate. Aspartate, in estimated concentrations from 7 to 140 m*M*, and glutamate, in estimated concentrations from 20 to 170 m*M*, rapidly depressed the b-waves of the ERGs, with abolition at high doses; the a-waves were not affected or showed a slight increase in their amplitudes. The optic nerve responses revealed decreases in their amplitudes parallel to the changes of the b-waves, but in a slower time course. These effects were dose-dependent and, in most instances reversible. – The data indicate, that aspartate and glutamate block the transmission of light-evoked signals from photoreceptors probably to all second order neurons and, thereby, to the ganglion cells in the retina of the cat in vitro.

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The Role of Conditioning in Determining 'plasticity' of Stimulus-Bound Behavior

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Stimulus-bound behaviours such as feeding and drinking, that are elicited by electrical stimulation of the lateral hypothalamus, show 'plasticity', i.e. are subject to change. E.g. if a rat eats in response to stimulation, the removal of food can result in a gradual switch from elicited eating to elicited drinking. That experience plays an important role in stimulus-bound behavior is also apparant from the fact that elicited behavior gradually stabilizes over trials. The purpose of the present study was to evaluate the role of classical conditioning as one determinant of stability and plasticity of stimulus-bound behaviour. Rats in which lateral hypothalamic stimulation did *not* elicit either eating or drinking were used. Classical conditioning consisted of pairing deprivation-induced eating or drinking (UCS) with electrical stimulation of the lateral hypothalamus (CS). The effect was that the incidence and duration of eating or drinking were increased during lateral hypothalamic stimulation when the animals were satiated on food and water. Conditioning of eating led to a significant increase in elicited eating, but not drinking, and vice versa. Conditioning was optimal in those animals in which stimulation through the same electrodes at higher current levels was rewarding.

Evaluation and Biological Significance of two Ca-sensitive Sites in the Human Red Cell Membrane

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The Ca-dependent specific increase in the K permeability of human red cell ghosts is mediated by a Ca-Sr-sensitive site on the internal surface of the membrane. The dissociation constant (K'd) for Ca at this site is $3–5 \times 10^{-7}$ *M* (pH 7.2). A pH shift from 8.5 to 6 causes the K'd to increase from 1.5×10^{-7} to 2×10^{-6}. Two different pK' values of this site were found at pH 8.3 and 6.3. The Ca-activated K efflux but not the influx is inhibited by increasing intracellular Na from 2 to 70 m*M*. Extracellular Na (0.1–140 m*M*) or K (0.1–6 m*M*) are without effect on net K efflux. A Hill coefficient of 2 is observed under all conditions but is changed to 1 in the presence of the inhibitor oligomycin. The K 'channel' controlled by this site has properties similar to the K channel in exitable tissues. The resealing of ghosts after hypotonic hemolysis is controlled by a second site sensitive to Ca, Sr, Ba and Mg with a K'd for Ca of 2×10^{-6} *M* and a Hill coefficient of 2. The K'd for Ca is barely affected by a pH change from 8 to 6. The site is exposed only during the hemolytic process but is unavailable in the native or resealed membrane. It controls a transmembrane pathway which is unspecific with respect to cations.

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Decreased Na^+-K^+-ATPase Activity in Canalicular Plasma Membranes of Cholestatic Rat Liver

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Active transport of Na^+ across the canalicular membrane (CM) of the hepatocyte may represent an important mechanism for the formation of bile. The evidence for this hypothesis, however, is scarce. The activity of Na^+-K^+-stimulated ATPase has, therefore, been investigated under normal conditions and during ethinyl-estradiol (EE) induced cholestasis. Male Sprague-Dawley rats were treated with a cholestatic dose of EE (0.5 mg/100 g b.w.) s.c. daily for 5 days. Control rats received the solvent, propanediol, only. On the sixth day, bile flow (BF) was measured under pentobarbital anesthesia, the liver removed and hepatocellular fractions rich in CM prepared according to Song (J. Cell Biol. *41*, 124, 1969). Treatment with EE decreased BF from 1.64 ± SD 0.30 to 0.92 ± 0.31 µl/min g liver. This reduction in BF, which was mainly due to a diminution in the bile salt independent fraction, was paralleled by a decrease of the Na^+-K^+-ATPase activity from 144 ± 55 to 50 ± 18 µmole P_i liberated/h g liver. These results demonstrate, that inhibition of Na^+-K^+-ATPase may be involved in EE-induced cholestasis. They are consistent with the contention, that the bile salt independent fraction of BF originates from active transport of Na^+.

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Stimulation Induced Glycine Release in Pigeon Tectum with Push-Pull Cannula

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In the pigeon, a pathway with origin in the midbrain (nucleus isthmi, pars parvocellularis = Ipc) and endings in the tectum opticum has been shown to have a specific affinity to glycine. The hypothesis that glycine is involved in neurotransmission in this system was tested by studying the release of glycine during electrical stimulation of Ipc. For this purpose the in vivo method giving the most reproducible results was a perfusion technique with a push-pull cannula, allowing to collect continually the radioactivity released from the cells previously loaded with labelled glycine, leucine or urea. Glycine only had a marked increase of release during 3 min periods of electrical stimulation (0.5 mA, 20 Hz) of the nucleus Ipc. Such an efflux increase was not observed when stimulation was applied around the nucleus or in the optic nerve papilla. The specificity of this release supports the hypothesis that glycine is a transmitter in this Ipc-tectal pathway.

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Inhibition of Transport Processes in the Dog Colon

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The dog colon mucosa, unlike that of other mammals, accumulates sugars and amino-acids actively by Na^+-dependent mechanisms, sensitive to ouabain and harmaline, which resemble the corresponding systems in the ileum. There is a partial inhibition of uptake by ethacrynic acid, but not by *n*-butyl-biguanide. Efflux experiments suggest that ethacrynic acid affects the permeability of the membrane. Net sodium and chloride fluxes across sheets of dog colon mucosa in vitro are abolished by ouabain in the serosal or harmaline in the mucosal solution. Ethacrynic acid increases the serosal-mucosal flux of sodium. Acetazolamide, oxyphenisatin, and dibutyryl-cAMP, which are active in the rat colon, have no influence on sodium and chloride fluxes in the dog colon, but theophylline slightly stimulated the serosal-mucosal flux of sodium. There is always a close correlation between sodium and chloride fluxes. Thus the principal sodium pump in this tissue appears to be a ouabain-sensitive Na^+-K^+-ATPase, and any secretory pump, analogous to that of the rat colon, is of minor importance.

Effects of 3′Deoxyadenosine (Cordycepin) and 3′Deoxycytidine on RNA in Toad Bladder: Analysis of Aldosterone Action

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Previous studies showed that aldosterone augments transepithelial active Na^+ transport and the incorporation of [^{3}H] uridine into Poly(A)(+)RNA (putatively mRNA) early in the latent period. Soon thereafter, incorporation of [methyl-^{14}C] groups, as well as [^{3}H] uridine into rRNA is also increased. To evaluate the relative roles of these pathways in mineralocorticoid action, two inhibitors, 3′deoxyadenosine (3′dAdo) and 3′deoxycytidine (3′dCyt) were used in studies on the epithelium of the urinary bladder of the toad *Bufo Marinus*. Both inhibitors suppressed the incorporation of [methyl-^{14}C] groups and [^{3}H] uridine into nuclear rRNA precursors and cytoplasmic rRNA subunits by 80% to 90%. 3′dAdo also effectively inhibited the incorporation of [^{3}H] uridine into cytoplasmic Poly(A)(+)RNA (e.g. the 8S to 18S species by more than 70%). In contrast, 3′dCyt inhibited incorporation of [^{3}H] uridine into this fraction minimally (e.g. the 8S to 18S by less than 15%). In control experiments, neither inhibitor (30 µg/ml) had a significant effect on Na^+ transport when given alone. 3′dAdo inhibited the Na^+ transport response to aldosterone significantly whereas 3′dCyt had no apparent effect on the hormonal response. We conclude that, during the first 3 h, the mineralocorticoid action of aldosterone depends on intact pathways for mRNA synthesis but not on rRNA synthesis.

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Ontogeny of the Potentiating Effect of Estrogen on the LHRH-Response in the Rat

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Prior exposure of adult females to estrogen leads to enhanced responsiveness of the anterior pituitary when challenged in vitro with LHRH (Hobson and Hansel, Proc. Soc. exp. Biol. Med. 470, 1974). The ontogeny of this estrogen priming effect has been studied in sexually immature female rats normally reaching puberty at 35 ± 3 (S.D.) days of life. After quick dissection, pituitary halves were placed individually on stainless steel grids and incubated in medium 199 (Gibco; 0.5 ml) at 37 °C in an atmosphere of 95% air/5% CO_2. After 2 preincubation periods of 60 min each, the halves were 'pulsed' with 2.5 ng LHRH for 15 min and the medium was discarded. After a further 60 min incubation period (without additional LHRH) the medium was collected and assayed for LH content. Animals were injected with 20 µg Estradiol Benzoate (EB) on days 18, 21, 24 and 30 and sacrificed 15 h later. In EB pretreated animals of all ages tested, the addition of LHRH led to a significantly ($p < 0.05$) greater LH release, the secretory response being increased by an average of 152%. These results indicate that prior injection of EB is able to enhance the sensitivity of the anterior pituitary to LHRH in vitro well before puberty. This sensitization may be an important component of the process of sexual maturation.

Structure-Activity Relations for Angiotensin II Action in Subfornical Organ

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The fact that Angiotensin II elicits short latency drinking can be linked to the action of microelectrophoretically applied hormone on single SFO (subfornical organ) unit discharges (D. Felix, Naunyn-Schmiedebergs Arch. Pharmak., in press). By structure-activity studies we tried to find out whether the pharmacology of the action in CNS parallels the effects on blood pressure and smooth muscle. 24 single neurons were tested. We found that Des-Asp^1-Val^5-Angiotensin II ('Angiotensin III') shows a slightly shorter latency and a significantly higher stimulation of firing rate compared to Angiotensin II. Both the action of Angiotensin II and Angiotensin III were blocked by Sar^1-Ala^8-Angiotensin II (8 single neurons tested). Other peptides of similar length lack any action of SFO single neurons. – These studies are interesting because of their specificity, the unique possibility of applying a peptide hormone directly to a single cell and the fast reaction times. They may give new insight in structure-activity relations for Augiotensin II which are obscured by side effects in other bioassay systems.

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Birefringence Changes and Murexide Signals from Frog Skeletal Muscle Fibres

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Measuring birefringence changes during activation of frog single muscle fibres, Baylor and Oetliker (Nature *253*, 97, 1975) suggested that component 2 of the obtained transient is due to Ca-release from the SR. To further test this hypothesis, an attempt was made to compare the time relation of this birefringence signal with changes in internal Ca-concentration. Small fibre bundles from frog ilio-fibularis were soaked (1 h) in a murexide solution, based on Jöbsis and O'Connor's experiment (Biochem. Biophys. Res. Comm. *25*, 246, 1966). Absorption changes related to murexide-Ca-binding were measured, light scattering being eliminated by recording differentially at 437 − 471 + 509 nm, and 509 − 544 + 624 nm. The earliest signals obtained started less than 1 msec after the birefringence signal, were well developed at the onset of contraction and peaked during the rising phase of twitch tension. These results support the hypothesis that the second birefringence component is related to Ca-release from the SR and therefore provides a tool for investigation of excitation contraction coupling.

Konformationen der Kohlenwasserstoffketten in Lipidmembranen

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Dipalmitoyl-3-*sn*-phosphatidylcholin und 1-Palmitoyl-2-oleyl-3-*sn*-phosphatidylcholin wurden in verschiedenen Positionen der Fettsäureketten spezifisch deuteriert. Aus den deuteriummagnetischen Resonanzmessungen der entsprechenden Lipidmembranen lassen sich die Kettenkonformationen bestimmen. Es wurde gefunden, dass sich die zwei chemisch äquivalenten Ketten des Dipalmitoyl-3-*sn*-phosphatidylcholin-Moleküls in der Membran physikalisch unterschiedlich verhalten. Für beide Systeme lassen sich mit Hilfe eines statistisch-mechanischen Modells die Messergebnisse interpretieren. Dabei zeigt es sich, dass die Einführung einer Doppelbindung in einer Kette des Lipidmoleküls zu einer Verkleinerung sowohl der Van-der-Waals-Energie (V_0) zwischen den beiden Ketten als auch der Oberflächenenergie (γ) führt.

The Permeability of Dog Mesentery to Water-Soluble Molecules

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The fluxes of ^{22}Na, ^{14}C-glucose, ^{14}C-urea and inulin-^{14}COOH across dog mesentery in diffusion chambers at 37° obeyed the laws of free diffusion. Addition of a tracer amount to one side of the membrane resulted in an approach to equilibrium by a simple exponential with half-time, t. The permeability coefficients were calculated by: $p = 0.693 \cdot V/(2 \cdot A_m \cdot t)$, where A_m is the surface area of the membrane and V the volume of the chamber. The average values of p for sodium, urea, glucose and inulin were 25.9, 26.3, 15.4 and 6.6×10^{-5} cm/sec. The linear correlation between these permeability coefficients and the corresponding diffusion coefficients in free solution, concerning non-electrolytes ranging in size from urea (r = 2.8 Å) to inulin (r = 12–15 Å), suggests that pores larger than 50–60 Å provide a pathway for the movement of such lipid-insoluble molecules across the tissue. The fact that the regression line does not pass through the origin indicates that hindrance to free diffusion occurs for molecules of higher molecular weight.

The Effect of Surgical Denervation on Metabolic Function of Brown Adipose Tissue in vitro

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Brown adipose tissue responds to potassium by a large increase in O_2 consumption, resulting from a release of endogenous noradrenaline, as demonstrated pharmacologically. Surprisingly, surgical denervation did not affect the response (Barde et al., J. Physiol. *252*, 523, 1975). This was reinvestigated using the Falck-Hillarp histochemical fluorescence technique. Unilateral (i.e. unilobular) surgical denervation of the bilobular interscapular brown fat pad 8 weeks before sacrifice decreased significantly the specific catechol fluorescence on both lobes (60%, 25%), whereas metabolic responses (O_2 consumption and glycerol release) were unaffected. Bilateral surgical denervation caused a further decrease of specific fluorescence; remaining innervation was estimated at 15% of the control and again the metabolic responses were not significantly modified. Preloading with α-methyl-noradrenaline did not change qualitatively the results. We conclude that a small percent of innervation is sufficient to supports metabolic activity and that there is a significant cross innervation in interscapular brown fat.

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Effects of Amitriptyline and Harmaline on Sodium and Water Transport

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Available evidence shows that the antidepressant amitriptyline (A) and the hallucinogen harmaline (H) inhibit Na + K − ATPase. We examined their effects on frog skin, taking the short circuit current (SCC) as a measure of net Na flux. At 2 to 5 m*M*, both A and H did decrease SCC and blocked its stimulation by oxytocin and norepinephrine. However, at 0.03 to 0.5 m*M*, both substances elicited a sustained increase in SCC, inhibitable with amiloride. These effects were seen whether the drugs were added to the outer or the inner bathing solution. A different pattern of response was found with water transport across toad skin. Added to the inner side, H induced a marked hydro-osmotic response, similar to and competitive with that of vasopressin or norepinephrine. In contrast, A produced only a small and transient hydro-osmotic effect but blocked the hormonal stimu-

lation of water flow. These results indicate that both A and H affect systems other than the Na pump, possibly the cAMP and the microtubular systems, but further work is needed to elucidate this point.

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GABA Inhibition on Central Neurones: Antagonistic Effects of Benzodiazepines

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Electrophysiological single unit recordings were carried out on neurones of the feline Deiters nucleus and on Purkinje cells of the cerebellar cortex (cat and rat). Antidromically activated Deiters neurones were inhibited by preceding stimulation of the GABAergic inhibitory input from the cerebellum as well as by iontophoretically applied GABA (2–20 nA). Preceding injection of diazepam (0.5 mg/kg i.v.) antagonized the synaptically and also the locally induced GABA inhibition of antidromically activated Deiters neurones. Purkinje cells, identified on the basis of the spontaneous 'inactivation responses' could be inhibited by iontophoretically applied GABA. Preceding injection of bromazepam (2 mg/kg i.v.) antagonized the GABA induced inhibition. Furthermore, the GABA mediated inhibition of Purkinje cells by basket cells as well as the direct inhibitory GABA effects were antagonized by iontophoretically applied chlordiazepoxide HCl (20–50 nA). These findings demonstrate an antagonism between the inhibitory neurotransmitter GABA and the benzodiazepine derivatives.

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Influence of Dietary Calcium, Phosphorus, and Vitamin D on the Conversion of 25-Hydroxyvitamin D_3 to 1,25-Dihydroxyvitamin D_3 by Kidney Tubules of Diphosphonate-Treated Quails

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Treatment of animals with large doses of ethane-1-hydroxy-1,1-diphosphonate (EHDP) is known to reduce bone mineralization, intestinal calcium absorption, and 1,25-dihydroxyvitamine D_3 [1,25-$(OH)_2D_3$] production by the kidney. In vitro no inhibition of 1,25-$(OH)_2D_3$ formation in renal tubule suspensions was observed at 5 m*M* EHDP. The reduced 1,25-$(OH)_2D_3$ levels may reflect a secondary response to the inability of the skeleton to retain calcium and phosphate. If this were true the drug's effects should be modulated by dietary manipulation. We studied the effect of EHDP on the 1-hydroxylation of 25-hydroxyvitamin D_3 by kidney tubules taken from quails fed diets of different calcium and phosphorus content, with and without vitamin D. – With vitamin D and high mineral diet (1.24% Ca; 1.10% P) the inhibition was 44%. With low calcium diet (0.25%), which increased the 1-hydroxylation in control animals, it was only 19%. Vitamin D depletion nearly abolished the inhibition to 4%. Lowering the phosphorus intake (0.39%) increased the 1-hydroxylation in control animals, but inhibited it in EHDP-treated animals by 83%. These results would indicate that the renal 1-hydroxylation is indirectly influenced by EHDP. Furthermore they suggest that dietary calcium and phosphorus influence the renal 1-hydroxylation by two distinct mechanisms.

Measurement of Platelet Surface Interaction by Morphometry or Platelet Label

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Collagen surfaces were exposed to citrated blood of rabbits in a perfusion chamber at a flow rate similar to that found in arteries. Platelet deposition at the surfaces as evaluated by a morphometric technique was compared with the deposition using 51Cr or 14C-serotonin labelled platelets. Fibrillar collagen e.g. was covered by an average number ($\times 10^3$) of 259, 504, 980 and 1789 51Cr-platelets/mm² or by 73, 119, 289 and 319 14C-serotonin platelets/mm² after perfusion periods of 2.5, 5, 10 and 20 min respectively. The number of deposited platelets based on the 14C-label therefore is underextimated if compared with the number based on 51Cr, most likely due to the adhesion-induced release of 14C-serotonin from the platelets. This falsely low 14C-platelet number however was found to correlate well with the number of platelet thrombi (platelet accumulations > 5 μm) as measured morphometrically. A distinction between platelet adhesion and thrombi requires morphometry. However, labelled platelets can be used to estimate the extent of platelet thrombus formation and its inhibition by drugs.

Analysis of Exploratory Behavior in the Rat

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Simple activity measures cannot be expected to give a more than crude picture of the psychological processes during exploration and inspection of an unfamiliar environment. Hence, activity measures are not very satisfactory indicators of drug and lesion influence on such processes. – A more detailed picture can be obtained by the variables investigated in the present study: turns, decisionfrequencies, transition times among other 'macro-variables' showed a strong dependency on environmental complexity and experience. (Uster and Bättig 1976, in press) During repeated exposure to the hexagonal mazes (Bättig and Wanner, Helv. Physiol. Acta 1967) the exploration showed an increasing complexity of locomotion with increasing number of turns, less constant velocity and weakening of the forward tendency. In contrast to these qualitative changes the amount of activity remained almost unchanged. The results suggest that these qualitative changes depend upon complex interactions between environmental complexity and experience. Therefore the exploration experiment might become an usefull tool for psychopharmacalogical and neurophysiological research.

The Role of Calcium in the Development of Hyposensitivity of Pineal β-Receptors

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The responsiveness of the pineal enzyme *N*-acetyltransferase (NAT) to β-stimulation both in vivo and in vitro varies considerably depending on the degree of previous exposure to agonists. Thus, repeated stimulation leads to hyposensitivity of the β-receptor whereas stimulus deprivation is followed by a hypersensitive state. In incubated rat pineals we have found that manipulation of cellular calcium by the ionophores X-537A and A23187 leads to a (reversible) inhibition ($p < 0.001$) of the elevation of NAT by small stimulatory concentrations of isoproterenol (10^{-7} *M*) or norepinephrine (10^{-6} *M*). Dibutyryl cyclic GMP (1 m*M*) also inhibits this response ($p < 0.005$); likewise, complete removal of calcium from the culture medium by EGTA 0.5 m*M*) inhibits the rise in NAT, whilst as little as 0.01 m*M* Ca + + almost completely restores sensitivity of the receptor. The results indicate a possible role of calcium in controlling the sensitivity of the β-receptor.

Electrical Uncoupling of Heart Muscle Cells in Hypoxic, Glucose-Free Tyrode

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Weidmann's method of measuring internal longitudinal resistance (J. Physiol. *210*, 1041, 1970) was modified to allow simultaneous recording of action potentials, tension and internal longitudinal resistance (r_i) in cow ventricular trabeculae. A combination of hypoxia and glucose-free Tyrode induced an increase in r_i, the increase being 36 ± 8,9% after 30 min and 87 ± 25% after 60 min. The increase in r_i was associated with an increase in diastolic tension (DT). The increase in r_i and DT could be potentiated by (1) increasing frequency of stimulation, (2) application of adrenaline and (3) a second exposure to hypoxic, glucose-free Tyrode. These changes were partly reversible. It is suggested that the increased r_i might be due to calcium accumulation inside the cell, leading to an uncoupling of the nexus structure. This effect could play a role in causing cardiac arrhythmias.

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An Estrogen-Dependent Priming Effect of LHRH in the Immature Female Rat

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The anterior pituitary gland of the adult female rat is most sensitive to Luteinizing Hormone Releasing Hormone (LHRH) during the day of proestrus. At this stage, LHRH is able to prime the gland so that further stimulation produces an enhanced LH response; this potentiation is presumably dependent on estrogen (Aiyer and Fink, J. Endocr. *62*, 573, 1974). We have investigated pituitary sensitivity to LHRH in immature female rats (26 days) which were either untreated or in which precocious sexual maturation had been initiated by Pregnant Mare Serum (PMS 10 IU, 48 h earlier) or by unilateral brain stimulation (80 h earlier). In untreated controls, plasma LH increments seen after two consecutive LHRH injections (100 ng/100 g b.w., s.c., separated by 60 min) were of comparable magnitude. In contrast, in rats pretreated with PMS and brain stimulation, the second plasma LH increment exceeded the first by respectively 111% and 94% ($p < 0.02$). The results indicate that the priming effect of LHRH is absent in the immature rat but can be induced by procedures known to stimulate ovarian steroidogenesis. They also show that the priming effect of LHRH on the anterior pituitary is independent of the overall responsiveness of the gland to the decapeptide, as in the PMS treated animals (but not in brain stimulation) a decrease in the responsiveness has been observed.

BIOCHEMIE – BIOCHIMIE – BIOCHEMISTRY

Inactivation, Regeneration and Hybridization of Human Erythrocyte Catalases

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Molecular hybridization has been suggested as an explanation for the properties of the erythrocyte catalase found in individuals heterozygous for the unstable enzyme variant of Swiss Type Acatalasemia. The feasibility of this hypothesis has been explored with model experiments in which hybridization has been accomplished between dimer subunits of normal human erythrocyte (HEC) and heterozygote acatalasemic (HET) catalases to give a hybrid tetramer. Treating HEC with increasing amounts of urea resulted in a progressive loss of catalatic activity accompanied with the generation of peroxidatic activity toward guaiacol. Complete dissociation into dimer subunits was accomplished with 8 *M* urea. The loss of catalatic and generation of peroxidatic activity was more rapid in identically treated HET, revealing an increased sensitivity of this enzyme toward urea. A reversal of these processes, the recombination of the subunits, could be effected by a rapid dialysis of the inactivated samples. Double immunodiffusion tests demonstrated complete antigenic identity of the native and regenerated tetramers but confirmed a difference in antigenic properties between the tetramer and the dimer particles. Hybridization was accomplished by a mixing of the urea-treated HEC and HET prior to recombination by dialysis. Starch-gel electrophoresis revealed that the migration of the hybrid differs visibly from the HEC and HET regenerated tetramers.

Structure of the Activated Form of Clr

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Assimeh and Painter (J. Immun. *115*, 488, 1975) have shown that both Clr and Clq bind independently to IgG and hold the other Cl subcomponents Cls and Clt by Ca^{2+} dependent bonds. To elucidate the role of Clr in the activation of Cl, activated Clr ($\overline{Clr}$) was isolated from human serum by affinity chromatography on Sepharose-IgG. It was dissociated from the bound Cl macromolecular complex by treatment with EDTA and separated from Cls and Clt by ion exchange chromatography. $\overline{Clr}$ activity was determined by its ability to activate Cls and to hydrolyze *N*-acetyl-L-arginyl-methyl ester. Isolated $\overline{Clr}$ was found to be a protein of about 200,000 daltons consisting of two noncovalently linked polypeptide chains of the order of 100,000 daltons. Each chain is composed of disulfide linked subunits of 67,000 and 37,000 daltons respectively. The precursor form of Clr is thought to be converted to the activated protein by proteolytic cleavage. Activation may occur spontaneously but is greatly accelerated by the presence of immune complexes or of insoluble polycations such as QAE-Sephadex.

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Isolation of Epoxide Hydratase and Properties of the Pure Enzyme

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The microsomal enzyme epoxide hydratase (EC.4.2.1.63) was solubilised using the non-ionic detergent cutscum and purified by a method involving ammonium sulphate fractionation, ion-exchange chromatography and hydrophobic chromatography. The product was detergent free and shown to be homogeneous by SDS-gel electrophoresis, analytical ultracentrifuge and immunological criteria. The enzyme appears to consist of a single polypeptide chain (mol wt 49,000; S_{20w} 3) which forms high molecular weight aggregates in aqueous solution (S_{20w} 14.5). The preparation hydrates a wide variety of epoxides including those derived from carcinogenic polycyclic hydrocarbons and is inhibited to the same extent as microsomal preparations by various inhibitors. These observations together with immunoprecipitation studies suggest that a single microsomal protein is responsible for the hydration of styrene oxide (which is generally used as a substrate to assay epoxide hydratase) and the highly mutagenic benzo(a)pyrene 4,5(K-region)-oxide.

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Affinity Chromatography of Glycogen Phosphorylase from Crayfish Tail Muscle and Properties of the Pure Enzyme

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Crayfish phosphorylase can be extracted at neutral pH with the soluble protein fraction from a muscle homogenate in 50 m*M* potassium chloride in the presence of EGTA and mercaptoethanol. Chromatography of these soluble proteins through a column of Ultrogel ACA 34 yields an enzyme fraction which elutes in a mol wt range between 40,000 and 190,000 (16% of the total soluble proteins). Filtration of this fraction, containing 5–10% phosphorylase, through a column of 5'AMP Sepharose 4 B at low ionic strength brings about the complete adsorption of the enzyme which can be eluted 90–95% pure with 0.4 *M* potassium chloride. A last chromatography on DEAE cellulose allows the ultimate purification of the enzyme. At this stage, gel electrophoresis of phosphorylase in SDS gives the same monomer mol wt as in the instance of rabbit; similarly, the active AMP-sensitive form is a dimer. Crayfish phosphorylase is extremely labile, the activity is destroyed at pH's lower than 6, upon ammonium sulfate precipitation or overnight storage in 1 *M* potassium chloride. Unless reducing agents are present, rapid polymerization occurs with inactivation and precipitation.

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Antigenicity of Human Glycophorin Constituents

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Purified human glycophorin can be dissociated into three glycoprotein bands; PAS-1, PAS-2 and PAS-3, by polyacrylamide gel electrophoresis in the presence of SDS. Previous observations have suggested that these proteins were subcomponents of the major red cell membrane glycoprotein (H. Furthmayr et al., B.B.R.C. *65*, 113, 1975); J. D. Berthoud and C. Bron, Experientia *31*, 717, 1975). In order to further investigate the structural relationship between these proteins, antisera were prepared in rabbits against glycophorin, PAS-1, PAS-2 and PAS-3. When tested in complement mediated cytotoxicity using ^{51}chromium-labelled targed red cells, each of these reagents was lytic, thus indicating that the three subcomponents have antigenic sites expressed on the cell surface. Furthermore, when tested in inhibition of cytotoxicity, the three subcomponents completely abrogated the cytotoxic activity of all antisera studied. These results, suggesting the presence of an identical constituant with common antigens in PAS-1, PAS-2 and PAS-3, are in agreement with the data available, indicating that glycophorin is composed of two glycopeptides, with a high tendency to form aggregates. – Using the same serologic test, the presence of blood group A antigens in all of these proteins could be confirmed. These co-purified antigens could not be dissociated from the above glycoproteins on affinity columns of bound anti-glycophorin or anti-A antibodies, which is suggestive of a strong linkage between these glycoproteins and the A blood group antigens.

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Correlation of Changes in the Content of ^{14}C-ATP, [ATP], and of ^{14}C-cAMP in Prelabelled Human Platelets

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In human platelets [ATP] falls after addition of inhibitors of phosphodiesterase. The effect is more pronounced with papaverine (pav) than with 3-Isobutyl,1-methyl-xanthine (IBMX). Pav. is known to inhibit respiration at site one, IBMX is not. The fall of [ATP] is more severe if platelet adenyl cyclase was stimulated by Prostaglandine-E1 (PGE-1), before the addition of the PDE-inhibitor. Activation of adenine cyclase by PGE-1 can by itself lead to changes in [ATP]. The poster demonstrates these observations and attempts to correlate them in the context of platelet physiology and of purine nucleotide metabolism.

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Chemical Mapping of Protein-Protein and Protein-Nucleic Acid Complexes

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Amino groups of protein components in protein-protein and protein-nucleic acid complexes can be acetylated by acetic anhydride (Ac_2O) either when free or in complex (differential labelling). Reactivities towards Ac_2O of amino groups that are involved in complex formation are expected to differ in the free protein and in complex. Amino groups of differing reactivity can be localized in the primary and, possibly, tertiary structure. Such information may be used to delineate contact areas in complexes and/or conformative responses consequent to complex formation. Acetylation of one group may affect reactivities of adjacent groups, jeopardizing results. This danger can be kept minimal by using protein in excess of ^{3}H-Ac_2O of high specific activity followed by complete acetylation with excess unlabelled Ac_2O. Mixing the resulting heterogeneously ^{3}H-labelled but homogeneously acetylated protein with homogeneously ^{14}C-acetylated protein and determining the ^{3}H/^{14}C ratios for each acetylated amino group facilitates the analysis of groups of altered reactivity. This technique has been applied to the complexes between $tRNA^{Tyr}$ and tyrosyl-tRNA synthetase and between cytochromes c and aa_3. Lysine residues of altered reactivies have been localized within the amino acid sequence of tyrosyl-tRNA synthetase, and based on the findings, a tRNA binding site has been tentatively demarcated on the synthetase molecule.

Uptake and Subcellular Distribution of ^{125}I-Myelin Basic Protein (MBP) in Rat Cortex Slices

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Purified bovine MBP was labelled with ^{125}I by the standard Chloramine-T procedure. The uptake of ^{125}I-MBP into rat cortex slices was tested at 1.10^{-5} and 1.10^{-6} *M* concentrations with MBP preparations not older than 4 days after labelling. The tissue/medium ratios after 10 min incubation at 25 °C were 2.1 ± 0.3 ($n = 6$) for 10^{-5} *M* and 1.4 ± 0.3 ($n = 4$) for 10^{-6} *M* MBP. The homogenate was fractionated on a 10-step discontinuous sucrose density gradient. Distribution of ^{125}I-MBP label was nearly identical for 10^{-5} and 10^{-6} *M* MBP: 18% 'mitochondrial' fraction, 47% 'synaptosomal', 6% 'myelin' and 29% 'cytoplasmic'. 31% of the label in the synaptosomal fraction were located in the heavy (catecholaminergic) subfractions. These results demonstrate a low uptake in the myelin and a high uptake into synaptosomes, possibly of catecholaminergic type. Lower MBP concentrations and pure histone fractions (to test the specificity) as well as the interaction of MBP with the uptake of ^{3}H-noradrenaline and ^{3}H-dopamine are under investigation.

Quantitation of Chicken Creatine Kinase Isoenzymes

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Purification of rabbit antibodies against chicken BB creatine kinase (BB-CK) was achieved by immunoadsorption of antisera on BB-CK coupled to Sepharose-4B by the CNBr method. Purified monospecific antibodies could be eluted most efficiently from these immunoadsorption columns with 1 *M* propionic acid, other eluants were less effective. These antibodies were coupled to Sepharose beads and retained their immune reactivity and specificity. BB and MB-CK were bound quantitatively (> 97%) but MM-CK did not interact with this matrix. The immunoadsorbent specific for BB-CK and the one previously described for MM-CK allow the quantitation of the three isoenzymes MM, MB and BB, involving CK determinations of a sample before and after immunoadsorption on anti-MM and anti-BB-CK matrices. The CK activities in the flowthroughs were only BB- or MM-CK respectively. The method is fast and its accuracy could be demonstrated on mixtures of purified homogeneous isoenzymes. It was also used for the determination of the CK isoenzymes in crude extracts of embryonic chicken muscle and differentiating myogenic cell cultures. Homomeric isoenzymes could be eluted with high ionic strength buffer to yield homogeneous CK preparations.

