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PHYSIOLOGIE - PHYSIOLOGY

Effect of a 500 g carbohydrate load upon glycogen synthesis and lipogenesis in man

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Metabolic rate and substrate utilization of 8 male subjects (21.8 years; 70.6 kg) was measured for 10 h after ingestion of a 500 g carbohydrate (CHO) meal (bread, jam and fruit juice). Peak values of glycaemia (115±10 mg% mean ± SEM) and insulinaemia (130±20 µm units/ml) were observed after 90 min at which time the nonprotein respiratory quotient (NP-RQ) was 0.98. During the next 8 h glucose levels remained near 100 mg% whilst insulin decreased progressively to 22 µm units/ml; the NP-RQ remained in the range 0.95-1.0, exceeding unity only for short periods; during which, fat synthesis surpassed fat oxidation by only 4 g. Assuming complete absorption of CHO after 5 h the subjects 'CHO balance' (ingested-oxidized) had increased by 418±18 g. 10 h after the meal 146 g CHO, 9 g fat and 39 g protein had been oxidized with a specific dynamic action of 7.2±1.2%. The data imply that the glycogen storage capacity in man is large and that CHO conversion to fat does not exceed fat oxidation, even after large CHO intakes.

Effect of surfactant on alveolar geometry

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To assess the influence of alterations of lung surfactant on the geometry of peripheral air spaces the morphology of air-filled normal and detergent-rinsed rabbit lungs was studied after vascular perfusion fixation at different points on the deflation P-V curve. The most important changes in rinsed lungs are: 1. With decreasing lung volume there is progressive collapse of alveoli; at low lung volume (40% of TLC) most alveoli are collapsed, and the air is contained in overextended ducts. 2. The alveolar surface area to volume ratio is considerably smaller particularly at low volumes; it changes linearly with lung volume. 3. There is only a slight change of mean air space curvature between 80 and 40% TLC. The results indicate that in detergent rinsed lungs volume changes are brought about predominantly by recruitment and derecruitment of alveoli. It appears that both a normal surfactant and the mechanical interdependence within the fibrous continuum are required to maintain a normal respiratory surface area within the lung volume range of normal breathing.

Control of adenohypophysis by posterior lobe of the pituitary gland?

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The discovery of a growing number of neural lobe peptides and of short portal vessels between anterior and posterior pituitary suggest that both lobes may influence their activity. To test this hypothesis, the neural lobes of intact (I) and median eminence lesioned (ME) anaesthetized rats were electrically stimulated (400 µA, 1 msec biphasic pulses, 30 Hz). 6 successive arterial blood samples of 2 ml were collected over 18 min, analyzed for plasma ACTH (RIA) and replaced by continuous infusion of donor blood. In I-rats, ACTH increased 22% and 17% ($p < 0.05$, $n = 7$) in the first and second plasma sample following initiation of electric stimulation (5 sec on - 5 sec off for 3 min). In ME-rats, ACTH increased 14, 36 and 29% ($p < 0.025$, $n = 4$) in the first, second and third plasma sample following initiation of the same stimulus. Electric stimulation of anterior lobe was ineffective. Thus *endogenously* released posterior lobe compound(s) affect(s) anterior lobe activity.

Size alterations of hypothalamic neurons in the genetically obese mouse (ob/ob)

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The genetically obese mouse (ob/ob) is characterized by excessive weight gain, hyperphagia and hyperinsulinemia. It has been suggested that a primary defect may be of CNS origin, possibly within the hypothalamus. We now report that: 1. Wet and dry brain weight of ob/ob mice is decreased compared to lean controls, no change in water content. 2. Brain volume is also decreased. 3. Morphometric analyses reveal a dramatic decrease in ob/ob individual neuronal cell surface areas, especially in VM hypothalamic neurons. 4. Only lateral hypothalamic (LH) neurons of ob/ob mice were as large as those of lean controls. 5. Preliminary Golgi-Cox analysis of VM neurons revealed no major change in the number of dendrites or dendritic orientation of ob/ob mice. Conclusion: relative to lean mice, ob/ob's have smaller VM neurons. Relative to their own brains, ob/ob's have enlarged LH neurons. The VM-LH interaction has been previously implicated in disturbances of food intake and b.wt control.

Neural control of insulin secretion: The effect of electrical stimulation of hypothalamic areas on peripheral plasma levels of insulin and glucose in the anesthetized rat

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Monophasic stimulation (250-500 µA, 0.2 msec, 50/sec) via stainless steel electrodes (tip diameter 0.2 mm) in nembutal anesthetized male rats bearing jugular catheters for frequent blood sampling revealed: 1. Bilateral 20 min VMH stimulation increased plasma glucose significantly, which was not accompanied by the expected increase in plasma insulin. 2. Unilateral 3 min LH stimulation produced only small and unsignificant changes in plasma levels of insulin and glucose. Elevation of baseline insulin by continuous glucose infusion did not change this finding. 3. Unilateral stimulation of the anterior far-lateral hypothalamic area produced significant increases in plasma insulin, which were not due to changes in glucose. These findings suggest a) that there is not a simple reciprocal influence of the VMH and LH on insulin secretion and glucose homeostasis and b) that increased insulin secretion is not the cause of elicited feeding from the perifornical area.

Spectral sensitivity in the drone retina

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Microspectrophotometrical investigation of the drone retina showed that the photoreceptors have a thermostable photopigment with 2 states interconvertible by light with peaks of absorption at 446 and 505 nm (Muri, 1977). The correlation between the absorption of the photopigment

and the electrical responses to flashes of low intensity shows that the phototransduction is initiated by light absorption of the 446 state, while absorption of light in the 515 state does not induce any electrical response. Reduction of the rhodopsin content by a blue light decreases the sensitivity of the cell but does not affect the time course of its responses. The content of rhodopsin can be restored by a new light conditioning at longer wavelengths, which leads to a complete recovery in sensitivity.

Circadian rhythm of sleep and motor activity of the rat maintained under various lighting schedules

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We present normative data of vigilance states and motor activity (MA) of the rat for the following lighting schedules: a) 12 h light - 12 h dark (LD 12:12), b) continuous darkness except for two 20-min L-pulses at 12-h-intervals (skeleton photoperiod; SP 12:12), c) continuous darkness (DD); and d) continuous light (LL). Schedules b-d were maintained for 6-9 days and preceded by 3 days LD 12:12. Waking and MA were generally increased during SP 12:12 and DD, and transiently reduced during LL. A decreasing trend in the duration of slow wave sleep (SWS)-episodes, but not of paradoxical sleep (PS)-episodes, was seen within the L-phase of LD 12:12 as well as the 12-h inactivity phase of SP 12:12. A dissociation between the circadian rhythms of SWS and PS was present under all conditions, indicating a partial independence of the processes controlling the 2 sleep states.

Rapid gating of synaptic channels in ectopic neuromuscular junctions

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Ion channels associated with extrajunctional acetylcholine (ACh) receptors have been found to have slower gating kinetics than synaptic channels. Therefore, the kinetics of channels found in ectopic neuromuscular synapses formed de novo in extrasynaptic membrane areas were investigated. ACh induced current noise was measured in ectopic neuromuscular synapses formed by the tibialis nerve after its implantation into the distal end-plate-free region of the crushed sartorius muscle of frog, *Rana temporaria*. The autocorrelation functions of the current fluctuations were exponential with decay constants $\tau = 1.6 \pm 0.05$ msec (SEM), $N = 12$). Increasing the membrane potential by 70 mV prolonged τ e-fold. The mean conductance γ of single channels was estimated as $\gamma = 21 \pm 1.6$ pS ($N = 5$). These values agree well with data reported from the normal frog endplate.

[^{14}C]-2-Deoxyglucose labeling in the pigeon visual system

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Patterns of functional activity in the pigeon visual system were mapped by means of the [^{14}C]-2-deoxyglucose method (Sokoloff, J. Neurochem. *29*, 13 1977). In awake animals with one eye covered heavy labeling was observed in most of the known areas receiving retinal projections from the exposed eye. The higher centers on both the retino-tecto-rotundo-ectostrial pathway and the retino-thalamo-hyperstriatal pathway were asymmetrically labeled as well. This asymmetry was less pronounced in the hyperstriatum which receives a bilateral projection from the visual thalamus. Heavy symmetrical labeling was observed in animals with both eyes exposed. In pigeons with both eyes covered during the experiment some visual centers were still faintly more labeled than the surrounding tissue. The olfactory bulb and the centers of the auditory system were heavily labeled in all these experiments, the very highest activity being found in the N. mesencephali lateralis dorsalis which corresponds to the mammalian inferior colliculus.

Spread of the receptor potential in the retinula cells of the honeybee drone

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Spread of the receptor potential due to a local light stimulus has been investigated in the retinula cells of the honeybee drone by intracellular recording. It has been found that the spread from the cornea to the basement membrane occurs without significant loss of amplitude, whereas the loss is highly significant for a spread in the opposite direction. These results could be explained if the membrane resistance were relatively high all along the cell except for a region of much lower resistance near the cornea.

Deprivation and recovery of desynchronized sleep

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The influence of different durations of desynchronized sleep deprivation ($\leqslant$ 19 h, at -10 °C ambient temperature) on the hourly amount of desynchronized sleep during recovery (5 h, at neutral ambient temperature) was studied in 3 unrestrained cats. In control conditions at neutral ambient temperature, the hourly amount of desynchronized sleep was 696 ± 30 sec (mean and SE). In recovery conditions, such control value was increased by 32 ± 3 sec $\cdot$ h^{-1} of deprivation. These results point to the existence of a very precise regulation of the circadian amount of desynchronized sleep.

Saturation of the response to light in the ventral photoreceptors of *Limulus*

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The light-induced membrane current for brief stimuli was recorded in *Limulus* ventral photoreceptors voltage clamped with 2 intracellular microelectrodes. In the dark-adapted cell the peak light-induced current reached almost its maximum value (saturated) when about 10^3 of the 10^9 rhodopsin molecules in the cell were photoisomerized. Injection of EGTA into the cell, so that light adaptation was inhibited, did not increase the saturating light-induced current. When current pulses were passed into the cell during the response to light and the resulting potential deflexions measured with a fast amplifier it was found that the saturating current was apparently limited by the resistance of the surface membrane, and not by a resistance in series with it. It was estimated that the rhabdome contains at least 10^5 microvilli. The results suggest that an amplifying mechanism exists such that absorption of a photon in only one microvillus in a hundred causes almost the

maximum possible change of the conductance of the surface membrane.

Inorganic phosphate uptake in isolated rabbit ileum

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Mucosal scrapings of rabbit ileum were incubated at 37 °C in a modified Krebs solution containing ^{32}P-Pi and ^{14}C-polyethylene glycol as an extracellular space marker. Phosphate was accumulated in intracellular water. The accumulation of phosphate in the cell was a saturating function of extracellular phosphate with a K_m of 2.5 mM; accumulation was Na-dependent and inhibited by ouabain. At the mucosal border, unidirectional influx of phosphate was a linear function of phosphate concentration in the medium but highly temperature-dependent.

Continuous and discontinuous acceleration of breathing in rabbits

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It is generally accepted that inhalation of histamine-aerosol stimulates lung irritant receptors and results in an acceleration of breathing. More detailed analysis reveals this can be of 2 different types. In the first, continuous type, both inspiratory time (t_I) and expiratory time (t_E) shorten gradually but not necessarily by the same proportion. Changes of t_E precede changes of t_I. In the second, discontinuous type, t_E shortens gradually, but t_I remains unchanged until an augmented breath occurs. t_I is immediately shortened and then remains substantially the same until recovery begins. The results reported here provide evidence for some independence of the 2 phases of respiratory cycle. Experiments are in hand, using a method of selectively inhibiting lung stretch receptors, to determine the relative roles of these and irritant receptors in the genesis of the 2 patterns of accelerated breathing we have described.

Shock-induced fighting differences in roman high- and low-avoidance rats

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RHA/Verh. and RLA/Verh. rats have been selectively bred at our institute for rapid- vs nonacquisition of 2-way-avoidance in the shuttle box since 1972. In this study, 36 pairs each of naive, 6-month-old RHA and RLA males and 12 pairs each of naive, 6-month-old RHA and RLA females were tested for shock-induced fighting in a closed chamber. Each pair was given 2 series of 100 foot-shocks, 1 week apart, with a shock intensity of 3 mA, shock duration of 0.5 sec and an intershock interval of 10 sec. The following scoring system was used: 1 = freezing, 2 = running and/or jumping, 3 = posturing, 4 = 1 rat attacking and 5 = both rats fighting. The RLA pairs showed no posturing or fighting, with the male pairs freezing 88% of the time and the female pairs 63%. The RHA male pairs scored 3-5 36% of the time and the RHA female pairs 25%, with the primary difference being more posturing by the males. Not only has this study demonstrated strain differences in shock-induced fighting, but also the total absence of that behavioral trait in RLA rats.

The effect of photostimulation on glycogen turnover in the retina of the honeybee drone

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The drone retina is composed of 2 types of cells: the photoreceptors, in which virtually no glycogen has been detected by electron microscopy, and the glial cells, which have abundant glycogen stores. To investigate a possible interaction between these 2 cell types, we have studied the incorporation of glucose into glycogen by the glial cells when the photoreceptors were stimulated or resting. ^{3}H-glucose (1.47 nmoles, 50 µCi) was injected into the heart and one eye was stimulated with intense light flashes for 15, 25, 30, 45 or 60 min. The other eye was maintained dark adapted. The radioactivity associated with glycogen (per µg of protein) in each eye was measured. The ratio of the radioactivities in the stimulated and unstimulated eyes had a maximum at 30 min, at which time the stimulated eye had incorporated 2.5 times as much glucose as the unstimulated one. We conclude that stimulation of the photoreceptors accelerates the synthesis of glycogen in the glial cells.

Putative neurotransmitters in bulbar respiratory neurons

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6 putative neurotransmitters and their antagonists were applied microelectrophoretically to spontaneously bursting neurons. Units were classified according to their discharge within the respiratory cycle. The various neuron types differ in their receptor properties. Expiratory-inspiratory cells were excited by NE. Inspiratory neurons were inhibited by GABA, glycine and DA, but were excited by NE. Inspiratory-expiratory cells were excited by NE and by 5-HT. Expiratory neurons were inhibited by GABA and excited by glutamate, while DA-receptors are apparently absent. With NE part of these cells were excited and others were inhibited. In continuously discharging 'unspecific' bulbar reticular neurons glycine and DA induced inhibition and NE activation. Apart from NE activation of some expiratory neurons probably mediated by alpha-receptor excitation, all other NE effects are apparently caused by stimulation of receptors other than of alpha- or beta-type. 2 types of 5-HT-receptors are probable in cells of the expiratory group.

Enkephalin and morphine in the pigeon optic tectum

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Specific opiate receptors could be demonstrated in tectal membrane preparations. The kinetic constants for [D-ala^2]-Met-enkephalin and etorphine in this order were: K_D (nM) $5.6 \pm 1.1/0.19 \pm 0.01$ and B_{max} (fmoles/mg prot.) $350 \pm 44/172 \pm 1$. The effects of iontophoretically applied Met-enkephalin and morphine on the spontaneous firing rate, the L-glutamate-evoked activity and the synaptic excitation of tectal neurons were tested in the anaesthetized pigeon. Morphine and enkephalin depressed both spontaneous and chemically enhanced firing on the majority of neurons. This effect was antagonized by naloxone on about 50% of the cells tested. Very often the sensitivity to glutamate increased following the ejection of the opiates. Morphine and enkephalin showed strong depressant effects on the synaptic activation of the tectal neurons. The present

results suggest that these substances can modulate activity of the optic tectum.

Insulin-like effect of cold adaptation in brown adipose tissue (BAT)

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O_2 uptake and glycerol release were measured simultaneously in BAT pieces from cold-adapted rats (CAT) and from their control littermates. Previous experiments (Friedli et al., Experientia Suppl., in press) showed that the threshold for a respiratory response to added noradrenaline (NA) was increased in CAT. A concomitant increase in the threshold for NA stimulated glycerol release was found. This effect could not be attributed to a more efficient sequestration of NA in CAT, as it was still present under blockage of both neuronal and extraneuronal NA uptake. Addition of insulin 150 mU/l to the medium also shifted both lipolytic and respiratory dose-response curves to the right in controls. The effects of insulin and cold adaptation were nonadditive for glycerol release and less than additive for O_2 uptake. Respiratory responses to prolounged supramaximal NA stimulation (10^{-6}M) were maintained at a higher rate in CAT (81% max after 90 min) or under insulin 300 mU/l (87%) than in controls (48%).

Influence of enriched environment on exploratory activity in roman high- and low-avoidance rats

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14 RHA and 15 RLA rats of both sexes were placed together in an enriched environment (e.e.) for 30 days (Kuenzle et al., Physiol. Behav. *13*, 205, 1974), after weaning at 21 days of age. 2 mixed cages (5 RHAs, 5 RLAs and 4 RHAs, 4 RLAs, resp.) and 2 separate cages (10 RHAs and 10 RLAs), also started at weaning, served as controls. The activity of each rat was measured in a labyrinth (Bättig et al., Pharmac. Biochem. Behav. *4*, 435, 1976) 3 times during 1 day, between 49 and 59 days of age. All RHs were significantly more active than their RLA counterparts, but this difference was smaller in the e.e. rats. Between the mixed- and separate-cage control conditions, there were no differences in activity within either strain. Finally, both strains from the e.e. were significantly less active than their corresponding controls. These results show that exposure to e.e. reduced subsequent exploratory activity, and that being housed together did not affect inborn differences in activity RHA and RLA rats.

Electrophysiological properties of cultured hypothalamic neurons

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Cultures of hypothalamic tissue including the supraoptic nucleus area were prepared from 1–7-day-old rats by the roller tube technique. EM studies revealed the existence of neurosecretory granules both in neuronal perikarya as well as in axons. When hypothalamic tissue was co-cultured with a neurohypophyseal explant, unmyelinated fibres originating in large nerve cells could be observed crossing the gap between the 2 explants. Bioelectric activity was recorded intra- as well as extracellularly from hypothalamic nerve cells in 3–11-week-old cultures. Many neurons with large resting potentials displayed spontaneous EPSP's, IPSP's and action potentials, which could also be elicited by field stimulation of the surrounding tissue. The spontaneous activity of 40% of the neurons was characterized by a phasic firing pattern as demonstrated by the occurrence of prominent peaks in their autocorrelograms. Crosscorrelation studies indicated that the majority of neuron pairs had a tendency to fire synchronously.

Measurement of frog skeletal muscle intracellular buffer value by CO_2-titration of homogenates

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It is generally accepted that specific mechanisms such as transmembrane exchanges or net acid production are involved in the response of intracellular pH (pHi) to a PCO_2-change. However, any assessment of their part in the pHi-response requires knowledge of the intracellular buffer value (B_i). In frog skeletal muscle only indirect estimates of B_i are available and I have attempted to measure this value directly by CO_2-titration of muscle homogenates at 5 °C: On correcting the measured buffer of the homogenates for the dilution effect of the extracellular space (measured with ^{3}H-Inulin) I obtained a value of 91.8±2.26 Sl. in the pH-range 7.05±0.3, i.e. substantially higher than previously estimated (21.9 Sl., Boyle and Conway, J. Physiol. *100*, 1, 1941; 40.3 Sl., Reeves and Malan, Resp. Physiol. *28*, 49, 1976). These findings suggest that the pHi response to CO_2 observed in frog skeletal muscle with a pH-microelectrode could be largely determined by nonbuffering mechanisms.

Paradoxical stimulation of water transport by alloxan in toad skin

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Alloxan (Ax) is a well-known inhibitor of adenylyl cyclase. We used this agent to examine the applicability of Sutherland's postulates on cAMP to the hormonal stimulation of Na and water transport in amphibian epithelia. Continuous monitoring of net fluxes of Na and water was done with standard techniques. In frog skin, Ax (5 mM) added to the inner medium inhibited the natriferic effects of oxytocin and norepinephrine ($p < 0.01$). In toad bladder *(Bufo marinus)*, Ax induced a reversible, 97%, block of the hydrosmotic effect of vasopressin ($p < 0.001$). Unexpectedly, Ax stimulated water flow in toad skin ($p < 0.001$); besides, there was mutual inhibition between the hydrosmotic effects of Ax and vasopression. The block of hormone action described here is consistent with an inhibition of adenylyl cyclase. The hydrosmotic effect of Ax in toad skin, however, is paradoxical. Collectively, the results suggest that, depending on the particular cell system used, Ax may have opposite effects on the activity of membrane adenylyl cyclase.

Projections of the monkey precentral motor cortex to the midbrain

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4 monkeys *(Macaca fascicularis)* obtained small injections of ^{3}H-proline and ^{3}H-leucine (40–70 µCi) into the main body representation areas of Woolsey in the precentral gyrus. Somatotopic projections were found bilaterally in pars parvocellularis and ipsilaterally in pars magnocellularis of the red nucleus. The latter was spared by projections from the face region. Arm and leg areas project to

N. Darkschewitsch and N. accessorius oculomotorius of Bechterev (visceral nucleus of Carpenter) bilaterally, to the ipsilateral zona incerta, N. subfascicularis and praetectalis anterior. The face region projects to the ipsilateral subthalamic nucleus and S. nigra. Terminals were found in all cases in circumscribed regions of the mesencephalic reticular formation. The cortical fibres reach the midbrain via pathways descending from the subthalamus and crossing in the dorsal tegmental decussation and via connections emanating from the cerebral peduncle coursing through S. nigra. No projections from postcentral gyrus to N. ruber were found.

Synaptic input to the giant dopamine-containing cell of *Planorbis corneus*

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The dopamine-containing neurone situated posterio ventrally in the left pedal ganglion of *Planorbis corneus* was assessed by scanning electron microscopy and electrophysiology. Excitatory synaptic potentials were obtained by stimulation of 3 repeatedly identifiable cells in the ventral part of the right pedal ganglion. The EPSPs had latencies of about 20 msec and lasted 350-500 msec. Inhibitory responses were found following stimulation of 2 neurones in the left pedal ganglion. The duration of the IPSPs were 0.5-5 sec and usually 5-6 mV in amplitude. High Ca-concentrations applied to the bath and high frequency stimulation resulted in a loss of transmission, suggesting that both postsynaptic responses were mediated polysynaptically.

Neuronal population related to force in motor cortex of awake monkeys

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The activity of single neurons within the hand region of precentral motor cortex was recorded in monkeys trained to squeeze a force transducer between thumb and index finger. Changes in firing frequency related to small variations in force were investigated by requiring the monkeys to hold successively 2 force levels. The analysis of the spike trains during the force trace revealed 4 neuron classes. Cells in the tonic and phasic-tonic classes increased monotonically their discharge frequency with force. Both phasic and phasic-tonic neurons showed activation during the force ramps related to the rate of force change. A fourth class of neurons decreased their firing frequency when force increased. The question of the connectivity between the neurons was investigated by examining pairs of neurons recorded simultaneously and related to the motor task. The connectivity was tested by means of cross correlation technique. Preliminary results disclose short latency excitatory and inhibitory connections between the neurons.

Angiotensin II increases electrical communication in cardiac muscle

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Electrical measurements of current flow were made in trabecular strips of calf ventricular muscle to study the effect of angiotensin II on electrical coupling. The experiments were carried out because my previous experiments have shown that angiotensin II increases electrical coupling between embryonic chick myocardial cells in culture by decreasing the coupling resistance. During the first 2 min after exposure to angiotensin II (100 nM), the ratio of intracellular to extracellular resistance (r_i/r_o) decreased while conduction velocity increased. There was no change detected in maximum upstroke dV/dt or input resistance. Therefore, angiotensin II appears to increase electrical coupling between myodardial cells by decreasing the coupling resistance.