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Active-Site-Directed Modification of Pyruvate Decarboxylase by Activation of the Carbanionic Enzyme-Substrate Intermediate with Extrinsic Oxidants

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The combination of substrate and an extrinsic oxidant has recently been recognized to constitute a highly specific binary system for the active-site-directed chemical modification of enzymes that form oxidizable carbanion intermediates (Eur. J. Biochem., in press). Applying this approach to pyruvate decarboxylase from yeast (PDC) we have found that the oxidation of the hydroxyethyl-thiamine diphosphate intermediate of PDC to acetate is

in fact accompanied by a progressive inactivation of the enzyme. In the presence of pyruvate (10 m*M*) and of an oxidant such as 2,6-dichlorophenolindophenol (0.1 m*M*) PDC (in citrate buffer, pH 6.0) loses 90% of its enzymatic activity within 15 min whereas in the presence of the oxidant alone only 40% and in the presence of the substrate alone only 15% are lost. Enzymatic activity is not restored by gel filtration. The rate of inactivation obeys saturation kinetics with respect to substrate concentration and is independent of enzyme concentration. Thus, in analogy to the findings with other carbanion forming enzymes the oxidation of the hydroxyethylthiamine diphosphate intermediate of PDC generates a transiently reactive product (of to date unidentified nature) which without being released from the active-site covalently modifies neighboring groups in an intramolecular reaction.

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Inhibition of Horse Liver Alcohol Dehydrogenase by Trifluoroethanol

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The progress curve for the oxidation of ethanol is concave upwards (lag) when ethanol is added to horse liver alcohol dehydrogenase (E) premixed with NAD^+ (O) and the substrate analogue inhibitor trifluoroethanol (I). Premixing conditions which do not allow the preformation of an EOI-complex yield progress curves concave downwards (burst). After this transient period of up to 2 min the curves become linear (steady-state). The steady-state inhibition patterns are uncompetitive versus NAD^+, NADH and acetaldehyde, and competitive versus ethanol, when corrected for product inhibition. The time course studies and the inhibition patterns indicate the formation of an EOI-complex which slowly isomerizes to a tighter E*OI-complex. Parabolic double reciprocal plots are found for the inhibition versus ethanol during the transient period. These nonlinear plots suggest a mechanism, in which the formation of EOI or E*OI at one of the subunits of the dimeric enzyme changes the kinetic properties of the adjacent subunit.

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Kinetic Studies on the Low K_m-Phosphodiesterase Inhibition by DH-Ergot Alkaloids

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The cAMP-Phosphodiesterase (PE) shows anomalous kinetic pattern (Proc. Nat. Acad. Sci. *69*, 1791, 1972). The low K_m PE regulates the cAMP splitting under physiological conditions by way of a negative cooperativity expressed by a Hill coefficient $n < 1$. Some DH-ergot alkaloids were proved to be stronger inhibitors of the low K_m- than of the high K_m PE in homogenates of rat and cat brains (IRCS Med. Sci. *3*, 403, 1975). Studies on a purified low K_m enzyme fraction of cat also showed inhibition by DH-ergot alkaloids such as DH-ergotoxine, DH-ergotamine. The DH-ergot alkaloids tend to lower the affinity of the low K_m PE for the substrate. The Hill interaction coefficient n was changed by these inhibitory effectors towards 1, meaning decreasing negative cooperativity, dose dependent. This kind of PE inhibition is of special interest at the normal cellular cAMP level, without hormonal activation.

Chemical Nature of Hemoglobin A_{1C}, the Minor Hemoglobin Massively Increased in Diabetes mellitus

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The amino acid sequences of Hb A_{1C} and Hb A are identical, but in A_{1C} both β *N*-terminals are blocked by an as yet unknown group. In normal human erythrocytes it constitutes $\sim$ 5% of all hemoglobin. In diabetics its level is 2–3fold higher. Experiments were designed to analyse synthesis and structure of A_{1C}: (1) In vitro incubation of erythrocytes, hemolysates or pure Hb A (8h, 37 °C) in the presence of labelled glucose (1000 mg%) led to marked formation of labelled Hb A_{1C} as judged by charge properties. Two moles of glucose were incorporated per Hb-tetramer. (2) Normal and diabetic Hb A_{1C} were physico-chemically identical. Both gave positive reactions with thiobarbituric acid after treatment with oxalic acid, in keeping with the postulated liberation of 5-hydroxy-methylfurfural. This was confirmed spectroscopically. (3) Hb A_{1C} from both sources incorporated tritium from labelled Na BH_4. (4) Tests for reducing sugars gave values of less than 0.2 *M* per β chain. – We conclude that in Hb A_{1C} from both sources the *N*-terminal blocking group is *N*-glycosidically bound glucose and is thus prone to Amadori rearrangement. This concept is capable to explain all of the conflicting results above.

A Gaschromatographic Assay for the Determination of Isoniazid *N*-Acetylation; Observation in Rats with Induced Constant Urine Flow

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An assay for determination of the degree of enzymatic *N*-acetylation in rats is presented. A simple method for inducing a continuous urine flow from rats was applied to measure the degree of acetylation at definite intervals. A rapid gaschromatographic method for the simultaneous quantitative determination of isoniazid as the substrate for polymorphic *N*-acetyltransferase and its metabolite, acetyl-isoniazid in urine is reported. Isoniazid and acetyl-isoniazid samples were derivatized with a silylating reagent. The gaschromatographic species were identified by GC-mass spectrometry as di-trimethylsilyl derivatives of isoniazid and acetylisoniazid. Significant dose-dependent and age-dependent changes in the degree of acetylation were observed in female rats. For example, relatively more acetyl-isoniazid was excreted after a 50 mg/kg isoniazid dose, than after treatment with 100 mg/kg isoniazid. Young rats of 6–7 weeks acetylated the same dose to a much higher degree than 8-month-old rats.

Reversible Cytolysis of Yeast Plasma Membrane Vesicles

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Yeast plasma membrane vesicles were isolated from mechanical disrupted yeast cells by filtration, differential centrifugation and aggregation of the mitochondrial vesicles at pH 4 (G. F. Fuhrmann, C. Boehm and A. P. R. Theuvenet, Biochem. Biophys. Acta, in press). As judged by biochemical, cell electrophoretic and electron microscopic criteria a pure plasma membrane vesicle preparation was obtained. – The yeast plasma membrane vesicles were tested for reversible cytolysis in analogy to the preparation of red cell ghosts. It could be demonstrated that the vesicles can be filled for example with hemoglobin or ATP during reversible cytolysis. The fraction of plasma membrane vesicles sealed to ATP was dependent on temperature, the best results for sealing were obtained at 0 °C. As in red cell ghosts plasma membrane vesicles sealed for K^+ can be separated on a sucrose density gradient.

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Release of 5-Thio (2-nitrobenzoate) from the Mixed Disulfide Derivative of Mitochondrial Aspartate Aminotransferase: A Probe for Syncatalytic Conformational Changes

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In the mitochondrial isoenzyme of aspartate aminotransferase from both chicken and pig heart the single reactive sulfhydryl group per subunit may be modified without any loss of enzymatic activity. The rate of its modification with 5,5′-dithiobis-(2-nitrobenzoate) is subject to syncatalytic alterations (Biochem. Biophys. Res. Commun. *63*, 441, 1975). Similarly, the rate of the reverse reaction, the release of 5-thio(2-nitrobenzoate) from the mixed disulfide derivative of the enzyme by excess 2-mercaptoethanol, dithiothreitol, reduced glutathione, or coenzyme A, is altered syncatalytically. The rate of the mixed disulfide-thiol exchange is at a minimum in the free pyridoxal and pyridoxamine form of the enzyme and is not markedly changed in the (unproductive) adsorption complexes (pyridoxal form with 2-ketoglutarate and pyridoxamine form with aspartate or glutamate). However, it attains a maximum ($\sim$ 10fold increase) in the covalent enzyme substrate intermediates. The striking similarity of the syncatalytic alterations in both the rate of formation of the mixed disulfide of the enzyme with 5-thio(2-nitrobenzoate) and of its cleavage by excess thiol compounds constitutes compelling evidence that the syncatalytic reactivity changes are due to conformational adaptations of the enzyme during catalysis.

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Mapping of the Primary Bilirubin-Binding Site of Human Serum Albumin

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Affinity labeling of the primary bilirubin-binding site of human serum albumin (584 amino acid residues) was performed using a reactive *bis*-enol ester of [^{14}C]bilirubin (C. C. Kuenzle et al., J. Biol. Chem. *251*, 1976, in press). The labeled protein was fragmented into 7 peptides by CNBr-cleavage followed by reduction and carboxymethylation (R. H. McMenamy et al., J. Biol. Chem. *246*, 4744–4750, 1971). The bilirubin label was located by monitoring all peptides for radioactivity and was found to be covalently attached to peptide 3 (residues 124–297, containing the lone tryptophan at position 213) and peptide 6 (residues 446–547). Further analyses presently underway will help to define the high-affinity bilirubin-binding site in more detail.

Discrimination of Active Metabolites of Aminostilbenes as Mutagens

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Mutagenic activation of aminostilbenes may be expected to proceed either by metabolism at double bonds (epoxidation) or at the nitrogen function. 4-Acetylamino-*trans*-stilbene (AAS) reverted histidine auxotroph *Salmonella typhimurium* only after activation by mammalian tissue preparations. Epoxidation of the olefinic double bond did not yield ultimate mutagens and even prevented the activation. The same was found for the corresponding dihydrodiol and dihydro derivatives, indicating that the electronic conjugation of the two benzene rings is essential for mutagenicity. Omitting or replacing the (acetyl)-amino-group also led to products which were not mutagenic nor were activated indicating that the nitrogen function is also essential. *N*-Acetoxy-AAS, a proposed ultimate carcinogen, was only weakly mutagenic. Liver microsomes or soluble proteins with or without NADPH increased its mutagenicity strongly. 4-Nitroso-trans-stilbene was a potent mutagen not requiring activation by liver preparations. Its role as active metabolite of AS, AAS, and acetoxy-AAS will be discussed in terms of mutagenicity ratios for different strains and the effect of glutathione.

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Experimental Myasthenia in Balb/c Mice Immunized with Rat Denervated Muscle Acetylcholine Receptor

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It has been recently shown that an autoimmune disease of the neuromuscular junction can be induced in animals such as rabbits, monkeys, guinea-pigs and rats by injection in complete Freund's adjuvant of acetylcholine

receptor (AChR) purified from electric fish. These models share many similarities with myasthenia gravis. Data so far suggest that both cellular and humoral responses to AChR, either sequentially or in combination, contribute to the pathogenesis. By using an antigen more closely related to the recipient, we have developed a new experimental model: AChR extracted from rat denervated muscle is injected into mice. These animals develop progressively high levels of rat and mouse antiAChR antibodies and signs of muscle weakness. Electromyograms reveal a bloc of myasthenic type, reversed after Edrophonium injection. The AChR used is purified according to a procedure based on solubilization of membrane fragments with Triton X100 followed by repeated affinity chromatography. A starting material of 25 g muscle gives a final yield of 65 µg AChR displaying a binding capacity for α-bungarotoxin of 317 pmole.

Characterization of Human T-Lymphocyte Membrane Antigens

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Crude membrane preparations of human thymocytes were used to raise rabbit antisera which could be rendered specific for T cells by appropriate absorptions (Experientia *31*, 720, 1975). When tested in complement mediated cytotoxicity using ^{51}chromium labelled target cells, such reagents were lytic for 55–65% of peripheral blood leukocytes (PBL), 50% of tonsil cells and 100% of thymocytes, bud did not react with five different B cell lines. In order to identify the specific membrane antigens detected by these antisera, PBL and thymocytes were labelled with ^{125}I by the lactoperoxidase method. After solubilization of the membrane with a non ionic detergent (Nonidet-P 40), the antigens were specifically precipitated with the antisera and analysed on polyacrylamide gel electrophoresis in the presence of 6 *M* urea and 1% SDS. One major peak of radioactivity could be detected on the polyacrylamide gels having an apparent molecular weight of 150,000–200,000 daltons, which could represent the membrane components specific for thymocytes and thymus-derived cells. Such proteins will be used as immunogens for the preparation of xenogeneic antisera specific for human T-cells.

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Isolation of the Third Component of Mouse Complement

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C3 fulfills a pivotal role in the complement system. The structure and function of human C3 is already well described. For use in defined, homologuous systems, we have purified C3 from mouse plasma by euglobulin precipitation and cation and anion-exchange chromatography. The resultant C3 native gave one immunoelectrophoretic line with anti-whole mouse serum antibodies. It was identified by immune adherence using EAC142 and human erythrocytes, and by anti-C3 antiserum. One SDS-polyacrylamide gel electrophoresis, C3 native was found to be an approximately 220,000 daltons protein composed of two chains of about 150,000 and 80,000 daltons. This structure appears to be similar to that described for human C3. C3 tryptic fragments, C3b and C3c, analogous to fragments generated by C3 convertase and C3 inactivator, have molecular weights of about 205,000 and 180,000 daltons respectively. Preliminary experiments indicate that mouse C3 can directly induce blast transformation of cultured murine lymphocytes.

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Parvalbumins from Chicken Muscle

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Parvalbumins (PV) are a well-known class of proteins abundant in the white muscle of fish and amphibians. They were recently also detected in sizable amounts in higher vertebrates. PV were isolated from chicken leg muscle (20 mg/kg) and from white back muscle (latissimus dorsi, 40 mg/kg) using published isolation procedures (P. Lehky et al., J. biol. Chem. *249*, 4232, 1974). Mol wt was 12,000, estimated from SDS-electrophoresis. The aminoacid composition (chicken leg) shows a high Glu, Asp content and 1 Tyr. Copurified with PV was a monometic, watersoluble protein (mol wt 60,000, 50 mg/kg) which was eluted from a DE-52 cellulose column at concentrations of 110 m*M* NaAc. Its amino-acid composition is different from proteins of comparable size such as the 55,000 dalton component from the SR (N. Ikemoto et al., J. Biol. Chem *249*, 2357, 1975) and the γ-component (L. W. Lee and S. Watanabe, J. Biol. Chem. *245*, 3004, 1975). Water extracts of chicken heart, kidney, brain, pancreas, lung, smooth muscle showed immunological cross-reactivity with rabbit anti-chick leg PV, suggesting proteins with common structures to PV. In cultures of differentiating breast and leg muscles the presence of PV was also demonstrated (by SDS-electrophoresis, immunoelectrophoresis and -diffusion). Enhanced accumulation occured in fused myotubes after 120 h coincident with other muscle proteins (e.g. myosin, actin, creatine kinase, aldolase).

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UDP-Galactose 4′-Epimerase of Human Erythrocytes: Apoenzyme-Coenzyme-Substrate Interactions

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Epimerase accepts NAD as the coenzyme. The binding of NAD to highly purified epimerase was studied and the behaviour of the enzyme during affinity chromatography with picolylamine-adenine-dinucleotide-Sepharose (R. Langer and L. Glaser, J. Biol. Chem. *249*, 1126, 1974) was tested. In binding epimerase, NAD keeps its active center distant from the binding site of the enzyme. This stands in contrast to the way NAD binds to several dehydrogenases. Using substrate analogues it was observed that UDP-galactose most probably binds in a very similar way, i.e. by that part which is distant from its site of action. It does not, however, compete with the

NAD binding site of the enzyme. – A molecular model for the interaction of apoenzyme, coenzyme and substrate reconciling these findings is proposed. We postulate that substrate and coenzyme reach their optimal conformation for catalysis by pairing their uracil and adenine bases.

M-Line Proteins from Chicken Muscle

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Proteins having mol wt of 160,000, 94,000 and 40,000 as judged by SDS-gel electrophoresis are considered to by specifically bound in the M-line of chicken skeletal muscle. Isolation using high ionic strength as well as low ionic strength conditions (0.6 *M* KCl buffers and 5 m*M* Tris pH 8, respectively) resulted mainly in the extraction of these 3 components. Separation on DE-52 cellulose (2 m*M* Tris, 0.5 m*M* ATP, 0.2 m*M* $CaCl_2$, 1 m*M* βME pH 8) or on DEAE A-50 Sephadex (50 m*M* Tris, 0.1 m*M* DTT pH 8) with a linear gradient (0–0.4 *M* KCl) resulted in the elution of proteins with chain weights of 160,000 (at 0.16 *M* KCl), 94,000 (0.19 *M*) and 40,000 (0.22 *M*, being a M-subunit of creatine kinase). The 94,000 component comigrated with rabbit glycogen phosphorylase b on SDS-gel electrophoresis, contained 1 mole PLP/94,000 g protein, as determined fluorometrically. The 94,000 component showed phosphorylase b activity (inactive without AMP; spec. activity comparable to rabbit enzyme). The 160,000 and 94,000 components are bound in a glycogen complex and released by α-amylase treatment. The 160,000 protein migrated exactly with a sample of purified debranching enzyme (1,4 glucan-4-glucosyltransferase plus amylo 1.6-glucosidase) from rabbit on SDS-electrophoresis and showed comparable debranching activity.

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Serine-Uptake in Pigeon Optic Tectum

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After superficial intratectal injection of ^{3}H-serine, autoradiographies revealed specific labeling of perykaria in a subtectal nucleus suggesting a selective uptake in nerve terminals followed by a cellulipetal transport of label (Hunt et al., Exp. Brain Res., in press). – Labeled serine was taken up into tectal P_2 fractions by 'high' and 'low' affinity uptake systems. The apparent K_m was approx. 2×10^{-5} *M* and 10^{-3} *M* respectively. The high affinity uptake was strictly sodium and temperature dependent and was partly inhibited by glycine. On a linear sucrose gradient, the label is localised in the synaptosomal B-band. Hypoosmotic shock lead to a complete loss of taken-up label and uptake capacity. Addition of a 50fold amount of serine to a P_2 fraction incubated at 5×10^{-5} *M* serine lead to massive loss of labeled serine, suggesting that part of the uptake was due to an exchange mechanism. – These results support the existence of a selective uptake of serine in the tectum, presumably in nerve endings.

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Interaction of Bovine Myelin Basic Protein (MBP) with the Uptake of H^3-5HT in Rat Cortex Slices

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MBP was prepared from bovine spinal cord and purified by gel filtration. The MBP was tested for the inhibition of 5HT high-affinity uptake: Rat cortex slices were incubated with 1×10^{-7} *M* H^3-5HT in the presence of 1×10^{-7} to 1×10^{-4} *M* MBP. The tissue/medium ratios (t/m) after 10 min incubation at 25 °C showed a significant inhibition of 5HT uptake by 1×10^{-4} *M* MBP [controls ($n = 20$) 8.0 ± 0.5, MBP ($n = 3$) 6.3 ± 0.2, = −21%] and by 5×10^{-5} *M* MBP ($n = 4$, 6.9 ± 0.4, = −13%). No significant effect on t/m ratios was observed at lower MBP concentrations. – The incubated cortex slices were homogenized and fractionated on a 10-step discontinuous sucrose density gradient. H^3-5HT content was reduced significantly after incubation with MBP only in the synaptosomal fractions (at 1.1–1.3 *M* sucrose): MBP 1×10^{-4} *M*, −34%; MBP 5×10^{-5} *M*, −22%. At lower MBP concentrations the inhibition was not significant. In the cytoplasmic fraction, H^3-5HT content was increased 17% by MBP at 1×10^{-4} *M*, while the other MBP concentrations had no significant effect. Our results demonstrate an interaction of MBP at 1×10^{-4} *M* and 5×10^{-5} *M* with the transport of 5HT, mainly into presynaptic terminals.

Retrograde Axonal Transport of Adenosine in the Central Nervous System

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Anterograde axonal transport of adenosine and transfer to postsynaptic neurons has recently been demonstrated in rabbits by Schubert and Kreutzberg (Brain Res. *76*, 526, 1974). In an attempt to assess the secondary anterograde transport of labelled compound along the postsynaptic axon, ^{3}H-adenosine was injected into the pigeon eye, optic tectum and telencephalic wulst. Although confirming primary anterograde axonal transport followed by axo-somatic transfer onto the postsynaptic neuron we were unable to discover a significant labelling of axon terminals within the secondary system. Perikaryal labelling after ocular injection, however, was not only found within the contralateral tectum, but also within the n. isthmo-opticus, which projects upon the retina, and within the n. spiriformis lateralis, which projects upon the tectum but does not receive retinal or tectal input. Labelled material failed to reach the n. isthmo-opticus after sectioning the isthmo-optic tract. Thus, retrograde axonal transport in primary and after axo-axonal transfer in secondary neurons is likely to occur.

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Effects of some Biogenic Amines on the (Na^+K^+)-ATPase-System (K^+-pNPPase, Step 3)

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Membranous fractions of beef brain were prepared according to the method of Goldman (JBC 248, 867, 1972). This procedure enriched the activities of (Na^+K^+)-ATPase (per mg of protein) and K^+pNPPase (compared with other p-nitrophenylphosphatases) by about $^2/_3$. The activity of K^+-pNPPase may be measured both with and without ATP. The presence of ATP diminishes the rate of pNPP-splitting since the physiological substrate of the phosphorylated ATPase-molecule also takes part in the reaction. As in the (Na^+K^+)-ATPase itself, the K^+-pNPPase is also activated by catecholamines. The enzyme without ATP is not so significantly affected by these substances. The range according to the activation is the following: noradrenaline $\approx$ adrenaline $\approx$ isoproterenol $\approx$ tyramine $>$ dopamine. Histamine, serotonine, γABA, glutamate and glycine showed no activation of K^+-pNPPase under these conditions, nor did they influence considerably the raise of the enzyme due to catecholamines. Similar results have also been shown on the (Na^+K^+)-ATPase itself by Hexum (6th International Congress of Pharmacology, Helsinki 1975).

pH-Dependent Inactivation of Hepatic Phosphorylase Phosphatase by ATP

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The activation of hepatic glycogenolysis without concomitant enhancement of cyclic AMP formation may result from an inhibition of phosphorylase phosphatase. The glycogenolytic response of perfused rat livers to alpha adrenergic agonists and to an interference with cellular energy balance was previously suggested to occur by this mechanism (Biochim. Biophys. Acta *362*, 469, 1974 and *404*, 57, 1975). The effects of ATP, Mg^{2+} and pH upon three different preparations of partially purified phosphorylase phosphatase from rat livers were studied. Phosphorylase phosphatase activity was assayed by measuring the rate of inactivation of added phosphorylase *a* that was also purified from rat livers. Phosphorylase activity was measured by the rate of [U-^{14}C]-glucose 1-phosphate incorporation into glycogen. The inactivation of phosphatase by ATP was time- and pH-dependent. The same concentration of total ATP was less inhibitory at pH 8.5 than at pH 6.5. At the lower pH increasing concentrations of Mg^{2+} progressively suppressed the effect of ATP, suggesting that $ATPMg^{2-}$ was not inhibitory. The phosphatase activity was correlated with calculated ATP^{4-} and $ATPH^{3-}$ concentrations at pH levels of 6.5, 7.5 and 8.5. The pH dependence of the inhibition in the presence of constant amounts of total ATP may be accounted for, if $ATPH^{3-}$ is the inhibitory form of the nucleotide. However, inhibition by ATP^{4-} is not excluded if the K_i values vary with pH.

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Liver Metabolism Abnormalities Following Hypothalamic Lesions

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Liver metabolism of rats made hyperinsulinemic by bilateral lesions of the ventromedial hypothalamus (VMH) has been studied, using an in situ perfusion technique. Following hypothalamic lesions and adequate prevention of increased food intake, VMH-lesioned rats had hyperinsulinemia, hypertriglyceridemia, hyperuricemia and decreased plasma FFA levels. Glucose-induced insulin release was higher in WMH-lesioned than in control rats. Perfused livers from VMH-lesioned rats secreted more triglycerides, produced more urea but less glucose and ketones than control animals. Lipogenesis and lipogenic enzyme activities were higher in perfused livers from VMH-lesioned than in untreated controls. Fasting as well as anti-insulin treatment abolished partly these metabolic differences. It is concluded that hyperinsulinemia is responsible, in part, for the abnormalities of livers from VMH-lesioned rats.

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Inhibition of Clq Fixation to Immune Complexes (IC) by Amino Acids and Derivatives

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Diamino and dicarboxylic amino acids were found to be effective inhibitors of Clq fixation to immune complexes: lysine up to concentrations of 0.025 mg/ml, ornithine, glutamic and aspartic acid up to 0.25 mg/ml, pH and ionic strength being maintained at constant values. The finding that neither lysine-ethyl ester, nor ornithine-methyl ester, nor ε-aminocaproic acid produced an inhibition up to 2.5 mg/ml suggests a contribution of the two amino groups in the non-esterified compounds. The inhibition by the decarboxylated amino acids lysine and ornithine (1,5-diaminopentane, 1,4 diaminobutane) at concentrations as low as 0.01 mg/ml confirms this suggestion. – In a previous communication (Experientia *30*, 691, 1975) inhibitory activity was also demonstrated in derivatives of vitamin B6. The configurational relationship of the latter with lysine and ornithine indicates that a similar mechanism of action may be shared by these substances, the presence of an aldehyde in pyridoxal contributing to the stabilization by the formation of Schiff bases. – Studies on Clq fixation to IC containing tryptophanmodified IgG combined with the finding that tryptophan does not inhibit Clq-binding to normal IC (Allan and Isliker) show that tryptophan is not directly involved in the interaction of Clq with IC, but affects the conformation of the Clq-binding site in IgG.

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Partial Correlation between in vivo and in vitro Resistance to Insulin in Experimental Obesity

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Previous studies carried out in spontaneous obese mice have suggested that the concomitant presence of hyperglycemia and hyperinsulinemia could be due to a resistance of muscle to insulin. The present experiments were designed to investigate how insulin resistance develops following onset of obesity. Mice were made obese by injection of goldthioglucose (GTG). In vitro muscle metabolism, in vivo glucose tolerance test (GTT) and the ability of insulin to produce hypoglycemia were studied 2 and 12 weeks after onset of obesity. Two weeks after the beginning of obesity (GTG mice 48 g body weight; controls 38 g) GTT was identical in both groups; the in vivo insulin secretion produced by glucose was higher in GTG than in controls. In both groups hypoglycemic response to i.v. insulin was identical. Basal glucose metabolism of soleus muscles was identical in both GTG and lean mice, with a nearly normal response to insulin in GTG mice. After 12 weeks of obesity (GTG 65 g bw, lean, 43 g) GTT was abnormal despite a marked secretion of insulin. Insulin administration failed to lower blood sugar in GTG mice while marked hypoglycemia was elicited in controls. Soleus muscles of obese mice had lower basal glucose metabolisms, and exhibited insulin resistance that was not, however, sufficient to account for complete lack of effect of exogenous insulin. This study suggests that: (a) insulin resistance develops early following onse of obesity; (b) it must be a generalized phenomenon that cannot be explained on the sole basis of defects in muscles, other tissues being likely to be involved as well.

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Structure of Agglutinated Sheep Red Blood Cell Membranes

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Regularly stacked arrays of isolated sheep erythrocyte (SE) membranes of high water content are obtained by agglutination with phytohemagglutinin M (PHA) which are seen by electron microscopy. By X-ray diffraction lamellar reflections of an about 180 Å period with two asymmetric membranes are recorded. The membrane lipids produce an equatorially accentuated 4.6 Å ring; a lipid phase with chain axes preferentially oriented normal to the membrane is part of the structure, but the membrane profile differs from that of a simple lipid bilayer which is observed with dried membrane pellets. Diffraction from intact SE is dominated by the scattering of intracellular hemoglobin. The observed five broad diffraction rings are replaced by a series of sharp rings in patterns from agglutinated SE in hypertonic medium. By SDS-gel electrophoresis and titration large differences between cells of individual sheep are observed with regards to agglutinability, binding of hemoglobin and sheep serum albumin to membranes, and interference of albumin and PHA in the agglutination.

Coupling of Fructose-1,6-P_2 to Aminated Sepharose by Schiff Base Reduction. Affinity Chromatography of Yeast Aldolase

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The application of sugar phosphates as ligands in affinity chromatography of enzymes requires a coupling method that avoids hydrolysis of the labile phosphate ester bonds by, e.g., alkaline pH or higher temperature. A procedure complying with this precondition has been used to link the aldolase substrate fructose-1,6-P_2 to AH Sepharose 4B. This type of Sepharose contains primary amino groups on 6 carbon long 'spacers'. The Schiff base imine obtained from the amino group of the 'spacer' and the keto group of fru-1,6-P_2, the latter added in excess, was reduced with $NaBH_4$ to a stable secondary amine. Reduction 2 h after addition of the fru-1,6-P_2 gave maximum incorporation of 0.1 mmole ligand per g dry AH Sepharose 4B. The liganded Sepharose is stable in 1 *M* NaCl at pH 5.0 and 4 °C for 2–3 weeks. For the isolation of fru-1,6-P_2 aldolase from yeast a prepurified preparation (50 units/mg) free from phosphatase activity was applied onto a fru-1,6-P_2 Sepharose column (1000 units per g dry bed material corresponding to a third of its total capacity). Elution with a phosphate gradient (0–100 m*M*) yielded electrophoretically homogenous aldolase with a specific activity of 100 units/mg. Following chromatography the column may be regenerated for reuse by 1 *M* NaCl. Coupling of sugar phosphates to an insoluble matrix by Schiff base reduction might be adaptable to the isolation of other glycolytic enzymes.

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Demonstration of Two Separate Binding Sites for Insulin and NSILA-S in Perfused Rat Heart Muscle

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Nonsuppressible insulin-like activity of human serum (soluble in acid ethanol) (NSILA-S), has been purified and could be identified as a definite molecular entity of a mol wt around 7500. The purest preparation has an insulin like activity of 350 mU/mg as measured by net gas exchange of rat epididymal adipose tissue. – In isolated perfused rat heart, equimolar concentrations of insulin and NSILA-S elicit the same metabolic responses. They stimulate glucose uptake and lactate production and they increase the efflux of 3-O-methyl glucose from preloaded heart muscle. – I-125 labeled insulin and I-125 labeled NSILA-S bind specifically to heart muscle. Binding of both substances shows saturation in the same concentration range, where glucose uptake is stimulated maximally. Whereas it is possible to displace bound I-125 labeled insulin with unlabeled NSILA-S, the reverse is not observed. Dissociation of I-125 labeled insulin and I-125 labeled NSILA-S from heart muscle shows first order kinetics. The half lifes of the insulin acceptor complex and the NSILA-S acceptor complex are 17 and 26 min, respectively. – These data indicate,

that two separate binding sites for insulin and NSILA-S exist in rat heart muscle. The question, whether NSILA-S mediates its insulin like effects by binding to the insulin acceptor or to the NSILA-S acceptor, cannot yet be answered.

Isolation and Identification of a Juvenile Hormone (JH) in Termites

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Adult termite females (queens) of *Macrotermes subhyalinus* provide an opportunity to detect large amounts of gonadotropic hormone(s), since they lay about 40,000 eggs each day. In one sample 120,000 *Galleria* units of biological JH-activity/ml hemolymph were measured with the *Galleria* wax test. The half-life of endogenous JH-activity is about 5 h as judged from bioassays of hemolymph incubated in vitro. The active material was purified by extraction and 3 thin-layer chromatography steps. Each purification step was monitored with bioassays. The resulting active zone was subjected to high-pressure liquid chromatography on 2-μ-Porasil columns using hexane: ether 95:5. The active fraction was analyzed with combined gas chromatography–chemical ionization mass spectrometry and the material identified as JH-III [methyl(2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyldodeca-2,6-dienoate]. The bioassay indicated the presence of 2 to 5 μg JH-III/ml hemolymph.

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Hormone-Like Action of Ca^{++} in Adrenal Steroidogenesis

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In adrenocortical cells Ca^{++} can replace ACTH as first messenger if the cells derived from adrenals of hypophysectomized or Dexamethasone treated rats and isolated under appropriate conditions are preincubated in a specific ionic environment. The most important feature is the adjustment of the pCa between 6 and 7 at 37 °C and then at 0 °C. After this pretreatment calcium ions, preferably in the presence of P_i, are able to activate the cells inducing the formation of corticosterone similar to that due to ACTH or cycl. AMP. Data are presented suggesting that Ca^{++} is the second messenger for ACTH.

Light Chain Pattern of Developing Fast and Slow Rabbit Skeletal Muscles

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Extensively washed myofibrils of the rabbit soleus (slow) and gastrocnemius (fast) muscle have been subjected to SDS-polyacrylamide gel electrophoresis, in order to study the myosin light chain pattern during postnatal development. On the basis of the low molecular weight subunits it could be demonstrated that at birth the gastrocnemius contains exclusively fast type myosin with the light cahins LCf1 (mw ~ 25,000), LCf2 (mw ~ 18,000) and LCf3 (mw ~ 16,000). The light chain pattern of the neonatal soleus, however, is composed of LCs1 (mw ~ 27,000) and LCs2 (mw ~ 20,000) of the slow type myosin plus LCf1, LCf2 and LCf3, indicating that at birth the soleus contains both types of myosin. At this stage the proportion of slow to fast myosin was calculated as 1:2. During development fast myosin is gradually eliminated from the soleus, and at the age of 30 days virtually slow myosin only is seen to be present. Quantitative evaluation of the light chains of the gastrocnemius revealed that at birth the ratio of LCf1 : LCf2 : LCf3 is 17.8 : 64.2 : 18.5, which continuously changes to 33.7 : 50.8 : 15.6 in adult muscle. No such developmental change is observed for LCs1 and LCs2 of the soleus.

Syncatalytic Conformational Adaptations in Aspartate Aminotransferase Determined by Hydrogen Deuterium Exchange

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Conformational transitions of cytosolic aspartate aminotransferase occurring during catalysis have been probed by infrared spectrophotometric measurement of hydrogen-deuterium exchange, a method singularly sensitive to changes in protein motility. In the unliganded pyridoxal form of aspartate aminotransferase (pD 6.0, 20 °C) approximately 128 (31%) of the total 411 peptide hydrogen atoms per subunit exchange within 6 h. In the free pyridoxamine form of the enzyme an additional 5–6 hydrogens per subunit exchange. Formation of the (unproductive) enzyme-substrate adsorption complexes (pyridoxal form with 2-ketoglutarate and pyridoxamine form with glutamate) or of the covalent aldimine intermediate (pyridoxal form with the substrate analog 2-methylaspartate) does not markedly alter these exchange rates. However, in the presence of glutamate plus 2-ketoglutarate when an equilibrium of all covalent transamination intermediates is formed the number of hydrogens exchanging within 6 h is decreased by 17–18 hydrogens per subunit (4% of the total hydrogens). The data demonstrate unambiguously that the syncatalytic sensitization of Cys 390 toward chemical modification (J. Biol. Chem. *248*, 1751, 1973) is the result of a substantial structural change of the enzyme during substrate turnover and suggest that conformational transitions of the enzyme are an integral feature of aspartate aminotransferase catalysis.

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Subcellular Localization of Isoenzymes of Aldehyde Reductase and Dehydrogenase in Rat Brain

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A major NADPH-dependent isoenzyme 1 and a minor NADH- and NADPH-dependent isoenzyme 2 of aldehyde reductase have been isolated from rat brain (Eur. J. Biochem. *37*, 69, 1973). Based on the different coenzyme,

substrate and inhibitor specificity isoenzyme 1 was localized in the cytosol, isoenzyme 2 in mitochondria, isolated from brain homogenates or from isolated synaptosomes. A third isoenzyme was found in microsomes. Aldehyde dehydrogenase activity was determined with both NAD^+ and $NADP^+$ as coenzymes and propionaldehyde and *p*-nitrobenzaldehyde as substrates in subcellular and subsynaptosomal fractions. At least two cytosolic, two mitochondrial and one microsomal enzyme forms were discerned. Aldehyde dehydrogenase activity from 'glial' and 'synaptosomal' mitochondria differed with respect to their substrate and coenzyme specificity. In vitro NAD-dependent oxidation of *p*-nitrobenzaldehyde was inhibited about 80% by disulfiram in 'glial' mitochondria, and only about 15% in 'synaptosomal' mitochondria.

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Syncatalytic Exposure of Thiol Groups in Myosin and their Subunit Distribution

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The duplex myosin molecule consisting of 6 subunits, 2 heavy chains (HC) and 4 light chains, contains 2 active sites. Associated with each site are 2 essential thiol groups. Blockage of one such group per active site destroyes the K ion stimulated ATPase whereas both need to be blocked to inactivate the divalent cation stimulated ATPase. Out of the total of 42 thiol groups per myosin these 4 essential ones are the most reactive in the presence of Mg-ADP. With Mg-ATP, however, when hydrolysis takes place between 15 and 35 °C, an additional number of about 5 non-essential thiol groups becomes as or even more, reactive than the essential ones. Both types of thiol groups reside in the HC. The two enzymically active globular head portions of myosin which each contain a fragment of the HC (mol wt ca. 90,000) and a total of about 10 thiol groups, may be separated from the rod portion by limited proteolysis. The essential thiol groups, now 2 per head, are located in this HC fragment but become the most reactive thiol groups in such head preparations even in the presence of Mg-ATP. The non-essential thiol groups exposed during hydrolysis of Mg-ATP by intact myosin are not in the HC fragment of heads but are lost during digestion. The results indicate that these non-essential thiol groups probably reside in the region of the HC linking the heads with the rod portion of the myosin molecule.

Hydration of K-Region Epoxides Derived from Different Polycyclic Aromatic Hydrocarbons (PAH's)

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Mutagenic effects of epoxides metabolically formed from various PAH's have been observed. Such epoxides may also be responsible for the carcinogenicity of some of these compounds. The microsomal enzyme epoxide hydratase (E. C. 4.2.1.63) plays a critical role in the further metabolism of epoxides. To investigate the hydration of such reactive intermediates we developed an assay which allows a rapid and easy measurement of the epoxide hydratase activity with respect to K-region epoxides from several PAH's. Some intriguing correlations will be discussed such as benzo(a)pyrene 4,5-(K-region-)-oxide representing a much better substrate for epoxide hydratase than 3-methylcholanthrene 11,12-(K-region-)oxide which agrees with our earlier observation of epoxide hydratase modulators dramatically influencing the mutagenicity of bioactivated benzo(a)pyrene but not 3-methylcholanthrene. This assay also proved to be very sensitive thus allowing determination of epoxide hydratase activity in organs with very low enzyme levels such as lung and skin, which are known to be susceptible to carcinogenic PAH's. This method is therefore useful in the investigation of the mechanism of tumorigenesis evoked by PAH's in such organs.

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Antibody Formation in Mice as a Function of the Concentration of Various Pathological Immunoglobulins

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Cells of the plasmocellular tumors x5563 and x5647 producing myeloma proteins of the IgG and IgA class respectively were inoculated into syngeneic mice of the C3H/He strain. These mice were immunized with sheep red blood cells, bovine serum albumin or horseradish peroxidase. In following the immune responses of the various groups of mice, a considerable decrease in antibody activities was observed for all three antigens in mice which carried the IgG producing tumor, whereas mice with an IgA producing tumor manifested also a decrease but less pronounced. No interference at all was apparent in the formation of antibodies of the IgM class. The avidity of the various IgG antibodies was neither influenced by the IgG not by the IgA secreting myeloma tumors. Two mechanisms can be shown to be responsible for a decrease in antibody concentration in mice which bear either an IgG or an IgA plasmocellular tumor. First, the total number of plaques forming cells are decreased and second, the catabolic rate of IgG antibodies is changed and the half life becomes shorter by an increased concentration of IgG myeloma proteins; the effect of IgA myeloma proteins is less specific.

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Binding of Wheat Hemagglutinin to Carcinoembryonic Antigen (CEA)

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Analysis of purified preparations of carcinoembryonic antigen (CEA) by gas-liquid chromatography has shown that *N*-acetyl-D-glucosamine (GlcNac) is the major carbohydrate component of CEA. The binding of wheat germ hemagglutinin (WGA) to ^{125}I labelled purified CEA was demonstrated by adding increasing amounts of purified WGA to labelled CEA and by precipitating WGA with 50% saturated ammonium sulfate. In presence of an

excess of WGA, up to 95% of the labelled CEA was precipitated whereas only 6% was precipitated in the absence of WGA. The binding between CEA and WGA could be specifically inhibited by purified GlcNac or by different purified CEA preparations. It has been claimed by Banjo et al. that the immunodominant antigenic site of CEA is related to the glycosidic linkage found between GlcNac and the amide group of asparagine. Therefore, we tried to inhibit the binding of CEA to goat anti-CEA antibody, as demonstrated using a double antibody radioimmunoassay, by adding increasing amount of WGA. Preincubation of labelled CEA with an excess of WGA was unable to inhibit the binding of a limited amount of anti-CEA antibody, suggesting that the major antigenic site of CEA is not related to GlcNac. The absence of inhibition of WGA in the CEA radioimmunoassay may, however, be explained by the relatively weak affinity of WGA to GlcNac.

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Einfluss von Lipiden auf die Aktivität der Cerebrosid-Sulfotransferase

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Im ZNS spielt die Synthese von Sulfatid während der Entwicklung (Myelinisierungsphase) eine wichtige Rolle. Die Synthese dieses Sphingolipids im Maushirn beginnt am 8. Lebenstag, erreicht am 16. ihr Maximum und sinkt am 20. auf einen Grundwert ab. Die Aktivität eines an dieser Synthese beteiligten Enzyms, der Cerebrosid-Sulfotransferase (CST), zeigt dieselbe Altersabhängigkeit. Da in der gleichen Zeitspanne auch grosse Veränderungen im Lipidmuster des ZNS feststellbar sind, wurde der Frage nachgegangen, ob das Totallipid des Gehirns einen Einfluss auf die CST-Aktivität und damit auf die Sphingolipidsynthese allgemein haben kann. Mit kaltem Azeton delipidierte, mikrosomale CST-Präparationen werden tatsächlich durch die Zugabe des extrahierten Totallipids um etwa 100% stimuliert. Damit kann die nach der Delipidierung auf 38% des ursprünglichen Wertes abgesunkene Enzymaktivität wieder auf 80% des Ausgangswertes angehoben werden. Zudem hängt diese Reaktivierung vom der Lipidquelle ab: Aliquote Lipidmengen von 11- bzw. 16tägigen Maushirnen stimulieren die CST des ZNS einer 17tägigen Maus bedeutend stärker als solche von 7- bzw. 23tägigen Gehirnen. Es ist deshalb nicht auszuschliessen, dass das Lipidmuster auch in vivo einen Einfluss auf die CST-Aktivität und damit auf die Regulation des Sphingolipidstoffwechsels hat.

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Modification of Myelin Basic Protein: Effect on Lipid Protein Interaction

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The basic protein (BP) of CNS myelin has been shown to form complexes with acidic lipids in vitro. While it has been suggested that these complexes are stabilised by electrostatic bonds, no direct experimental evidence supporting this concept has been available so far. The BP contains a number of amino acid residues which are the prospective counterions in the binding of acidic lipids. In order to investigate further the mechanism of complex formation, selective acetylation of these amino groups was carried out using a purified BP from bovine brain (H. Fraenkel-Conrat, Methods Enzymol. *4*, 247, 1957). The acetylated BP migrated more slowly than the native BP on polyacrylamide gel at acid pH indicating the conversion to a less basic form. CD spectra measurements indicated that the predominantly coiled structure does not show any transformation into ordered structures upon acetylation. Complexes between the acetylated BP and acidic myelin lipids contained significantly less lipids than those with native BP. These results demonstrate the importance of free amino groups of BP in lipid protein interaction and support the concept that the acidic lipids interact electrostatically with these groups.

Male Hypogonadism in a Rodent. In vitro Testosterone Synthesis

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A defect of testicular endocrine function was postulated to explain many abnormalities of the testis and genital tract in *Ellobius* l. (Microtinae). In vitro studies of testosterone synthesis using ^{3}H-dehydroepiandrosterone and ^{14}C-androstenedione demonstrated a reduced activity of 17β-hydroxysteroid oxidoreductase. This is confirmed in experiments with ^{14}C-pregnenolone and ^{3}H-progesterone. Biosynthesis proceeds mainly through the Δ^{5}-pathway-(pregnenolone - hydroxypregnenolone - dehydroepiandrosterone). Moreover, a product having the same chromatographic properties as hydroxyprogesterone in five different systems and after derivative formation also accumulates, indicating an other possible enzymatic defect at this level, but further studies are needed to elucidate this point.

Sustained Oscillations in Mitochondrial Pyruvate Metabolism

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The dynamic behaviour of a simplified model of mitochondrial pyruvate metabolism was studied by computer simulations and stability analysis of the linearized model. The model contained the reactions catalyzed by the pyruvate dehydrogenase, the pyruvate carboxylase, the malate dehydrogenase, the citrate synthase and the oxidation of NADH in the respiratory chain. The stability behaviour of the system was investigated with a newly developed FORMAC program. This program algebraically calculates the characteristic equation of the model which was linearized about a steady state. These calculations revealed the existence of oscillatory and nonoscillatory critically stable states. Numeric integration of the non-linear model demonstrated the existence of limit cycles. The essential parameters to obtain these phenomena were the allosteric activation of the pyruvate carboxylase by acetyl-CoA, the rate of malate uptake by the mitochondria and the rate of NADH oxidation in the respiratory chain. The results showed that far from thermodynamic

equilibrium the stable nonoscillatory steady states can bifurcate into stable oscillatory and unstable nonoscillatory steady states. These singularities can coalesce into stable nonoscillatory states under special conditions.

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Properties of Soluble and Particulate Protein Kinases in Calf Ovaries

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Protein kinase (PK) activity was present in each of the 4 main subcellular fractions isolated from calf ovaries. The PK activity in the particulate fractions was stimulated only slightly by cAMP as compared to a 5fold stimulation in the cytosol. In the presence of 0.2% Triton X-100 the PK activity in the mitochondrial and microsomal fractions was increased 3fold, while little effect was observed in the cytosol and nuclear fractions. Following Triton-treatment 30–50% of the PK activity was solubilized from the particulate fractions. Sucrose gradient analyses of the Triton-solubilized fractions revealed that a major cAMP-independent PK peak sedimented at 3.5 S in all three fractions; the sedimentation value of the catalytic subunit of the cytosol cAMP-dependent PK. A cAMP-dependent PK peak was also observed in the microsomal fraction at 7.0 S. Similar substrate specificities were observed for the particulate fractions and the cytosol PK, with protamine being the best substrate. These results suggest a possible biochemical similarity between the PK in the particulate fractions and the catalytic subunit of the soluble enzyme. However the cAMP-independent PK activity in the particulate fractions could not be inhibited with an excess of the rabbit muscle inhibitor and therefore it would appear that these particulate activities are not derived from the soluble cAMP-dependent PK.

Regulation of Myosin Crossbridge Attachment to Actin

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The essential thiol groups which are most reactive in isolated myosin and whose blockage inactivates its enzymic function, are well protected from alkylation when the myosin crossbridges interact with actin as they do in rigor. The presence of Mg-ADP renders these essential thiol groups reactive again. Thus the binding of Mg-ADP to the crossbridges changes their rigor state conformation. This binding proceeds in two steps with equilibrium constants $K_1 = 3.32 \times 10^4\ M^{-1}$ and $K_2 = 6.61 \times 10^2\ M^{-1}$. Nevertheless the crossbridges remain attached to actin since in centrifuge studies at low ionic strength myosin sediments as a complex with actin. In the additional presence of the regulatory proteins (tropomyosin plus troponin) which form a complex with actin, the Mg-ADP mediated crossbridge interaction with actin, unlike the rigor state, becomes sensitive to trace amounts of Ca ions. In the presence of Ca ions the crossbridges remain still attached, whereas without Ca ions they detach and myosin may be separated from actin.

Partial Purification of Soluble High-Affinity Ca^{2+}-ATPase of Human Erythrocyte Membranes

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High-affinity Ca^{2+}-ATPase (adenosinetriphosphate-phosphohydrolase, EC 3.6.1.3), which has been solubilized in the presence of 1.0 m*M* diisopropylfluorophosphate (DFP) to prevent proteolytic digestion by membrane proteases, can be purified partially on a Sepharose 6B-CL column equilibrated with 200 m*M* K^+, 1 m*M* Mg^{2+}, 0.5 m*M* Ca^{2+}, 10 m*M* MES-buffer, 10 m*M* TES-buffer (pH 6.9), 0.1 m*M* DFP and mixed micelles composed of Triton X-100 and crude phosphatidylcholine in a molar ratio of 2. The activity appears after an elution volume corresponding to a mol wt of approximately 700,000. The specific activity of the partially purified enzyme amounts 6.5 U/mg protein. In SDS-gelelectrophoresis the subunit pattern of the active material consists of several bands including a doublet of mol wt $\approx$ 140,000, which can be phosphorylated by γ-^{32}P-ATP. The phosphorylation occurs in the presence of 50 m*M* Na^+, 20 m*M* K^+, 1 m*M* Ca^{2+}, 0.1 m*M* Mg^{2+}, and 10 μ*M* ATP at pH 7.5. It can be prevented almost totally either by 1 m*M* ATP, by an excess of EGTA or by hydroxylamine. There is no indication that the high-affinity Ca^{2+}-ATPase is in part or totally identical with spectrin. – The properties of the partially purified enzyme do not differ essentially from those of the membrane-bound enzyme with respect to its K_m-value, Ca^{2+}-dissociation constant, pH-profile, activation by Na^+, K^+, NH_4^+, and Rb^+, and its sensitivity to chemical modifiers.

PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

La spectrométrie d'émission dans l'étude du métabolisme des médicaments marqués à l'azote 15

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La technique de spectrométrie d'émission permet de déterminer l'enrichissement en azote 15 des molécules marquées par cet isotope stable. Elle est basée sur la différenciation du spectre d'émission des molécules $^{14}N^{14}N$, $^{14}N^{15}N$, $^{15}N^{15}N$ à 297,68; 298,20 et 298,86 nm respectivement. La technique de Dumas modifiée et l'utilisation de gaz rares tels que l'Hélium et le Xénon permettent la détermination d'enrichissement dans les limites de 0,37 à 99% sur des quantités de 0,1 à 5 µg d'azote. Par dilution isotopique, il est possible de quantifier 2 à 5 µg d'azote avec une précision de l'ordre de 8%. – En tant qu'exemple d'application dans le métabolisme des médicaments, le phénobarbital-^{15}N a été synthétisé et mesuré au niveau sanguin chez le rat. Dans l'état actuel

de nos travaux, la détermination des molécules marquées à l'azote 15 s'effectue après leur séparation par des techniques chromatographiques. La principale difficulté de cette application est la contamination par l'azote extérieur et l'azote présent aux différents niveaux biologiques. Cette technique n'atteint pas la sensibilité des molécules marquées au carbone 14 mais permet par contre l'utilisation d'isotopes stables chez l'homme sans problème éthique.

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Cyclosporin A: A New Antilymphocytic Agent

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The metabolite cyclosporin A derived from 2 fungus species represents a novel antilymphocytic agent. It is a small molecular weight ring-peptide. Cyclosporin A suppresses humoral as well as cellular immunity in several animal models. It also inhibits specifically in vitro proliferation of murine and human blood lymphocytes. This compound is furthermore highly effective in the experimentally induced polyarthritis in rats, but not in acute inflammation. It is orally active and its effects are comparable to those obtained with appropriate reference compounds. In contrast to other immunosuppressive agents and to cytostatic drugs, the weak side effects on the haemopoietic tissues suggest that its action may be directed mainly towards the immunocompetent lymphocytes. It is speculated that cyclosporin A interferes at an early stage of mitogenic stimulation of the lymphoid cells.

The Role of Parietal Cells for the Gastrotoxicity of Salicylates

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Basing on the clinical evidence which implicates parietal cells in the development of gastric side effects of salicylates we performed the following studies: (a) Morphological studies: Electron microscopical evaluation of gastric mucosa of rats revealed early and preferential damage of parietal cells occurring already minutes after oral administration of acetylsalicylic acid. (b) Biochemical studies: Measurement of radioactivity in samples of rat gastric mucosa 1 min after administration of C^{14} labelled acetylsalicylic acid revealed about 4 times higher concentrations in parietal cells containing areas as compared to samples devoid of parietal cells. – From these and other results it is concluded that parietal cells which are unprotected by mucus may serve as an entrance for (non-ionized) acetylsalicylic acid. They trap these molecules, get destroyed and open a gateway to further tissue damage.

The Influence of Intracerebral Cyclic AMP on Drinking Responses to Angiotensin (AT)

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Enhancement of drinking by intrahypothalamic or systemic beta-adrenergic agents and the second messenger role of cAMP at other beta-adrenergic effector sites suggested an investigation of the role of cAMP. – When injected unilaterally into the preoptic brain area of water-satiated rats neither cAMP (non-acylated; 0.004 to 4.0 µg), nor the phosphodiesterase inhibitor theophylline (0.002 to 2.0 µg), nor both agents combined elicited any drinking response. When injected 15 min after 2.5 mU of partially purified hog renin, cAMP (4 µg) significantly increased the drinking response from 5.1 ± 0.9 to 7.4 ± 1.1 ml/3 h ($n = 17$); similarly water intake after 125 ng AT II-amide rose from 2.3 ± 0.5 ml to 4.2 ± 1.0 ml/3 h ($n = 19$). Neither systemic (i.p.) nor intracerebral theophylline (2 µg: THE) influenced drinking to AT II. Paradoxically, intracerebral cAMP plus THE injected 2 min before 125 µg AT II depressed the drinking response from 4.0 ± 0.9 to 1.0 ± 0.4 ml/3 h ($n = 16$). I.p. THE (75 mg/kg) injected 30 min before access to water increased drinking in rats kept thirsty for 21 h from 16.3 ± 0.6 to 18.1 ± 1.0 ml/3 h.

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Effect of *p*-Nitrophenyl Diazonium Fluoroborate on Cholinergic Mechanisms

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Electrophysiological experiments were done to investigate the effect of *p*-nitrophenyl diazonium fluoroborate (*p*-NPD) on the motor endplate of the frog. When added to the bath *p*-NPD stabilizes the postsynaptic membrane. Longtime iontophoretic ejections of *p*-NPD produce a biphasic effect: initially a potentiation of the depolarization due to ACh and subsequently an inhibition. When the AChE is inactivated previously only the inhibition is demonstrable. Ionophoretic ejections of *p*-NPD on cholinergic neurons in the hippocampal cortex of the cat produce also a biphasic effect on ACh-induced activity. – The association constant of *p*-NPD to purified AChE and to membrane fragments of electroplax was determined biochemically. Its affinity to the AChE is about 20 times greater than to the ACh-receptor. – The biphasic effect seems to depend on the capacity of *p*-NPD to combine with both the AChE and the ACh-receptor. The AChE is first inhibited thereby potentiating the ACh-response. The ACh-receptor is then inhibited too thereby blocking the postsynaptic excitability.

Differential Effects of Neuroleptic Drugs on Hyperactivity and Stereotyped Behaviour Induced by D-Amphetamine in the Rat

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Low and high doses of D-amphetamine induce two distinct types of behavioural excitation: hyperactivity and stereotypies. These are thought to be mediated by

dopaminergic receptors situated in mesolimbic and nigrostriatal areas respectively (Kelly et al., Brain Res. *94*, 507–522, 1975). Several neuroleptics and related drugs were assayed for their inhibitory action against these two types of behaviour in order to determine whether one or the other behaviour will be inhibited preferentially. Differences in the potencies of inhibitory actions (expressed in terms of ED_{50}) were found among different neuroleptics. Haloperidol was markedly more active in suppressing stereotypies than hyperactivity. Chlorpromazine showed almost equal activity in respect to both behaviours, whereas clozapine and sulpiride were more potent in inhibiting the hyperactivity. Promazine and baclofen (Lioresal®) failed to block stereotypies specifically, but reduced the hyperactivity dose-dependently. These results indicate that a pharmacological distinction of preferential site of action of neuroleptics in the brain is possible.

The Effect of Diphosphonates on Cartilage Cells in Culture

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Diphosphonates, compounds related in structure to pyrophosphate but resistant to metabolic destruction, prevent calcification of soft tissues and resorption of bone in vivo. Previously these effects were attributed to physicochemical interactions. To test the possibility of altered cellular activity, the effect of two diphosphonates, disodium dichloromethylene diphosphonate (Cl_2MDP) and disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP), on glycogen metabolism of cartilage cells in culture was studied. Both Cl_2MDP and EHDP increased the amount of glycogen in cells. Cl_2MDP was more potent, however, and at 0.25 m*M* also decreased consumption of glucose. Production of lactate was decreased by Cl_2MDP both in absolute amounts and in relation to glucose consumed. EHDP had no such effects. Under N_2, these changes in glucose utilization and lactate production caused by Cl_2MDP were not observed. These results indicate that Cl_2MDP may alter the metabolism of the cartilage cell such that the energy source, glucose, can be used more efficiently.

The Effect of Diphosphonates on Bone and other Cells in Culture

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Diphosphonates characterised by a P-C-P bond are known to interfere in Ca metabolism. The classical explanation lies in its effect on apatite formation and dissolution. However some indirect evidence points to some influence on cellular mechanisms. Therefore the effect of disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) and disodium dichloromethylene diphosphonate (Cl_2MDP) on the growth of cultured cells obtained by digestion with collagenase from rat calvaria, rat skin and rabbit ear cartilage was studied. Both compounds had no influence as long as the cells were in the log-phase. In the plateau where the cells were still slightly growing Cl_2MDP decreased the number of cells in the case of rat calvaria and rat skin, but not in the case of rabbit ear cartilage. EHDP had no marked effect. Preliminary studies have shown that Cl_2MDP interferes in the glucose metabolism.

Nuciferine as an Antagonist of Excitant Amino Acids in Pigeon's Optic Tectum

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Nuciferine (1-5,6Dimethoxyaporphine) an alkaloid with depressant central nervous effects has been shown to antagonize the excitant action of L-glutamate on neurones of the feline cuneate nucleus and to block the synaptic evoked activity in thalamocortical relay cells (Ben-Ari and Kelly, J. Physiol. *251*, 25P, 1975). Since L-glutamate may play an important role as an excitant amino acid in the pigeon nervous system we were interested to see whether such an antagonism could take place on neurones of the optic tectum. The glutamate evoked firing of ganglion cells was antagonized by nuciferine in 85% of tested neurones. An interaction with L-aspartate although to a weaker extent was observed in 75% of cells. When log-dose-response curves were constructed, the curve relating excitation was shifted to the right, thus indicating that the interaction of glutamate and nuciferine was competitive. Preliminary results with acetylcholine showed no comparable antagonism, suggesting that nuciferine is a reasonably good tool investigate the nature of excitatory amino acid transmitters within the pigeon optic tectum.

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Tolerated Doses of an Extract from *Amanita phalloides* and Ethanol Protect against Lethal Doses of the Mushroom

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In continuation of previous work reporting a protective effect of hepatotoxic agents such as carbon tetrachloride on the toxicity of the death cap poison phalloidin (G. L. Floersheim, Biochem. Pharmac. *15*, 1589, 1966), it was shown that tolerated doses of phalloidin itself protect mice against lethal doses of this poison. Furthermore, it was demonstrated that pretreatment with repeated tolerated doses of a lyophilisate prepared from the whole mushroom *Amanita phalloides* (APL) protect mice against a lethal challenging dose of the mushroom. An immunological mechanism was excluded to account for the resistance. Single tolerated doses of APL as well as prophylactic application of ethanol failed to confer tolerance to a lethal challenge of the mushroom. However, after a combined pretreatment with APL and ethanol, mice became resistant against a lethal dose of *Amanita phalloides*.

Micropuncture Study of Fluid Handling in the Cat Kidney

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Renal tubular function in cats was studied by free-flow micropuncture. Domestic cats of either sex (1.2–2.5 kg) were anesthetized with pentobarbital (40 mg/kg i.p.) and infused with 2.5% polyfructosan in Ringer's solution at 0.45 ml/min. Either left or right kidney was prepared for micropuncture. One kidney GFR averaged 3.9 ± 0.2 (SEM) ml/min ($n = 7$). Superficial single nephron GRF (SNGFR) was 15.0 ± 0.67 nl/min ($n = 42$): proximal collection. A significant correlation ($r = 0.90$, $p < 0.005$) was found between the mean value of SNGFR in individual cats (14.7 ± 1.2 nl/min) and their one kidney GFR. Values for SNGFR estimated from one kidney GFR and the reported 2.15×10^5 glomeruli per kidney (E. M. Renkin and J. P. Gilmore, in *Handbook of Physiology, Section 8: Renal Physiology*, 1973, p. 229) were some 9–25 nl/min. Absolute fluid reabsorption was 1.90 ± 0.26 nl min/mm tubular length ($n = 11$): the distance from glomerulus to point of punture was measured by latex localization and dissection. Proximal fluid samples were isotonic (TF/Posm 1.02 ± 0.01, $n = 19$), whereas fluid entering the distal tubule was hypotonic (TF/Posm 0.56 ± 0.10, $n = 3$). Late distal samples appeared to be isotonic (TF/Posm 1.03 ± 0.03, $n = 4$).

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Biochemical Response of Dopaminergic (DA) Nerve Cells to Acute Changes in Activity

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Quantitative histochemical studies have revealed a close correlation between unit activity and fluorescence intensity of nigral DA nerve cell populations in rats (Lichtensteiger, Lienhart, Hefti and Felix, Exp. Brain Res. *23*, Suppl., 124, 1975). The biochemical nature of this reaction was investigated, using nicotine and cold exposure as stimulatory and gammabutyrolactone as inhibitory agents. DA and DOPA were both determined by enzymatic-isotopic assays in tissue cylinders of standardized topographical position containing mainly zona compacta. The absence of a detectable change in total DA and DOPA concentrations of substantia nigra after nicotine (1 mg/kg s.c., 30 min) or cold exposure (4 °C), when histochemical fluorescence intensity increases markedly, suggested that the biochemical processes underlying the histochemical response would be more complex. Synthesis rate in the DA nerve cell area was studied by DOPA accumulation after decarboxylase inhibition. Possible subcellular changes in the nigral DA neuron group were studied by two miniaturized differential centrifugation techniques and compared with the subcellular distribution observed in the nerve terminal area of caudate-putamen.

The Effects of the Ionophore X-537A (Lasalocid) on the Heart and on Vascular Smooth Muscle

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The properties of a calcium ionophore and a positive inotropic action are attributed to the antibiotic X-537A (Lasalocid, Ro 2-2985). In isolated perfused cat hearts, X-537A (3×10^{-8} to 10^{-6} *M*) increased coronary flow as well as rate and force of contraction. These effects were abolished by destruction of cardiac adrenergic nerve endings or by blockade of cardiac β-adrenoceptors. Higher concentrations of X-537A (3×10^{-6} to 10^{-4} *M*) produced cardiac arrest in diastole followed by a slowly developing contracture; the rate of rise of the contracture was linearly related to the logarithm of the concentration of X-537A. The development of contracture did not depend on intact adrenergic nerve endings or β-adrenoceptors. In strips of adrenergically denervated rabbit main pulmonary artery, X-537A (3×10^{-5} to 3×10^{-4} *M*) produced depolarization of the smooth muscle cells and contraction. Only the latter was absent after omission of $[Ca]_0$. It is concluded that low concentrations of X-537A release catecholamines and that in higher concentrations X-537A transports both mono- and divalent cations.

Central Dopaminergic Stimulation and Avoidance Performance under Hypoxia in Rats

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In rats trained to work in a Sidman avoidance schedule, reduction of O_2-content of inspired air from 21% (normoxia, NO) to 9% (hypoxia, HO) reduces the number of avoidance lever presses (ALP) from 163 ± 23 to 92 ± 10 per 20 min. Similar performance decrements under hypoxia have been suggested to be related to inhibition of dopaminergic transmission in the CNS (Brown et al., Brain Res. *85*, 491, 1975). L-Dopa (100 mg/kg i.p.) with 50 mg/kg i.p. benserazid hcl significantly increased ALP both under NO (+ 118 ± 38) and HO (+ 189 ± 26) conditions. Similarly, after 0.5 mg/kg i.p. *d*-amphetamine the number of ALP was significantly enhanced by + 44 ± 12 and + 47 ± 9 under NO and HO conditions. Apomorphine hcl (5 mg/kg i.p.) reduced NO-ALP (− 33 ± 57), but enhanced HO-ALP (+ 148 ± 62) significantly. Bromocriptin mes (CB 154, 2-bromo-α-ergokryptine) or dihydroergotoxine mes (Hydergine®), at a dose of 3 mg/kg i.p.., did not increase the number of ALP under NO. Under HO, either compound significantly increased ALP by + 32 ± 11 and + 22 ± 5, respectively. – These results indicate that central dopaminergic stimulants in rats partially restore behavioral depression induced by a lowering of the O_2-content of the inspired air.

Decline of the K^+-Induced cAMP Accumulation in Decentralized Superior Cervical Ganglion

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The concentration of cyclic AMP in the superior cervical ganglion of the rabbit increases when the tissue is exposed for short periods to depolarizing media. This increase is not affected by agents that are known to potentiate or to antagonize the effects of acetylcholine. – It was found that the depolarization-induced cAMP accumulation did not occur in ganglia that had been decentralized prior to isolation and K^+-stimulation of the tissue. When the preganglionic nerve was severed one day before the experiment the response to high K^+ was reduced, it declined gradually during the second and the third day after the operation. Similarly, the increase of the cAMP level due to high potassium can be reduced in intact ganglia by preincubation of the tissue in glucose-free Locke solution. After 3 h of incubation the response to depolarizing media starts to decline, it is abolished after approximately 7 h. – Both procedures are known to bring about degeneration of the presynaptic terminals in the ganglion and in both cases the damage develops with a time course closely resembling that of the observed decreases of the cAMP response. The effect of depolarization on the cAMP level does thus appear either to be confined to the presynaptic terminals or to be dependent on continuous presynaptic activity.

Induction du métabolisme in vitro des Stéréoisomères de l'amphétamine-^{14}C chez le rat

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Des rats Wistar mâles adultes ont été traités pendant trois jours par du phénobarbital dans l'eau de boisson à raison de 100 mg/kg/jour. Les microsomes hépatiques ont été obtenus par centrifugation fractionnée et utilisés pour l'étude in vitro du métabolisme de la (+)-, de la (−)- et de la (±)-amphétamine-^{14}C. Les métabolites formés ont été séparés par des méthodes chromatographiques et quantifiés par la mesure de leur radioactivité. – Le traitement au phénobarbital a pour conséquence d'augmenter la quantité du principal métabolite, la *p*-hydroxyamphétamine, par rapport à celle trouvée chez les animaux témoins. Pour la (+)-amphétamine, cette augmentation est faible, tandis que pour l'isomère (−) elle est de l'ordre de 30%. – La *p*-hydroxylation de la (−)-amphétamine est plus importante que celle de l'isomère (+), aussi bien chez les animaux témoins que chez les animaux traités au phénobarbital. Cette stéréosélectivité est particulièrement évidente dans le dernier cas. – En outre, la β-hydroxylation in vitro de la (+)-*p*-hydroxyamphétamine en *p*-hydroxynoréphédrine est deux à trois plus élevée que celle de l'isomère (−). – Il s'avère donc que le phénobarbital induit de manière modérée le métabolisme in vitro de l'amphétamine.

Stereoselective Hepatic Metabolism of D- and L-Nirvanol® as a Research Tool

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Biotransformation of drugs is known to occur mainly in the microsomal enzyme system of the liver. Investigation of hepatic factors influencing metabolism of different drugs in vivo, however, is complicated by physicochemical dissimilarities simultaneously influencing extrahepatic handling of these compounds. The enantiomers of D- and L-phenyl-ethyl-hydantoin (PEH, Nirvanol®) having identical physicochemical properties were, therefore, studied in 4 boxer dogs after i.v. injection of 20 mg/kg of each enantiomer according to a cross over design. The enantiomers, separated in our laboratory, were at least 95% pure. Plasma levels and urinary concentrations of PEH and of its major metabolite, hydroxy-PEH (HPEH), were determined by GLC. Despite similar average volumes of distribution (D: 936 ml/kg, L: 937 ml/kg) plasma half lives were 16.3 and 23.3 h for D- and L-PEH and the metabolic clearances 15.8 ± 0.9 (SEM) ml/min and 10.3 ± 0.5 ml/min respectively. Within 5 days urinary output of D-HPEH amounted to 25 ± 3.6% of the dose after D-PEH and 3.9 ± 0.2% after L-PEH. These data may be explained by preferential aromatic hydroxylation of D-PEH. Consequently, the use of enantiomers allows the study of different rates of hepatic drug metabolism in vivo of otherwise physico-chemically identical compounds. Stereoselectivity, therefore, appears to be an interesting research tool for investigation of drug metabolism at the molecular level.