Viscous and elastic properties of the normal and the hypertrophied left ventricle

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In chronic heart disease myocardial hypertrophy and admixture of fibrous tissue are thought to alter the diastolic properties of the left ventricle. Therefore, left ventricular (LV) myocardial stiffness and viscosity were determined in 25 patients (6 controls=CO, 11 with aortic insufficiency = AI, 8 with congestive cardiomyopathy = CMP) by the diastolic stress(S)-strain(E) relationship. LV-pressure (micromanometer), minor axis diameter and wall thickness (echocardiography) were determined at 20 msec intervals and were fitted to the equation: $S = b \cdot e^{K \cdot E} + y \cdot \dot{E}$; whereby b = intercept, K = constant of myocardial stiffness, y = constant of myocardial viscosity and $\dot{E}$ = strain rate. LV muscle mass (LMMI, g/m^2) was determined angiocardiographically.

	CO	AI	CMP
b	1.06	0.95	2.62
K	0.12	0.17	0.28
		*	
	CO	AI	CMP
y	0.67	2.99	3.23
		*	
LMMI	92	166	136
	**	*	

*p<0.05, **p<0.005

It is concluded that a) LV hypertrophy per se, such as in AI, does not necessary lead to an increase in myocardial stiffness, although in LV hypertrophy due to CMP stiffness is increased, and b) myocardial viscosity appears to be an important determinant of LV diastolic function in myocardial hypertrophy.

Uptake of ^{3}H-GABA and L-^{3}H-glutamic acid in cultured rat dorsal root ganglia

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The cellular localization of the uptake of ^{3}H-GABA and L-^{3}H-glutamic acid was studied in organotypic cultures of fetal rat dorsal root ganglia (DRG) using autoradiography. Both amino acids (10^{-6} M, incubation time 1-5 min) were taken up in isolated neurones which are lying in the outgrowth zone and are deprived of their glial envelopment. In contrast, in the explant and dense zones of the cultures, where neurones are completely surrounded by satellite glial (SG) cells, ^{3}H-GABA and L-^{3}H-glutamic acid were accumulated exclusively by SG cells suggesting that these cells form an effective barrier preventing the uptake of the amino acids into neurones. The uptake of both amino acids was sodium- and temperature-dependent. Similar observations were made in the cerebellum in tissue culture, where uptake of ^{3}H-GABA into Purkinje cells was only found when the glial barrier was disrupted or absent (E. Hösli and L. Hösli, Exp. Brain Res. *26*, 319, 1976).

Facilitation of contrast responses of single cortical visual cells during induced gazing

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Gazing evoked by electrical stimulation of the thalamic intralaminar system in cat enhances the potential recorded in the visual cortex in response to optical gratings. In order to assess the type of cortical cell facilitated a single unit study is being undertaken in the alert animal by recording the responses to stationary or drifting gratings of optimal orientation and spatial frequency during gazing and in controls. Facilitation during gazing is found in cells with tonic response to slowly drifting gratings, mean latency 50 msec, but not in those with phasic response, mean latency 35 msec. Such cells can be better locked to the optical stimulus. The facilitated cells have been located in layers IV and II/III of area 17 and 17/18 border. Results suggest that intralaminar afferences to the visual cortex exert a facilitatory action on cells involved in pattern vision.

Step-up avoidance: A new paradigm for studying passive avoidance learning

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When placed head downward on an inclined plane many animals, including rats, have an innate tendency to turn around and climb up. This response provides an ideal new procedure for investigating passive avoidance learning in rats. A suggested procedure is to first fit the animal with the tail-electrode, then place it with nose facing the bottom onto a grid that is inclined at a 35° angle. Within seconds the animal makes a 180° turn and climbs up the encline. A 1 mA 1 sec duration tailshock is administered after the turn contingent on the first climbing response. Learning consists of the inhibition of the innate climbing response.

The effect of myelin basic protein (MBP) on the bioelectrical activity of the frog spinal cord in vitro

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The effect of bovine MBP on the activity of the isolated hemisected spinal cord of the frog was compared with that of L-glutamate. With 10^{-4}M MBP there is an elevation of the DR-VR reflex, followed by an inhibition lasting 1 h or more. The elevation is comparable to that of 10^{-3} M Glu but the inhibition is more marked and lasts longer. In unblocked spinal cord (DC recording) MBP causes a dose-dependent depolarization of the ventral and the dorsal roots. The height of the depolarization with 10^{-4} M MBP corresponds to that with 10^{-3} M Glu; however the effect only reaches a maximum after 6 min and lasts for 26-60 min. The depolarization is still present, though weaker, when synaptic transmission has been blocked by $MgSO_4$ and tetrodotoxin. We can therefore assume that MBP has a direct depolarizing effect on the motoneurones and the primary afferents, although part of the original depolarization may be attributed to an effect on the presynapses and interneurones.

Der EEG-Schlaf der Ratte in unterschiedlich grossen Käfigen

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Charles-River-Ratten, bei denen von 8 bis 16 Uhr das EEG abgeleitet wurde, zeigten in Käfigen von $20 \times 29 \times 29$ cm Grösse nur etwa 6% PS, während von anderen Laboratorien etwa 10% angegeben werden. Erfolgte die Ableitung in Käfigen von $25 \times 35 \times 35$ cm Grösse, so betrug der PS-Anteil ebenfalls rund 10%. Um diese Beobachtung zu überprüfen, wurde bei 4 Ratten jeweils Montag, Mittwoch und Freitag das EEG abgeleitet. In der 1. und 3. Woche waren die Tiere in den grösseren Käfigen, in denen sie auch sonst lebten, in der 2. in den kleinen. Tatsächlich zeigten die Ratten in den kleinen Käfigen nur halb so viel PS und mehr Wach als in den grossen. Die verglichen mit der 1. Woche höheren Werte für Wach und Dösen in der 3. Woche sprechen für einen länger dauernden adaptiven Prozess des tieferen Schlafes im grösseren Käfig.

Electrophysiological investigations on the ocelli of the honeybee

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Intracellular recordings from ocellar photoreceptors (REC) and extracellular recordings from second order neurons (SON) were done. The REC potential shows a short on-spike (ca. 5 ms). 2 spectral (λ) types were found: a) max. sensitivity (sens.) at ca. 350 nm and b) at ca. 500 nm. SON types exhibit a) decrease (inhibition) and b) increase (excitation) of spontaneous spike frequency, c) strong on- and off-excitation. The λ-sens. can be ascribed to the combined action of the 2 REC types when the ocelli are stimulated by orthodromic whole field illumination (head in the centre of a homogeneously lighted hemisphere). However, small field (light guide directed on ocelli) or antidromic stimulation (light guide directed on a caudal window cut in the head capsule) result in depression of UV-sens., and max. sens. is shifted to 550-580 nm. So far, this shift cannot be explained by effects of the red ocellar screening pigments as the λ-sens. of ortho- and antidromically elicited ERG matches the λ-sens. of the REC at long wavelengths.

Respiratory, circulatory and ECG changes at 6000 m and 7000 m

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After stepwise ascent (30 or 40 min) in a low-pressure chamber, healthy, unacclimatized, young male subjects were examined at rest during 30 min at 6000 m and 7000 m. All the subjects (20) tolerated well the halt at 6000 m. At 7000 m (15 subjects) physical and mental reserves were spent. During the halt at both altitudes the hyperventilation decreased as a result of falling PA_{CO_2}, so that PA_{O_2} also fell. The resultant hypoxemia may underlie the cardiovascular changes observed: A tendency for cardiac output to increase, for diastolic pressure to decrease, for T-wave flattening and S-T depression to augment, all effects already noted during ascent. The T-wave flattening lessened, however, after 20 min at 6000 m. At 7000 m 6 subjects showed acute signs of deterioration - unconsciousness, ectopic heart beats and rhythms, cardiac arrest - which disappeared during rapid descent. - In conclusion: Hypoxemia resulting from acute ascent augments during the halt at both altitudes, owing to insufficient respiratory readjustment and reaches critical values at 7000 m.

A relationship between behavioral and physiological responses to i.v. 1-5HTP

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The dark adapted pupil diameter, blood pressure, heart rate and skin temperature were measured after i.v. 1-5HTP (100-200 mg following peripheral decarboxylase inhibition) in 16 healthy subjects (11 ♂; 6 ♀). Although no systematic effect of 1-5HTP was found in the group as a whole, analysis according to behavioral response patterns revealed differences. A first group was characterized by marked mood elevation followed by psychic withdrawal, a second had only mood elevation, and a third showed no effects. Whereas the behavioral pattern of the first group was associated with increased pupil diameter and decreased skin temperature, the inverse was found in the second group, and minimal physiological changes occurred in the third one. Furthermore, predrug heart rate and systolic blood pressure were highest and pupil diameter smallest in the first group. A given individual reacted analogously to any of these groups after repeated 1-5HTP. The initial physiological state thus may determine the direction of both inter- and intraindividual pharmacological responses.

Vestibular projections to the monkey thalamus

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The ascending vestibular pathways to the thalamus of the rhesus monkey were studied by injections of radioactive amino acids (3H (proline and leucine) into the vestibular nuclear complex. The majority of thalamic projections were situated in the Nucleus ventroposterior lateralis pars oralis, and some in the Nucleus ventroposterior inferior. This location agrees well with previous electrophysiological studies, where vestibularly driven cells were found at similar sites. Thus these projections may be considered as thalamic relays in a vestibulo-cortical pathway, and are probably sites of convergence of somatosensory and vestibular afferents.

Correlation of firing rate and histochemical fluorescence intensity in individual giant dopamine neurons of the water snail *Planorbis corneus*

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The relationship between firing rate and fluorescence intensity previously established by us in the nigral dopamine (DA) neuron group of rats, has been studied on individual giant DA neurons (GDN) of *Planorbis*. Spontaneous activity recorded with intracellular microelectrodes ranged from 0 to 10/sec. Nicotine caused depolarization and variable changes in firing. 1-1.5 sec after termination of recordings, the preparation was frozen and processed for microfluorimetry. Intensities of GDN ranged from levels of rat DA neurons to values several times higher. Firing rate and intensity were positively correlated; so far, the correlation has been established for the last 60 sec. These results provide the first evidence for a correlation of activity and DA fluorescence in individual neurons, and indicate that similarities exist in the reaction of mammalian and invertebrate DA neurons.

Subdiaphragmatic vagotomy eliminates drinking induced by i.v. dipsogens in rats

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Vagotomy has previously been reported to disrupt drinking inducted by regulatory challenges. In this experiment, recovered vagotomized rats (vagx) were implanted with chronic hepatic-portal cannulae and subsequently infused with buffered Ringer's, or vehicle plus 0.38 M NaCl, 0.75 M NaCl, 0.75 M sucrose, 0.75 M glucose, or 0.75 M urea (0.5% b.wt). Latency and 0.5 h water intake were measured after daily infusion in the homecage. Control rats ($N = 8$) drank more after 0.38 M NaCl, 0.75 M NaCl and 0.75 M sucrose and had a shorter latency after 0.75 M NaCl and 0.75 M sucrose than following infusion of vehicle ($p < 0.05$). Latency and intake values in electrophysiologically-verified vagx rats ($N = 5$) were not different following vehicle and experimental infusions. Vagx and control rats did not differ in latency or intake following vehicle infusion, indicating that the insensitivity of vagx rats to osmotic challenge was probably not the result of debilitation produced by the denervation procedure.

Gold ions as a tool for morphofunctional studies in frog skin

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Reports about the effects of metal ions (e.g. Cu and Ag) on bioelectric parameters of the frog skin in vitro are found in the literature. Morphofunctional evaluation of these effects in our laboratory brought the following results (metal added to the outside solution only): a) A reproducible dose-dependent effect can be found with gold ions with *R. esculenta* kept at 4 °C early winter: A sustained stimulation of PD and SCC at low dose, followed by an irreversible inhibition of these parameters at higher dose (critical limit 5×10^{-4} M). Morphological survey of these effects show that in the stimulated skin gold precipitates can be found in the subcorneal space only, intimately associated to the apical membrane. As soon as inhibition occurs the metal has also penetrated into the cells of the first living cell layer. Indication of animal species, season and temperature is critical in the evaluation of these effects.