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Effect of Diazepam and Oxprenolol on the Conditioned Emotional Response in the Pigeon

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Estes and Skinner (1941) reported an experimental procedure to develop a state of anxiety in the experimental animal and to monitor the effects of this on the normal behavior of the organism. The present study was undertaken to investigate the influence of diazepam and oxprenolol to alleviate the schedule-induced suppression on the retention of this response in pigeons. After 12 sessions of conditioning and stabilisation, diazepam was administered in the first group of 6 pigeons at doses of 0.50, 1.0, 2.0 and 1.50 mg/kg, 30 min before each session, at a maximum rate of 2 treatments a week and in a random order. The diazepam treatment was followed by oxprenolol in the same manner at doses of 5.0, 10.0 and 20.0 mg/kg. In group II ($n = 6$) treatments were reversed, i.e. oxprenolol was administered before diazepam. Every animal was used as its own control: the average performance on the day preceeding drug treatment (saline treatment) was compared with the performance following treatment. The results showed, that in 5 of 6 birds (group I), diazepam produced a disinhibition of the conditioned suppression, not significant with 0.50 mg/kg, but quite clearcut with 1.0, 2.0 and 1.50 mg/kg. In group II there was a slight disinhibition only in 2 of 6 birds at 2.0 mg/kg. Oxprenolol did not show this effect.

Glucocorticoids as Modulators of Trans-Synaptic Enzyme Induction in Organ Cultures of Rat Sympathetic Ganglia

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After in vivo experiments (Proc. Nat. Acad. Sci. *72*, 1415, 1975) had provided indirect evidence that glucocorticoids act as modulators in trans-synaptic enzyme induction this aspect was studied in more detail in organ cultures of rat sympathetic ganglia. It was shown that the selective induction of tyrosine hydroxylase (TH) and dopamine β-hydroxylase is mediated via nicotinic receptors and that an induction was also possible in organ cultures of sympathetic ganglia originating from animals which had been adrenalectomized 2 weeks before. However, the selective enzyme induction was markedly potentiated by glucocorticoids in a dose-dependent manner. Under these conditions the time of exposure to nicotinic agonists, necessary to obtain maximal TH induction after 48 h, could be reduced from 4 h to 5–10 min. Thus, the primary factor in trans-synaptic enzyme induction is the cholinergic transmitter acetylcholine acting via nicotinic receptors and glucocorticoids play an important modulatory role.

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Benzodiazepines and Spontaneous Activity of Cerebellar Purkinje Neurons (PN)

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In rats immobilized with tubocurarine and respirated with air enriched to 50% oxygen, with a normal arterial pO_2, the spontaneous firing of PN from the vermian lobules VI–VIII was recorded. Chlordiazepoxide 3 mg/kg i.v. did not decrease PN discharge rate, whereas at the dose of 10 mg/kg i.v. a clear-cut reduction was obtained. Diazepam 2 mg/kg i.v. induced a comparable reduction in intensity and duration of PN firing; the dose of 0.1 mg/kg i.v. was only slightly effective, whilst in rats respirated with oxycarbon, giving an extremely high arterial pO_2, this dose markedly abated PN activity. In both cases the range of firing frequency and the average mean rate were the same. It seems therefore that in hyperoxemia diazepam is more potent than in normoxemia. Assuming an interaction of benzodiazepines with GABA-ergic transmission to explain their effect on PN firing, the observed discrepancy in potency might be due to differences in GABA content and metabolism in the cerebellum, since GABA is known to be decreased in the brain of rats exposed to oxygen at high pressure.

Degradation of Insulin by Plasma of Rats with *N*-Monomethylacetamide-Induced Diabetes

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In NMMAA-induced diabetes, the plasma IRI concentration has been shown to rise to levels corresponding to increased blood glucose concentration (BG), while the plasma suppressible insulin-like activity (SILA) measured by the fat-pad assay, remained low (R. Guidoux, Diabetologia *5*, 11, 1969). The new data presented show that: (1) Crystalline pork insulin incubated at 37 °C for 2 h with plasma from NMMAA-poisoned rats progressively looses both its immunological and biological properties: the rate of decrease of both IRI and SILA is proportional to the BG of the donor rats. (2) Insulin added to plasma from NMMAA-poisoned rats just before an immunoassay is fully recovered, while insulin added just before a fat-pad assay is only partially recovered. We conclude that plasma from NMMAA-treatment rats contains a system which destroys insulin at 37 °C (condition of the bioassay) but not at 0 °C (condition of the immunoassay). The apparently low levels of SILA found in NMMAA-induced diabetes in rats presumably are an artifact due to destruction of plasma insulin during bioassay incubation at 37 °C. The relationship of the occurrence of an insulin-degrading system in the plasma from NMMAA-poisoned rats to the development of hyperglycemia remains to be defined.

Fluorimetric Determination of Tetracyclines in Biological Materials

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The method is based on solvent extraction of mixed tetracycline-calcium-trichloroacetate ion pairs from aqueous solutions. Extraction of TC and CTC is almost quantitative whereas only very poor extraction occurs with OTC. However, about 70% of the OTC can be extracted after addition of salicylate ions. Saturation of the aqueous phase with sodium chloride results in 100% extraction into the organic phase. Based on this effect, OTC can be determined besides TC and CTC. Ethylacetate was found to be the most suitable extractant. Fluorescence of the organic phase is measured after addition of magnesium ions and a base. Emission maxima are at about 500 nm, excitation maxima at 400 nm. They differ only very slightly between the 3 tetracyclines. In biological materials, the following detection limits for CTC were found: serum 0.05 µg/ml, muscle tissue 0.125 µg/g, kidney 0.15 µg/g, liver 0.3 µg/g, fattened milk powder 2 µg/g. Recovery values are between 30–45%. Losses occur during the protein precipitation step.

Is Baclofen an Antagonist of the Excitatory Transmitter in the Cat Cuneate Nucleus?

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In decerebrate cats, the GABA derivative baclofen given intravenously induced the following dose-dependent effects on the synaptic transmission in the cuneate nucleus: presynaptic inhibition, assessed by the P wave, the dorsal column reflex and the increase of excitability of primary afferent endings evoked by volleys in the median nerve, was reduced after 1 mg kg^{-1} and abolished after 10 mg kg^{-1}. Baclofen did not affect the resting excitability of cuneate primary afferent terminals. Within

the same dose range, baclofen depressed the N wave elicited by volleys in the median nerve, which is indicative of orthodromic excitation of the cuneo-thalamic relay (CTR) cells, and reduced the resting excitability of CTR cells. These results do not support the view that baclofen acts as a GABA agonist, but rather suggest that baclofen inhibits the release of the excitatory transmitter or blocks its receptors in the cat cuneate nucleus.

Accumulation of Cyclic AMP in Rabbit Vagus Nerve by Fluorine and Adenosine

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The effects of some pharmacological agents on the content of cAMP were studied in desheathed vagus nerves. – Application of sodium fluoride (10 m*M*) or adenosine (100 μ*M*) during 6 min produced a 180% increase in the level of cAMP. In presence of 0.9 m*M* $CaCl_2$, 5 m*M* fluoride was inactive, but when calcium was omitted, 0.1 m*M* fluoride raised the cAMP level to about 150% of controls. An inhibitor of phosphodiesterase, theophylline, is known to inhibit the effect of adenosine on adenylate cyclase in the brain but not in the superior cervical ganglion. In our system 10 m*M* theophylline inhibited both the fluoride and adenosine effects. Changes in the cAMP content have been reported in several nervous tissues after application of drugs or electrical stimulation, but so far no similar effects have been observed in peripheral nerves.

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Serotonin (5HT) Depletion in Rat Thrombocytes (T) by *P*-Chloro-D,-L-metamphetamine (PCMA)

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Maximal 5HT depletion (> 90%) was obtained in rat T 16 h after i.v. injection of 35 mg/kg PCMA or 0.5 mg/kg reserpine (R). With both drugs recovery to normal levels occurred with a similar time course in about 7 days. Intestinal 5HT or T adenosine-5′-triphosphate (ATP) were unchanged by PCMA (70 mg/kg i.p.). In vivo, PCMA was unable to reduce the radioactivity of T loaded with ^{3}H-PCMA, and in vitro PCMA, tyramine and R did not lower the radioactivity of T isolated from rats injected with 35 mg/kg ^{3}H-PCMA 15 h before exsanguination. The disappearance of the radioactivity from T loaded with ^{3}H-PCMA (35 mg/kg i.v.; about 90% decrease in 3 days) was more rapid than that of T labelled with ^{3}H-R (0.5 mg/kg; about 6 days for a 90% decrease). It is concluded that (1) PCMA, like R, causes a long-lasting decrease of 5HT not due to unspecific damage, since ATP is normal in T, (2) PCMA seems to liberate directly 5HT from the T and to induce an irreversible impairment of 5HT storage, (3) the 5HT-depleting effect is possibly due also to some metabolite(s) of PCMA.

Maze Familiarity and the Effect of Nicotine on Maze Exploration

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In an earlier study (Bättig et al., Pharmac. Biochem. Behav., accepted for publ.) nicotine (0.2 mg/kg, 30 min injection test interval) was found to stimulate exploratory behavior only after several successive maze exposures. The present study was undertaken to see whether this delayed effect of nicotine might be due either to a development of tolerance against possible depressive effects of nicotine or to the nonfamiliarity with the maze during the first runs. Four groups of 9 female RHA rats each and 5-month-old received differential treatments for the first and second block of 6 experimental days each. The conditions were for group 1 saline injections and maze exposures for both blocks; for group 2 saline injections–maze exposures and then nicotine injections–maze exposures; for group 3 saline injections and then saline injections–maze exposures and for group 4 nicotine injections and then nicotine injections–maze exposures. The results showed, that previous nicotine injections did not abolish the delay of the onset of nicotine stimulation were as with preliminary maze exposures subsequent nicotine injections produced an immediate onset of stimulation.

Effects of Antiparkinsonian Drugs on cAMP Accumulation in Rabbit Retina in vitro

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Dopamine or apomorphine, each at 5×10^{-7} to 10^{-4} *M* concentration increased the cAMP content of intact retinae of the rabbit or the cAMP production in retinal homogenates (Biochem. Pharmac. *23*, 3079, 1974; Naunyn-Schmiedebergs Arch. Pharmak. *288*, 103, 1975). The accumulation of cAMP in response to maximal doses of dopamine or apomorphine was blocked by 5×10^{-4} *M* haloperidol, chlorpromazine or fluphenazine. Similar studies were undertaken with clinical or potential antiparkinsonian drugs, which are supposed to mimick the agonist at dopamine receptor sites of the CNS. L-dopa or *N*-methyldopamine, each at 10^{-4} *M* concentration, were found to be as effective as dopamine by producing a 2.5fold stimulation of cAMP content in intact retinae. Similar increases were observed after exposure to 10^{-4} *M* ergometrine, agroclavine or CB-154, detectable effects being already observed at 10^{-6} *M* concentration. Furthermore, the dopaminomimetic effects, at 10^{-4} *M*, were blocked by 5×10^{-4} *M* fluphenazine. These results suggest that isolated retinae of the rabbit may be useful for testing in vitro interaction of drugs with postsynaptic receptors for dopamine.

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Role of Gangliosides in Retrograde Axonal Transport of Tetanus and Cholera Toxin

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In previous studies it has been shown that the retrograde axonal transport of nerve growth factor (NGF) is restricted to adrenergic and sensory neurons whereas tetanus toxin (TT) is transported in all peripheral neurons studied so far (adrenergic-, sensory-, motoneurons). The present experiments have shown that cholera toxin (ChT) and wheat germ agglutinin (WGA) are transported in the same set of neurons as tetanus toxin. The retrograde transport of both TT and ChT was abolished by a mixture of bovine brain gangliosides whereas that of WGA and NGF was not affected. It is concluded that the membrane binding and subsequent retrograde axonal transport of TT, ChT and WGA depends on membrane properties which are common to all peripheral neurons. However, only the retrograde transport of TT and ChT seems to be linked to gangliosides.

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Factors in Cerebrospinal Fluid as Regulators of the Motor Activity Level

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The rat exhibits high motor activity (MA) at night. We investigated the possibility that humoral factors present in the cerebrospinal fluid (CSF) regulate the level of MA. Donor rats kept at a 12 h:12 h light-dark cycle were anesthetized with ether, either 90 min before or 90 min after the onset of the light period. CSF was removed from the cisterna magna, centrifuged, exposed to vacuum to evacuate the ether, and frozen for storage. Recipient rats with cannulae chronically implanted in the lateral ventricle were infused with CSF (90 µl during 30 min) obtained from either light (L-CSF) or dark exposed donors (D-CSF). The infusion was started either 150 min before or 90 min after the onset of the light period. Rats infused in darkness exhibited for 2 h a significantly lower MA with L-CSF than with either D-CSF or artificial (a-)CSF. Rats infused in light were for 1 h significantly more active with D-CSF than with either L-CSF or a-CSF. The results support the hypothesis that effects of illumination and/or of a circadian 'clock' on MA are mediated by humoral factors.

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Gender and Hormonal State Modify Monoamine Uptake Inhibition by Antidepressant Drugs

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Analagous results with two different monoamines, two different antidepressant drugs and two different models (rat brain or human platelet), indicated that sex and endocrinological state modify a pharmacological effect. Adult male and prooestrus female rats were killed at 10 a.m. The uptake of ^{14}C-noradrenaline (NA, 1×10^{-7} *M*, 10′ incubation at 37 °C) in regional brain slices was studied (with or without 5′ preincubation with maprotiline, a specific NA uptake inhibitor, 1×10^{-6} *M*). NA uptake was significantly higher in prooestrus females than in males in all regions investigated. The %NA uptake after maprotiline was higher in prooestrus females than in males in cortex (♀: 50 ± 6%, $N = 8$; ♂: 37 ± 7%, $N = 9$; $p < 0.05$) and medioventral hypothalamus (♀: 43 ± 8%, $N = 9$; ♂: 32 ± 7%, $N = 10$; $p < 0.01$). EDTA plasma was collected at 8 a.m. (fasting) from healthy subjects. The uptake of ^{14}C-serotonin (5HT, 2.8×10^{-6} *M*, 3′ incubation) in platelets was studied (with or without 5′ preincubation with chlorimipramine, a potent 5HT uptake inhibitor, 1×10^{-8} *M*). 5HT uptake was similar in men and women. The % 5HT uptake after chlorimipramine was higher in women (51 ± 11%, $N = 19$) than in men (43 ± 12%, $N = 22$; $p < 0.05$). Furthermore, a correlation was found between individual values and the day of the menstrual cycle ($r = -0.667$, $N = 15$, $p < 0.01$). High values of % 5HT uptake after chlorimipramine were found during the preovulatory phase (56 ± 11%, $N = 9$), with lower values, during the luteal phase (40 ± 5%, $N = 6$).

Circulating Platelet Aggregates Induced by ACTH, Lauric and Arachidonic Acid

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Blood obtained from the carotid artery of anaesthetised guinea pigs was diluted 1:400 in Dilusol containing 1% EDTA with and without 1% formalin and vibrated for 5 min. Platelet aggregates were counted in a counting chamber. Immediately after i.v. injection of 50 and 100 U/kg of ACTH there was a significant increase in reversible platelet aggregates (demonstrable only in formalin-containing dilution fluid). It was not accompanied by a drop in total platelet count. Serum free fatty acid levels (FFA) rose slowly, reaching 1.8–3.0 mEq/l 1 h after injection. Slow i.v. infusion of lauric (LA) and arachidonic acid (AA) caused a rapid increase in reversible and irreversible platelet aggregates and a marked drop in platelet count, indicating that aggregates were retained in the capillary bed. It is concluded that ACTH caused reversible platelet aggregation which did not correlate with rising FFA levels. In contrast, LA and AA caused irreversible platelet aggregates which were dose-dependent and led to thrombocytopenia due to microembolization.

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

Kinetics of Synthesis and Transport of Polyoma Virus-Specific RNA in Productively-Infected Mouse Cells

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Late during productive infection of mouse cells, polyoma-specific RNA is synthesized in the nucleus as a heterogeneous population of molecules with sedimentation coefficients from 20 to 80S. However, polyribosome-associated messenger RNA consists of 3 or 4 species with sedimentation coefficients from 16S to 19S. We have measured the relative rates of synthesis and transport of polyoma-specific RNA by labeling infected cells with ^{3}H-uridine for times ranging from 5 min to several hrs and separately analysing nuclear and cytoplasmic RNAs. Ten to twenty times as much polyoma-specific RNA is synthesized in the nucleus as is transported to the cytoplasm. Polyoma-specific messenger RNA, which is polyadenylated, appears in the cytoplasm approximately 20 min after it is synthesized. Although most of the polyoma-specific nuclear RNA is rapidly degraded (half-life about 1 h), polyoma-specific messenger RNAs have much longer lifetimes (half-life $>$ 3 hr).

Capsid Maturation Pathway of Bacteriophage T2 and T4

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The principal protein-protein interactions of T_{even} bacteriophage capsid maturation have been studied on giant phage and polyhead tubes (Aebi et al., J. Supramol. Struct. *2, 253,* 1974) by optical diffraction and filtering of electron micrographs, complemented with biochemical analysis. Therefrom we infer that capsid maturation follows sequential transformations between states which are recognizable by distinct capsomere morphologies and lattice parameters (Steven et al., submitted to J. Mol. Biol., 1976). The earliest detectable precursor is very fragile with 112 Å lattice constant whose major structural protein P23 is specifically cleaved by a phage-coded protease to P23*, triggering a cooperative lattice transformation which is characterized by 130 Å lattice constant and a distinct capsomere morphology. This stable intermediate state is essentially that of the mature T2 phage capsid. It can be transformed to the T4 phage capsid state by adding further structural proteins which first trigger a transformation of the T2 phage capsomeres which allows stoichiometric incorporation of these proteins, to give rise to the final capsomere morphology leaving the 130 Å lattice constant unaffected. We are able to simulate individual steps of this pathway in vitro.

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Solitary Membrane Associated Particles in Synaptic Vesicles as Possible Calcium Binding Sites

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Freeze-etch replicas of synaptic vesicles in nerve terminals of the vertebrate central nervous system as well as in the frog neuromuscular endplate have revealed the presence of solitary particles which seem to be attached to the P-face of the vesicle membrane. These findings bear a striking resemblance with the observations of cytochemically identified calcium binding sites which seem to occur in the form of single membrane bound granules of similar size (Politoff et al., J. Cell Biol. *61,* 818, 1974). The particles in our material were especially evident in the initial stages of Wallerian degeneration when the synaptic vesicles are abnormally large. The role of these particles in the calcium dependent phase of transmitter release remains to be further elucidated.

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Sedimentation Studies on Giant DNA from *E. coli* and *D. melanogaster*

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We have subjected the Klotz and Zimm lysis of *E. coli* cells and the Kavenoff and Zimm lysis of *D. melanogaster* cells to analysis by sedimentation velocity. The work with *E. coli* lysates serves to define the sedimentation conditions required for the preparative determination of $S20_1w$. These conditions differ from the normal operation of the preparative centrifuge in several important aspects. We show that the Klotz-Zimm lysis procedure yields a sample of DNA molecules with a very broad distribution of $S20_1w$. The system applied to the Kavenoff-Zimm lysate of *Drosophila* cells shows that (1) the lysate is highly variable in the size of DNA it produces; (2) the DNA is practically uniformly distributed between very low values of $S20_1w$ ($<$ 50s) and about 450s; (3) the largest DNA we measure by this technique is 10^{10} g/mole. Implications of these data will be discussed.

Entrée présynaptique de calcium pendant l'activité: démonstration directe par radio-autographie

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La stimulation des nerfs cause une augmentation nette du calcium cellulaire et accélère son échange avec le milieu extérieur. Cette entrée de calcium s'observe aussi

lorsque la transmission est bloquée par le curare qui agit au niveau des électroplaques. Il semble donc qu'elle concerne avant tout les terminaisons nerveuses présynaptiques (E. Babel-Guérin, J. Neurochem. *23*, 525, 1974). Cette hypothèse est confirmée ici par une démonstration directe. Des fragments de tissu électrogène de Torpille sont incubés en présence de ^{45}Ca, puis stimulés à 10/s pendant 10 min. Après lavage de la radioactivité externe à 2 °C, on mesure le ^{45}Ca restant et on fixe le tissu pour la radio-autographie. Le ^{45}Ca cellulaire des fragments stimulés est environ le double de celui des témoins. On peut voir sur les radio-autographies, que cette accumulation de calcium concerne presque uniquement les terminaisons nerveuses.

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Reactioncenter Complexes (RC) from Chromatophore Membranes of *Rhodospirillum rubrum*

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Chromatophores are prepared from the green mutant of *R. rubrum* (G-9$^+$) which contains only little carotenoid. Membrane proteins are partially solubilized by a mild treatment (0.25–0.35%) with the zwitterionic detergent LDAO. RC-complexes are purified to a protein to pigment ratio (OD_{280}/OD_{865}) of ≤ 1.25. The photochemical activity of the pigments is tested by the reversible bleaching at 865 nm when illuminated with actinic light of $\lambda = 600$ nm. Oxidation with $K_3[Fe(CN)_6]$ shows that 100% of the absorption at 865 nm are due to RC-pigments. Our RC-preparations yield a single band in LDAO-polyacrylamide electrophoresis (LDAO-PAGE) and 3–4 bands in SDS-PAGE. They stain with Coomassie-Brilliant-Blue (CBB) unequally and exhibit molecular weights between 20 and 30 kilodaltons. Three additional bands which stain with the PAS-method but do not coincide with the CBB-stainable bands are detected. Iodination of whole chromatophores shows that at least part of the light-harvesting-pigment-protein-complex (LH) and of the RC-complex is exposed at the membrane surface. The protein subunits and the interaction of pigments and proteins are presently investigated further.

Bovine Pancreatic Polypeptide (BPP) in the Pancreas and in the Gastro-Intestinal Tract of the Dog

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In the course of a systematic study, by immunofluorescence, of the endocrine cell content of pancreatic and gastro-intestinal tissues in several animal species, we were able to localized cells fluorescent with a specific bovine pancreatic polypeptide (BPP) antiserum (courtesy of R. E. Chance, Eli Lilly, Indianapolis, USA). In the dog, BPP-containing cells are present in the islets of Langerhans, among exocrine cells and in the gastro-intestinal mucosa, mainly in the antro-pyloric and ileal regions. In the pancreas, we found that the fluorescent cells are particularly numerous in the uncinate process as compared to a low content of such cells in other regions of the organ. The abundance and the preferential location of anti-BPP-containing cells in the exocrine tissue of the uncinate process may render particularly easy the identification of the fluorescent cells at the ultrastructural level. A candidate for being the BPP-producing cell may well prove to be the so-called F-cell (B. L. Munger, F. Caramia and P. E. Lacy, Z. Zellforsch. *67*, 776, 1965).

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A Particulate, Thiol-Dependent Acid Aryl Phosphatase of Polymorphonuclear Leukocytes (PMN)

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We studied the properties of the acid *p*-nitrophenyl phosphatase which we originally found in rabbit and human PMN (J. Cell Biol. *45*, 586; *63*, 251), and which has now been demonstrated in the PMN of rat and guinea-pig. The enzyme hydrolyzes aromatic, but not aliphatic, phosphate esters, and therefore qualifies as an aryl phosphatase (ARP). In all four species, the ARP has a pH optimum between 5 and 5.5, and exhibits high specific activity as compared to other PMN enzymes. In contrast to the lysosomal acid phosphatase (EC 3.1.3.2), the ARP is not inhibited by fluoride or tartrate, but is very sensitive to pCMB, low pH, and heat. The ARP of human PMN was found to be insensitive to chelators, ouabain, tetramisole, and several concentrations of mono- and divalent cations. Subcellular fractionation shows the ARP to be bound to particles with low sedimentation rate and low density. These data and EM observations suggest that the ARP is a constituent of the PMN plasma membrane. This assignment, however, is still hypothetical since the enzyme is not accessible to substrate added to intact cells.

Isolation of Globin Messenger RNA from *Xenopus laevis*

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We have isolated globin messenger RNA from red blood cells of anaemic *Xenopus laevis*. The RNA sedimenting in the 9S region of a sucrose product was characterized by cell-free translation in a wheat germ system. The polypeptides synthesized co-migrated with the adult globin proteins when chromatographed on CM52 columns or electrophoresed in acid-urea acrylamide gels. The α and β RNA chains of the globin messenger were separated on denaturing formamide-polyacrylamide gels and used as template for the synthesis of highly labelled complementary DNA.

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Surface Labeling of Membrane Galactosyl Residues from Chick Fibroblasts

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In a search for biochemical events in cells stimulated to proliferate, cell surface glycoproteins of synchronized chick fibroblasts were labeled and analyzed in either whole cell homogenates or isolated plasma membranes. – Monolayers of chick fibroblasts were labeled by the galactose-oxidase/ $NaBT_4$ method with or without prior treatment with neuraminidase. Plasma membranes were isolated by either an aqueous two phase system or isopycnic gradient centrifugation. Labeled glycoproteins were separated on polyacrylamide slabs according to Lämmli and visualized fluorographically. – A drastic reduction of unspecific labeling of preexisting aldehyde groups was achieved by treatment with cold $NaBH_4$ prior to the galactose-oxidase/$NaBT_4$ treatment. Neuraminidase treatment approx. doubled the overall labeling of membrane glycoproteins. The average specific activity of glycoproteins increased during the first 22 h after stimulation to proliferate. Qualitative differences in the labeling pattern during the various phases of the cell cycle are currently investigated.

Polyoma Virus Transcription Complex

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Some if not all of the polyoma virus-specific RNA synthesised late in productive infection is known to be copied from a viral DNA template which is not integrated covalently into host cell DNA (Gariglio and Mousset, FEBS Lett. *56*, 149, 1975). Preliminary experiments suggest that a complex containing viral DNA template, newly-synthesised RNA, and associated proteins, can be isolated from infected cells and separated from the bulk of viral DNA by density gradient centrifugation. The nature of the DNA template and attached proteins, as well as the size of the RNA and the origins of transcription on the template, are being studied in the isolated complex.

Drosophila Cells: Fusion of Somatic Cells by Polyethylene Glycol

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Polyethylene glycol (PEG) has been reported as a potent fusing agent for plant protoplasts, yeast cells, avian erythrocytes and mammalian cells. We report the formation of dikaryons in somatic *Drosophila* cells by PEG and we provide evidence that the dikaryons arise by cell fusion and not as a result of incomplete cytokinesis. Cloned *Drosophila* tissue culture cells (K_c) were labelled with ^{3}H-thymidine and the labelled cells were mixed with equal numbers of unlabelled K_c cells or imaginal disc cells (ID) isolated from 3rd instar larvae. Mixed cultures were incubated for 10′ at 25 °C in a 50 m*M* solution PEG (MW 4000) in balanced insect salt solution (BSS). PEG was gradually diluted with BSS at 5′ intervalls and the cells were seeded in fresh culture medium. After 24 h the cultures were harvested, fixed, and autoradiographed. The frequency of heterokaryons was computed by dividing the number of cells containing one labelled and one unlabelled nucleus by the number of cells scored. Most heterokaryons contained two nuclei but polykaryons were also observed. In both combinations $K_c \times K_c$ and $K_c \times ID$ the estimated frequency was 5%. In contrast to most fusing agents PEG does not impair cell viability. Our results do not show the survival of such heterokaryons and it will be important to demonstrate that they can give rise to viable progeny.

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The Relationship between Recognition and Cleavage Sites of the Restriction Endonuclease from *E. coli* K

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The restriction endonuclease from *E. coli* K (R.K) will cleave DNA containing recognition sites for the enzyme provided the sites are not methylated (modified). These recognition sites are few in number and may be lost by mutation to yield a DNA molecule that is resistant to digestion. Cleavage on the other hand may occur at a relatively large number of sites. Bacteriophage λ DNA for example, containing 5 genetically mapped recognition sites, gives a pattern of fragments upon digestion that is too complicated to resolve by agarose gel electrophoresis. – We have investigated some aspects of the relationship between these two kinds of sites. We have shown that cleavage is not completely random: the enzyme shows a strong preference for cleavage within the early melting regions of DNA. This was shown by (1) a comparison of the lengths of the fragments produced by the enzyme from phage PM2 DNA and *Eco*RI fragments of λ DNA with the distances between the early melting regions and (2) direct denaturation mapping of R.K-nicked PM2 supercoiled DNA. R.K can cleave DNA in regions separated from the nearest recognition site by at least 5000 bases pairs. This finding was the result of sequential digestion experiments designed to determine which *Eco*RI fragments could be rescued from R.K digests of DNA of wild type λ and λ mutants containing only one recognition site.

Influence of Medium Composition on the Behaviour of Chicken Heart Cells in Culture

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The behaviour of cells derived from hearts of 9-day-old chicken embryos was studied in vitro. Glycogen content was demonstrated with the PAS-staining technique and used to characterize myocytes (PAS⊕-cells). Cultures in Eagles medium supplemented with various amounts of fetal calf serum (FCS) and/or chicken embryo extract (EE) (each in dialyzed or undialyzed form) were followed over a 5-day period. Total cell number increased only in media with undialyzed FCS and/or EE. An increase in the number of PAS⊕-cells was never observed. However, autoradiography revealed that more than 50% of the PAS⊕-cells had synthesized DNA during 5 days of culture with

continuous ^{3}H-thymidine labelling. Since in several media the number of PAS$^{\oplus}$-cells declined, we are investigating whether such cells are lost or converted to PAS$^{\ominus}$-cells. Also, studies are in progress to determine whether PAS$^{\oplus}$-cells undergo mitosis.

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Fast and High Sensitive Trace Element Analysis of Biological Samples

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If characteristic X-rays are excited with protons in the MeV range and measured with a high resolution Si(Li)-detector, all trace elements with $Z > 12$ can be determined simultaneously in any specimen with a sensitivity of 0.1–1 ppm. Since the method is fast, accurate and only small amounts of material (few μg) are needed, it is especially well suited for an investigation of the role of trace elements in biological samples. The advantages and limits of this new analytical method are discussed using the case of blood serum as an example.

TEM, Cytochemistry and SEM of Na-TL-Induced Acute Cholestasis in Rats

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After a single injection of Na-Taurolithocholate (Na-TL), severe and acute cholestasis is observed in rats, from 10 min to 6 h, before restauration of basal choleresis. In various experimental schemes, Miyai et al. (Lab. Invest. *24*, 212, 1971, and *32*, 527, 1975) showed ultrastructural alterations to be characteristic for cholestasis induced by monohydroxylated biliary salts. They noticed specially the presence, both in hepatocytes and in biliary canaliculi, of peculiar 'crystals', which they interpreted were constituted by biliary acids. Using Williamson's digitonin-osmium reaction in TEM, we ascertain the participation of *free cholesterol* in these crystals. A very high concentration of free cholesterol is also found in cytoplasm and in canaliculi. In SEM, after the same reaction, crystalline structures are observed, obliterating biliary canaliculi. Moreover, in vitro prepared cholesterol and cholesterol-digitonin complexes show in SEM a tendency to form similar crystals, whereas biliary salts do not.

Extrinsic Signals for Monitoring the Association Reaction of Proteins

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Strong extrinsic signals for monitoring the association reaction of proteins were introduced by covalent binding of fluorescent and nonfluorescent dyes to the reaction partners. The association of α-chymotrypsin with basic pancreatic trypsin inhibitor was used as a test system. The contact regions were protected by complex formation. The labelled complex was then dissociated and separated by gel chromatography. Suitable reactive dyes were 5-dimethylamino-naphtalene-1-sulfonic acid-(3-methoxy-3-iminopropylamide) and azodye aniline-4-sulfonic acid → 1-hydroxy-8-(α-bromacrylamino)-naphtalene-3,6-disulfonic acid. Changes in fluorescence and absorbance upon complex formation were due to changes in extinction coefficients, quantum yields and energy transfer. An average distance between donor labels at PTI and acceptor labels at α-chymotrypsin was measured to be 32 Å. Kinetic and equilibrium measurements of the differently labelled proteins were found to correspond well with the data for the unlabelled system. Measurements were possible at so far unaccessible nanomolar concentrations. Equilibrium binding constants could therefore be obtained by fluorimetric titration. The values were in agreement with those calculated from the rate constants. At neutral pH the binding constant is in the range of 10^{-8} M^{-1} and the reaction enthalpy was found to be 4 kcal/mole. Even the pH dependence of the dissociation rate constants was essentially unaltered by the introduction of the labels. Therefore the labelling method can be applied to other associating systems with some confidence.

Mapping of Restriction Fragments by DNA Hybridization

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Ordering of restriction fragments with the classical methods may sometimes lead to ambiguous results, especially when the electrophoretic mobility of a fragment does not correspond to its molecular weight. We have developed a method for mapping the position of restriction fragments by electron microscopy which is based on the specific hybridization of fragments to intact molecules. This method has several advantages: (1) it requires a minimal amount of DNA, (2) it permits very accurate measurements and localization of fragments, (3) it allows to distinguish between fragments of equal mobility or equal molecular weight. The restriction fragments produced by Hind III on PM2 DNA were mapped against the unique cleavage site for Hap II on this circular molecule. Purified restriction fragments were hybridized to full length linear molecules and the hybrids (double stranded linear molecules with double stranded loops) were analysed in the electron microscope. The percentage of hybrid molecules depends on the length and the molar excess of the fragment used, and varies between 1 and 5%.

NMR-Studien der Molekülkonformationen der synthetischen Teilsequenz 1–34 des menschlichen Parathyroidhormons

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Das Fragment 1–34 des menschlichen Parathyroidhormons wurde entsprechend der von Brewer et al. (Proc. Nat. Acad. Sci. US *69*, 3585, 1972) angegebenen Amino-

säuresequenz synthetisch hergestellt, ebenso die Fragmente 1–12, 18–34, 19–24, 20–24, 22–24 und 25–34. Das ^{1}H-NMR-Spektrum des Fragmentes 1–34 enthält eine auffallend nach höherem Feld verschobene Methylresonanz, entspricht aber im übrigen weitgehend dem für die Random-coil-Form einer Polypeptidkette dieser Aminosäuresequenz erwarteten Spektrum. Durch vergleichende Studien der oben angegebenen Reihe von Teilsequenzen des Parathyroidhormons konnte gezeigt werden, dass die Aminosäurereste 20–24 eine relativ stabile räumliche Struktur ausbilden, die sich in den NMR-Spektren vor allem durch die obenerwähnte Hochfeldverschiebung einer Methylresonanz von Val 21 manifestiert. In wässriger Lösung konnte diese lokale räumliche Struktur in allen Peptiden nachgewiesen werden, die das Peptidsegment 20–24 enthalten; sie ist jedoch im Peptid 21–24 nicht vorhanden. Auf Grund der Abhängigkeit von der Kettenlänge und weiterer Beobachtungen über die NMR-Parameter in den kleineren Peptidfragmenten ergab sich eine ziemlich weitgehende Charakterisierung der Molekülkonformation im Bereich der Aminosäurereste 20–24.

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Slow DNA Increase during Postnatal Development of Neurons from Rat Brain Cortex

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The DNA content of isolated neuronal nuclei was assayed in developing rat brain cortex (4 days prenatally to 60 days postnatal age). Determinations were carried out using cytophotometric techniques (fluorimetry with Feulgen type stains such as BAO and pararosaniline and UV-absorbance scanning following RNase treatment) and a biochemical method (diphenylamine reaction). The results show a slow and continuous DNA increase from a 2C complement prenatally to a value approaching 4C at age 30 days. The DNA content then remains constant up to at least 60 days, after which age no further measurements were performed.

Serine Proteinases from Azurophil Granules of Human Polymorphonuclear Leukocytes (PMN)

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Neutral proteinases were extracted from granules of human blood PMN by treatment with 0.2 *M* acetate buffer pH 4.5 at 4 °C. The enzymes were characterized by cathodic polyacrylamide gel electrophoresis using specific substrates and inhibitors, and by kinetic studies with purified fractions. Two groups of proteinases have been identified as lysosomal elastase and cathepsin G (see also J. Exp. Med. *141*, 709, 1975). A third group of proteinases was identified which is also contained in the azurophil granules. Like the former, these enzymes are sensitive to phenylmethyl sulfonyl fluoride and to phosphonates, and may, therefore, also qualify as serine proteinases. In contrast to elastase and cathepsin G, they are inhibited by *p*-chloromercuribenzoate although SH-reagents are not required for maximum activity. They further differ by their lower electrophoretic mobility, higher molecular weight, preferential hydrolysis of α-naphthyl acetate, and a pH optimum of 6.0 for the hydrolysis of casein. Identification with any of the well characterized proteinases has not as yet been established.

Terminal Deoxynucleotidyl Transferase Activity of Reverse Transcriptase

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The addition to detergent disrupted Rous sarcoma virions of a low molecular weight (LMW) fraction of the nucleic acids extracted from the virion leads to extensive stimulation of deoxynucleoside triphosphates incorporation into trichloroacetic acid (TCA) insoluble material; this incorporation is observed in presence of one deoxynucleoside triphosphate alone. Reverse transcriptase purified from avian myeloblastosis virus is also able to catalyse the incorporation of anyone of the four deoxynucleoside triphosphate into TCA insoluble material in presence of the LMW nucleic acid fraction even in absence of 60–70S viral RNA. This enzymatic activity therefore resembles that of terminal deoxynucleotidyl transferase in its lack of requirement for template and in its need for primer. The nature of the primer and the evidence that reverse transcriptase itself has terminal transferase activity will be discussed.

Erythroblasts versus Erythrocytes: Nuclear Transplantation in *Xenopus laevis*

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Non-proliferating erythroblasts obtained 14–16 days after phenylhydrazine treatment of adult *Xenopus* were individually injected into nucleated- or enucleated *Xenopus* eggs, immediately after 'opening' the cells by osmotic shock. Eggs containing the pronucleus injected with single broken or unbroken erythroblasts developed into tailbud stages whereas enucleated eggs injected with the same cell type developed into neurulae. In contrast, with broken erythrocytes the nucleated- or enucleated eggs only developed into gastrulae. In addition, single erythrocytes injected unbroken into eggs never promoted development. The difference between erythroblasts and erythrocytes in promoting development seems to depend on the differentiated state of the cell used. The results provide evidence that differentiation of the blood cells leaves not only cytoplasmic traces but changes the cell surface in a way that the egg is not able any more to open the membrane of erythrocytes. Whether the restriction in developmental capacity of erythrocyte nuclei as compared to nuclei of erythroblasts is or is not related to the smaller diameter of erythrocyte nuclei and the higher degree of heterochromatization remains an open question.

Isolation and Identification of Histone mRNA from *Drosophila melanogaster*

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To study the regulation of histone mRNA synthesis during the early embryogenesis of *Drosophila*, histone mRNA was isolated and characterized. The RNA was extracted from small polysomes of pulse labelled, exponentially growing tissue culture cells at 25 °C and of cells labelled for 1 h at 37 °C (heat shock cells), then passed through an oligo-dT column and fractionated by 15–30% sucrose gradient centrifugation. The 5–15S polyA$^-$ fractions contained ^{3}H-uridine labelled RNA sequences which migrated in 6% SDS-polyacrylamide gels with a rate comparable to that of histone mRNA's of sea urchins. The unlabelled polyA$^-$ RNA of heat shock cells was translated in a wheat germ cell-free system. Most products were identified as histones in a 14% SDS-polyacrylamide gels using as standards calf thymus histone proteins, unlabelled embryonic and in vitro labelled histones of *Drosophila* cell cultures.

Jean Burckhardt is a fellow of the Julius-Klaus-Stiftung. Supported by the SNSF, 3.8630.72

The Effect of Oxygen on the Cell Cycle in vitro

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In the present study of our institute on cellular growth we wanted to see the effect of different oxygen concentrations and of anoxia on chick embryo fibroblasts cultivated in vitro. The cells cultivated in a 20% O_2 atmosphere (control) showed an active growth (mitotic index(MI) 18‰, duration of mitosis 55–70 min). Feulgen cytophotometry (by scanning microdensitometer) showed 80% cells in G1 phase, 4% in S and 16% in G2. Histoautoradiography (Th-^{3}H) showed the presence of synthesizing cells. After 24 h in hyperoxic atmosphere (80% O_2) MI decreased (5‰); mitosis and chromosomes showed anomalies, cells accumulated in G2 (23%). After 48 h MI was zero, cell accumulation in G2 even more evident (44%) (premitotic block). DNA synthesis (Th-^{3}H) was blocked after 48 h in hyperoxic atmosphere. In cells cultivated in anoxic atmosphere mitochondria began to vacuolise and to disappear already after 6 h. Proliferative cycle was completely blocked after 8 h: MI zero, great cell accumulation in G2 (39%), large part of which was affected by nuclear degeneration of granular and thready type; no DNA synthesis (TH-^{3}H).

Affinity Chromatography of Proteins Using Homopolymer RNA-Cellulose

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I subjected extracts of bacterial and mammalian cells to affinity chromatography on columns of cellulose to which polyC or polyA were coupled. In addition to previous results using bacterial extracts (J. Biol. Chem. *250*, 6160, 1975). I found that in buffers containing 30% glycerol, the *E. coli* RNA polymerase *holoenzyme* binds to poly C-cellulose and is greatly purified simply by salt elution from the column. The termination factor rho binds more tightly than the polymerase, and can be isolated at the same time. – Primary baby mouse kidney cells were lysed and separated into crude nuclear and cytoplasmic fractions; bulk nucleic acids were then removed by liquid polymer (polyethylene glycol/Dextran) phase partitioning, or sometimes for the cytoplasms, by LiCl precipitation. The extracts were then analyzed by affinity chromatorgraphy. A small subset of cellular proteins is selected by these techniques, and the patterns of RNA-binding proteins are different for nucleus and cytoplasm, and using polyC- or polyA-cellulose. Several major cellular proteins have been greatly enriched by this affinity chromatography.

Part of this work was done in the laboratory of Walter Gilbert, Harvard University. Supported by SNCF, grant 3.3070.74, to B. Hirt, and by a postdoctoral fellowship from the Jane Coffin Childs Fund

A Freeze-Fracture Study of the Influence of Lipolysis on Adipose Cell Plasma Membrane

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The freeze-fracture technique was used to study the ultrastructural organization of the adipose cell plasma membrane during lipolysis. Lipolysis was induced in male rats by fasting (3 and 6 days) and by experimental diabetes (6 days after a single injection i.v. of 75 mg/k streptozotocin). The number of surface invaginations of the plasma membrane and of the intramembranous particles (proteins) was quantified in epididymal adipocytes. All results were expressed per μm^2 of membrane. 3 and 6 days fasting increased membrane invaginations from 46.2 $\pm$ 1.1 (control) to 54.0 $\pm$ 1.2 ($p < 0.001$) and 57.8 $\pm$ 1.3 ($p < 0.001$), respectively. After 6 days of streptozotocin diabetes, membrane invaginations increased from control values to 64.9 $\pm$ 2.0 ($p < 0.001$). The number of protein containing particles on the cytoplasmic leaflet of the plasma membrane was 592 $\pm$ 24 in control adipocytes; it raised to 690 $\pm$ 28 after 3 days fasting ($p < 0.001$) to 764 $\pm$ 30 after 6 days fasting ($p < 0.001$) and to 896 $\pm$ 26 after 6 days of streptozotocin diabetes ($p < 0.001$). These results indicate that lipolysis modifies significantly the organization of the adipose cell plasma membrane.

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Rous Sarcoma Virus RNA-Directed DNA Synthesis Initiates at one Major and Several Minor Sites

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The origin of in vitro DNA synthesis by oncorna virus RNA-directed DNA polymerase was investigated by annealing DNA synthesized in vitro to [^{32}P] viral RNA and determining which regions of the RNA were hybridized. Viral DNA ($S_{20,w}$ = 10 S and below) synthesized by disrupted Prague B RSV particles was hybridized to [^{32}P] PrB RSV RNA, the preparation was treated with RNase T1 and the RNase-resistant fraction isolated.

Following denaturation, the [^{32}P] RNA was completely digested with RNase T_1 and the characteristic large T_1 oligonucleotides were identified. After annealing at a low DNA-to-RNA ratio only 2 of 30 large T_1 oligonucleotides were detected; T_{13} in high and T_1 in lower yield. T_{13} carries the capping group and therefore constitutes the 5′ terminus; T_1 is derived from the middle of the RNA. The major initiation point is located within less than 200 nucleotides from the 5′ end of the molecule; however, a second initiation point apparently occurs in the middle of the molecule. After hybridization at high DNA-to-RNA levels most large T_1 oligonucleotides were recovered in varying molar ratios. Thus, several minor initiation points give rise to DNA complementary to most of the viral RNA.

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Amplification of Eukaryotic Genes for Histone Proteins, 5S RNA and tRNA via a Phage Lambda Receptor

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The genes for histone proteins of *Psammechinus miliaris* and for 5S RNA and for some tRNAs of *Xenopus leavis* have been isolated as purified *Hind*III restriction fragments and then inserted into phage lambda DNA containing only a single *Hind*III site in the C_1 gene. Phage T4 ligase (a gift of F. Rougeon) has been employed for the insertion reaction, which can be achieved within a few minutes. Ligated DNA molecules are detected as phage plaques following transfection of calcium-treated $C600r_K^-$ m_K^- *rec*B$^-$C$^-$ *E. coli* cells. Typically, 25–50 plaques have been obtained per ng of ligated receptor DNA. Rejoined vector molecules give rise to turbid plaques whereas those with inserted DNA fragments are easily identified as clear plaques. Using a favourable ratio of 5S DNA to receptor DNA 20% clear plaque recombinants were obtained, of which 85% were positive for 5S RNA genes by hybridization. Nanogram quantities of histone DNA have yielded 2–5% clear plaques; of the 40 recombinants so far tested, 38 contain histone genes. Similar amounts of tDNA have yielded 5% recombinants whose hybridization properties are being investigated.

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The Chromatin Subunit Structure in Metaphase Chromosomes

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The structure of interphase chromatin and metaphase chromosomes has been examined in Chinese hamster ovary cells by nuclease digestion and electron microscopy. Interphase nuclei and metaphase chromosomes were separately purified and resuspended in 1m*M* Tris pH 8.0, 1m*M* $CaCl_2$ at 37 °C. Micrococcal nuclease was added and the digestion subsequently stopped with EDTA. Material was placed directly onto EM grids or was deproteinised for electrophoresis. – Electrophoresis (2.5% polyacrylamide, 0.5% agarose) of material from both interphase and metaphase digestions showed that high molecular weight DNA was converted to discrete multiples of about 185 base pairs by the nuclease. The sizes of the different components are identical for interphase and metaphase material during all phases of digestion. – Examination of the digested material in the electron microscope showed the presence of spheres approximately 125Å in diameter. The average number of spheres per chromatin molecule decreases with increasing extent of digestion. There are no apparent differences between the subunit structure of free chromatin molecules from interphase and metaphase chromatin.

Mutagenic Specificity in *E. coli*

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The study of mutagenic specificity requires a method for the recognition of the specific base changes caused by mutations. Our studies of the lacI gene and its protein product the lac repressor allow us to identify the exact codon involved in the creation of nonsense mutations in the I gene. The correlation of the sequence of the repressor protein with the genetic map enables us to assign each nonsense mutation to a specific residue. We have analyzed more than 4000 nonsense mutations in the I gene, each of independent origin, and have identified 83 different nonsense sites. (The lacI gene codes for a protein 347 amino acids long.) We have used these sites to determine the mutagenic specificity of 2-aminopurine, Ethyl methylsulfonate, nitrosoguanidine, 4-nitroquinoline-1-oxide, *mutT* (a mutator gene), ultraviolet light, and spontaneous mutagenesis.

A Bacteriochlorophyll-Containing Protein (Light Harvesting-Complex) from *Rhodospirillum rubrum*

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A bacteriochlorophyll-containing protein (LH-complex) is obtained from chromatophores of the green mutant of *R. rubrum* (G-9$^+$). The LH-complex is solubilized with the zwitterionic detergent LDAO (*N,N*-dimethyllaurylamine oxide) from the chromatophores after the reaction center complexes had been removed completely. After sedimentation (210,000 *g*, 60 min) of the resulting membranes the supernatant fluid shows an optical absorption spectrum with maxima near 820, 770, 590, 370 and 280 nm. This supernatant is fractioned by ammonium sulfate precipitation. The resuspended (in the presence of 0.05% LDAO) green, floating pellet is found to have the same optical properties as the supernatant fluid described before. Disc electrophoresis under alcaline conditions in 5% polyacrylamide gels containing 0.1% LDAO shows three protein bands: One major pigmented component (LH-complex) and two minor faster moving bands. The pigmented protein is presently being isolated on a preparative scale for characterization.

Isolation of a Globin mRNA Precursor from DMSO-Induced Friend Cells

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^{32}P-labeled globin mRNA was purified from DMSO-induced Friend cells, labeled for 24 h with [^{32}P] phosphate, by hybridization to polydC-globin cDNA and isolation of the hybrid on poly I-Sephadex. The [^{32}P] globin mRNA, which was over 83% pure, was characterized by T_1 fingerprints. In order to search for a precursor to globin mRNA, induced Friend cells were labeled for 16 h with [^{3}H]uridine and for 20 min with [^{32}P]phosphate. Purification was carried out as above. Sedimentation analysis showed that the ^{32}P-labeled RNA sedimented with a peak at 15 S and one at 10 S, while the ^{3}H-labeled RNA sedimented at 10 S, the position characteristic for mature globin RNA.

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Characterization of Membranes Isolated from Rabbit Polymorphonuclear Leukocytes (PMN)

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Azurophil (Az) and specific (Sp) granules and a membrane fraction of low density, presumably plasma membranes (M), were isolated from rabbit PMN either by zonal differential centrifugation or by zonal isopycnic equilibration. The purity of the three fractions was assessed by the relative concentration of marker enzymes (myeloperoxidase for Az, alkaline phosphatase for Sp, and acid *p*-nitrophenyl phosphatase for M). Membranes were prepared from each fraction and analyzed for phospholipid and protein. The three membrane types were remarkably similar in their phospholipid composition. Notably, a high content of sphingomyelin (SM) was observed. The following values were determined for the major phospholipids: PC 16–20%, PE 21–26%, PS plus PI 11–13%, SM 30–40%. The protein components of the solubilized membranes were analyzed by SDS gel electrophoresis. Distinct differences in the Coomassie brillant blue staining patterns were observed. The membranes prepared from M exhibited the highest number of polypeptides which, unlike those from Az and Sp, were more prominent in the high molecular weight region.

Activation of RNA Polymerases by 17 β-Estradiol-Receptor Complex on Liver Chromatin from Immature Roosters

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In liver nuclei from immature roosters treated with 17β-estradiol for 24 h (E-24) the endogenous RNA polymerase I and II activities are increased by more than 100% over that found in control nuclei. During purification of the RNA polymerases over 70% of this increase in activity was lost at the moment when the RNA polymerases were solubilized from the nuclei. In an attempt to explain this loss we thought that E-24 chromatin might contain a factor responsible for the activation of the polymerases. Chromatin was made from freshly prepared nuclei and used immediately. Endogenous RNA polymerase I and II activities were always very low in control chromatin but much higher in E-24 chromatin. The difference however was less than that obtained during in vitro transcription of control and E-24 nuclei. E-24 chromatin used as a template for semi-purified homologous RNA polymerases produced an increased rate of transcription of 40% by comparison with that obtained with control chromatin. Addition of saturating amounts of semi-purified receptor to control chromatin also increased transcription by 40% when homologous polymerases were used. The homologous polymerases could not be substituted by *E. coli* polymerase. These experiments in part confirm the data by Gschwendt (BBA *361*, 84, 1974) that E-24 chromatin contains 17β-estradiol-receptor complex. We conclude that (1) the activation of the RNA polymerases after estrogen treatment is highly specific and (2) that the 17β-estradiol-receptor complex on the chromatin is involved.

Synthesis of Structural Viral Proteins in Avian Myeloblastosis Virus Infected Chicken Embryo Fibroblasts

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In order to determine the molecular structure of nascent viral polypeptides on polysomes of Avian Myeloblastosis Virus infected cells, we isolated free cytoplasmic and membrane bound polyribosomes following a 60 second pulse-label with ^{35}S-menthionine. The separated polyribosomes were then fractionated by sedimentation on sucrose gradients. The fractions were tested for the presence of Pr 76 (the 76,000 mol wt precursor to the 4 group specific proteins of the virus) and gp 85 (85,000 mol wt envelope glycoprotein of the virus) by indirect or direct immunoprecipitation of the released nascent chains with the corresponding antisera. – Our results suggest that both viral polypeptides are synthesized on free cytoplasmic polysomes. Experiments are in progress to identify the size of the mRNA responsible for the synthesis of the two viral polypeptides. – In addition we were able to demonstrate the presence of a slightly slower migrating unstable polypeptide (90,000 mol wt) in immunoprecipitates from pulse-labeled cells using the anti gp-85 antiserum. The relationship between this polypeptide and the viral glycoprotein is being examined.

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X-ray Diffraction Studies of Peptide Ionophores

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The activity of antibiotics of the Valinomycin group (valinomycin, enniatins, actins) and of the Nigericin group (nigericin, grisorixin, monensin, dianemycin and others) depends on the presence or absence of specific alkali cations and is probably associated with transport of cations across biological membranes. The structure determination of a number of theses ionophores in the form of alkali cation complexes shows that oxygen atoms of the ionophore from a cavity which encloses the unhydrated cation. The surface of theses complexes is mainly hydrophobic and enables solubility of the cation

in lipids. The K^+, Na^+ and NH_4^+ complexes of nonactin show S_4 symmetry with nearly cubic coordination for the cation. The structure of uncomplexed nonactin shows that only 8 of 32 torsion angles are significantly changed upon complex formation. Models suggest a detailed, but tentative, mechanism for the complexing process.

Nucleotide Sequence Heterogeneity in the RNA of Phage Qβ

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On digestion with T_1 RNase, Qβ RNA yields a set of characteristic, large T_1 oligonucleotides which have been separated by polyacrylamide gel electrophoresis and sequenced (Billeter et al., Experientia *31*, 735, 1975). We have examined the RNA of 18 clones of Qβ phage picked at random from the progeny of spheroplasts infected with Qβ RNA derived from uncloned phage stocks. In 4 clones oligonucleotide 10* was replaced by 11-1, due to a G→A transition in one and the same position of the A_2 cistron, and two further clones showed other unique modifications of their oligonucleotide pattern. In a second experiment 22 clones, obtained by infecting spheroplasts with Qβ RNA synthesized in vitro, were analyzed. Half of the clones showed a substitution of oligonucleotide 10* by 11-1, and 4 further clones showed unique modifications of their oligonucleotide pattern. Since the large T_1 oligonucleotides represent less than 10% of the phage genome, these results suggest that RNA prepared from uncloned phage stocks contains several non-lethal mutations per strand. It is at present not yet clear whether these mutations are due to errors during RNA replication or whether they are generated during extraction and purification of the viral RNA.

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Heavy/Light Atom Discrimination in Electron Microscopy

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Attempts to deduce the sequence of nucleic acids by electron microscopy of molecules with specifically labeled bases will be highly dependent on the ability to see the labeling atoms in the noisy background of the supporting film. To this end we have developed methods for discriminating between components of the image arising from heavy and light atoms, allowing an enhancement of the image of the heavy atoms by supressing the background noise from the light support film. Two micrographs of the same specimen are used, taken with opposite halves of the diffraction plane blocked. Appropriate computer processing then yields separate images of the heavy and light components. The methods have been demonstrated using several model systems and are currently being tested for sensitivity to single atoms and suscpetibility to radiation damage of the specimen.

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Relation entre l'acétylcholine et les phospho-esters de thiamine

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Les résultats que nous présentons ici démontrent que le métabolisme de l'acétylcholine (ACh) est étroitement lié avec celui de la thiamine (Thia). Des fragments d'organe électrique de Torpille sont soumis à diverses conditions expérimentales, puis la Thia et ses esters phosphoriques sont extraits et séparés par électrophorèse; l'ACh est dosée dans les mêmes extraits par une méthode radiochimique. Au cours d'une stimulation à 5/s, le taux d'ACh décrit des oscillations non amorties d'une période de 4–6 s; la phosphorylation de la Thia oscille en phase avec l'ACh, les esters étant en excès au moment des pics d'ACh. On obtient la même relation entre esters et ACh en incubant le tissu dans des milieux où le rapport Ca/Mg est abaissé: l'ACh diminue, les esters également au profit de la Thia. On sait que, dans ces conditions, les terminaisons nerveuses libèrent une moins grande quantité de transmetteur. Ces résultats sont du point de vue fonctionnel d'autant plus intéressants que la relation entre ACh et esters de Thia ne concerne que le pool «libre» du transmetteur, celui qui est immédiatement disponible pour la libération.

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Studies on the Adenyl Cyclase, cAMP, Protein Kinase System in Neoplastic Human Breast Tissue

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We have determined the levels of cAMP, adenyl cyclase (AC), phosphodiesterase (PD) and cAMP-dependent protein kinase (PK) in primary carcinomas, different dysplasias and in normal human breast tissue of 105 patients. No significant differences of PD activity was found in the various tissues examined. In the primary carcinomas increased levels were observed for cAMP (162 ± 45 pmole/g tissue), AC (12,3 ± 4.8 pmole cAMP min/mg prot.) and PK (198 ± 58 pmole ^{32}P incorporated/mg prot.) as compared to normal breast tissue (cAMP: 50 ± 19; AC: 7.0 ± 0.36; PK: 80 ± 35). Intermediate levels were found for simple and proliferative dysplasia. The differences observed for the specific activity of cAMP, AC and PK were not due to the menopausal status of the patients but due to the different properties of the normal and neoplastic cell: The levels found for cAMP, AC, PD and PK per cell were 40–60% lower in 85% of the primary carcinoma as compared to simple dysplasia and normal breast tissue. In the remaining 15% of the primary carcinomas 2fold increased levels of cAMP per cell were found but similar levels for AC and PK as compared to normal cells. – The cases of simple and proliferative dysplasia which exhibited the elevated levels of PK and AC activity found in primary carcinomas might be considered as high risk groups.

Effect of Age on in vitro Phosphorylation of Histones in Chromatin from Mammalian Skeletal Muscle

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Chromatin and histones from skeletal muscle nuclei of young (2 years) and old (over 14 years) dogs were incubated with histone kinase and ATP-γ^{32}P. After the reaction the histones were isolated and electrophoresed on urea-polyacrylamide gels. Quantitative densitometry and determination of the content of ^{32}P within the single histone fractions revealed that in old age the relative content of phosphate is reduced in histone 4 from 30% to 10% of total histone phosphate. This might be indicative for age-related structural changes of the chromatin complex in postmitotic cells.

Fractionation of Template-Active and Template-Inactive Chromatin

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Deoxyribonucleoprotein (DNP) prepared by shearing chromatin of mouse cells may be fractionated in a 2-phase aqueous dextran-polyethyleneglycol mixture. Countercurrent distribution of DNP in this system allows resolution of a spectrum of fractions with different non-histone protein/DNA rations. DNP containing labeled nascent RNA can be separated from the bulk of the chromatin; they are found in the region of the distribution profile with the highest non-histone protein/DNA ratio. The template-active and -inactive DNP fractions can be freed of the aqueous polymers by gel exclusion chromatography on Sepharose columns. The template-active fraction represents 5–10% of the total chromatin DNA. Present evidence indicates that the pulse-labeled RNA in this fraction represents nascent RNA attached by the RNA polymerase to the DNA template. Polyacrylamide gel analysis of the extracted chromosomal proteins shows an enrichment of non-histone proteins in the template-active fraction.

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Localization and Characterization of Newly Transcribed Hepatocyte Nuclear RNA

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Ultrastructural localization of rapidly labeled nuclear RNA is studied using isolated rat hepatocytes in vitro. Cells labeled with ^{3}H-5-uridine are either fixed in glutaraldehyde and processed for electron microscopy, or their RNA is examined by electrophoresis on exponential polyacrylamide gels under denaturing conditions. Autoradiographs of ultrathin Epon and frozen sections of cells labeled for 2 or 5 min demonstrate, after staining with EDTA, that the majority of extranucleolar label, frequently found over the periphery of condensed chromatin regions, is associated with perichromatin fibrils. The major part of this label is characterized as growing chains of pre-m RNA. When the labeled cells are incubated for 2 or 4 h in cold medium following the radioactive pulse, the labeled perichromatin fibrils appear rather homogeneously throughout the nucleoplasm, suggesting a possible migration of a part of them into the interchromatin space. At this time, the label is represented by pre-m RNA of intermediate size.

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Ricin- and Con A-Binding Sites of Rabbit Polymorphonuclear Leukocytes Have no Receptor Function in Phagocytosis

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It is assumed that recognition and specific binding of a particle to the membrane of a phagocyte is a prerequisite for its ingestion. As part of a study of surface receptors responsible for particle recognition, we investigated the effect of lectins on phagocytosis of yeast by rabbit polymorphonuclear leukoxytes (PMN). PMN were incubated at 4 °C with an excess of ferritin-conjugated ricin or concanavalin A, and subsequently brought to 37 °C, thus inducing capping of the lectin-binding sites. PMN were allowed to phagocytose yeast for 15 min, processed for electron microscopy and evaluated by morphometry. – Lectin pretreatment did not affect phagocytosis; the relative phagosome surface area of PMN bearing a lectin cap was the same as that of control cells. Capped PMN engulfed yeast only with a lectin-free portion of the plasmalemma, clearly indicating that ricin- and concanavalin A-binding sites are not involved in particle recognition and uptake. The absence of lectin-tagged membrane in the phagosomes suggests that the capped cell is functionally polarized, and only able to phagocytose at the pole opposite the cap.

Isolation and Electron Microscopic Visualization of DNA Extracted from Bands of Polytene Chromosomes

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Polytene chromosomes of *Chironomus* larvae show characteristic decondensation of different bands depending on the larval stage. It is not known, which mechanisms are responsible for the control of the chromosomal structure. There is some evidence that certain interspersed DNA sequences are involved in this control. In order to get information about the arrangement of such DNA sequences we developed a method to isolate DNA from single bands of *Chironomus* polytene chromosomes and to prepare this DNA for electron microscopical characterization. – Salivary glands of *Chironomus tentans* and *C. thummi* are explanted and single chromosomes layed out onto a plexiglas microscope slide. The isolated chromosomes are then fixed in 0.3% formaldehyde. By vertically lowering a conventional glass EM knife from an LKB Ultramikrotome onto the chromosome by means of a micromanipulator the chromosome is cut at

two determined positions. The cutting procedure is controlled through an inverse microscope. The excised piece (2–3 bands) is brought onto a bit of parafilm for further processing. The proteins are digested by protease and the DNA spread onto a hypophase of distilled water. DNA filaments upto 120 μm in length are thus obtained.

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Modulated Excitation, Infrared Spectroscopy

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The technique of Meier-spectroscopy has been used recently to study the dynamic behaviour of nematic liquid crystals in a modulated electric field. (U.P. Fringeli, M. Schadt and Hs. H. Günthard, J. Phys. Chem., to be published.) The kinetics of realignment was found to be complex due to cooperative interaction between the molecules. Moreover it was shown that this interaction results mainly from the rigid parts of the molecules. Hydrocarbon chains are not directly involved in this process. – Furthermore the dynamic process of the hydration of oriented lecithin and lysolecithin multilayers is studied by modulating the relative humidity. This results in more detailed informations on the sites of hydration as well as on hydration induced conformational changes of the lipid molecules, than obtained by conventional infrared spectroscopy.

Bedeutung der räumlichen und zeitlichen Energieverteilung für den strahlenbiologischen Effekt

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Die Abhängigkeit der strahlenbiologischen Effekte von der zeitlichen (Verdünnung, Fraktionierung gegen Konzentration der Dosis) und der örtlichen Energieverteilung («ungenau» beschrieben mit dem Faktor des LET = «linear energy transfer») ist entscheidend für die Beurteilung von Risikofaktoren (Strahlenschutz) und für die Belange der Strahlentherapie. Es wurde untersucht die relative biologische Wirksamkeit von hochenergetischen Elektronen und Photonen (niedriger LET), 100-kV-, 200-kV-Röntgenstrahlen und negativen Pionen (hoher LET) des 590-MeV-Protonenbeschleunigers (S. I.N.). Benutzt wurden verschiedene biologische Systeme, die sich in ihrer Milieuabhängigkeit und Strahlenempfindlichkeit voneinander unterscheiden. Besonders wird auf präklinische Experimente mit Pionen Wert gelegt, die eventuell eine hochwirksame Strahlung für die Bekämpfung des Krebses darstellen, da sie sich durch eine verschiedene räumliche Energieverteilung im zu bestrahlenden Volumen (Tumor) und dem umliegenden Gewebe auszeichnen. Die Resultate werden anhand der möglichen Schädigungsmechanismen (singuläre und multiple Ereignisse, Erholungsfaktoren) diskutiert.

Early Synthesis of the Precursor to RNA Tumour Virus Internal Proteins

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The synthesis of the 76,000 dalton precursor (Pr 76) of the avian tumour virus group specific antigens has been studied as a function of time after infection of chick embryo fibroblasts with avian myeloblastosis virus. During the course of infection, cells were pulse-labelled with ^{35}S-methionine, lysed, and the labelled viral precursor was precipitated with antibody against AMV. Pr 76 was detected by SDS gel electrophoresis of immune precipitates. – Pr. 76 synthesis began at 3 h after infection but was undetectable in uninfected cells. Pr 76 synthesis remained low – about $^1/_{100}$ that of fully infected cells – and constant from 3 until about 9 h after infection, at which time there began a large increase in its synthesis. Precursor synthesis occurred early (3–7 h after infection) in cells treated prior to and during infection with actinomycin D or cytosine arabinoside. The amount of precursor synthesized early in the presence of inhibitors was similar to that synthesized in the absence of inhibitors, suggesting that the incoming RNA served as a messenger RNA for Pr 76.

Polare Kopfgruppen in Membranen

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Phospholipid-Doppelschichten sind gute Modelle für die Struktur der Lipide in biologischen Membranen. Die polaren Kopfgruppen beeinflussen die Temperatur des Übergangs von der Geldphase in die flüssig-kristalline Phase und zeigen spezifische Wechselwirkungen mit Ionen und Proteinen. Wir haben daher Phosphatidylcholin und Phosphatidyläthanolamin spezifisch deuteriert. Mittels Kernresonanzspektroskopie haben wir die Quadrupolaufspaltung des Deuteriums und die Anisotropie der chemischen Verschiebung des Phosphors an entsprechenden Doppelschichten gemessen (Gally et al., Biochemistry *14*, 3647, 1975). Aus diesen Daten erhält man ein detailliertes Bild der Konformation dieser beiden Kopfgruppen. Binäre Mischungen dieser beiden Lipide zeigen Koexistenz von Gelphase und flüssig-kristalliner Phase im Übergangsbereich. Die Signale der Cholingruppe in äquimolaren Mischungen von Phosphatidylcholin und Cholesterol weisen auf das Verschwinden eines Phasenüberganges hin.

Intermolecular Disulfide Bond Formation of Histone 3 in Chromatin

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The present research was undertaken to determine if significant disulfide bond formation of histone 3 occurs chromosome condensation at mitosis, and whether phosphorylation of H3 might be a signal for such dimer

formation. Using monolayer cultures of Chinese hamster cells, mitotic cells were obtained by selective detachment, either in the presence of absence of Colcemid, and were compared with interphase cells by double label experiments. Chromatin was isolated at high or low ionic strengths, and histones were resolved on 25 cm urea-acrylamide gels. When chromatin is prepared with great care to avoid aerial oxidation, both mitotic and interphase cells contain less than 7% of their total H3 as a disulfide dimer. However, up to 5fold higher H3 dimer levels are observed when chromatin is prepared without precautions against oxidation. This 'induced dimerization' occurs during chromatin purification and not during subsequent protein processing steps. Phosphorylated H3 deos not preferentially dimerize in this manner. Except for phosphorylated H1 and H3 of mitotic cells, the levels and mobilities of histones of mitotic and interphase cells are identical.