Possible modulation of cholinergic transmission by brain extract

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There is some evidence that learning may be correlated with a modification of cholinergic neurons in CNS. However, there is no information available on underlying mechanisms responsible for this modification. In 3 separate experiments we tested our hypothesis that synaptic transmission can be modified by molecules occurring in the brain. Similar to the method of Ungar in each experiment a peptide fraction was obtained from 50 brains of naive rats. The effect of these extracts were tested on nicotinic synapses by intracellular recording of frog muscle endplates. The following reversible effects were observed: a) decrease of resting potentials; b) abolition of action potentials with successive diminution of endplate potentials amplitude; c) decrease of depolarization caused by iontophoretic application of ACh. The observed effects support the assump-

tion that our brain extracts contain substances which may act as cholinergic neuromodulators. Whether these effects are caused by known neurohormones is under investigation.

Improvement of retention by posttrial morphine

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Earlier findings have shown that retention of a behavioral task can be improved by giving a reinforcing stimulus (food reward or rewarding brain stimulation) during a critical interval immediately after the learning situation. The existence of morphine receptors in brain raised the question as to how endogenous morphines may be involved in the physiology of reinforcement. To investigate this question 144 mice were subjected to a one trial passive avoidance task and injected either with morphine sulphate (40 mg/kg) or saline 5-10 sec after the footshock. Morphine injected animals showed better retention compared to the controls. This finding is interpreted in terms of a possible involvement of the morphine system in reward mediation. It should be noted that morphine is given only to activate the morphine system and it is therefore rather unlikely that a single injection of morphine is acting as a 'traditional' reinforcer (the mice are not addicted).

Cholinergic antagonists fail to block S-potentials in the cat retina

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Cholinergic transmission of light-evoked signals has been postulated for the inner nuclear and plexiforme layers of mammalian retinas. We reported depressant effects of cholinergic antagonists on electroretinogram, optic nerve action potential and ganglion cells in the cat (Experientia *33*, 83, 1977; Docum. Ophthal. Proc. Ser. *13*, 307, 1977). The present study is confined to intracellularly recorded, physiologically identified signals from horizontal cells (S-potentials) in the isolated, perfused feline eye. Atropine, mecamylamine and dihydro-β-erythroidine were injected into the perfusion in concentrations from 0.1 to 3×10^{-3} M. S-potentials, recorded from 9 horizontal cells, were neither blocked nor reduced in amplitude by the cholinergic antagonists. These data suggest, that transmission from photoreceptors to horizontal cells is *noncholinergic* in the cat, substantiating further the concept that cholinoceptive sites are located more proximally.

Early birefringence signal and latency relaxation in single fibres of frog skeletal muscle

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Since the first presentation of the large early birefringence signal (BS) (supposed to reflect potential changes of the SR membranes associated with Ca^{++} release) in single fibres of skeletal muscle (Oetliker and Baylor, Experientia *30*, 682, 1974) it has been suggested several times that this signal could simply reflect latency relaxation (LR). Evidence against this hypothesis in addition to the one presented in Baylor and Oetliker, J. Physiol. *264*, 156, is obtained from the following experiments. 1. During posttetanic potentiation LR is absent while BS is unchanged. 2. Double stimulation (intervals 4 to 100 ms) elicits a BS on both pulses but LR preceds only the first contraction. 3. Nitrate abolishes LR and potentiates BS. 4. Caffeine (1-2 mM) suppresses LR and leaves BS normal. These findings strongly suggest that the large early birefringence signal and latency relaxation are caused by different processes in EC-coupling, which follow each other in rapid succession, the birefringence signal preceding latency relaxation.

Exogeneous ^{13}C-glucose (^{13}C-G) utilization during prolonged exercise in glycogen depleted (GD) and control subjects (C)

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5 subjects whose glycogen stores were depleted by a 3-day low carbohydrate (CH) diet were compared with 5 C consuming a normal diet. Total CH utilization was calculated from the nonprotein RQ and ^{13}C-G oxidation from expired $^{13}CO_2$ using mass spectrometry. 1 h after ingestion of 100 g naturally labelled ^{13}C-G, the subjects pedaled for 2 h at 33% of their VO_2 max. In both groups, expired CO_2 reached similar peaks of enrichment with ^{13}C after 75 min exercise. At this time the energetic contribution of CH, lipids and proteins was, respectively 27, 69 and 4% for GD and 69, 28 and 3% for C. The ^{13}C-G oxidation rate was 398±14 mg/min and 425±25 mg/min for GD and C, respectively, representing 20 and 24% of the total energy expenditure. GD oxidized an average of 38±2 g and C 41±1 g of ^{13}C-G during the 2 h exercise period. Inspite of depleted CH stores, GD did not utilize the exogenous glucose to a greater extent than C, the lipids remaining the major energy source.

Action of taurine and glycine on strychnine-induced convulsions in the rabbit

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There is evidence that alterations in the concentration and metabolism of various amino acid transmitters in the CNS might be involved in the origin of epilepsy. We have tested the action of i.v. applied taurine and glycine, 2 suspected inhibitory transmitters, on convulsions induced by strychnine - an antagonist of these amino acids - using EEG and EMG recordings. The strychnine dose necessary to produce a generalized seizure was 0.55±0.15 mg/kg i.v. for rabbits pretreated with taurine (4×1.60 mmoles/kg, $n = 10$) which was significantly higher ($p < 0.05$) than for control animals (0.41±0.11 mg/kg, $n = 10$). After pretreatment with glycine (4×1.60 mmoles/kg, $n = 11$), the strychnine dose necessary to evoke convulsions (0.51±0.22 mg/kg) was slightly but not significantly higher than the control values. Our results indicate that taurine exerts a more pronounced anticonvulsive action in strychnine-induced seizures than glycine.

α-Sympathetic nerve control of glucose output in in situ perfused mouse liver

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Electrical stimulation of perivascular nerve bundles of mouse liver perfused in situ at constant flow resulted in an increase of glucose production which was found to be maximal at 20 Hz. The neurally-induced glucose output was only slightly modified by the β-blocker, propranolol, but greatly inhibited by the α-blockers phenoxybenzamine and phentolamine. The effect of 20 Hz electrical stimula-

tion could be matched by an infusion of norepinephrine at a concentration of 5×10^{-7} M. It is suggested that the carbohydrate metabolism of the liver is controlled by its own nerve supply rather than by circulating catecholamines and that this control is mediated principally by the α-adrenergic receptors.

Optical properties of birefringence- and transmission spike in the olfactory nerve of pike

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The action potential in the pike olfactory nerve is accompanied by a retardation change ('optical spike', von Muralt et al., Pflügers Arch. *367*, 67, 1976). Due to the very high membrane to volume ratio of this preparation, with the nerve placed between crossed polarizers, Δ I/I is close to 1%. With additional retardation introduced by means of a Babinet-compensator, resting light intensity and optical spike amplitude follow expectations [$I = k\ (1-\cos\varnothing)$, $\Delta I = k \cdot \sin\varnothing \cdot \Delta\varnothing$, $\Delta R/R$ estimated to be $\sim 1\%$]. Simultaneously the nerve shows a decrease in light transmission of about 10^{-4} (546 nm) with exactly the same time course. The amplitude of this 'transmission spike' decreases with increasing wavelength, suggesting a change in light scattering. The propagation velocity of these optical action potentials is about 6 cm/sec at 1 °C. In addition, transparency of the nerve increases slowly by up to 10% with repetitive stimulation, time course and plateau value depending on the rate of stimulation.

Task-dependent effects of posttrial reinforcing or subreinforcing stimulation of the substantia nigra on avoidance learning

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To evaluate a possible role of the substantia nigra in memory formation the influence of 30 sec long posttrial electrical intermittent (0.2 sec on/1.8 sec off) stimulation of the substantia nigra on passive avoidance learning was investigated. Using a step-down avoidance-task, reinforcing stimulation (at current levels adequate to maintain optimal self-stimulation) presented 30 sec posttrial, impaired learning, whereas subreinforcing stimulation (at 25% of reinforcing current level) did not. Opposite effects were attained using a small-chamber passive avoidance-task. Therefore, the effect of posttrial substantia nigra stimulation on learning depends on a) whether the stimulation is below or above the current threshold for reinforcement, and b) on the nature of the learning task.

Long-term measurement of energy expenditure in obese subjects on an ad libitum diet and during a protein sparing modified fast (PSMF)

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An air-tight respiration chamber (2.5 × 5.0 m) was constructed to obtain continuous long-term measurements of energy expenditure in human subjects performing their usual activity. The air from the chamber is renewed at a precisely determined rate and the F_{O_2} and F_{CO_2} of the incoming and outgoing air are measured. Response time of the chamber is 7 min. Each subject performed a light exercise of 38 W for 15 min on a bicycle ergometer 5 times daily, in order to mimic the everyday life activity. From 11 p.m. to 6 a.m., the metabolic rate (MR) lies between 35 and 40 W/m^2 in both control and obese subjects. Diurnal MR of controls averaged 70 W/m^2, and that of the obese was about 10% lower. Under PSMF, a protein diet: 1.3 g per kg ideal b. wt daily, the 24-h energy expenditure decreased progressively, reaching -15 to -20% of the control value after 20 days. This shows a metabolic adaptation to the reduced food intake, decreasing the daily energy deficit.

GABA-specific presynaptic dendrites of local circuit neurons

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After microinjection of ^{3}H-GABA (γ-aminobutiric acid) into the pigeon optic tectum some labelled cell bodies and a widespread plexus of horizontally oriented labelled processes were detected in sublayer IId(5) by means of light microscopic autoradiography thereby reproducing the results of Hunt and Künzle (J. comp. Neurol. *170*, 173-190, 1976). In electron microscopic autoradiography the following structures were covered by silver grains: a) postsynaptic elements with and without polyribosomes, b) few presynaptic elements - probably axon terminals - filled with pleomorphic synaptic vesicles, c) fibrous elements with some pleomorphic vesicles scattered in the cytoplasm or clustered at the plasmalemma and d) elements which are both postsynaptic and presynaptic, the latter forming symmetrical junctions. These post- and presynaptic structures are most likely presynaptic dendrites of almost axonless local circuit neurons which might play a role in mechanisms of lateral inhibition.

Afferent connections to the paraventricular nucleus studied by the retrograde transport of horseradish peroxidase

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Information on afferent connections to the paraventricular nucleus (PVN) of the hypothalamus is still scanty. An attempt was therefore made by means of the horseradish peroxidase (HRP) method to determine which areas of the central nervous system project to PVN. A 30-50% solution of HRP, 0.05-0.1 µl, was injected into the PVN region of adult rats and the animals allowed to survive 24-48 h, without having access to drinking water. After glutaraldehyde-formaldehyde fixation, cryostat sections were cut, exposed to diaminobenzidine and examined. To date, 2 animals had injections apparently confined to 1 PVN; 3 others, to PVN and a region just dorsal to it. Preliminary observations of the brains, serially cut at 40 µm and counterstained with cresyl violet, showed transported HRP in cells in the septum and in periaqueductal gray. No reaction product was seen in the contralateral PVN, the supraoptic nuclei and the neurohypophysis.

Inhibition by strophanthidin of the uptake of potassium by pigment cells in the drone retina

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A double-barrelled K^+-sensitive microelectrode has been used to measure changes in intra- and extracellular K^+-activity (a_K) in the drone retina during photostimulation. Stimulation with a standard train of flashes, 1 per sec, caused a_K in the photoreceptors to fall by 21 mM,

SD = 9 mM, N = 11, $t_{1/2}$ = 30 sec. In the extracellular space there was an increase in a_K with a transient maximum (a_K reaching as much as 42 mM) followed by a plateau. In pigment cells with membrane potentials > 50 mV, a_K in the dark was 52 mM, SD = 13 mM, N = 11: photostimulation caused an increase of 14 mM, SD = 5 mM, $t_{1/2}$ = 21 sec. It is estimated that the quantity of potassium taken up by the pigment cells was approximately equal to that lost by the photoreceptors. When strophanthidin-k, 2×10^{-5} M was included in the superfusate for 2-min-periods, the rate of uptake of K^+ by the pigment cells was reversibly inhibited by up to 78%. We conclude that in this retina there is a pump-mediated transport of K^+ across the glial cell membrane during quasi-physiological stimulation.