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Inhibition of Antigen-Induced Lymphocyte Proliferation by Strain-Specific Anti-Idiotypic Sera

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The in vitro T cell proliferation induced by penicilloylated bovine IgG (BPO-BGG) in sensitized strain 2 and strain 13 guinea-pigs can be specifically blocked by strain-specific antisera directed against immunoglobulin idiotypes. The anti-idiotype anti-sera were prepared in strain 2 and strain 13 guinea-pig against antigen immunosorbent-purified anti-BPO-BGG antibodies which had been raised in strain 2 and strain 13 animals. Strain 13 anti-strain 13-anti-BPO-BGG (a strain 13 BPO-BGG) suppressed the in vitro BPO-BGG response of cells from immunized strain 13 animals but failed to inhibit the response from immunized strain 2 animals. Conversely, the corresponding antiserum raised in a strain 2 combination (a strain 2 BPO-BGG) only inhibited the in vitro BPO-BGG response of strain 2 cells. The inhibitory activity of the antisera could only be absorbed by immune cells from the syngeneic strain. The activity of the a strain 13 BPO-BGG serum was highly specific: the inhibitory activity could only be absorbed by BPO-BGG strain 13 cells. The experiments suggest that(a) the production of anti-idiotype antibodies play a role in the regulation of the immune response, (b) T cell receptors carry similar idiotypes to immunoglobulins.

Molecular Forms of AChE in Intact and Denervated Sympathetic Ganglion

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Four species of acetylcholinesterase activity with sedimentation constants of 4S, 6,5S, 10S and 16S were observed when proteins, extracted from the superior cervical sympathetic ganglion of the rat, were subjected to velocity sedimentation. The solubilization of the 6,5S and 10S forms required 1% Triton and the 16S form a high ionic force in addition to the detergent (1% Triton $\pm$ 1 *M* NaCl). Three days after the section of the preganglionic nerve, the total AChE activity decreased suddenly, to about 50%. Afterwards the activity recovered progressively, up to 80–90% of the initial value. These variations of the total activity could be ascribed mainly to the variations of the activity of the 10S form. The denervation increased the activity of the 16S form, in contrast with the loss of this form observed by Hall in the denervated neuro-muscular junction (J. Neurobiol. *4*, 343, 1973). The cytochemical study showed that the AChE activity decreased sharply in the denervated sympathetic neurones and remained low, even during the recovering phase. During this period an AChE activity appeared in the perineuronal glia.

Preliminary Results on Human Mesothelial Cells Growth

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DNA content measurements on mesothelial cells smears showed the presence of diploid, tetraploid and polyploid cells. No cell was labelled after 1 h incubation with Th-^{3}H. Cellular amount of proteins varied from simple (2p) to double (4p) and more. Nuclear histones content had the same behaviour of DNA content. No mitosis was observed. The results showed that the proliferative cycle was suppressed and cells were in R1 or R2 phases. These cells lived 4–5 months in culture. At the beginning they had a mitotic activity (number of mitoses $3^0/_{00}$). After 1 h incubation with Th-^{3}H 5–10% of nuclei were labelled. The histograms of DNA content showed a growing cell population, the cells in G2 of which increased in number till the end of the first month. From this moment an autoregulation by proliferative cycle inhibition was established, with a constant number of cells in G1 and G2 (cells which could be resting cells [R1 and R2]). During this period no mitose neither Th-^{3}H labelled nuclei could be seen. Histones content was coupled together with DNA content during the all culture period. Cellular amount of total proteins progressively increased during culture. Cell ageing was observed during the all period of culture (amitosis, polyploid cells, nucleolus going into cytoplasm accompanied by chromatin, abnormal synthesis of cytoplasmic-mitochondrial DNA). Nuclear amitosis was observed in tetraploid cells. The adjunction of β-estradiol in vitro did not modify cell growth.

Freeze-Fracturing in Ultrahigh Vacuum

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Experience has shown that with standard freeze-etching techniques the resolution of the method in recording structural details of frozen specimens is limited to about 3 nm. In the endeavour to push this limit forward, a new apparatus has been developed which allows fracturing and coating (i.e. evaporation of Platinum-Carbon) of specimens at –196 °C maintaining a vacuum better than 1×10^{-9} Torr. This machine consists of a Balzers BA 350 UHV unit equipped with an airlock which enables the in-

put of un-hoar-frosted specimens directly into the evacuated chamber. A new fracturing system and improved electron guns have been included which, together with film-thickness and deposition rate measuring devices, make possible a completely reproducible coating. – A comparison of yeast plasmalemma structures portrayed by standard and UHV-freeze-fracturing shows clearly visible progress which can be explained by less contamination of the cold specimen surface under UHV and less distortion of structures during fracturing at lower temperature. A more detailed analysis of the progress has been achieved by appliying the methods of image analysis and reconstruction.

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Anatomy of the Gene Cluster Coding for the Five Histone Proteins in the Sea Urchin

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The repeated genes coding for the 5 major histone proteins in the sea urchin *Psammechinus miliaris* have been mapped by molecular techniques using restriction, resection and RNA/DNA hybridization. The 5 genes are contained in a repeating unit of 6 kb. Within this unit, the genes are not contiguous, rather they alternate with spacer DNA. All coding sequences are arranged asymmetrically on the same DNA strand. Their arrangement in the direction of transcription is H4, H2Y, H3, H2X, H1.

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The Determination of the Molecular Structure of Adenovirus Type 2 Hexon

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Adenovirus Type 2 hexon is the major structural component of the virion. 240 hexons at the faces and 12 pentons and fibres at the corners form the icosahedral virusshell. Hexon has a molecular weight of 360,000 and is composed of 3 identical subunits (Grütter and Franklin, J. Mol. Biol. *89*, 163–178, 1974). Hexon crystallizes at pH 3.2 with space group $P2_13$ and a cell edge of 150.4 Å. An increase of the ionic strength enabled both a change of pH to 5, facilitating platinum binding, and a concurrent increase in crystal life of 50%. The binding sites of a mercury PCMS derivative were identified from difference Patterson projection at 3.5 Å. These sites were used to phase difference Fourier projections of other derivatives. Refinement of the binding site parameters for PCMS, $PtCl_4^{2-}$ and $Pt(en)Cl_2^{2-}$ gave a figure of merit of 0.75 for the 3.5 Å centric projection. – The 3-dimensional data is being collected with an Arndt-Wonnacott oscillation camara.

Ribosomal RNA Synthesis during the Mitotic Cycle in *Physarum polycephalum*

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An isotope dilution technique has been used to analyze the synthesis of metabolically stable nucleic acids during the mitotic cycle in surface plasmodia of the slime mould *Physarum polycephalum*. Microplasmodia that had been labelled with ^3H uridine were used to prepare a surface culture, after a period of growth long enough to ensure that radioactivity was present only in tRNA, rRNA and DNA. The synthesis of rRNA during the growth of the surface plasmodium was then followed by measuring the specific activity of the nucleic acid. – Synthesis of rRNA during the mitotic cycle shows the following characteristics: (a) it is low during the immediate period of nuclear division, (b) synthesis is then continuous throughout interphase and (c) the *rate* of synthesis increases 5–6fold between the beginning and end of interphase. These results are discussed in relation to the pattern of replication of the genes for rRNA.

Properties of Chromatin Structures from Interphase Cells

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The chromatin of interphase cells of a number of cell lines grown in culture is released as a discrete structure by the nonionic detergent Nonidet P-40 in low ionic strength medium without divalent cations. Electron microscopy shows that the chromatin structures thus released retain no nuclear membrane (which is dissociated by the detergent), and that the pore-complex layer which lies under the nuclear membrane is also removed as sheets of pores with interconnecting fibrils, probably due to the expansion of the chromatin – Fragmentation of these structures releases soluble chromatin molecules showing the characteristic nucleosome subunit structure, of about 10–20 nucleosomes in length. Those chromatin molecules which bear nascent RNA chains in transcription may be separated from nontranscribed molecules as described by Faber (these abstracts).

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The Binding of Rifampicin to RNA Polymerase at Various Transcription Steps

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Two separate features must be distinguished in the action of the antibiotic rifampicin on bacterial RNA polymerase: (1) The rate of binding (k_a) of rifampicin to RNA polymerase at the various transcription steps; (2) the effect of rifampicin on transcription when bound to RNA polymerase. We have investigated the first question using T7 DNA as template. It was found that the k_a decreases with increasing stability of the enzyme-nucleic acid complex. Thus, a nonspecific complex between T7 DNA and holo or core enzyme binds rifampicin 10 times slower than the free enzyme, whereas a stable and specific holo enzyme-T7 DNA promoter complex

binds the drug 1000 times slower. The rate of binding to the even more stable ternary enzyme-T7 DNA-RNA complex as it is found during elongation is about 100,000 times slower. These direct measurements of k_a explain the earlier observations that preincubation of RNA polymerase with DNA can lead to a protection against inactivation by rifampicin, and that during elongation the enzyme is resistant towards the drug.

Efficiency of Energy Conversion in Whole Cells of *Paracoccus denitrificans*

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Efficiency of conversion of metabolic energy into ATP is investigated with intact, resting cells of *P. denitrificans* grown aerobically. A new reaction chamber is described which allows for rapid withdrawal of samples from a homogeneous cell suspension. Changes in cellular nucleotide pools are induced through rapid transitions between anaerobiosis and aerobiosis. Nucleotide pool sizes are determined with high performance liquid chromatography (HPLC) and with the sensitive luciferin-luciferase assay. – Actual ATP/O ratios between 1.5 and 2 are calculated from the rates of ATP production and AMP utilization during the transition to aerobiosis and the maximal steady state oxygen consumption rate. – An electron transport chain similar to that of mitochondria but with only two energy coupling sites is proposed for *P. denitrificans*. Uncertainty exists about a functional site III in this bacterium. – Varying energy charge in vivo and following its effects on selected biochemical reactions or 'pulse energizing' the cells for desired periods of time and monitoring the consequences on metabolism are two possible applications that emerge from this study.

A Fast and Simple Method for the Preparation of Histological Cryostat Sections for Ultrastructural Investigation

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The normal collection method for cryostat sections using a temperature gradient causes the tissue water to thaw. Therefore a tight adhesion of cryosections to the supporting glass slide follows, making it very difficult to separate the fixed sections from the slide. However, if the slide is first coated with a 0.3% solution of Parlodion (nitrocellulose) in amyl acetate, a supporting film is formed which allows for easy removal of the sections from the glass after the first fixation step. The parlodion film can be dissolved in propylene oxide just before embedding. The Parlodion film forms an elastic substrate, which can expand and contract with the tissue, whereas cryosections picked up on glass slides are compressed and partially destroyed by osmotic changes. For histochemical procedures, there is no chemical interference, nor are the structural details obscured. Using this technique complete fixation of a 20 µ liver tissue cryosection was achieved after only 30 sec in 2.5% glutaraldehyde. The described method allows an exact correlation between histochemical and ultrastructural findings.

Polypeptides of the in vitro Translation of Rous Sarcoma virus RNA

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The 30–40s subunits of the Rous Sarcoma virus (RSV) genome can be translated in a cellfree system of ascites Krebs II cells, preferentially into a polypeptide of 75,000–80,000 dalton. Immunochemical and tryptid fingerprint analysis shows that this in vitro made polypeptide contains peptide sequences identical to the group-specific (gs) antigen of the RSV. – A minor product of the in vitro translation of the 30–40s RSV RNA is a polypeptide of 140,000 dalton, which has little or no peptide sequences with the gs antigen. – The posibility of the viral RNA as a polycistronic messenger RNA will be discussed.

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Studien der dynamischen Eigenschaften von globulären Proteinen mittels semi-empirischer Berechnungen der Konformationsenergien: Aromatische Aminosäurereste in BPTI (= basischer pankreatischer Trypsin-Inhibitor)

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NMR-Untersuchungen in Lösung haben gezeigt, dass die aromatischen Ringe der Phenylalanin- und Tyrosin-Seitenketten in der globulären Form von BPTI sehr rasche ($\gtrsim$200 s^{-1}) 180°-Rotationsbewegungen um die C^β–C^γ-Bindung ausführen können. Mit Konformationsenergieberechnungen, basierend auf semi-empirischen «klassischen» Potentialfunktionen, wurde gezeigt, dass die beobachteten Energiebarrieren für diese Rotationsbewegungen nur verstanden werden können, wenn man Fluktuationen der Positionen aller Atome um ihre röntgenkristallographisch bestimmten Mittelwerte zulässt. Die Arbeit ergab auch einige Einsicht in die Natur dieser intramolekularen Fluktuationen, die notwendig sind, damit die aromatischen Ringe umklappen können. Die Ergebnisse weisen darauf hin, dass sich die Rotationsachse (C^β–C^γ-Bindung) selber nur sehr wenig verschieben muss ($\Delta\chi^1 \lesssim 15°$). Die mit Abstand wichtigsten Fluktuationen sind diejenigen jener Atome, welche in der starren Röntgenstruktur die unmittelbare Umgebung der Ringe bilden. Diese bewegen sich dann vom Ringzentrum weg und machen so die Übergangskonformationen sterisch möglich.

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Purification of Globin-Specific DNA from Rabbit Liver DNA

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High molecular weight rabbit liver DNA was cleaved by $EcoR_1$ restriction endonuclease and denatured. In order to purify the plus strand globin DNA, the DNA was hybridized in the presence of oligo C with dC-elongated rabbit globin cDNA (obtained by transcribing rabbit

mRNA with RNA-dependent DNA polymerase and elongating the DNA with dC residues, using terminal transferase) and passed through a poly I-Sephadex column (Coffin et al., J. Mol. Biol. *86*, 373, 1974). The hybrid was eluted with formamide, self-annealed and purified on hydroxyapatite. As assayed by hybridization to [^{125}I] cDNA about 5–10% of the globin specific DNA was recovered, with a purification of about 200- to 500fold. To purify the minus strand globin DNA, denatured restriction fragments were hybridized to globin mRNA and the resulting hybrid purified on poly U-Sephadex. As assayed by hybridization with [^{125}I] mRNA, a 100fold purification with a yield of 5–15% was achieved.

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Localization by SEM of Wheat Germ Agglutinin Receptor Sites on Erythrocyte and Yeast

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Colloidal gold of a size suitable for SEM (Horisberger et al., Experientia *31*, 1147, 1975) was coated with WGA cross-linked to bovine serum albumine. When erythrocytes were incubated with the marker, and examined by SEM, the marker was distributed homogeneously over the surface. Labelled erythrocytes were lyzed and the isolated membranes reincubated with a smaller size marker. Receptor sites appeared to be on both sides of the membranes when examined by TEM. The smaller size gold granules were distributed in patches. – *Saccharomyces cerevisiae* cells had to be first treated with an α-mannanase to interact with the WGA marker, thus exposing chitin and chitin oligomers. When examined by SEM, the marker was found on the chitin ring of bud scars and randomly distributed on the surface of young cells thus confirming that chitin oligomers are present in the yeast cell wall (Bauer et al., Arch. Mikrobiol. *85*, 202, 1972).

Progesterone and Estradiol Receptors in Human Breast Cancer

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Sera of 200 breast tumor patients have been screened for alterations in the level of Estradiol (E_2), Progesterone (P), Prolactin and Growthhormone. Our results as well as those of other workers have shown the plasma levels of above hormones to be normal in breast cancer patients. Therefore the reason for hormone dependency of breast carcinoma growth has to be looked for within the carcinoma cell itself. In our experiments we studied the content of estradiol receptors in 29 cases of human breast tumors and found that 40% contained the E_2-binding protein. The receptor content was measured in 14 cases and 70% contained progesterone receptor. The tumor tissue which bind E_2 and P had binding constants (K_d) of 0.04 n*M* and 0.03 n*M* respectively. In certain cases sucrose gradient studies carried out showed 4S and 8S binding for estradiol, which was completely abolished by U-11100 (an antiestrogen) indicating the specifity of E_2-binding to both forms of the receptor. Similar experiments are presently carried out on the progesterone receptor using the highly specific progestin R5020 (Roussel-Uclaf). These experiments indicate that progesterone is bound to a component which is different to the estradiol receptor, but has similar properties. These findings suggest that not only E_2 and its receptor have to be taken in account in hormonal therapy of human breast cancer but the progesterone receptor content as well.

Inhomogeneity of Renal Glomerular and Tubular Membranes as Detected by Freeze-Fracture

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The different parts of the nephron and of the collecting tubule perform complex and specific functions, many of them thought to involve the plasma membrane of epithelial cells lining each segment. A counterpart for such specificity has been seeked by freeze-fracture, since it is believed that the degree of functional complexity of a given membrane is related somehow to the amount of protein (particles) present in the membrane. Indeed, freeze-fracture reveals membrane proteins as distinct subunits, the intramembranous particles, whose size and number can be quantitated. With this technique, it can be shown that the epithelial cell plasma membranes of kidney glomeruli and tubules have a population of intramembranous particles characteristic for each segment. The number of intramembranous particles on the cytoplasmic leaflet of the plasma membrane was found to vary from a maximum of 3500/μ^2 (A-face) in the descending branch of Henle's thin loop to a minimum of 250/μ^2 in the foot processes of podocytes. Similarly, the size of individual particles varied from 13 nm (podocytes) to 8.7 nm (papillary ducts), as measured in the same leaflet of the plasma membranes. These results indicate that the functional specificity of the various kidney plasma membranes is well reflected in their morphological inhomogeneity.

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Chemical Dehydration of Microorganisms for Scanning Electron Microscope Study

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Repeated mechanical treatments, such as centrifugation, can destroy fine details of microorganisms during sample preparation for scanning electron microscopy. Classic solvent-water exchange based methods claim at least 9 resedimentations, and about 2 h of preparation time. When dealing with small amounts of cells, repeated changings of baths may also lead to a loss of material. Chemical dehydration based on destruction of water molecules by reaction with 2,2-dimethoxypropane (L. L. Muller, T. J. Jacks, J. Histochem. Cytochem. *23*(2), 107, 1975) or 2-methoxyethanol, followed by a washing in absolute acetone, reduce the number of manipulations to two and the time to half-an-hour. These techniques have been successfully applied to preparation of glutaraldehyde fixed *E. coli*, *Proteus vulgaris*, *Clostridium perfringens*, *Clostridium sporogenes*. Comparison is made with classical acetone dehydration. All samples have been critical point dried in CO_2.

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Crystallographic Studies on Thermophilic LDH from *B. stearothermophilus*

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Large single crystals of the thermophilic lactate dehydrogenase from *B. stearothermophilus* (we are grateful to Prof. H. Zuber, ETH Zürich, for generous gifts of the purified enzyme) have recently been grown. Preliminary X-ray crystallographic studies have determined the space group to be $P6_1$ (or $P6_5$), $a = b = 86.2$ Å, $c = 358$ Å, $V = 2.3 \times 10^6$ Å^3 with one tetramer (mol wt 133,00) per asymmetric unit. Crystallization conditions will continue to be screened for additional crystal forms and for maximum stability of the present form. Also, we are now studying data collection techniques suitable for large unit cells. These include modifications of the Arndt oscillation camera which will allow greater crystal to film distances without loss of resolution.

Partial Genetic Map of Rous Sarcoma Virus RNA

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Complete digestion of oncorna virus RNA with T_1 RNase yields a set of large oligonucleotides which can be isolated by 2-dimensional polyacrylamide gel electrophoresis. RNAs from different strains of oncorna virus yield different and characteristic sets of T_1 oligonucleotides which will be described. The relative order of the large T_1 oligonucleotides (physical map) has been established for several strains by the method of Coffin and Billeter (J. Mol. Biol., in press). Crosses were carried out between pairs of strains differing substantially in their T_1 oligonucleotide pattern and in one or more genetic properties. Analysis of the RNA of several recombinants from each cross allowed the correlation of genetic properties and T_1 oligonucleotides characteristic for the one or the other parent. A cross between Prague strain RSV (subgroup B, transforming) and RAV 1 (subgroup A, non-transforming) allowed location of subgroup specificity (env) in about the middle of the genome, while the transforming capacity (trf) mapped close to the 3′ terminus. A cross between wild type RAV 6 and Prague C RSV carrying a temperature sensitive mutation in reverse transcriptase (pol) showed that this mutation was located at the 5′ end of the RNA molecule. The order of the genes located so far is 5′-pol-env-trf-(poly A)-3′.

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Immunochemical Isolation and Characterization of Vitellogenin mRNA from Estradiol Treated Chicks

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Purification of vitellogenin mRNA was achieved through 3 steps. (a) A heavy polysome fraction was obtained by discontinuous sucrose density gradient centrifugation. (b) Vitellogenin polysomes were immunoprecipitated with anti-vitellogenin IgG and goat anti-rabbit IgG. (c) The poly-A containing mRNA was isolated on a poly-U Sepharose column. Anti-vitellogenin IgG and goat anti-rabbit IgG were purified on CM and DEAE cellulose. In addition very specific anti-vitellogenin IgG was obtained by affinity chromatography on a lipovitellin-Sepharose column. As judged by its specific activity in a reticulocyte lysate system, vitellogenin mRNA has been purified a 1000fold with a recovery of 30%. On 99% formamide-polyacrylamide gels vitellogenin mRNA has a mol wt 2.5×10^6 and codes for a main polypeptide of a mol wt 240,000. As measured by urea-polyacrylamide gel electrophoresis of the ribonucleases T_1 and A digests, the polyadenylic segment of vitellogenin mRNA has about 200 nucleotides.

Genetic Studies on the Heat-Induced Loci of *Drosophila melanogaster*

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X-irradiation of *Drosophila melanogaster* have yielded a number of chromosomes deficient for a region of the third chromosome that 'puffs' on heat-treatment. Two of these, Df(3R)kar^{3J} and Df(3R)kar^{3Q}, lack the 87Cl heat-induced puff. Homozygotes for kar^{3J} die as first-instar larvae lacking malpighian tubules. This permits ready identification of mutant larvae that are completely deficient for the 87Cl region normally activated by heat-shock. SDS-polyacrylamide gel electrophoresis of the heat-induced proteins of individual homozygous kar^{3J} and wild-type larvae does not reveal any qualitative differences. The relationship between the heat-activtated loci and subsequently synthesized proteins is being investigated.

Freeze Drying-Shadowing of Supramolecular Crystalline Structures

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A new freeze-drying and shadowing technique was developed which is suitable for structural analysis of 2-dimensional protein crystals with subsequent application of optical diffraction and filtering methods. In addition to standard negative staining where only integrated mass densities in the direction of the electron beam are recorded, shadowed pictures reveal 3-dimensional aspects of the structural surface, depending on the elevation angle of the evaporation source. Resolution limits are set up by the specimen preservation (thermal vibration and collaps phenomena during sublimation). The grain size and the distribution of the shadowing deposit and above all by the elevation angle of the evaporation source which has to be determined according to the structural features of the specimen. Since unidirectional shadowing causes a superimposed resolution polarity which can be easily shown in diffraction, one has to select out particles with varying azimuthal angles to the shadowing direction. Systematic data have been collected on T-layers of *Bac. Brev.* which are built of a tetragonally arranged protein (mol wt = 140,000). The data from freeze dry-shadowing are presently compared

to other preparation methods such as replica-negative staining and one-sided preferentially stained structures. Thus some information about the 3th dimension should get available.

The Swiss STEM Project

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The goal of the Swiss STEM project is to investigate experimentally the advantages of a Crew-type scanning electron microscope (Crewe 1970) for biological research. The system, based on the microscope HB 5 from Vacuum Generators, which is interfaced to a mini computer V73 from Varian has been deviced with the aim to help overcoming the damaging effects of the electron beam, which is at present the first limitation to the capability of electron microscopes (Beer et al. 1975). In this respect the development are along two lines: (1) Optimization of detectors and data acquisition system such as to detect and process most electrons that have interacted with the specimen, thus reducing the electron dose required to record an image. (2) The damaging effect of a given electron dose depends on molecules adsorbed onto the surface of a biological specimen (Dubochet 1975). Therefore, our STEM is equiped with a pretreatment chamber where the specimens can be treated either by removing the adsorbed molecules (by heat, light, ion or electron bombardement) or by replacing them by some nonreactive gas.

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Relationships of Parental Components in Mouse Zygote Genome Using BudR-Containing Spermatozoa

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Exposure of cells to 5-bromodeoxyuridine (BudR) during two rounds of replication results in chromatids either unifilarly or bifilarly substituted. These can be morphologically distinguished using a combined stain (fluorochrome Hoechst 33258 followed by Giemsa; P. E. Perry and S. Wolff, Nature *251*, 156, 1974). This technique has been adapted to mouse spermatogonia in vivo, according to results previously obtained with tritiated thymidine. The dose of BudR has been carefully selected at 0.25 µg/testes, based on the observation of minimal damage expressed either at male meiosis or on fertilizing ability of resulting spermatozoa, as well as on the proportion of normal viable offsprings following fertilization. The BudR-containing paternal strands have been observed in fertilized zygotes at various stages of cleavage (pronuclei to 16 cells embryos) in the chromatin of interphase nuclei and in chromosomes. In-vivo exposure conditions do not produce a uniform incorporation pattern, however, a statistical proportion of cells is obtained which allows recognition of localized paternal chromatin in suitably well spread nuclei and of sister-chromatid exchanges as a function of zygote mitosis in metaphase chromosomes.

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Langmuir-Blodgett Layers Instability Shown by EM and ATR-IR

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Mono- and multimolecular layers of saturated lipids, deposited on a solid substrate by the method of Langmuir and Blodgett, may undergo ultrastructural and molecular transitions in the course of which the original films brake down and the substance rearranges into randomly distributed microcrystals. With the aid of electron microscopy and attenuated total reflexion infrared spectroscopy (ATR-IR), it can be demonstrated that in the case of tripalmitin a transition from a liquid crystalline state to a crystalline state takes place. In order to get more details on the time course of the rearrangement process, the time dependend changes in the CH_2-bending region were analyzed. Concomitant with the rearrangement, the methylene-bending band is shifted from 1469 to 1473 cm^{-1} and a reduction of its half width is observed. Since oriented layer assemblies, built up by the Langmuir-Blodgett technique, have widespread applications in physico-chemical studies, such an instability could lead to severe implications and should therefore be considered.

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Human Pineal Acervuli: Ultrastructural and X-Ray Energy Dispersive Microprobe Analysis

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The pineal acervuli obtained from seven 65–75 year old humans of both sexes were studied with both scanning (SEM) and transmission (TEM) electron microscopes and with an X-ray energy dispersive spectrometer. – Under the SEM the acervuli show a lobated structure. The lobes can measure 135–800 µm in diameter. Between the lobes are localized clustered groups of globuli of 4–14 µm in size. A 'bas-relief' of corresponding dimensions was observed on the convexity of the lobes. The globuli are bound together and with the lobes by 0.2–1.5 µm thick bridges. Both of these facts suggest that an aggregation of globuli form the acervular lobes. – In the TEM the acervular mineral is composed of randomly set needle-shaped crystals of dimensions identical to hydroxyapatite. – The X-ray microprobe analysis reveals the existence of Ca and P as main components of the acervuli. Small quantities of Cl, S, Mg and Sr were also detected. – Our results confirm earlier X-ray diffractographic studies of Angervall et al. (Acta Pathol. Microbiol. scand. *44*, 113, 1958) and Earle (J. Neuropath. Exp. Neurol. *24*, 108, 1965) about hydroxyapatite nature of human pineal acervuli.

Processing of Electron Microscopical Images

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Resolution in bright field phase contrast electron microscopical images can be extended beyond the conventional limit by imaging at special optimal defocus values and subsequent processing of the images. Proper account must be taken of the defects of the transfer function by correcting for sign reversals and attenuation of the spatial frequencies transmitted into the image. The improvement in image quality, the accuracy and the relative merits of processing by analog and digital methods have been assessed by processing images of the same object taken at different optimal defocus settings. Areas of 1024×1024 picture elements are used. Visual assessment of the results as of the modifications due to the processing steps is made possible by displaying the digital data in an optical density representation. The gain in image quality is quantified by the use of auto- and cross-correlation functions. The problems of generating a gapless transfer function by combining two or more images are investigated. The trade-off of having a perfect transfer function whilst the object has suffered changes due to radiation damage and the effects on the image are studied.

Artificial Swelling of λ Preheads

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Preheads of bacteriophage λ are small DNAfree particles that can be transformed to larger DNA containing heads by providing the appropriate DNA and maturation proteins. The enlargement of preheads can however be induced artificially, i.e. in absence of DNA. Different conditions as pH, temperature, presence of certain concentrations of urea have been found under which this process takes place. A number of physical methods was used to characterise preheads and enlarged (empty) preheads. Diffusion constants and Stokesradii were determined by Laser light scattering using a 24 channel Malvern correlator. (Radii are 280 Å and 320 Å for preheads and enlarged preheads resp., 3% error.) In combination with sedimentation constants (s = 138 and 124 in H_2O at 20 °C, 3% error) the molecular weight can be calculated to be 17 10^6 (6% error) for both types of particles. This is in good agreement with independent mw determination by sedimentation equilibrium. Classical light scattering was used to determine radii of gyration (220 Å and 270 Å at 20 °C, 4% error). Together with estimates of thickness of the shell from electron microscopy of thin sections these data allow determination of approximative radii to be about 240 Å and 280 Å resp., which upon comparison with Stokesradii can be used to estimate the hydration of the particles.

Sequence Organization of $tDNA_1^{met}$ of *Xenopus laevis*

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DNA containing the reiterated genes for $tRNA_1^{met}$ has been partially purified from *X. laevis* by equilibrium centrifugation techniques. Further purification and evidence for long range periodicities within this DNA has come from the combined used of restriction enzymes and DNA-RNA hybridization. The $tDNA_1^{met}$ comprises tandemly-linked 3.1 kb repeat units which are defined by the regular spacing of sites recognized by *Eco*RI, *Hpa* I and *Hind*III. The repeat units can be further subdivided by digestion with *Hpa*II and *Hind*II. The combined effects of these enzymes provide a detailed map of this repetitive DNA and generate short DNA fragments that are suitable for detailed sequence analysis.

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Determination of Na^+ in Single Isolated Nuclei of *Chironomus* Salivary

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The ionic environment influences the structural and functional states of specific regions of isolated chromosomes. It is therefore important to know the ionic milieu inside the cell nuclei and its probable change under varying physiological conditions. The principal problems with determining this milieu are: (1) translocation of ions during sample preparation, (2) contamination, (3) quantitation, (4) sensitivity of the detection method. In order to overcome these problemes a new procedure has been developed. *Chironomus* larvae were rapidly frozen and lyophilized at −60 °C specimen temperature. The lyophilized material was then penetrated with silicone oil, in which the glands and their nuclei were extracted by micromanipulation. After removal of the oil the dry weight of each nucleus was determined by use of a quartz fibre balance, and the Na^+ was measured in a flameless atomic absorption apparatus. The Na^+ content of nuclei varies between 1 and 5 mg/g dry weight. Presently, we are investigating the correlation between this parameter and the physiological state of the nuclei.

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Isolation and Partial Purification of Glial Factor

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Morphological transformation of cultured neuroblastoma cells induced by glial factor has been reported (Monard et al.). The importance of this factor is related to its possible biological role in the glial-neuronal relationship in developing brain. Conditions to maximise the yield of glial factor from C-6 glial cells have been investigated. After an initial frowth period in serum containing medium, glial cells were maintained for 4 days on serum free medium, with a medium change every 24 h. Glial conditioned medium was concentrated 20fold and a

substantial purification of glial factor was achieved by a combination of DEAE-cellulose chromatography and gel filtration on Sepharose-6B. The glial factor is stable under physiological conditions, in high salt and urea, and on storage at neutral pH at 4° or −20 °C, but is inactivated by proteolytic enzymes. The apparent mol wt of the factor is in the 10^6 range, but aggregation is suspected.

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Effect of a PG-E_2 Analogue (PG-E_2′) on the Vascularization of the Gastric Mucosa in the Rat[1]

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We have quantified the effects of PG-E_2′ on the vascularization of the gastric mucosa by determining the relative volume occupied by erythrocytes (V_{ve}) as a measure of the capillary volume in the zones of the acid- and mucus-producing cells (A- and M-zones). Two ulcer-inducing treatments were adopted: immobilization stress and oral administration of 150 mg/kg phenylbutazone (PB). PG-E_2′ was given 1 h after starting the ulcerogenic treatment at the fully protective oral dose of 1 mg/kg. In non-treated rats, V_{ve} was the same in the A- and M-zones. Immobilisation resulted in a 50% increase of V_{ve} in the A- and a 60% decrease in the M-zone, whereas PB caused a decrease in both zones. Treatment with PG-E_2′ resulted in a 3- to 4fold V_{ve} increase in the M-zone of both immobilized and PB-treated rats. – It appears that the anti-ulcer activity of PG-E_2′ is related to an increase in vascularization in the zone of the mucus-producing cells of the gastric mucosa.

[1] PG-E_2′ is 11,15-dihydroxy-9-keto-16-dimethyl-2,3-methylene-prosta(5,13)dienic acid

In vitro Transcription of Amplified rDNA by the RNA-Polymerase C from *Xenopus laevis* Ovaries

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RNA-polymerase C, sensitive to high concentrations of α-amanitin, represents 70% of the total RNA-polymerase activity solubilized from *Xenopus leavis* ovaries. After partial purification, it can be resolved in two forms by chromatography on DEAE-Sephadex. Unlike RNA-polymerase A, RNA-polymerase C is able to transcribe efficiently high molecular weight DNA and intact adenovirus DNA. Transcription of amplified rDNA by the RNA-polymerase C is not random: 90% of the RNA synthesized in vitro hybridizes to the H-strand, which serves as template in vivo. In contrast, both strands are transcribed with equal efficiency by RNA-polymerase A. On denatured rDNA, RNA-polymerase C no longer discriminates between the two strands. The region of the gene where transcription takes place has been localized by hybridisation of the in vitro product to restriction fragments of rDNA cultured on plasmids and by competition hybridization with 18S and 28S RNA.

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Do Supra-Ependymal Serotonergic (5-HT) Nerves Regulate Ciliary Activity in the Rat Brain?

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The hypothesis was tested that supra-ependymal 5-HT nerves exert a regulatory role on the activity of ependymal cilia measuring the velocity of particle transport. A suspension of erythrocytes (1 µl) was carefully introduced above the exposed fourth ventricle which was maintained at 37 °C in Krebs-Ringer bicarbonate (+ glucose) saturated with 5% CO_2 in O_2; the time required for their extrusion out of the ventricle was measured. No correlation with 5-HT mechanisms was found: the transport of erythrocytes was unchanged after reserpine (10 mg/kg i.p. 18 h before sacrifice), DL-*p*-chlorophenylalanine (3 × 100 mg/kg i.p. 72, 48, 24 h) or intraventricular injection of the neurotoxic agent 5,6-dihydroxytryptamine (25 µg 4 days). 5-HT added to the medium (1 m*M* 5-HT + 1 m*M* pargyline) and attempts to release 5-HT from supra-ependymal nerves (using 10 µ*M* reserpine or 0.1 m*M* D-amphetamine + pargyline), also proved ineffective. Nevertheless, inhibition of ciliary activity can be readily demonstrated with low temperature (22 °C), saturation of buffer with N_2/CO_2, 2,4-DNP (0.1 m*M*); KCN (0.1 m*M*), NaF (1 m*M*) and iodoacetic acid (1 m*M*). Stimulation, however, of ciliary activity by 5-HT mechanisms cannot be excluded since adenine nucleotides (1 m*M* ATP or 0.1 *M* cyclic AMP), known to stimulate activity in various ciliary systems, did not influence the transport of erythrocytes.

Outside-Out Orientation of Plasma Membrane Vesicles Revealed by Freeze-Fracturing

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The orientation of the plasma membrane (PM) in vesicles formed during homogenization of liver tissue was found identical to the orientation in intact cell. A plasma membrane rich fraction was isolated from rat liver microsomes by flotation through a gradient of 3 steps containing 57%, 34% and 0,25 *M* sucrose in 3m*M* imidazole-HCl buffer pH 7,4. With respect to the starting material (100%) this membrane fraction contained 5,8% of proteins, 1,4% of glucose-phosphatase and 28% of the 5′-nucleotidase indicating an richement of PM fragments. The specific activity of the 5′-nucleotidase was 0.417 U/mg in the purified fraction as compared to 0.087 U/mg in the starting microsomal preparation (mean of three experiments). Samples of this fraction were submitted to freeze-fracturing. The particle density on concave vesicle profiles (EF_v) was determined. 85–90% of the total surface area of the concave vesicles had a particle density less than 2000/µm², which is consistent with the density determined on face B (EF_{pm}) of the PM in intact tissue. 6–10% of the vesicle surface corresponded to endoplasmic reticulum ($N_p/\mu m^2$ = 2000–3500) and only 3% of the A face of PM or of the outer mitochondrial membrane ($N_p/\mu m^2 > 3500$). This ananlysis leads to the conclusion that PM vesicles produced by homogenization are oriented in the same way as intact plasma membrane, i.e. with the external membrane leaflet towards the outside.

Histone Phosphorylation in Human Lymphocytes as Early Event Following Stimulation by PHA

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Lymphocytes were isolated with the Ficoll-Hypaque technique and washed twice in medium 199 (HEPES buffered). After preincubation for 20 h in medium 199 (sodium bicarbonate buffered) to one half of the cells PHA-M (Difco) was added, followed by a 30–60 min pulse with ^{32}P-orthophosphate for both stimulated and control cells. – Cells were washed twice with saline phosphate buffer and the histones extracted with 0.4 *N* H_2SO_4. The Histones were precipitated in 25% TCA, washed in acetone and electrophoresed on urea-polyacrylamide gels. The gels were scanned, the peaks integrated and the radioactivity determined by liquid scintillation counting. The histones of the stimulated lymphocytes contained 2.27 times more ^{32}P than those of the controls. – In order to study the influence of the donor age on the response to stimulation these experiments are also performed with lymphocytes from subjects of more than 65 years of age. First results indicate that during ageing histone phosphorylation in response to PHA stimulation is diminished.

A New Method for the Isolation of Polytene *Chironomus* Salivary Gland Nuclei

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The polytene nuclei of *Chironomus* salivary glands are a good system for studying the regulation of transcription. However for many biochemical aspects milligram quantities of polytene nuclei would be desirable. Therefore we developed a new method for the isolation of such nuclei. Larvae are frozen in liquid propane, brocken in a precooled mortar and thawed in a Mg free sucrose medium under controlled conditions. This suspension is pressed through a glass tube with regulary spaced capillary constrictions. After a short settling time the sediment consists only of salivary glands, malphigian tubes and guts (recovery of glands: 80%). The glands are finally cleaned under a dissecting microscope. Nuclei are extracted from the glands by suction through an injection needle in the presence of digitonine and filtration through a nylon net. By this technique purified nuclei show normal chromosome morphology and the known response to changes of the ionic milieu. The electrophoretic pattern of chromosomal proteins and the transcriptional activity corresponds to that of fresh nuclei.

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Kinetics of Dimerization of the Variable Fragment of the Bence-Jones Protein Au

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The dimerization of the variable fragment of the Bence-Jones protein Au was examined in phosphate buffers at pH 6.8–6.9 and ionic strength of 0.1–0.2 at 20 °C. The dimerization constant from sedimentation equilibrium was about $1 \times 10^5\ M^{-1}$. On dimerization molar absorption coefficient per monomer at 235 nm increased by $1.6 \times 10^4\ M^{-1}\ cm^{-1}$. Fluorescence decreased down to about 58% of the monomer together with a blue shift of emission maximum from 350 to 345 nm. Temperature jump experiments from 16 to 21 °C exhibited the presence of two isomers of the dimer with an equilibrium constant of about unity. The association and dissociation rate constants were $9 \times 10^6\ M^{-1}\ sec^{-1}$ and $1.5 \times 10^2\ sec^{-1}$, respectively. The dimerization step was a fast pre-equilibrium of the isomerization which occurred with a half life time of about 0.1 sec. The enthalpy and entropy changes (per dimer) for the dimerization step were 6.4 kcal/mole and 44 e.u. and those for the isomerization were −1.4 kcal/mole and −5 e.u. The dimerization was an entropy-driven process.

Transplants of Primordial Germ Cells to Distinguish Somatic versus Germ-Line Contributions to Egg Shape

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The *Drosophila* ovary consists of germ-line (oocytes and nurse cells) and somatic tissue (follicle cells). Follicle cells are involved in forming the exterior egg membranes (chorion and vitellin membrane). The egg cytoplasm is formed primarily by the oocyte and nurse cells, whereas yolk is synthesized in the fatbody and transported to the oocyte. We have isolated a female sterile mutant, *fs(1)K10*, which produces abnormally shaped eggs, and have asked whether proper egg shape depends upon the genotype of the follicle cells or that of the oocyte and nurse cells. Reciprocal transplants of primordial germ cells between mutant and wild-type allowed the recovery of females with mosaic ovaries in which wild-type germ cells are surrounded by mutant follicle cells and vice versa. Wild type recipients receiving some mutant germ cells carrying genetic markers yielded wild-type but no mutant progeny. However, they produced abnormally shaped mutant eggs as well as normal eggs. In reciprocal transplants, mutant recipients receiving wild-type germ cells produced some normal eggs which gave rise to progeny of the donor genotype. Therefore, the abnormal egg morphology in this mutant depends upon the genotype of the germ-line rather than of the follicle cells.

Electrophoretic and Volumetric Characterization of Cultured Lymphocyte Subpopulations

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Nylon wool purified and unpurified mouse spleen cells were cultured for 4–6 days in the presence of irradiated allogeneic target cells. After culture the cells were purified of dead cells and separated electrophoretically. The cell fractions obtained were tested for (a) percentage of Θ-bearing cells (T cells), (b) percentage of surface Ig + cells (B cells), size distribution and cytotoxic activity towards fresh chromium labelled target cells. A 3-dimen-

sional computerised plot ('finger-printing') allowed the characterisation of 4 subpopulations according to size and charge, namely (a) unstimulated small T cells, (b) cytotoxic T blasts (varying in size according to culture period), (c) unstimulated small B cells, (d) stimulated B cells. The latter 2 classes appear only in vigorously proliferating cultures starting with unpurified spleen cells.

Phagocytosis of *L. enrietti* by Activated and Normal Macrophages: A SEM Study

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Mouse peritoneal exudate macrophages were cultured in Eagle's medium with syngeneic lymphocytes in absence (normal cells) or in presence (activated cells) of 5 µg/ml of Concanavaline A for 48 h. The cultures were then infected with flagellate forms of the protozoan parasite *Leishmania enriettii* for various lengths of time. Glutaraldehyde and osmium fixed cultures were dehydrated with acetone, critical point dried and observed by scanning electron microscopy. Parasites were seen to enter macrophages by their flagellum confirming earlier findings (H. Miller, D. Twohy, J. Protozool. *14* [4], 781, 1967). Activated cultures were characterized by extensive clumping of lymphocytes on and around macrophages, by an increased spreading and membrane activity of the latter cells and by their acquisition of the capacity to destroy engulfed parasites, as demonstrated by light microscopy.

Kinetics of Synthesis of Polyribosome-Bound and Free-Cytoplasmic mRNP in HeLa Cells

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Polyribosome bound messenger ribonucleoproteins and cytoplasmic messenger-like ribonucleoproteins not associated with polyribosomes were specifically labelled with 3H-Uridine in Hela cells in which rRNA synthesis was arrested with toyokamycin and mitochondrial RNA with ethidium bromide. The labelling kinetics of both RNP fractions were measured after CSCl buoyant density fractionation of the total cytoplasmic particle fraction (G. Spohr, N. Granboulan, C. Morel, K. Scherrer, Eur. J. Biochem. *17*, 296–318, 1970). – The incorporation of radioactivity into the free cytoplasmic mlRNP fraction follows exponential kinetics with mean half life of 0.5–1 h. – The incorporation of radioactivity into the polyribosomal mRNP fraction follows a complex exponential pattern which can be decomposed into a term with half life of the order of 24 h and into a term with half life of the order of 2–3 h. – RNA was extracted from polyribosomes after labelling with ^{3}H-Uridine for 1–8 h, and fractionated into polyadenylated and non-polyadenylated fractions. The polyadenylated fraction appears to contain the RNA of long half life, whereas the non-polyadenylated fraction appears to be kinetically heterogeneous and to contain RNA fractions of short half lives.

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The Chromaffin Granule Surface: Localization of Carbohydrate on the Cytoplasmic Surface of an Intracellular Organelle

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Intact chromaffin granules can be agglutinated in a sugar specific manner with Wheat Germ Agglutinin (WGA). In addition, significant amounts of sialic acid can be removed from the granule surface with neuraminidase. Taken together, these data indicate that complex carbohydrates are localized on the external (cytoplasmic) surface of chromaffin granules. WGA agglutination is mediated by glycoproteins in the granule membranes, not glycolipids. Models can be envisioned whereby such surface localized carbohydrates play a role in catecholamine secretion via exocytosis.

Chain Initiation by Qβ Replicase is Specifically Inhibited by Qβ RNA Fragments Containing Binding Sites for the Replicase

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Qβ replicase binds tightly to two regions of Qβ RNA, the S site at the beginning of the coat cistron and the M site near the beginning of the replicase cistron (Weber et al., Experientia *30*, 711, 1974; Meyer et al., Experientia *31*, 743, 1975). To ascertain whether interaction of Qβ replicase and these RNA regions is required for the formation of a productive initiation complex, S and M site RNA fragments were purified in 0.1 nmole quantities. Both (labeled) RNA species were selectively bound to Qβ replicase, as shown by binding competition with (unlabeled) random Qβ RNA fragments. In an initiation assay, using Qβ RNA as template, both S and M site RNA were strongly inhibitory: when present in amounts equimolar with Qβ RNA, inhibition was 50 and 46% respectively; at a 7fold molar excess the corresponding values were 88 and 83%. Ribosomal RNA fragments of similar length showed no inhibition at 7fold molar excess. Neither S nor M site RNA inhibited initiation of poly C-directed poly G synthesis. These findings suggest that when certain recognition sites on Qβ RNA are blocked, Qβ RNA can no longer be bound correctly. Utilization of poly C as template, which is far less efficient than Qβ RNA, appears not to require the same recognition mechanisms.

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Association in vitro of Semliki Forest Virus (SFV) Nucleocapsid (NC) Protein with Initiation Complexes for Virus Directed Protein Synthesis

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Using an extract from BHK21 cells obtained 4 h after infection, we have shown previously that all species of SFV RNA's are catalyzed in vitro. The in vitro system used allows the concomitant association of the newly synthesized 26S mRNA with ribosomal subunits to form functional initiation and elongation complexes directing viral protein synthesis. We have investigated the fate

of the NC protein synthesized in the cell between 3 and $3^1/_2$ h after infection. It is found to be firmly associated to the various initiation and elongation complexes being formed during incubation in vitro and is not removed by 0.5 *M* KCl. Upon high salt treatment or addition of chelating agents, the density shift in CsCl of the RNP's containing NC protein corresponds to that obtained for cellular mRNP's. Addition of NC protein to the in vitro system reveals a precursor-product relationship: The NC protein is associated first with a 40S ribosomal subunit-26S mRNA complex. Upon prolonged incubation in vitro, the NC protein is chased into a ribosome-mRNA complex and finally found on polyribosomes. The significance of these findings for translational events will be discussed.

Labeling Kinetics of the Nuclear and Cytoplasmic Nonmitochondrial DNA from Anemic Duck Erythroid Cells

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Anemic duck erythroid cells were labeled in vitro with ^{32}Pi (1 mCi/8 $\times 10^8$ cells) for 10–120 min, and nuclei, ribosomes and postribosomal particles were isolated. From these DNA was purified and their amounts and specific activity of labeling (cpm/μg DNA) was determined. The specific activity of all DNA species increased with time. As compared to the high mol. wt. nuclear DNA (> 16s) at every time period the specific activity of cytoplasmic nonmitochondrial DNA and small mol wt nuclear DNA (< 16s) was 10–15 times greather. Although the amount of high mol wt nuclear DNA cell remained unchanged, the amount of small mol wt DNA recovered from the nuclear and cytoplasmic nonmitochondrial fractions increased progressively, showing that during the labeling period the latter two species accumulate in the nucleus and the cytoplasmic-postmitochondrialparticle fraction. To test whether this is a consequence of radiobiological damage, cells were labeled for 120 min with ^{32}Pi at isotope concentrations varying between 0.002 and 1.0 mCi/8 $\times 10^8$ cells. It was found that the amount of labeled DNA found in the cytoplasmic nonmitochondrial fraction increased from 0.01% to 0.4%, with the increasing amount of the precursor. Thus, at higher doses of ^{32}Pi the nuclear DNA is damaged and labeled DNA appears in the cytoplasmic-nonmitochondrial fractions.

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Analysis of mRNA Induced by Heat Shock in *Drosophila melanogaster*

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Following a temperature treatment at 37 °C (heat shock) *Drosophila* cells synthesize a discrete class of 10 proteins. At the same time synthesis of the normal developmentally specific proteins is arrested. This phenomenon has been observed in all tissues examined so far (Tissières et al., J. Mol. Biol. *86*, 433, 1974). – In salivary glands and cells in culture we have observed the induction of new species of mRNA by heat shock. Other species of RNA are no longer synthesized following a heat shock. Following induction the heat shock mRNA's are synthesized for only a short time and are less stable than the bulk of the cellular mRNA. The partially purified messengers direct the synthesis of heat shock proteins in vitro. Evidence for the control of protein synthesis at both the translational and transcriptional levels will be discussed.

Mitogen-Induction of Endogenous C-Type Viruses

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Tuberculin, lipoprotein and lipopolysaccharide, three structurally different B-lymphocyte mitogens isolated from Gram-negative bacteria have been found to be efficient inducers of endogenous C-type virus from lymphoid cells of mice in vitro. A membrane-immunoglobulin-positive B-cell has been identified as a target for virus induction. Indirect evidence indicates that mitogenicity and virus induction are linked properties. Host-range studies with mitogen-induced virus from virus-free BALB/c cells indicated that mitogens induce exclusively xenotropic, and not mouse-tropic, virus. In contrast, in AKR cells which already express mouse-tropic virus a dual effect was observed: mitogens again induced xenotropic virus but in addition amplified release of mouse-tropic virus.

Freezing in a Propane Jet

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The main prerequisite for successful freeze-etching is vitrification, i.e. a type of freezing which avoids specimen destruction by hindering ice crystal formation and growth. Dilute solutions and most biological objects can only be vitrified if the temperature zone between 0 and −100 °C is passed in 10^{-2} to 10^{-4} sec. This speed of temperature lowering can be approached in specimens which do not exceed a thickness of about 20 μm, by the application of different techniques: with high-pressure freezing by using a FN_2-jet, and with spray-freezing by blowing small droplets into liquid propane. The former technique demands quite sophisticated machinery, the latter an unagreable handling of frozen droplets and glue. Propane-jet-freezing is a quite simple alternative to these techniques: A specimen, sandwiched between two gold platelets (as used in standard freeze-etching) is fixed with very fine-tipped tweezers and held between two nozzles through which liquid propane (−180 °C) is shot from opposite directions onto the sample. The propane is first liquified in a small container cooled with FN_2. After filling, the container is closed and then the propane is blown through the nozzles by the action of pressured N_2 (10 atm.). Using this technique, a freezing speed of more than 5000 °C/s can be achieved and most samples thinner than 20 μm are vitrified without introducing anti-freeze agents like glycerol.

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Xenotransplantation of Human Testicular Tissue in the Nude Mouse

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Human testicular tissue obtained from males of various ages was grafted into various sites, mostly subcutaneously, of male BALB/c-nu/nu mice which are deficient in thymus-dependent immunological functions. The transplants were well accepted and mouse tissue proliferated in the space between the seminiferous tubules as could be demonstrated by a newly developed cytogenetic staining technique which distinguishes heterochromatin differences. The experiments showed that spermatogenesis under these conditions ceases, however, primitive spermatogonia survive over periods of months.

Messenger RNA Synthesis during Different Phases of the Cell Cycle in CHO Cells

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Chinese hamster ovary cells were synchronized without inhibitors by mitotic selection and labelled in G1, S and G2 phases of the cell cycle by incubation for 90 min with ^{3}H- or ^{14}C-Uridine. Purified polyribosomes were extracted with phenol and the polyadenylated mRNA prepared by poly-U sepharose chromatography. Polyadenylated ^{3}H-Uridine labelled mRNA from the G1 phase of the cell cycle was compared by exponential polyacrylamide gel electrophoresis in dry formamide with ^{14}C-Uridine labelled polyadenylated mRNA from the S or G2-phase. The electrophoretic patterns obtained correspond to the size range expected for mRNA (7S-28S). No prominent differences were detected between mRNAs synthesized in different phases of the cell cycle. – From these data we conclude that the major size classes of polyribosomal poly-A containing mRNA are synthesized in equal ratios throughout the cell cycle.

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Cleavage Sites of Pancreatic DNase in the Chromatin Subunit

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The interaction of DNA and histones in the chromatin subunit can be studied by digestion of chromatin with pancreatic DNase. In a previous communication it was shown that such a digestion produces a regular pattern of DNA fragments which consist of multiples of 10 bases when analyzed under denaturing conditions (M. Noll, Nucl. Ac. Res. *1*, 1573, 1974). Further analysis reveals that of the 200 base pairs of DNA per subunit 140 base pairs are associated more tightly with the histone core. Evidence is presented that pancreatic DNase cuts the tightly associated DNA with a stagger on opposite strands and that the potential cleavage sites occur *every* ten bases on each strand. This proves that the DNA is on the outside of the chromatin subunit wrapped around the histone core.

Ribosomal DNA in *Allomyces arbuscula*

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The main nuclear DNA of *Allomyces arbuscula* has a buoyant density of 1.721 g/cm^{3} (Ojha et al., Mol. Gen. Genet. *136*, 151, 1975). The DNA from isolated mitochondria has a density of 1.710g/cm^{3}. The hybridization of purified ribosomal RNA (25 and 18 S) with the fractions of DNA collected after preparative CsCl centrifugation shows that rRNA genes are lighter than the main peak DNA. Their density is closer to 1.706g/cm^{3}. The Tm of the rRNA-DNA duplex is 85 °C. The amount of DNA coding for 25 and 18 S ribosomal RNA in an exponentially growing gametophytic mycelium is 0.3% of the total. There is no difference in the amount of rDNA between exponentially growing gametophytic and sporophytic mycelia. The reassociation kinetics of *A. arbuscula* DNA gives a genome size of about 1.4×10^{10} daltons. This corresponds to approximately 20 copies of the ribosomal cistrons per haploid genome.

The Location of the Deletion in Transformation-Defective Rous Sarcoma Virus RNA

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Transformation defective (td) mutants of Rous sarcoma virus RNA lack an RNA segment, as shown by the decreased chain length of their RNA (Martin and Duesberg, Virology *47*, 494, 1972) and the absence of 3 characteristic large T_1 oligonucleotides (Coffin and Billeter, J. Mol. Biol., in press). Since these oligonucleotides are located close to the poly A segment, the question arose as to whether the deletions were close to the 3′ end, but internal, or whether the whole RNA region immediately adjacent to the poly A was eliminated. [^{32}P] RNA from td and wild type Prague B RSV was partially digested with T_1 RNase, and poly A-containing fragments were isolated by poly U-Sephadex chromatography. These were between 150 and 300 nucleotides in length as revealed by polyacrylamide gel electrophoresis, and contained on the average 80% AMP residues. The T_1 and pancreatic fingerprints of the fragments derived from both viruses were identical, suggesting that a region of at least 100 nucleotides immediately adjoining the poly A segment was not deleted in transformation defective virus, in agreement with conclusions by Wang et al. (J. Virol. *16*, 1051, 1975).

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The Role of Buffer Osmolarity in Fixation for SEM and TEM

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The chick embryo hearts on the 4th day of incubation were perfused in ovo with 2% glutaraldehyde and 1% formaldehyde in cacodylate buffer whose osmolarity was adjusted with NaCl to 100, 150, 200, 250, 270, 290 and 310 mOsm/l (isotony = 250 mOsm/l). Morphometric investigations (P. Chavaz and T. Pexieder, Acta anat., 1976)

in TEM have shown that the percentage of the hypo- and hypertonically altered mitochondria depends uniquely on the buffer osmolarity of the fixative and is sensible to modifications of 20 mOsm/l. Qualitative SEM investigations of the endocardial lining of the embryonic heart indicate lesser sensitivity (20–50 mOsm/l) of surface ultrastructure to varying buffer osmolarities. Previously described features like microvilli, intercellular openings, exocytose vesicles, ruffling edges and dying cells remain present under all circumstances. The cell size and form as well as the number and form of microvilli seem to be most sensitive to variations in buffer osmolarity. Surprisingly lower degree of reactivity of surface of the embryonic endocardium to buffer osmolarity changes may be explained by the instantaneous effect of perfusion fixation in comparison with the diffusion phenomena taking part in the fixation of cellular organelles.

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The Interaction between Immune Complexes and Human Platelets

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Immunoglobulin G (IgG) coupled covalently by bis-diazobenzidine to form high molecular weight aggregates (BDB-IgG) has been used as a model compound to study the interaction of antigen-antibody complexes or IgG aggregates with human platelets. Using BDB-IgG labelled with H-aniline by diazotisation, binding of H-BDB-IgG could be examined. A minimum of 0.1–0.2 μg ^{3}H-BDB-IgG bound per 5×10^8 platelets was needed for the release of platelet granule contents and cell aggregation. In plasma, although neither release nora ggregation were observed, binding still occurred, albeit at a much slower rate. Ca^{2+} was inhibitory to binding in plasma but not in the washed platelet system. Human monomeric IgG in 150 fold excess inhibited binding by 50% while bovine IgG was less than one fourth as effective. A wide variety of compounds which inhibit the release reaction failed to affect ^{3}H-BDB-IgG binding. When the major surface glycoproteins of platelets were removed by proteolytic digestion binding of H-BDB-IgG was enhanced suggesting that such glycoproteins may mask underlying receptor sites.

There are 7–8 *E. coli* RNA Polymerase Binding Sites in the Early Promotor Region of T_7 DNA

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Complexes of T_7 DNA and DNA-dependent RNA polymerase from *E. coli* were analyzed by electron microscopy. Measurements on over a thousand molecules were made with a precision down to 2.5 nm and the final results were obtained with a standard deviation of about 15 base pairs. – In the promotor region for the early genes of T_7 DNA there are four major binding sites for RNA polymerase[1]. At saturation, binding site I contains one enzyme molecule, site II takes up three, site III takes up one to two and site IV takes up two RNA polymerase molecules. The findings that saturation T_7 DNA binds specifically 7–8 RNA polymerase molecules in the early promotor region is in excellent agreement with Hinkle and Chamberlin[2] and Schäfer et al.[3]. It has not yet been decided whether the three to four additional binding sites to the four major ones are storage regions. – All the data published so far fit nicely into a model of four major binding regions of different sizes for E. coli RNA polymerase in the early promotor region of T_7 DNA.

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Interaction of α-Chymotrypsin or β-Trypsin with Basic Pancreatic Trypsin Inhibitor

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Kinetic studies of the interaction of α-chymotrypsin or β-trypsin (E) with basic pancreatic inhibitor (I) led to the followed minimal mechanism: $I + E \rightleftharpoons L \rightleftharpoons C \rightleftharpoons X \rightleftharpoons L^* \rightleftharpoons I^* + E$. I* is the inhibitor in which the reactive peptide bond Lys 15–Ala 16 is cleaved. L and L* are labile pre-complexes (binding constants about $10^3\ M^{-1}$) which are formed with diffusion controlled rates. The final complex C (which is the tetrahedral complex according to X-ray studies) is formed from L with a rate constant of 350 sec^{-1} at pH 7 for α-chymotrypsin. Starting with I* a second kinetic intermediate X was found. Experiments with various chemical modifications of I* indicate that X is an intermediate on the way to the acyl complex.

Ultrastructural Localization of Calcium in Rat Adrenal Medulla, Pancreatic Islets and Parathyroid Glands

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The pattern of calcium localization in the endocrine cells of the rat adrenal medulla, pancreatic islets and parathyroid glands was studied using potassium pyroantimonate during fixation. This treatment resulted in the precipitation of calcium-containing electron-dense deposits in various sites of the endocrine cells. In adrenal chromaffin cells, the main sites of calcium deposition were represented by the secretory granules and mitochondria. Plasma membranes were practically free of deposits. In pancreatic islets, deposits were mostly associated with secretory granules and the plasma membrane. In parathyroid glands, where secretory granules are extremely rare, abundant precipitates were found in the rough endoplasmic reticulum and Golgi cisternae and applied to the external aspect of the plasma membrane. The morphological identification of different patterns of calcium precipitation in various types of endocrine cells may be important in assessing the respective role of cellular compartments in secretory processes.

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In vitro DNA Replication of Gently Lysed Mouse P-815 Cells

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Two different procedures have been developped: hypotonic treatment for 10–60 min and lysis with Brij-58 for 5–15 min both at 0 °C. Cells gently lysed by either one of these methods use ^{3}H-TTP as DNA precursor and continue semiconservative DNA replication in vitro for more than 30 min at 30 or 37 °C. In both systems, the requirements for optimal activity and the characteristics of newly synthesized DNA are similar. No repair replication is detectable. Okazaki fragments (50–200 nucleotides) synthesized just before the lysis procedure are chased into high molecular weight DNA (40–50 S) during in vitro incubation. To study DNA chain initiation and elongation in vitro, cells were prelabeled with bromodeoxyuridine, lysed and incubated with ^{3}H-TTP. Analysis of 10–20 S ^{3}H-DNA molecules in alkaline CsCl-Cs_2SO_4 density gradients suggests that initiation of new DNA chains in vitro occurs at least during the first 20 min of incubation.

Cleavage of SV40 DNA by the P1- and P15-Specific Restriction Endonucleases

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Supercoiled SV40 DNA in the presence of *S*-adenosyl methionine (Ado-Met) is cleaved by the P1- and P15-specific restriction endonucleases (R.P1 and R.P15) into full length linear molecules. These are permuted as shown by further digestion with *Eco*RI or *Hpa*II, both of which make cuts at unique sites on SV40 DNA. A minimum of 5 cleavage sites for R.P1 and 7 for R.P15 have been found on SV40 DNA and these sites have been mapped relative to the *Eco*RI site. In the absence of Ado-Met smaller fragments are produced which fit the proposed map. The limited cutting in the presence of Ado-Met is explained by a protecting modification methylase activity which is expressed in parallel with the endonuclease activity. The 5'-terminal sequences produced by these enzymes were analysed after labeling with polynucleotide kinase and ^{32}P-γ-ATP. At each site the enzymes cut a specific sequence but the sequences differ from one site to another. Furthermore, they seem to be different from the nucleotide sequence methylated by the P1-specific modification methylase.

SEM of Unicellular Alga *Treubaria*: Simplified CPD-Apparatus Using Solid CO_2

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In SEM of biological specimen, the importance of drying is unquestionable: the 'critical point method' is now generally prefered. A simplified apparatus, based on previously published work of Tanaka et al. (Stain tech. *59*, 1974) is described, which allows as good preservations of morphological features as more sophisticated installations. This apparatus consists of an 180 ml-hollow-cylinder, closed at the base with an hollow screw, used as specimen-holder, and at the top with a screw-cap fitted with manometer and vent valve. After introduction of specimen and minimal volume of acetone, the cylinders is filled with pieces of solid CO_2, then closed. Temperature is controled with a water-bath. In order to test the morphological preservation, an unicellular green alga was chosen: *Treubaria* Bernard. Its cell wall bears long and mucilaginous hollow cones made of a fibrillar network, which represent a very convinient test materiel.

Selective Uptake of ^{3}H- 5-HT by Supra-Ependymal Nerve Terminals: Localization by Autoradiography

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A network of supra-ependymal nerve fibres has been shown to exist in most ventricular regions of rat brain. These nerves possess a specific uptake mechanism for monoamines since they are able to selectively accumulate exogenous 5-hydroxydopamine. It was therefore reasonable to assume that 5-HT would also be taken up and, if radiolabelled, should be visualized by autoradiography. ^{3}H-5-HT (s.a. 12.6 Ci/m*M*; radioact. conc. 1.0 mCi/ml) was injected in 10 µl by slow infusion (5 µl/min) into a lateral ventricle of pargyline treated rats (100 mg/kg i.p. 18 h); for comparison, ^{3}H-DA and ^{3}H-NA (s.a. 10.0 and 25.0 Ci/m*M* respectively) were also investigated. Thirty minutes after administration of the amine animals were fixed by vascular perfusion with aldehydes and postosmicated. Semi-thin (1 µm) and ultrathin (60 nm) Epon sections of the caudate nucleus and lateral anterior hypothalamus were prepared for light and electron microscopic autoradiography; particular attention was paid to the ependymal region. In the caudate nucleus of 5-HT-injected animals radiolabel was observed not only in the parenchyma but also above the ependymal cells whereas in the hypothalamus only the parenchyma was labelled. Electron microscopic examination of the caudate nucleus revealed that the label was selectively localized to supra-ependymal nerve fibres (mainly terminals). ^{3}H-DA and ^{3}H-NA accumulated in the parenchyma of both regions although the ependymal surface was never labelled. Chlorimipramine and reserpine, which block neuronal uptake and storage respectively in serotonergic nerves, prevented the accumulation of ^{3}H- 5-HT in supra-ependymal nerve terminals. The physiological significance of the specific uptake mechanism in supra-ependymal nerves might well be the removal of 5-HT from 'synaptic areas' in order to terminate the possible neurotransmitter action of 5-HT.

Infrared and Raman Spectroscopy of Lecithin and Lecithin-Components

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Infrared ATR (Attenuated Total Reflection) and Raman spectra of egg lecithin, dipalmitoyl lecithin, glycerophosphoryl choline, phosphoryl choline, choline as well as of some deuterated modifications were measured. The normal coordinates analysis of choline was carried out

and the spectra were interpreted in terms of the calculated normal vibrations. – There exist characteristic group vibrations such as C=O stretch, PO_2 symmetric and antisymmetric stretch, CH_3 umbrella, CH_2 wag, etc., which result in polarized infrared bands. This enables the orientation of the molecule with respect to the ATR plate. – Hydrocarbon chains of dipalmitoyl lecithin at room temperature are found to be nearly pependicular, whereas the polar head group of lecithin lies approximately parallel to the surface of the ATR plate. – Some normal vibrations are shown to be conformation dependent e.g. $N^+(CH_3)_3$ symmetric stretch. – Comparison of the experimental results with calculated frequencies of different molecular conformations enables to determine the probable conformation assumed by the molecule under given experimental conditions.

Chromosome Bahavior during Prometaphase of Mitosis in Rat Kangaroo Cells

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Analysis of time-lapse films revealed three principal patterns of initial chromosome behavior: (1) movement to and/or association with the proximal spindle pole, (2) movement to and association with the more distant pole, (3) direct congression. The behavior of a given chromosome is primarily determined by the distance from its kinetochore region to the spindle poles. Chromosomes lying near a pole at the time the nuclear envelope begins to disintegrate exhibit the first pattern of behavior, whereas chromosomes lying approximately equidistant from the poles congress directly. As a rule, pole-associated chromosomes eventually congress. Ultrastructural observations suggest that pole-associated chromosomes are oriented only to the proximal pole (monotelic orientation) and they demonstrate that congressing chromosomes are oriented to both poles (amphitelic orientation). The different patterns of chromosome behavior can thus be interpreted as a result of synchronous or asynchronous formation and function of sister-kinetochore fibres.