The role of glutamate in pigeon optic tectum

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Superficial layers of the pigeon optic tectum contain high levels of endogeneous glutamate and accumulate L-[^{3}H] glutamate by a high affinity uptake process. After ablation of the pigeon retina the high affinity uptake for glutamate decreased suggesting a possible role in the optic nerve terminals. Using microiontophoretic techniques we were interested to see whether an antagonism exists between known glutamate antagonists and the synaptic-evoked activity of tectal neurones. The majority of tested neurones was located in the 'P-zone'. Nuciferine and glutamic acid diethylester reversibly blocked the synaptic excitation. On the other hand atropine and dihydro-β-erythroidine were without effects. The results strongly suggest the role of glutamate as excitatory amino acid neurotransmitter within the pigeon optic tectum.

Spectral cues in skylight navigation of insects

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Ants *(Cataglyphis)* are able to navigate by the polarized light in the sky (R. Wehner: Scient. Am. *235* [1], 106, 1976). What they also might exploit in navigation are differences in the hue of color or in the UV (or green) intensity of skylight. The quantum catch of UV and green receptors in the upper visual field of the ant has been calculated by photometric measurements of different points in the sky and at different times of the day. The photodiode was equipped with specifically designed color filters in which the transmission $T(\lambda)$ mimicked the sensitivities $S(\lambda)$ of the ant's UV, green (and blue) receptors: $S(\lambda) = k \cdot R(\lambda) \cdot T(\lambda)^{-1}$ [$R(\lambda)$ is the responsivity of the calibrated UDT-222 diode ($qu \cdot s^{-1} \cdot cm^{-2}$), k is a constant]. $S(\lambda)$ was scaled so that the area under the curve was equal for each receptor. Results: The green receptor is maximally stimulated when viewing the sun, the UV-receptor when viewing points that are 90° distant from the sun, i.e. where skylight is maximally polarized. With respect to trichromatic color vision the spectral loci and the hue loci of different points in the sky are mapped in the ant's color triangle.

Body temperatures after (+)-amphetamine in heat and cold exposed rats

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Experiments are being carried out to study the effect of heat and cold exposure on rats given drugs that affect thermoregulation. Results with amphetamine are reported here. Each set of experiments is based on a standardized procedure: 4 doses of (+)-amphetamine (A) (0.4, 0.8, 1.6, 3.2 mg/kg) are injected i.p. to 4 groups of 5 singly caged female rats (200 g b.wt), and T_{re} and T_s are measured at 30–60 min intervals for 7 h. Rats acclimatized to 21 °C were exposed to 30 °C in a climatic chamber for 1, 3, 5 and 10 days before A. In rats without heat exposure before A, T_{re} curves at 30 °C showed a steep increase (T_{re} max at ~ 2 h) and a long lasting plateau (LD_{50} 3.0 mg/kg). After 1 day preexposure to 30 °C there was a less pronounced hyperthermic response and no death; after 5 and 10 days preexposure T_{re} decreased linearly after T_{re} max was reached. This reduced hyperthermic response to A of 30 °C acclimatized rats persisted after 5 days reexposure to 21 °C. Repetition of the experiments in the cold (10 °C) resulted in an analogue type of response pattern.

Early ultrastructural and secretory responses of canine parathyroid glands to changes in serum Ca-concentration

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Serum PTH levels were inversely related to changes in serum Ca-concentration induced by 7- or 60-min-infusions with $CaCl_2$ or EGTA. External parathyroid glands were removed immediately before and after the infusions and morphometrically evaluated by electron microscopy. In normocalcemic dogs 5 stages of the secretory cycle were distinguished and the volume densities of the cells estimated. 1% of the cells were in transition from a resting to an early stage of the secretory cycle, 15% in an early and 35% in a late stage, and 15% involuting and 10% resting. The most striking alterations in hypocalcemic dogs were an increase of cells in the late stage of the secretory cycle by 10% after 7 and by 40% after 60 min whereas in hypercalcemic dogs involuting and resting cells increased by 10% after 7 and 25% after 60 min. These rapidly occurring shifts are probably related to changes in PTH secretion.

Fictive locomotion in curarized high spinal cats elicited with 4-aminopyridine and DOPA

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DOPA-activated spinal cats are known to generate stepping patterns in their hindlimbs which can be recorded as neurograms in the curarized state ('fictive locomotion' after functional deafferentation). It will be shown that the same preparation spontaneously produces coordinated fictive 4-limb locomotion after combined application of 4-aminopyridine (4-AP) and DOPA. This fictive locomotion appears at much lower doses of DOPA and is considerabely faster than with DOPA alone. 4-AP is known to enhance excitatory and inhibitory transmission on many different synapses of the spinal cord. This suggests that 4-AP increases the frequency of stepping by raising the excitability level in the spinal interneuronal network. An additional spinal transection at the first lumbar segment does not alter the hindlimb locomotion pattern, while the stepping of the forelimbs becomes considerabely slower and weaker.

BIOCHEMIE - BIOCHIMIE - BIOCHEMISTRY

A new form of rabbit skeletal troponin C

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When troponin from rabbit skeletal muscle is chromatographed on DEAE sephadex A-50 in 1 mM EDTA without prior treatment with urea, one obtains a major peak of nondissociated troponin followed by a minor peak containing an 18,000 mol.wt protein. Further purification of the latter by gel filtration yields a pure protein which resembles troponin C (TN-C) in mol.wt, pI and electrophoretic mobility. The recovery is 2–3% of the starting material, representing ca. 10% of the amount of TN-C present in the troponin complex. Similarly to TN-C, both the electrophoretic mobility and the far UV circular dichroism (CD) of this protein decrease upon removal of CA^{2+}. In contrast to TN-C, which possesses 2 types of Ca-binding sites, this protein has a single category of Ca^{2+} sites ($K > 10^5$ M^{-1}), as monitored by CD at 220 nm. Its amino acid composition differs significantly from that of TN-C, being richer in Cys, His, Tyr and Pro, and poorer in Asp, Gly and Phe. This suggests that TN-C exists in more than one form, as already reported for TN-T and TN-I.

Effects of s.c. adrenaline, isoprenaline and phenylephrine on blood glucose and lactate in the phenformin-treated streptozotocin-diabetic rat

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In diabetic rats oral phenformin causes hypoglycaemia and concomitant increase in lactataemia. Adrenaline, an alpha- and beta-receptor stimulant, abolishes the phenformin-induced hypoglycaemia; blood lactate increases transiently to return almost to initial levels. Isoprenaline, mainly a beta-receptor stimulant, has no effect on hypoglycaemia and hyperlactataemia caused by phenformin 4 h after administration. Phenylephrine, a relatively specific alpha-receptor stimulant, prevents the phenformin-induced hypoglycaemia; blood lactate increases only slightly. The hypoglycaemic effects of phenformin in diabetic rats appear mainly to be due to the inhibition of resynthetization of glucose from lactate. This metabolic disturbance is restored to normal by the alpha-receptor stimulatory component of catecholamines.

Structure and function of a copper-metallothionein from *Neurospora crassa*

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A low-mol.wt copper-binding protein has been isolated from *Neurospora crassa* grown in the presence of nontoxic doses of $CuSO_4$. Amino-acid sequence determination of the pure protein revealed a striking sequence homology to the Cd- and Zn-metallothioneins. The 7 cysteinyl residues present in *Neurospora* Cu-thionein match exactly the positions of the first 7 cysteinyl residues in the amino terminal region of human liver, equine kidney and mouse liver metallothioneins. The outstanding conservation of the cysteinyl residues points to an evolutionary relationship to these metallothioneins. *Neurospora* Cu-thionein is the smallest metallothionein found to date. The binding capacity of 6 copper ions per mol.wt of 2200 shows this protein to be an efficient chelator for this metal. It may therefore by generally involved in copper homeostasis or serve a detoxicating function in this eucaryote.

Isolation of a novel plasma macroglobulin by affinity chromatography on sepharose-IgG

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Normal human IgG covalently linked to derivatized sepharose has been recently used as an affinity ligand for the purification of the first component of complement (Cl). After elution of the Ca^{2+}-dependent subcomponents Clr and Cls, Clq is removed by chaotropic agents together with 2 antigenically distinct macroglobulins. – One of these has been identified as cold insoluble globulin, while the other has not revealed any antigenic relationship to known plasma proteins. This previously unrecognized glycoprotein has been purified to homogeneity and shown by immunoelectrophoretic and SDS-PAGE analysis to be a β1-globulin of apparent mol. wt 450,000 daltons, composed of similar, disulfide-linked subunits of about 100,000 daltons. The persistent association of this macroglobulin with Cl prepared from normal human sera by affinity chromatography or euglobulin precipitation suggests a possible functional relationship to the macromolecular complex of Cl.

Kinetic studies of the aerobic reduction of ascorbate oxidase

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Ascorbate oxidase (L-ascorbate: O_2 oxidoreductase, EC 1.10.3.3) from green zucchini squash, a 'blue' copper-containing enzyme, was investigated by stopped flow technique using reductic acid as a substrate under aerobic conditions. The time course of the reaction was monitored either at 610 and 330 nm (absorption of Type I and Type 3 Cu^{++}, respectively). With substrate enough to perform several catalytic cycles, Type I Cu^{++} enters the steady state after a fast initial phase, the amplitude of which corresponds to $\frac{2}{3}$ of the total absorbance at 610 nm. Type 3 Cu^{++} undergoes a biphasic initial absorbance increase, probably indicative of a reaction intermediate. With a large excess of substrate such as to deplete all oxygen present in solution, the enzyme, after the steady state, remains 'frozen' in a fully reduced state. These and other data are compared with those already present in the literature for other 'blue' copper-containing oxidases.

Attempt to estimate the degree of saturation of mitochondrial pyruvate carrier and pyruvate carboxylase by their substrate in liver of living mice

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The effect of pyruvate overloads on the incorporation of 2-^{14}C pyruvate into blood glucose was studied in fed and 24-h-fasted mice as well as on the activities of the liver pyruvate carboxylase and phosphoenol pyruvate carboxykinase in vitro. No modification of enzyme activities was observed compared to control whatever the importance of overload. In spite of a chemical dilution of 2-^{14}C-pyruvate by 45 and 77 µmoles of unlabeled pyruvate the incorporation of ^{14}C-pyruvate into blood glucose

was not modified. Only a 154 µmoles overload decreases the incorporation of this precursor both into blood glucose and liver $^{14}CO_2$. These results suggest that the liver intramitochondrial pyruvate is far to be sufficient to saturate the pyruvate carboxylase and that the utilization of pyruvate in vivo could not be limited by its intramitochondrial carrier as it was proposed by A.P. Halestrap, Biochem. J. *148*, 85, 1975.

Actin solubilization and 'supercontraction' in crayfish muscle as shown by immunofluorescence and electron microscopy

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During 'supercontraction' of crayfish myofibrils, a state not observed in vertebrate myofibrils under similar conditions, actin (by indirect immunofluorescence) and thin filaments (by electron microscopy) are frequently no more visible. Concurrently large amounts of solubilized actin (or actin like protein) appear in high speed supernatants of myofibril suspensions or muscle homogenates as seen by SDS-PAGE and double immunodiffusion. In a relaxing medium, myofibrils as well as muscle fragments of crayfish, retain the 'classical' structure while actin solubilization in myofibril suspensions and muscle homogenates remain low. This unusual behaviour of the crayfish muscle components is probably related to the structure of actin as well as to the particular organization of muscle fibres in this crustacean.

Uneven distribution of actin in the pituitary gland

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In view of the suggestion that actin might be involved in exocytosis, the actin content of pituitary glands in adult rats was determined by 3 methods: by SDS-PAGE for total actin, by the RNAse method of Lazarides and Lindberg (PNAS *71*, 4742, 1974) which measured predominantly monomeric actin and by immunofluorescence. The amount of actin present in the neurointermediate lobe (NIL), expressed per mg total protein, was found to be comparable to that found elsewhere in the central nervous system and in the liver. In contrast, the concentration of actin present in the anterior lobe (AL) was much lower. Thus, 3 independent, semi-quantitative methods all point to an uneven distribution of actin between NIL and AL, 2 tissues where secretion occurs by exocytosis. The bulk of the actin present in the cells is therefore more likely to be involved in intracellular translocation of secretory products rather than in exocytosis.

Primary structure of equine hepatic metallothionein: Comparison of renal and hepatic isoproteins

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The amino acid sequence of hepatic metallothionein, a major zinc metalloprotein from horse, was determined. The results document the existence of 2 homologous types (isometallothioneins) with chain lengths of 61 and 60 residues. Both contain the same characteristic metal-chelating oligopeptide sequences composed of Cys, Ser and basic amino acid residues but differ in the deletion of 1 cysteinyl residue and in 7 residues that are not involved in metal binding. The 2 sequences are completely identical with those of the 2 primarily cadmium-containing isometallothioneins from equine kidney. Thus, the organ-specific variation in the metal composition of the metallothioneins cannot arise from differences in metal-ion selectivity of the polypeptide chains but must be attributed, instead, to the dissimilar chemical environment in the different organs.

Extraction of peripheral proteins from intestinal brush border membrane vesicles

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Brush border membrane vesicles were prepared according to the method of M. Kessler et al. (Biochim. biophys. Acta *506*, 136, 1978). Starting with that preparation a method was developed to purify the membrane from adhering proteins and/or to separate peripheral from integral membrane proteins. The brush border membranes were treated with a chelating resin (Chelex 100) in a low ionic strength medium. The SDS-Polyacrylamide Gelelectrophoresis pattern of membranes treated in that way was greatly simplified showing the loss of mainly low mol.wt proteins. After removal of those proteins the purified membrane vesicles retained their specific transport functions.

Preincubation with Papaverine (PA) can lower the PGE-1-induced rise of cAMP in human platelets (platelet-rich plasma)

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In intact adenine-^{14}C-prelabeled PGE-1-stimulated platelets the steady-state cAMP-^{14}C concentration showed a biphasic response to rising concentrations of PA but a linear one with isobutyl-methyl-xanthine (MIX). When the rise (linear initial slope) of cAMP after adding PGE-1 simultaneously with MIX (phosphodiesterase-saturating) was set = 100%, preincubation with 0.5 mM PA for 1 min lowered the slope to 70±12%, $p < 0.05$. ATP was $\geqslant 90\%$ of control at 1 min after 0.5 mM PA (hence no substrate depletion for adenylyl cyclase, AC). The results suggest that 0.5 mM PA may lead to significant inhibition of in-situ stimulated AC-activity in a time-dependent manner (slow and/or indirect effect) in intact human platelets in addition to the well-known powerful inhibition of high-affinity phosphodiesterase. Consideration of these properties help to assess intracellular regulation of cAMP-metabolism.

Gelelectrophoresis-derived enzyme-linked immunosorbent assay (GEDELISA): A new technique for the characterization of antibody specificity and the detection of antigens in complex mixtures

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The antigen (AG) is solubilized with SDS and electrophoresed on 2 parallel polyacrylamide gels. 1 gel is stained with Coomassie-blue and the other is sliced into 1-mm-fractions. The slices are incubated in 1 ml of Na_2CO_3, pH 9,6, in polystyrene tubes until the proteins have diffused out and are bound to the surface of the tubes. Subsequently a conventional ELISA is performed, and the absorbance of the fractions correlated with the stained protein bands of the parallel gel. By this highly sensitive technique the specificity of antibodies (AB) may be easily characterized, as shown by a myosin-antimyosin system. With the GEDELISA it is further possible to recognize those antigenic

determinants, e.g. of bacterial cells, which induce AB in naturally occurring infections. This is demonstrated with M. suipneumoniae and sera of infected pigs.

Estimation of total estradiol receptors in the rat uterus

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This estimation is achieved by labeling free estradiol receptors (R^E) with ^3H-estradiol ($^3HE_2$) and exchanging E_2 bound to R^E with $^3HE_2$. The conditions of the incubation were chosen in such a way that the R^E in the cytoplasm and the nucleus remained stable but were able to exchange their endoginous E_2 with $^3HE_2$. The main steps in the procedure are as follows: The homogenate is preincubated for 2 h at 4 °C in 16 nM $^3HE_2$. Aliquots are then precipitated with protamine sulfate and bound E_2 is exchanged with $^3HE_2$ (24 nM) for 15–18 h in a shaking waterbath of 30 °C. The pellets are washed in a buffer containing 0.5% Triton X-100, brought on Whatman GF/C filters and analyzed for cpm. Parallel incubations in presence of excess diethylstilbestrol are performed in order to estimate the $^3HE_2$ bound by other binding sites than R^E. These unspecific binding sites comprise about 15% of the total $^3HE_2$ binding and are substracted from the total binding in order to calculate R^E. This procedure should help to elucidate the factors controlling the amount of R^E.

Sulfatide metabolism of the cerebrum and cerebellum of normal and myelin-deficient jimpy mice

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Sulfatide, a myelin lipid, is formed by microsomal cerebroside sulfotransferase (CST) and degraded by lysosomal arylsulfatase A (ASA). In developing mouse brain, their activity patterns seem to be correlated, suggesting functional cooperation. To study a metabolic cooperation of CST and ASA, we measured these enzymes and the sulfatide content in brain regions with differently timed myelination in normal and in jimpy mice with hypomyelination. CST and ASA patterns were synchronous in both brain parts, following the time sequence of myelination, thus indicating functional cooperation. In jimpy mice, CST was abnormal, but ASA followed normal pattern. Results and data previously shown indicate, that in normal myelination sulfatide is incorporated into a stabile myelin pool, but a large portion is degraded. In jimpy, most of the synthesized sulfatide is degraded by normal ASA. We speculate, that lysosomal degradation is involved in regulation of myelination.

Rotational mobility of Ca^{2+}-Mg^{2+}-dependent ATPase of sarcoplasmic reticulum

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The Ca^{2+}-Mg^{2+}-dependent ATPase of sarcoplasmic reticulum and the purified enzyme (in vesicular form) were labeled with iodoacetamido-eosin without loss of ATP-splitting activity. The tripletprobe eosin is a strong sensitizer for photooxidative inactivation of the splitting activity of the ATPase. Photooxidation could be completely prevented by working under dim red ligth or by bubbling the sample with argon. Rotational diffusion of the active enzyme was investigated by measuring the decay of dichroism of flash induced absorbtion changes of the eosin probe. Preliminary work shows a strong aggregation tendency of the Ca^{2+}-Mg^{2+}-dependent ATPase and a rotational relaxation time of less than 20 µsec at room temperature. Alternatively this fast relaxation time may apply to a part of the ATPase molecule which rotates independently.

Action of acetazolamide on liver pyruvate carboxylase (PCX) and acetyl CoA carboxylase (ACX) activities in mice

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Studies have been carried out on PCX-activity, gluconeogenesis and glycogenolysis in order to obtain some informations about the hyperglycemic effect of acetazolamide, a carbon anhydrase inhibitor. The incorporation of 2-^{14}C-pyruvate into blood glucose and the liver PCX-activity decreases in acetazolamide-treated mice while liver glycogenolysis increases. The hyperglycemic effect of acetazolamide seems to depend on this last action. Acetazolamide inhibits the activity of the purified ACX even in the presence of high concentrations of bicarbonate. The inhibitory effect of acetazolamide on ACX cannot be explained by an action on the phosphorylation of this enzyme, nor by preventing its polymerization by citrate, but it could act in facilitating its depolymerization. This effect is probably not the only reason of its inhibitory action on lipogenesis in vivo since this effect is suppressed by insulin and bicarbonate.

Mode d'action d'analogues de l'AMP-cyclique sur les phosphodiestérases du cerveau

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L'étude de l'hydrolyse de l'AMPc par les phosphodiestérases (PDE) à K_m élevé, en présence d'analogues de l'AMPc a permis de mettre en évidence:
- Le site catalytique, où certains dérivés entrent en compétition (dérivés 2'O-butyrylés de l'AMPc substitués en 8, N-butyryl-GMPc ...).
- Un site de contrôle allostérique négatif (inhibition non-compétitive). Le 2'O-butyryl-GMPc est parmi ceux étudiés, le plus puissant.
- Le 2'O-butyryl-IMPc, à concentration variable, semble se fixer successivement sur les 2 sites mentionnés.
- Un site de contrôle allostérique positif (activation). On l'observe en présence de faibles concentrations de dérivés de l'AMPc dibutyrylés ou N6-monobutyrylés substitués en 8 (thio, méthyl-thio ...).

Le site d'activation possède une affinité beaucoup plus élevée que le site d'inhibition pour les dérivés étudiés.

Study on insulin resistance in muscles from genetically obese (fa/fa) rats

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In obese hyperinsulinemic animals insulin-stimulated glucose utilization is impaired. In muscles of obese mice, the decrease in the number of specific insulin receptors was not sufficient to explain such a resistance (Y. Le Marchand et al., Am. J. Physiol., in press). We have therefore hypothesized that excessive lipid utilization could partially inhibit glucose metabolism via the well-known glucose-fatty acid cycle. Soleus muscles of normal and obese (fa/fa) rats were incubated to test this hypothesis. At 6 weeks, insulin-stimulated glucose transport and glycolysis, intracellular citrate, and G-6-P levels were similar in muscles from

control and obese animals. At 10–15 weeks, the effect of insulin upon glycolysis was now impaired in muscles of obese rats while glucose transport was little affected. However, citrate levels were higher than controls, concomitant with an increase in those of G-6-P. These experiments are consistent with the concept of an increased fatty acid utilization, in muscles of obese animals, with secondary increase in citrate inhibitory to glycolysis at the level of phosphofructokinase. These data suggest that, in muscles of obese rats, an intracellular defect, distal to insulin receptor, could contribute to the establishment of insulin resistance.

EHDP-effect on the synthesis of phosphophoryns and dentin collagen in the rat incisor

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Collagen and phosphophoryns are the principal proteins in the matrix of the rat incisor. EHDP (ethane-1-hydroxy-1,1-diphosphonate) given at high dose (10 mg P per kg) has been shown (Larsson, Calc. Tissue Res. *16*, 109, 1974) to inhibit the mineralization of dentin, however the effects of the diphosphonate on matrix formation are unknown. In our experiments, growing rats (150 g) were administered the high dose of EHDP for 30 days. The diphosphonate had no effect on the cross-linking or the NaCl solubilization of the dentin collagen. Collagen synthesis measured by the uptake of ^{3}H-serine after i.v. injection was only minimally affected, while the incorporation into the phosphophoryns was reduced greater than 50%. These results may be an illustration of the proposed relationship between phosphophoryn synthesis and mineralization.

Towards the spatial structure of mitochondrial aspartate aminotransferase

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Mitochondrial aspartate aminotransferase, a dimeric, pyridoxal phosphate-dependent enzyme with identical subunits (mol. wt $2 \times 45{,}000$), has been crystallized in the triclinic space group P1 with 1 dimer per unit cell. Single crystal absorption spectra with and without substrates show that both active sites are catalytically competent. A 4.5 Å resolution electron density map, based on 5 heavy-atom derivatives was calculated. It clearly reveals the molecular boundary of the dimer ($105 \times 65 \times 65$ Å) with the monomers related by a 2fold axis. A difference map (apo-holo) gave the coenzyme positions. The determination of the amino acid sequence is underway. To date, 70% of the about 400 amino acid residues per subunit have been identified. Collection of X-ray data to high resolution as well as studies of the catalytic features of the enzyme in the crystalline state are in progress.