Messenger RNA-Directed Synthesis of Double-Stranded Complementary DNA

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When rabbit globin or mouse Ig L chain mRNA is transcribed into cDNA by AMV reverse transcriptase, most of the product is single-strand DNA. Molecules corresponding in length to the entire mRNA sequence are obtained. The cDNA could then be replicated into double-stranded DNA, either with *E. coli* DNA polymerase or with AMV reverse transcriptase. The dependance upon various oligodeoxynucleotide primers and the effect of the two polymerases was studied. The reaction is primer-dependant with *E. coli* polymerase whereas with reverse transcriptase, replication takes place without primer. Resistance to single-strand-specific nuclease S1, of either the first or the second DNA strand was followed. The length of the ds-cDNA, following treatment with nuclease S1, was analysed on acrylamide gels under denaturing conditions. Replication of cDNA with reverse transcriptase results in ds-cDNA corresponding in part to the entire length of the ss-cDNA used and thus produces the equivalent of a specific gene sequence. – cDNA elongated with polydG has also been used for the synthesis of the second DNA strand using polydC-cellulose as primer, thus producing 'anti-cDNA'-cellulose.

Messenger RNA-Specific Insertion of Eukaryotic Gene Sequences into *E. coli* plasmid DNA

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The insertion of a rabbit globin gene sequence into an *E. coli* plasmid has recently been reported (Nucl. Acid Res. *2*, 2365, 1975). Improvements on our original procedure have now resulted in the requirement for less mRNA and in the reproducible production of chimeric plasmids with mRNA-specific sequences. Double-stranded DNA was synthesised from cDNA with AMV reverse transcriptase without the use of primer. The longest d-s molecules were elongated with homopolymeric tails and hybridised to linear *E. coli* plasmid DNA (kan-resistance), also elongated with homopolymeric tails. The procedure ensures that circularisation of plasmid DNA can occur only through the insertion of d-s cDNA. After bacterial transformation, kanamycin-resistant clones were selected and the plasmid produced were analysed for the presence of specific gene sequences by hybridisation. – This procedure has allowed the first insertion of a mammalian gene sequence into a bacterial vector. It is reproducible and applicable to other mRNAs. Chimeric plasmids carrying specific eukaryotic gene sequences constitute new tools for the purification of mRNAs, for the identification and quantitation of specific sequences and for the purification of specific genes from 'restricted' cellular DNA.

Quantitative Assessment of Support Films for Electron Microscopy

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The existence of several types of support films with different properties and different structural appearances in the electron microscope image has made it desirable to find a method for quantitatively assessing support films with a small number of easily understandable parameters. Several attempts are being made to characterize films using the power spectrum or correlation function of the image. These both clearly show the defocus dependence of the image and the dependence on film thickness and, to some extent, the type of film. However these parameters are insensitive to the phases in the image, which largely determine the visual appearance of the image. Procedures are now being used which analyze the films in terms of properties also characterizing the visual system and the actual appearance of the image.

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Localization of Secretin in Rat Pancreatic Monolayer Cultures by Immunofluorescence

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The availability of antibodies to secretin renders possible the localization of this hormone at the light microscope level by immunofluorescence. We studied 3-days-old pancreatic rat monolayer cultures (L. Orci et al., J. Ultrastruct. Res. *43*, 270–293, 1973) which were fixed in Zamboni's fluid and processed for indirect immunofluorescence with the following controls: (a) incubation with antisecretin adsorbed with an excess of synthetic secretin; (b) incubation with antisecretin adsorbed with either somatostatin, glucagon or gastrin and (c) incubation with normal rabbit serum instead of the specific antiserum. After incubation with the antisecretin serum, immunofluorescent cells were observed in endocrine clusters. The number of fluorescent cells was usually scarce and varied from one cluster to another. Controls a and c showed no fluorescence whereas the finding of positive cells in control b excluded a cross reaction of antisecretin with either glucagon, somatostatin or gastrin. The presence of cells containing secretin or a secretin-like peptide in neonatal rat pancreatic cultures adds a new functional cell type to the three already characterized by immunofluorescence (insulin, glucagon and somatostatin-containing cells) in the pancreas.

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Abnormal Microtubules in the Mutant '*lethal-polyploid*' (*lpl*) of *Drosophila hydei*

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The mutant gene *lethal-polyploid* (*lpl*) causes arrested mitosis in a limited proportion of those cells which divide during larval life. It leads to lethality at the onset of metamorphosis. At this stage, the ultrastructure of the microtubules seems to be normal. In *lpl* disks and gonads rescued from mature lethal larvae and cultured over a prolonged period in adult host flies, the effect of *lpl* becomes more pronounced, especially in tissues which need considerable amounts of microtubules for cellular differentiation. Germ cells of wildtype larval testes which are cultured in adult females pass through meiosis and differentiate into late elongated spermatids. By contrast, the ultimate stage reached in cultured *lpl* testes are early spermatids whose nuclei and nebenkern derivatives do not elongate. The flagella do not grow normally, and the arrangement of microtubules in the axialfilament is often disorganized. Furthermore, part of the cytoplasmic microtubules show various abnormalities, frequently an α-shaped crossection.

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In vivo Transcription of the rDNA Spacer Sequences in Amphibia

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Treatment of *Xenopus* cells with 5-fluorouridine results in the accumulation of RNA molecules heavier than 2.6 10^6 daltons which corresponds to the molecular weight of the first ribosomal precursor (40S) detectable as a descrete species. The accumulated heavy molecules are heterogeneous in size. By hybridisation to restriction fragments of *Xenopus* rDNA repeating units cultured in plasmids, it was shown that the heavy RNAs are in part molecules containing ribosomal sequences which are also complementary to the socalled nontranscribed rDNA spacer. Electron microscopy on spread preparations of *Triturus* oocytes shows an increase in frequency of transcriptional complexes in the rDNA spacer region upon drug treatment. Also extralong fibrils appear in the matrix units, interpreted to be uncleaved spacer transcripts. Some matrices reach the size of an entire repeating unit. On the basis of these results it is suggested that the RNA polymerase reads through the spacer sequences of rDNA, synthesizing unstable transcripts which are normally cleaved in statu nascendi.

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Complexity and Abundance of the Estrogen-Induced Polyadenylated RNA in *Xenopus* Liver

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The estrogen-induced poly(A)-containing RNA found in the liver of hormonestimulated *Xenopus* males was isolated and used as template to synthesize cDNA. Hybridization of this cDNA with the estrogen-induced RNA suggests a complexity characteristic of a single species of poly(A)-containing RNA. Hybridization of the cDNA with cytoplasmic poly(A)-containing RNA from *Xenopus* males treated for 7 days with estrogen and from control toads shows that the estrogen-induced RNA constitutes 12–15% of total cytoplasmic poly(A)-containing RNA, and is at least 2000fold less abundant in untreated males. The hormone-dependent increase of the estrogen-induced RNA is followed by hybridization of the cDNA with RNA isolated from toads after various periods of estrogen treatment. – Renaturation of the cDNA from the estrogen-induced RNA with sheared Xenopus DNA reveals that the sequence coding for the estrogen-induced RNA is a single copy gene.

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Lipid Bilayer Dynamics Studies Using Capacitance Relaxation

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Capacitance relaxation studies (Sargent, J. Membr. Biol. *23*, 227, 1975) reveal motion at the molecular level in black lipid membranes (BLM). Studies with oxidized cholesterol membranes show the influence of membrane-

active substances on the mobility of the BLM molecules. With valinomycin, the direction and magnitude of the changes depend on the kind and concentration of the complexing cation: 10^{-7} *M* val. + 0.2 *M* Na^+ cause the relaxation spectrum to be shifted to longer time constants; with K^+ both slower and faster components are found. These results are consistent with published structures for val./cation complexes and their effects on NMR spectra of lecithin films. A cation-complexing but non-transporting synthetic peptide also showed interactions with oxidized cholesterol BLM.

Comparative Studies on Bacterial Thermophilic and Mesophilic L-Lactate Dehydrogenases

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LDH was isolated by affinity chromatography from thermophilic (55 °C, resp. 70 °C) and mesophilic (37 °C) cells from *B. stearothermophilus* and *B. caldotenax*. Molecular weights, isoelectric points, amino acid compositions, *N*-terminal sequences, homology of sequences, maps of tryptic peptides, thermostabilities and some kinetical properties of the different LDH's were compared. To get some information about the efficiency of the different LDH's, the three thermodynamic activation parameters $\Delta G‡$, $\Delta H‡$ and $\Delta S‡$ were determined and compared with those from ectothermic, avian and mammalian species.

Genetic Studies of the Lac Repressor

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A large set of deletions with one terminus ending in the lacI gene of *E. coli* has been isolated. These enable the fine structure mapping of mutations in the I gene (which codes for the structure of the lac repressor). More than 400 deletions have been mapped against more than 6000 mutations to produce a map consisting of more than 100 deletion intervals. We have utilized a set of nonsense mutations which have been correlated with specific residues in the repressor to correlate the physical map with the deletion map. Different suppressors were used to insert one of five amino acids at each of 83 nonsense sites in the gene-protein system. This allows the production of more than 300 altered repressor molecules with known sequence alterations. The properties of these variant proteins have been correlated with the structural changes.

Surface Potentials of Asymmetric Charged Lipid Bilayers

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A simple method for the determination of surface potentials and hence charge density on asymmetric lipid bilayers is described. The method is based on the dependence of bilayer capacity on transmembrane voltage. The capacity, Cm, of Mueller-Rudin-membranes containing egg-lecithin with 15% cholesterol was measured at 100 or 1000 Hz. The current flowing from a constant voltage signal is rectified to give a convenient, direct measurement of Cm. Cm is raised by 10 or 26% at 100 or 160 mV, respectively. The kinetics of the adaptation to the applied voltage is complex. If a triangular wave with dV/dt variable between 1.0 and 80 sec per 100 mV is applied, hysteresis-like figures arise. Only symmetric membranes show the centre of these figures at zero applied voltage. With asymmetric charged membranes formed by addition of uranyl to one compartment the centre of the hysteresis figure is shifted along the coltage axis by an amount compensating the one-sided surface potential.

Glial Factor Activity in Rat Brain Primary Cultures

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The release of a macromolecular factor which induces morphological differentiation in neuroblastoma cells has so far only been detected in clonal glial cells[1]. The present study demonstrates such an activity in the conditioned medium of rat brain primary cultures. However, the amount of glial factor activity present is closely correlated with the developmental stage at the time of the biopsy. These results are compared with those obtained with the conditioned medium of corresponding kidney, liver, skin and spleen primary culture.s

Reference
[1] D. Monard, F. Solomon, M. Rentsch and R. Gysin, Proc Nat. Acad. Sci. USA, *70*, 1894–1897 (1973).

Selective Trans-Synaptic Migration of Tetanus Toxin in Rat Sympathetic Ganglia after Retrograde Axonal Transport

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Highly purified ^{125}I-labeled tetanus toxin (TT) or ^{125}I-Nerve growth factor (NGF) was injected into the anterior eye chamber of adult rats. Electron microscopic autoradiography of the superior cervical ganglion 14 h and 24 h after injection showed intraaxonal retrograde transport of both substances followed by a redistribution to various cellular organels in the perikaryon and transport into the dendrites. Whereas TT leaves the ganglionic cell and migrates trans-synaptically to the preganglionic cholinergic terminals where it is accumulated with high preference, *no* labeling of presynaptic terminals can be seen in the case of NGF. – Trans-synaptic migration of TT to the presynaptic terminals following retrograde transport has been demonstrated in spinal cord motoneurons (Schwab and Thoenen, Exp. Brain Res., suppl. 23, 187, 1975). The present findings show the occurrence of trans-synaptic migration in the peripheral sympathetic nervous system and indicate that this process occurs selectively for TT and not for NGF.

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Oxalacetate Control of Krebs Cycle Oxidations in Neurospora Mitochondria

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0.4 m*M* oxalacetate (OAA) causes a strong but transient inhibition of Krebs cycle intermediates respiration in *Neurospora* mitochondria. A similar observation was reported in plant mitochondria by Douce and Bonner (BBRC *47*, 619, 1972). These authors suggest that inhibition of NAD-linked substrates oxidation is due to the reduction of OAA by malate dehydrogenase (MDH), which causes a transient competition for NADH between this enzyme and the respiratory chain. – The fate of $_{14}C$ α-ketoglutarate, malate, aspartate and glutamate shows a reversible aspartate aminotransferase (AAT) activity. OAA concentration can thus be controlled by AAT and MDH activities. This view is consistent with a bifunctional AAT-MDH complex, as described by Munkres (BBA *220*, 149, 1970). This complex could in fact be the key enzyme for regulation of the electron flow in the respiratory chain, as well as of the distribution of carbon between the Krebs cycle and the biosynthetic pathways.

A Method for Isolating Specific Regions of Qβ RNA Minus Strands

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Qβ RNA (100 mg) was totally digested with T1 RNase and 44 large T1 oligonucleotides, most of which have been mapped, were purified by DEAE cellulose chromatography and two-dimensional polyacrylamide gel electrophoresis. About 80 μg of each fragment were recovered. The oligonucleotides were elongated with an average of 50 AMP residues by terminal riboadenylate transferase. These poly A-oligonucleotides could be hybridized to Qβ minus strand fragments containing the complementary sequence and the hybrids isolated by poly U-Sephadex chromatography. Using oligonucleotide T5, which is derived from the replicase cistron, 3 major and several minor fragments of [^{32}P] minus strands, ranging from 100 to 400 nucleotides, were isolated. Fingerprint analysis revealed that all of these fragments had one set of oligonucleotides in common, suggesting that they were derived from the same region of the Qβ minus strand and contained the sequence complementary to oligonucleotide T5. This procedure should be useful for the isolation and sequence determination of specific regions of Qβ minus strands.

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Cleavage of Phage T4 Proteins by a Fragment of the Gene 21 Protein

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A proteinase which cleaves specifically the T4 core protein P22 to TCA soluble peptides has been purified from *E. coli* B infected with an amber mutant in gene 24. It also cleaves other head precursor proteins, including P23, P24 and IP-III to the size found in mature phage. Its molecular weight is about 18,000 and isoelectric point is 5.25. – Immunological studies show this polypeptide to be derived from the product of T4 gene 21. Temperature sensitive and SUS mutants in gene 21 lack the 17,000 mol wt antigen, producing instead polypeptide antigens of a size which depends on the mutant, ranging from 27,000 for two ts mutants, through 15,000 for SUS N90 and N121, to no antigen for SUS E322. Mutants in other head genes all produce a series of 5 antigens ranging in size from the presumed 27,000 mol wt precursor to the active, 17,000 mol wt protease. Studies on these mutants strongly suggest that only the 17,000 mol wt species can cleave precursor proteins in vitro. Cleavage of P21 probably takes place on the prohead, since all the precursor antigens are found on 23 ts 'crummy heads'. The conversion from precursor to protease appears to be autocatalytic, the final step being inactivation by cleavage of the proteinase itself.

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Erythropoietin Dependence of Primitive and Late Red Cell Precursors in Culture

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Primitive erythroid progenitors (B) from mouse bone marrow require 10fold higher doses of crude erythropoietin for colony formation in culture than do more mature precursors (E). Since the differing requirements could reflect dependence on different factors, human urinary erythropoietin was extensively purified in an attempt to dissociate B- and E-stimulating activity. After adsorption onto benzoic acid, followed by chromatography on DEAE-cellulose, Con A-Sepharose, Sephadex G-100, calcium phosphate and Phytohemagglutinin-Sepharose, a final product was obtained which was purified 400fold over the starting material and had a specific activity of 4300 erythropoietin units/mg protein. B- and E-stimulating activities did not dissociate at any stage of the purification. This result is compatible with the view that both precursors are dependent on an identical factor – erythropoietin – for colony formation in culture, and that their differing dose requirements reflect their differing sensitivities to this hormone.

Haemoglobin Crystals in the Midgut of the Tick *Ornithodorus moubata*

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Dark red crystals appear in the midgut of the tick *Ornithodorus moubata* Murray within a week after a blood meal on guinea-pig (*Cavia porcellus* L.). These crystals were studied by scanning electron microscopy and X-ray diffraction. Single crystals of the major haemoglobin component (~ 80%) of guinea-pig blood were grown at

neutral pH. Crystallographic studies show the same diffraction pattern for both crystal forms, strongly suggesting that the 'thick-grown' crystals are composed of guinea-pig haemoglobin. Spectra in the region of 450–700 nm of single 'tick-grown' crystals show the normal haemoglobin absorption bands. PAA-SDS gel electrophoresis experiments indicate the same molecular weight for the crystalline material from the tick and the major haemoglobin component of guinea-pig blood. – Therefore we must conclude that the large dark red crystals found in the midgut of the tick *Ornithodorus moubata* are in fact composed of intact guinea-pig haemoglobin.

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Studies on two RNA Polymerases from the Nuclei of *Physarum polycephalum*

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We have attempted to study the purification of the RNA polymerases from kilogram quantities of *Physarum* microplasmodia in some detail. Under the conditions we employ, both known polymerases chromatograph together at about 0.15 *M* $(NH_4)_2SO_4$ on DEAE-Cellulose, but elute as separate peaks on DEAE-Sephadex at 0.2 *M* (α-amanitin sensitive polymerase) and 0.3 *M* (α-amanitin resistant polymerase) $(NH_4)_2SO_4$. In addition, a batchwise exposure to DEAE-Sephadex in the presence of 0.35 to 0.5 *M* $(NH_4)_2SO_4$, or an adsorption and stepwise elution with 0.4 *M* $(NH_4)_2SO_4$ appears to be a prerequisite for subsequent separation during DEAE-Sephadex chromatography. We employ the batchwise method since, in addition to removing more than 50% of the ploysaccharide and more than 90% of the nucleic acid in the extract, it results in a large activation of polymerase activity. Our present purification procedure employs a DEAE-Sephadex batch step, followed by DEAE-Sephadex, DEAE-Cellulose and Phospho-Cellulose chromatography to give nearly homogenous enzymes in about 1% yield.

Structural Information from Electron Micrographs of the Extended Tail Sheath and Polysheath of Bacteriophage T4

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We have carried out a 3-dimensional reconstructions of extended tail sheath and polysheath from bacteriophage T4 and have studied concomitantly the transfer of information from an electron micrograph to the 3-dimensional reconstruction obtained from it. Two methods have been developed to quantitatively assess micrograph images of helical particles and the reconstructions obtained from them. First, a filter has been designed which eliminates all structure in the image inconsistent with the symmetry of the helix. Individual micrographs can therefore be assessed with respect to their consistency with the symmetry prior to reconstruction. Second, the quality of an averaged reconstruction can be checked by producing a map of the root-mean-square deviation of the individual reconstructions from their average. – General observations on the structure of the tails in conjunction with information from our final reconstructions suggests that the P18 molecules of which the sheath is composed have a significantly different conformation in the sheaths' extended and contracted forms. We therefore favour a model which envisages a major conformational change in the P18 during the course of sheath contraction.

The Revised Aminoacid Sequence of Qβ Coat Protein

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The nucleotide sequence determined for the first half of the Qβ coat cistron (Escarmis et al., Experientia *31*, 737, 1975) was not compatible with the published sequence for Qβ coat protein (Konigsberg et al., Nature *227*, 271, 1970). The aminoacid sequence of the relevant region of the coat protein (positions 1–60) was reinvestigated and two differences were found: Asn rather than Asp in position 22, and an additional aminoacid, serine, between pro (position 55) and arg (position 56). The revised structure agrees with the nucleotide sequence.

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Study of the Mechanism of Immunoglobulin Secretion by Plasma Cells

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The mechanism of immunoglobulin secretion by plasmocytes is poorly understood and has often been considered free from short-term physiological control. The present study attempts to investigate the validity of this idea and to describe some of the biochemical parameters of secretion. To this end, malignant and normal plasmocytes are marked for 1 h with ^{3}H-leucine and returned to culture. During the ensuing 30'–6 h the release of specifically immunoprecipitable ^{3}H-immunoglobulin is followed. The rate of discharge is little influenced by (1) insulin, glucagon, carbamylcholine, adrenaline, histamine, serotonin, somatostatin, (2) bu_2cAMP, PGE_1, bu_2cGMP, (3) Ca omission, 50 m*M* KCl, 48/80, colchicine, cytochalasin B, (3) lowering of the energy supply of the cell. The rate of discharge is strikingly lowered by (1) reduction of temperature, (2) omission of KCl, (3) concanavalin A, ricin, (4) chlorpromazine, (5) the ionophore X537A in the absence or presence of Ca, Mg, K, (6) the ionophore A23187 in the absence of Ca. The effects of the two ionophores are reversible. When either ionophore is added to cells labeled 5–15 h with $^{45}CaCl_2$, the rate of Ca efflux is increased at least 5× relative to controls. – The ultrastructure of inhibited cells has been studied. Con A produces a striking infolding of the plasma membrane which accompanies cell-to-cell aggregation. Both ionophores produce characteristic alterations of the Golgi apparatus, which differ according to the ionophore. Thus, each may block a different step in intracellular immunoglobulin transport.

Role and Effects of Divalent Cations in Specific Tumour Cell Lysis by Sensitized Mouse T Lymphocytes

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The understanding of the mechanism of T cell cytotoxicity is still very limited. It has been shown that divalent cations are important for target cell lysis in vitro, since in the presence of EDTA no lysis occurred. We have analyzed the role of divalent cations in detail and found that both Ca^{++} and Mg^{++} are essential for full activity of cytotoxic T lymphocytes (CTL). A much higher concentration of Ca^{++} (0.2 m*M*) than of Mg^{++} (0.05 m*M*) was required for optimal lytic activity of CTL. On the other hand, the lytic activity of CTL was diminished in the presence of very high concentrations of Ca^{++} (up to 80 m*M* Ca Cl_2). This was not a toxic effect, since preincubation of tumour cells or lymphoid cells for 60 min at 37 °C in a medium containing 80 m*M* Ca Cl_2 did not affect the cell viability nor their proliferative capacity. As well, inhibition of CTL activity by 80 m*M* Ca Cl_2 was fully reversible.

Influence of Culture Medium on Differentiation in Myogenic Clones*

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Clonal cultures of 11-day chick embryo breast muscle cells were plated in standard medium containing 15% horse serum and 5% chick embryo extract. After 6 days the cultures were fixed and stained, and all nuclei in every clone were scored as being either in uninucleated cells or in myotubes. In control cultures (no medium changes) the unequivocally myogenic clones (those with 2 or more myotube nuclei) varied greatly in total nuclei/clone (mean: 121; s.d.: 173), myotube nuclei/clone (mean: 39; s.d.: 48) and fusion index (range: 1.7–100% myotube nuclei). Smaller clones consistently showed a higher percentage of fusion than larger clones. The variability of myogenic clones at 6 days with respect to size, myotube nuclei/clone and fusion index was reduced in parallel cultures given daily changes of an enriched medium containing 20% fetal calf serum and 8% embryo extract from day 1 to day 4. The average clone size increased greatly (mean: 491 nuclei; s.d.: 479), myotube nuclei/clone increased slightly (mean: 48; s.d.: 51) and values of the fusion index ranged from 0.5% to a maximum of only 39%. The data are interpreted in terms of a stochastic model of cell differentiation, in which frequent replenishment of enriched medium increases the probability that proliferative myogenic cells will self-renew instead of giving rise to postmitotic fusion-capable cells.

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Ribosomal DNA in *Physarum*

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The sequences coding for *Physarum* ribosomal RNA are localized on independently replicating, linear DNA molecules of a discrete size, 37×10^6 daltons. Restriction endonucleases $EcoR_I$ and $Hind_{III}$ each cut rDNA into one large and two small fragments. The latter are represented twice per intact molecule, once at each end. Sedimentation and electron microscopic analyses of intact rDNA that has been neutralized from alkaline solution indicate that the entire rDNA molecule has a rotational axis of symmetry near the center. Blocks of short, inverted repetitious sequences appear to be located at the center of the native rDNA and also at 3.7 to 11×10^6 daltons flanking the center.

The Interaction of G-Actin with ATP

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1:N^6-Ethenoadenosine 5′-triphosphate, a fluorescent analog of ATP was used to study the kinetics of the interaction of actin with ATP. Nucleotide free actin denaturates rapidly and irreversibly. Therefore the interpretation of the experimental results was based on a two step mechanism with three rate constants: k_+ and k_- for the association and dissociation process of actin and nucleotide and k_D for the denaturation of nucleotide free actin. At pH 7.5 and 50 μM Ca^{2+} the rate constants were found to be $k_+ \geqslant 8 \cdot 10^4\ M^{-1}\ s^{-1}$, $k_D \geqslant 2 \cdot 10^{-3}\ s^{-1}$ and $k_+/k_D = 4 \cdot 10^7\ M^{-1}$. With these values the binding constant of the ATP analog to actin becomes $K = k_+/k_- \geqslant 10^8\ M^{-1}$. The Ca^{2+} dependence of K_- was explained by a twofold lower dissociation rate constant for actin with Ca^{2+} bound as compared with Ca^{2+} free actin. The exchange of actin bound Ca^{2+} was found to be a fast process and the Ca^{2+} binding constant was $10^5\ M^{-1}$.

Characterization of an Estrogen-Induced RNA in Livers of *Xenopus*

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Estrogen treatment of *Xenopus* males leads to the appearance of a new species of poly(A)-containing RNA in the liver, at a stage when large amounts of the estrogen-induced yolk precursor protein, vitellogenin, is produced. This estrogen-induced RNA sediments at 28S and migrates on gels in aqueous solution with an apparent molecular weight of 2.0×10^6. Contour lenght measurements under denaturing conditions in the electron microscope reveal a molecular weight of 2.34×10^6 as compared to the mouse 28S rRNA. Labeling experiments show that the estrogen-induced RNA has a higher stability than the average liver poly(A)-containing RNA and that it constitutes 10% to 20% of the poly(A)-containing RNA found in the cytoplasm after 24 h labeling.

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Fine Structural Analysis of Wild-Type and sv^{de} Bristle Organs of *Drosophila melanogaster*

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The bristle organ of *Drosophila melanogaster* consists of the tormogen, trichogen, sense and neurilemma cells. Electron microscopic studies of the developing bristle organs on the tibia of wild-type and *shaven-depilate* (sv^{de}: 4–20) flies have revealed that all 4 cell types are also present in mutant bristle organs carrying an aberrant shaft. However, the typically regular arrangement of the 4 cells in the wild-type is not maintained in the mutant sv^{de}: The angle of inclination of the sense cell towards the epidermis varies to a wide degree and the trichogen cell is not always enclosed by the tormogen cell. In contrast to the wild-type, the branching of the neurilemma cell septate desmosomes in the mutant sv^{de} is either reduced or completely absent. Moreover, some neurilemma cells show aberrant protrusions containing abnormally arranged microtubules.

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The ATPase Activity of Actin Filaments

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The association of G-actin to actin filaments is accompanied by a conversion of ATP to ADP. The latter remains bound to the actin protomer in the filament. The ATPase activity of polymeric actin was therefore attributed to the association dissociation reaction which takes place at the filaments. The kinetics of actin association was successfully described by a mechanism in which it is assumed that association and dissociation takes place at the ends only. A quantitative evaluation showed however that the association dissociation steps at the ends can account only for a fraction of the ATP cleavage and incorporation of radioactively labeled nucleotide. This observation supports the view that ATP is continuously incorporated and split also at other sites along the filament.

NMR-Studien der molekularen Dynamik von globulären Proteinen in Lösung: BPTI

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Die Einkristallkonformation des basischen pankreatischen Trypsin-Inhibitors ist bekannt. Sie enthält als dominierende Konformationselemente eine α-Helix und ein antiparalleles β-Blatt. In NMR-Messungen wurden die Ergebnisse der Röntgenstrukturanalyse mit Daten über die Dynamik der Lösungskonformation ergänzt. Die Dynamik der Konformation des Molekül-Rückgrates wurde auf Grund der Austauschkinetik der Amidprotonen aus den intramolekularen Wasserstoffbrücken untersucht. Diese Amidprotonen zeigen gut aufgelöste NMR-Signale zwischen 6 und 11 ppm, und ihr Austausch gegen Deuterium in D_2O-Lösung kann in der Abnahme der Signalintensität beobachtet werden. Es wurden dabei Austauschzeiten zwischen 20 min und ~ 10 Jahren festgestellt. Dieses Ergebnis weist auf ein sehr differenziertes dynamisches Verhalten verschiedener Regionen des Molekülrückgrates hin, welche auf Grund der vorliegenden Daten durch thermodynamische Parameter näher charakterisiert werden konnten. Nach dem vollständigen Austausch der Amidprotonen gegen Deuterium enthält das NMR-Spektrum zwischen 6 und 8,5 ppm nur noch die Resonanzen der 8 Aromaten (4 Phe, 4 Tyr). Mittels der in modernen Spektrometern bei 360 MHz erreichbaren spektralen Auflösung war es möglich, das dynamische Verhalten der aromatischen Ringe in der globulären Form des Proteins zu untersuchen. Studien über den Temperaturbereich von 4 bis 80 °C zeigten ein individuell verschiedenes Rotationsverhalten dieser aromatischen Seitenketten mit Rotationsraten von 10^5 sec^{-1} bis <10 sec^{-1}. Die Analyse der Temperaturabhängigkeit ermöglichte die Ermittlung der Aktivierungsenthalpien und -entropien für diese dynamische Prozesse.

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Structural Studies on Phage λ Head

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Tubular aberrant phage λ capsids (polyheads) can be obtained by self-assembly of capsid protein. Three types of polyheads can be distinguished from negatively stained (mass density) and freeze-dried tungsten shadowed preparations (surface structure) which correspond in their structures to petit λ (pλ) enlarged pλ (D⁻head) and phage head, respectively. The type A polyhead related to pλ containing p*E* (gene E product) only, are tubes with rough surface appearance built from one type of cluster with a lattice constant of 10.8 nm. The type B related to D-head, is wider than type A (55 nm and 45 nm, respectively) and appears smoother with a lattice constant of 13 nm. The type C containing p*E* + p*D* is as wide as type B and has a rough surface appearance showing two types of clusters. From comparison of squashed λ particles, we have deduced that the pλ is structurally made of hexameric and pentameric clusters of p*E*, the D⁻head from the same clusterings, but in a flattened expanded form and the λ head of p*D* trimers added to D⁻head. This model is in contradiction to the alternative model previously proposed by Williams et al. and Hohn et al. in 1974 (pλ: p*E* hexamers; λ: p*E* trimers + p*D* hexamers) but our model has the advantage of requiring less rearrangement of the main capsid protein.

Reference
M. Wurtz, J. Kistler and T. Hohn, *Surface Structure of in vitro Assembled Bacteriophage Lambda Polyheads*, J. Mol. Biol. *101*, 36 (1976).
Supported by SNSF

Density Gradient Sedimentation to Equilibrium and Electron Microscopic Studies on Nuclear, Mitochondrial and Cytoplasmic-Nonmitochondrial DNA from Duck

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Nuclear DNA was prepared from erythroid cells and liver of ducks and purified on Cs_2SO_4-urea gradients. From the postmitochondrial supernatants of lysed erythroid cells the cytoplasmic DNA was also purified as described before (Modak et al., Eur. J. Biochem., 1975). DNA was extracted from duck liver mitochondria and the supercoiled form was purified on ethidium bromide-CsCl gradients. Samples along with the density-marker DNA from *Micrococcus luteus* were centrifuged to equilibrium in a Beckman model E analytical centrifuge. Both nuclear and cytoplasmic-nonmitochondrial DNA contain the same density classes with a main band of density 1.694. On the other hand, the mitochondrial DNA forms a single band at density 1.707. – Electron microscopic observation on the DNA spreads prepared by the aqueous Kleinschmidt monolayer technique showed that both nuclear and cytoplasmic-nonmitochondrial DNAs are linear forms. In case of mitochondrial DNA, 67% molecules were found to be supercoiled and the rest as relaxed circles. – From these studies we conclude that DNA isolated from nuclei and cytoplasmic postmitochondrial supernatant are different from the mitochondrial DNA.

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CONGRESSUS

Belgium

The 5th European Drosophila Research Conference

in Louvain-La-Neuve, 6–8 September 1976

This Conference will be held at the University of Louvain. Further information by: Prof. F. A. Lints, Laboratoire de Génétique, Université de Louvain, Place Croix-du-Sud 2, B–1348 Louvain-La-Neuve, Belgium.

France

17th International Congress of Physiological Sciences

in Paris, 18–23 July 1977

The first two days will be devoted to general lectures and during the last four days specialized meetings will take place. Further information can be obtained from the National Physiological Society of each country or by writing to the Congress Secretary: Prof. J. Scheerer, Secrétariat du 17. Congrès Int. des Sciences Physiologiques, U. E. R. Pitié-Salpêtrière, Cedex 1300, F–75300 Paris-Brune, France.

Federal Republic of Germany

6th International Colour Symposium

in Freudenstadt (Black Forest), 27 September to 1 October 1976

Nine main lectures and 28 discussion papers will be presented. The final programme may be asked for from the Gesellschaft Deutscher Chemiker, Secretariat, P. O. Box 90 04 40, D–6000 Frankfurt am Main 90, Federal Republic of Germany.

Canada

Third International Symposium on Pharmacology of Thermoregulation

in Banff, 14–17 September 1976

The Symposium will be held at the Banff Centre and further details about registration may be obtained by the organizers: Prof. K. E. Cooper, Division of Medical Physiology, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 1N4; or by Prof. P. Lomax, Department of Pharmacology, UCLA School of Medicine, Los Angeles, California 90024, USA; or by Prof. E. Schönbaum, Peelkensweg 4, 4274 Venhorst N. Br., The Netherlands.

France

29th International Meeting on Electrical Phenomena at Membrane Level

in Saclay, 12–15 October 1976

The main topics are: 1. Bioenergetical study of coupling mechanisms. 2. Electrical phenomena at exitable membrane level. The scientific program and registration information will be available by: Dr. C. Troyanowsky, General Secretary, Société de Chimie physique, 10, rue Vauquelin, F–75231 Paris Cedex 05, France.

Ruzicka-Preis 1976

Aus dem Fonds für den Ruzicka-Preis wird alljährlich einem jungen Forscher für eine hervorragende veröffentlichte Arbeit auf dem Gebiete der allgemeinen Chemie, die entweder in der Schweiz oder von Schweizern im Ausland ausgeführt wurde, ein Preis erteilt. Kandidatenvorschläge können bis spätestens 15. Juli 1976 dem Präsidenten des Schweizerischen Schulrates, Rämistrasse 101, 8006 Zürich, unterbreitet werden.