Phosphofructo-kinase activity in human autoptic brain

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The phosphofructo-kinase (PFK) plays, within the glycolytic chain a regulatory role for the energy supply of the brain. The catalytic activity of several glycolytic enzymes in human autoptic tissue changes with age (Experientia *33*, 794, 1977). Separating the human material into cases with a short premortal agony and long agony, the linear regression of PFK-activity as a function of age are: aPFK = 6.15–0.059 years ($r = 0.610$, $p < 0.001$) for short agony and aPFK = 0.217 + 0.004 years ($r = 0.118$, n.s.) for long agony. The age-dependent decrease of PFK is therefore more clearly demonstrated in cases with short premortal agony. On the other hand, short agony cases do not significantly depend on post mortem delay (PMD) in the determination of their activity. Whether the demonstrated effects are identical with real age-dependence of PFK in human brain remain to be proved in further experiments. However not only PMD, but also other factors, such as premedication or different causes of death for young and old people have to be considered in this argumentation.

Bovine brain glutamate dehydrogenase

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GluDH (EC.1.4.1.3) from central nervous system consists of identical subunits, 56,000 mol. wt. It accepts both NAD(H) and NADP(H) as coenzyme. An equilibrium constant of $3{,}25 \times 10^{-15}$ M^{-2} establishes that under standard conditions, the oxidative deamination of L-glutamate is the spontaneous reaction, but in vivo conditions satisfy near to equilibrium situation. Observed kinetic and regulatory properties are adapted to dynamic regulation of L-glutamate and ammonia levels in the CNS. Brain and liver enzymes differs in their isoelectric point and in some of their antigenic determinants, but have similar steady state kinetics parameters. In vitro responses to purine mononucleotides, cyclic AMP's, GTP, ADP and ATP show these metabolites are likely candidates for the in vivo regulation of glutamate synthesis and breakdown. A random substrates and coenzymes addition mechanism fits the kinetic data.

Characterization of protein kinases isolated from the cytosol of GH_3-cells

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Protein kinases have been partially purified and characterized from GH_3-cells. 80% of the cytosol kinase activity was cAMP-dependent and 20% cAMP-independent. Phosphocellulose chromatography revealed 3 cAMP-independent fractions designated as PC II, PC III and PC IV and a cAMP-dependent enzyme fraction (PC I) in the void volume. The cAMP-independent enzymes were different with respect to their mol. wt, substrate specificity and biochemical properties. PC II represents the free catalytic subunit (mol. wt 38,000), whereas PC III (mol. wt 20,000) and PC IV (mol. wt 200,000) are cAMP-insensitive protein kinases. The cAMP-dependent protein kinase can be resolved on DEAE-cellulose into 2 peaks, corresponding to type I (eluted at 0.1 M NaCl) and type II (eluted at 0.2 M NaCl). The mol. wts of these 2 enzymes have been determined by PAGE: Type II (representing the major cAMP-dependent protein kinase activity) has a mol. wt of 180,000, type I consists of 2 molecular sizes present in a ratio of 1:1 (mol. wt 90,000 and 240,000).

Spin-labelling technique applied to the problem of the control of fatty-acids translocation and oxidation in the heart

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A 12,000 daltons fatty acid-binding protein has been purified and characterized for the first time from rat and pig heart muscle. The fatty acid/protein interaction as analyzed by spin-labeling is characteristic of a strong immobilization of the fatty acid. The transfer of the fatty acid from the protein to the isolated mitochondria, where it undergoes β-oxidation, suggests that this protein could be the physiological transcytoplasmic carrier in the heart. A modulation of the β-oxidation is observed when this protein is present in the incubation medium. This control mechanism and the physiological significance will be discussed in the light of the results obtained with albumin used as a model carrier (Biochim. biophys. Acta *486*, 82, 1977).

Polymorphic inheritance of transcobalamin II

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Transcobalamin II (TC II) is a vitamin B12 binding protein in serum, essential for transporting B12 to the cells. TC II can be saturated with radioactive B12, split up into isoproteins by polyacrylamide gel electrophoresis and detected by autoradiography. 10 different phenotypes have been observed: 3 patterns appear at rather high frequency in a heterogeneous population, 40% TC II 3-3, 40% TC II 1-3, and 15% TC II 1-1. The variants are compatible with a 5 allel system with corresponding allele frequencies of: TC II-3 0.604, TC II-1 0.367, TC II-2, TC II-4, and TC II-5 < 0.015. Family studies including families with rare patterns, support autosomal inheritance according to the mendelian law. Homozygotes exhibit 2, and heterozygotes 2+2 isoproteins, yielding 3- (when overlapping) or 4-banded patterns. TC II variant 4-4 is a functionally deficient TC II and variant 2-2 is found in the mother of a child with congenital TC II deficiency, indicating that functional and probably selective differences accompany rare TC II variants.

Purification of glutathione transferases by affinity chromatography

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A simple method for the purification of glutathione transferases by affinity chromatography was developed. Glutathione was coupled to modified sepharose 4 B via the thiol group. Such glutathione sepharoses were relatively stable and bound glutathione transferase activity. When N-(2-aminoethyl) maleic acid amide was used as spacer and bound protein was eluted with 5 mM glutathione, a mixture of glutathione transferases which was free of contaminating enzyme activities was obtained. Analysis showed that the mixture contained at least 4 glutathione transferases with isoelectric points at pH 7.0, 7.75, 8.15 and 8.7. 2 of these were identified as glutathione transferases A and C by immunoprecipitation experiments. The other 2 enzymes did not react with antisera against transferases A, B or E.

The charge heterogeneity of pure galactosyltransferase from human milk

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With a new purification method consisting of acid casein precipitation and 2 affinity-chromatography steps on N-acetyl-D-glucosamin-p-aminophenyl-sepharose and α-lactalbumin-sepharose, respectively, pure galactosyltransferase from human milk was obtained in a yield of 2 mg/l. In contrast to earlier purification methods we found only the native enzyme form with a mol.wt around 55,000. The enzyme transfers galactose from UDP-galactose to ovalbumin, free N-acetyl-glucosamin, sialic acid-free ovine submaxillary mucin and, in the presence of α-lactalbumin, also to glucose. The purity of the enzyme preparation was checked on polyacrylamide gel electrophoresis with or without SDS. Isoelectric focusing of pure enzyme in presence and absence of 8 M urea yielded at least 7 distinct forms with IEP's ranging from pH 5.5 to 7.5. The charge heterogeneity was confirmed by preparative column focusing.

Role of the plasma membranes and of the intracellular membranes in adipocyte glycerolipid synthesis

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The incorporation of a cell-permeating esterification substrate (1-^{14}C palmitate) and 2 different nonpermeating substrates (1-^{14}C palmitoyl CoA and U-^{14}C α-glycerophosphate) into adipocyte subcellular fraction glycerolipids was compared. Results show that all 3 substrates are incorporated to the same extent into the glycerides and phospholipids of the 3 adipocyte subcellular membranes. In the storage glycerides, however, the incorporation of 1-^{14}C palmitate was found to be much greater than that of 1-^{14}C palmitoyl CoA and U-^{14}C α-glycerophosphate. This suggests that the plasma membranes mainly synthesize membrane glycerolipids and the intracellular membranes storage glycerides.

Human serum albumins from newborns and adults are structurally identical

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The reduced bilirubin-binding capacity of newborn plasma has been claimed to be due to the occurrence of a functionally immature fetal albumin. Therefore, human serum albumin was isolated from pooled umbilical cord and adult donor plasma and compared. Isolation was by affinity chromatography on Cibacron Blue coupled to sepharose 4B. The albumins were further purified by treatment with colloidal SiO_2 followed by defatting with charcoal and Millipore filtration. The 2 albumins were indistinguishable by polyacrylamide gel electrophoresis with and without SDS, isoelectric focusing, immunoelectrophoresis, immunodiffusion using specific antibodies and amino acid composition. Furthermore, the N-terminal 8 residues, the C-terminal leucine and the staphylococcal proteinase fingerprints were identical in both albumins. However, slight differences were observed in the bilirubin-binding properties as shown by CD, fluorescence and binding constants.

α-Galactosidase isozymes from *Bacillus stearothermophilus*

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A strain of *Bacillus stearothermophilus* is found to produce 2 isozymes of α-galactosidase (EC 3.2.1.22) constitutively. The relative amount of the isozymes depends on growth stage, growth temperature and medium composition. α-Galactosidase I possesses significant melibiase activity and with the substrate p-nitrophenyl-α-D-galactopyranoside (α-PNPG) has a pH-optimum of 6. It shows mixed-type inhibition kinetics with lactose and at 65 °C has a half-life of > 2 h at low protein concentration. α-Galactosidase II has very weak melibiase activity and with α-PNPG has a pH-optimum of 7. It is noncompetitively inhibited by lactose and has a half-life of about 3 min at 65 °C. Both enzymes are inhibited competitively by D-galactose, melibiose and Tris(hydroxymethyl)-aminomethane, and noncompetitively by cellobiose. Their mol. wts, estimated by disc gel electrophoresis, are: α-galactosidase I: 280,000, α-galactosidase II: 325,000.

Comparison of allogeneic and xenogeneic surface markers specific for murine T-cells

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Mouse T-lymphocytes are characterized by the presence on their surface of 2 sets of antigenic determinants detectable with xeno and alloantisera. Where as xenoantigens (MTLA) are common to all strains of mice, the expression of the 2 alleles of Thy-1 is strain-specific. Preliminary experiments have shown that the xeno and alloantigens have identical mol. wts and are adjacent on the cell surface. Immunofluorescent studies showed that when MTLA antigens were induced to cap, Thy-1 antigens were also found at the same pole of the cell. This effect was not observed when H-2 antigen was examined as a control. Immunoprecipitation of either antigen from a lysate prepared from surface labeled T-cells led to the co-precipitation of the other antigen. When anti-H-2 antiserum was used, only H-2 antigen was precipitated. Based on these results we conclude that xeno and alloantigenic surface markers of murine T-cells exist on the same membrane protein.

Identification of human T-cell specific antigen

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Crude membrane preparations of human thymocytes have been used to raise rabbit antisera which can be rendered specific for T-cells by absorptions with human red blood cells, plasma proteins, liver homogenate and a pool of 9 B lymphoid cell lines. The specificity is demonstrated by immunofluorescence, complement mediated cytotoxicity and by determining the absorptive capacity of various target cells, including lymphoid cell lines, PBL, tonsil and thymus cells as well as separated B- and T-cell populations. After selective labeling of the surface of lymphocytes by means of lactoperoxydase-catalyzed iodination, followed by immunoprecipitation and resolution on PAGE-SDS several membrane proteins (apparent mol. wt 160,000, 110,000 and 45,000) are detected on thymocytes, PBL and cultured T-cell lines which have been shown to be absent from B-lymphoblasts. Partial purification of the human T-cell specific antigens is obtained by gel filtration and ion exchange chromatography of a water soluble extract of human thymocyte membrane proteins.

The transport system for D-glucose is the rate limiting step of its metabolism in *Trypanosoma brucei*

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D-Glucose and 2-deoxy-D-glucose enter the long slender bloodstream form of *Trypanosoma brucei* only through a carrier mediated system. Free diffusion and pinocytosis can be ruled out during 1-minute uptake period. The permeation of sugar is driven by the downhill concentration gradient and therefore is not energy-dependent. The basic metabolic rate of the cell is of the same order of magnitude as the permeation rate; therefore the gradient is maintained by the continuous removal of free substrate. In the blood of the mammalian host the carrier of *T. brucei* works under saturation conditions, being the rate limiting step in the glucose metabolism.

The transport of cytoplasmically-synthesized proteins into mitochondria

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Only 10% of the mitochondrial protein mass is made by the mitochondria themselves. The remaining 90% is made by the nucleo-cytoplasmic system and imported into mitochondria. Some of the imported proteins are the mitochondrial F_1-ATPase subunits. Yeast cells were pregrown in $^{35}SO_4^{2-}$ (to label all proteins) and pulsed with ^3H-leucine. Part of the cells was 'chased' in the presence of unlabelled leucine or inhibitors of cytoplasmic protein synthesis. F_1-subunits were isolated from mitochondria and high-speed supernatant by a newly developed immuneprecipitation method. The precipitates were resolved by SDS-gel electrophoresis and the $^3H/^{35}S$ ratio in the 2 largest F_1-subunits was determined. Newly synthesized F_1-subunits were first detectable in the high-speed supernatant; they could be chased into the mitochondria. These preliminary data argue against vectorial translation across the mitochondrial membranes. An alterate model will be presented.

Enzyme-substrate binding reaction: Determination of activation energy and enthalpy change using the immobilized substrate

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UDP-galactosamine coupled by its amine via spacer to sepharose was highly biospecific and purified UDP-galactose 4'-epimerase of human erythrocytes and liver from a specific activity of 0.003 to 1.2 U/mg protein. Initial binding velocity of erythrocyte enzyme to immobilized substrate and the equilibrium between free and bound enzyme were measured at different temperatures from −1 to +10 °C. Activation energy (Arrhenius) and enthalpy change (Vant'Hoff) of the substrate-enzyme binding reaction were calculated. E_a was +37 kJ/mole, ΔH −42 kJ/mole. The temperature-dependence of the overall enzymatic reaction was also measured. The logarithm of the activity was proportional to the reciprocal of the absolute temperature from 15 to 37 °C only, and the slope corre-

sponded to an E_a of 50 kJ/mole. For lower temperatures higher activation energies were observed. We conclude that the binding reaction was not the rate limiting step in the overall enzymatic epimerization process.

M-line in chicken muscle

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The M-line component with a mol.wt of 166,000 was isolated from chicken breast muscle by low ionic strength extraction in 5 mM Tris-HCl, 1 mM DTT, 1 mM EDTA, 0.1 mM PMSF, pH 7.7. Purification was carried out by acid and ammonium sulfate precipitations and repetitive ion exchange chromatography on DEAE-cellulose in 50 mM Tris-HCl, 1 mM EDTA, 0.3 mM DTT, 0.1 mM PMSF, pH 8, applying a linear salt gradient (0–0.2 mM NaCl). The mol.wt was identical to that of chicken glycogen debranching enzyme on 8% SDS slab gel electrophoresis, but both proteins were immunologically distinct. The M-protein was also detected in liver, intestine, brain, skin, kidney, gizzard and chicken heart where no M-line can be found by electronmicroscopy. The M-protein could be localized (by the indirect immunofluorescent technique) in the M-line of isolated myofibrils.

Kainic acid binding in the pigeon CNS

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^{3}H kainic acid binding has been determined in the pigeon CNS using 10^{-3} M L-glutamate as displacer. At 50 nM kainic acid concentration the binding was (fmoles/mg prot.): telencephalon 230, tectum 190, pons/medulla 130, spinal cord 60, cerebellum 8300. Retinal ablation had no effect on the tectal binding (1–8 weeks of survival time). The effect of various chemicals on the binding suggests the presence of several, partially interacting binding sites. L-glutamate sensitive kainic acid binding was found also in other vertebrates and seems to be higher in phylogenetically older families.

Identification of the normal glycoprotein crossreacting with carcinoembryonic antigen in granulocytes and macrophages

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Using immunoabsorbent column containing IgG from rabbit hyperimmunized against Carcinoembryonic antigen (CEA), it was possible to purify from perchloric acid (PCA) extract of human lung a normal glycoprotein (NGP) containing common antigenic determinants with CEA. As previsously reported, the mol.wt of NGP, determined by its elution on sephadex G-200 and by sucrose density gradient, was found to range between 55,000 and 65,000 daltons. By double diffusion, using anti-CEA or anti-NGP specific antisera, it was demonstrated that NGP present in PCA extract of human colon carcinoma serially transplanted in nude mice was immunologically identical to NGP present in PCA extract of human granulocytes purified from human peripheral blood. By immunofluorescence and immunoperoxidase methods, it was found that NGP was present in the cytoplasm of granulocytes and macrophages. However, contrary to previous results from the literature, we found that NGP was present only in 1–3% of peripheral blood monocytes and only in a small percentage of myeloblasts from acute myelocytic leukemia. Future work will determine if NGP can be a useful additional marker for granulocytes, macrophages and subpopulations of monocytes or myeloblasts.

Effect of acute bilateral vagotomy on hypersecretion of insulin of ventromedial (VMH) lesioned rats

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Acute lesions of the VMH area in anesthetized rats followed by immediate i.v. glucose load resulted in a greater insulin secretion than in control animals. When pairfed VMH-lesioned rats (i.e. food intake restricted to that of controls) were anesthetized and tested in vivo 7 days after the lesion, a similar glucose-induced hypersecretion of insulin was observed when compared to controls. In this group of VMH-lesioned rats, bilateral vagotomy performed just prior to i.v. glucose administration reduced the insulin secretion to values that were similar to those seen in vagotomized unlesioned controls tested under the same conditions. It is concluded that acute or chronic lesion of the hypothalamic area results in greater than normal glucose-induced insulin secretion in vivo. It is further concluded that such abnormality is related, at least in part, to a possible overactivity of the vagus nerve.

5HT-receptors in brain of rats with experimental allergic encephalomyelitis (EAE) and after incubation with myelin basic protein (MBP) in vitro

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Whole brain membranes were incubated 60 min/22 °C with 1–20 nM ^{3}H-5HT. 10 min preincubation with bovine MBP. Specific binding and Kd by Scatchard plot. Results in the table for EAE with Lewis rats, for MBP with SIV rats. Maximal specific binding (MSB) in fmoles/mg protein, dissociation constants Kd in nM for high- and low-specificity 5HT receptors, ±SEM.

	N	MSB (high spec.)	Kd
Controls	14	536 ± 36	4.07 ± 0.50
Adjuvant-injection	10	480 ± 25	3.77 ± 0.43
EAE-animals	10	*410 ± 33	*2.54 ± 0.27
Controls	8	634 ± 53	5.98 ± 1.43
+ 10^{-9} M MBP	6	*444 ± 16	3.30 ± 0.87

	N	MSB (low spec.)	Kd
Controls	14	1421 ± 110	23.3 ± 2.2
Adjuvant-injection	10	1116 ± 108	20.4 ± 3.0
EAE-animals	10	995 ± 135	18.1 ± 3.7
Controls	8	1175 ± 142	18.9 ± 1.8
+ 10^{-9} M MBP	6	852 ± 42	10.9 ± 1.4

* Difference to controls sign, sign. $p < 0.01$.

Conclusions: EAE results in a reduction in high-specific 5HT receptors with an increase in specificity. Incubation of membranes with MBP has a similar effect. The molecular mechanisms may be the same in both conditions, as free MBP is increased in the CNS-tissue in demyelinating diseases.

Interrelations between the exchange of H^+, K^+, Ca^{2+} across the cell membrane and the control of hepatic glycogenolysis

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The redistribution of ions across the cell membrane is closely associated with the control of glycogenolysis in perfused livers from fed rats. A forced transient redistribution of H^+ (without pH-change in the effluent perfusate in the steady state) was used to trigger a disturbance of the distribution of other ions. K^+, Ca^{2+} and pH in the perfusate were continuously monitored by ion-specific electrodes. By switching from bicarbonate/CO_2 buffered perfusate to HEPES without CO_2 a transient decrease of the effluent pH (> 0.2 pH-units) was observed. O_2-uptake increased and Ca^{2+} was taken up while K^+ was released. This was paralleled by an increased rate of glucose, lactate and pyruvate production reflecting an activation of glycogenolysis. Neither the omission of K^+ from the medium (partial K^+-depletion of the liver) nor the replacement of Ca^{2+} by 0.3 mM EGTA suppressed the activation of respiration and glycogenolysis. The increased O_2-uptake may reflect enhanced ion pumping and ATP turnover.

Characterization of ^{125}I-labeled α-bungarotoxin (α-Bgt)

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Isotopically labeled snake venom toxins are used to purify and characterize the nicotinic acetylcholine receptor (AcChR). The labeling reaction must be such that modified toxin molecules may be readily separated from unreacted ones. This is essential when only a small percent of the molecules are labeled as in the case of the iodination reaction, and therefore the biological activity of the unfractioned reaction mixture may not be representative of the labeled molecules. The modified toxin molecules, once purified, must still be biologically active. Iodination of α-Bgt with ^{125}I was studied. Gel filtration on Biogel P4 is used to separate toxin molecules from excess ^{125}I. The mixed population of toxin molecules obtained is resolved by ion-exchange chromatography (sephadex CM 50) using a linear NaCl gradient (0-80 mM). Nonreacted, mono-iodinated and di-iodinated toxins are well-separated by this procedure. The major reaction product is homogeneous as shown by IEF (pI = 8.7). The iodinated toxin is quantified according to a procedure based on the Mancini single radial immunodiffusion technique using toxin-specific antibodies raised in rabbits. Complex formation between iodinated toxin and AcChR was studied a) in vitro by serial dilution with nonlabeled toxin, b) in vivo by determining the LD 50 in mice. According to these 2 criteria, the radiolabeled toxin is active.

Release of monosaccharides from *Candida albicans* compared to human serum glycoconjugates

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C. albicans is a yeast known to have cell wall antigenic components essentially constituted of mannan and glucan. The sugars of purified *C. albicans* analyzed by GLC consists of mostly mannose, glucose and an additional sugar not yet identified. The kinetics of sugar release by hydrolysis/methanolysis (4 N HCl) was studied. Under those conditions *C. albicans* releases very rapidly the unidentified sugar and mannose is released quicker then from the glycoconjugates of human serum, where the mannose is blocked in the internal oligosaccharide structure. Septicemia by *C. albicans* causes a severe clinical situation and necessitates a rapid diagnostic tool. Characterization of the unidentified sugar as well as the differential mannose release will allow the quantitation of *C. albicans* or released cell wall antigenic components in patients with septicemic candidiasis.

Effect of removal of cholesterol on microsomal enzyme-activity

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Cerebroside-sulfotransferase (CST) a mouse brain microsomal enzyme with a lipid requirement, catalyses the transfer of sulfate to cerebroside forming sulfatide. We have shown previously by removing lipids with acetone and reconstituting the membrane with artificial lipid-mixture, that the age-dependent CST-activity depends on the cholesterol-phospholipid ratio (C/P) in the membrane. In order to avoid membrane damages by acetone we now developed a procedure using phospholipid-micelles which removes only cholesterol from the membranes. 14-day-old microsomes (C/P = 0.60; specific CST-activity = 4900±530 dpm) were incubated with phospholipid-micelles for 120 min. The achieved C/P (0.53) and the resulting CST-activity (9800±1050 dpm) correspond to that of 18-day-old membranes. Concomitant ESR-studies of the membranes show an increase of fluidity. We therefore speculate that age-dependent CST-activity changes are partly due to membrane fluidity changes.

Paradoxical hyperexcretion of nitrogen in hyperinsulinemic rats

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Ventromedial hypothalamus (VMH) lesions in normal rats are known to result in hyperinsulinemia. Adipose tissue lipogenesis measured in vivo was found to be greater in VMH-lesioned rats than in controls. Perfused livers from VMH-lesioned animals revealed an increased lipogenesis from various substrates, a lower glucose output and a reduced ketogenesis. All these changes supported the concept of an overstimulation of liver and adipose tissue by excessive levels of insulin. Unexpectedly, VMH-lesioned rats were also characterized by high urea levels, increased ureogenesis and increased nitrogen excretion. In an attempt to understand those abnormalities (apparently unfitting with prevailing hyperinsulinemia) hepatic albumin synthesis, hepatic amino acid uptake and release were measured in vitro and found to be identical to those of control rats. The release of amino acids from muscle mass in vivo was similar in normal and VMH-lesioned rats. Thus, the cause of the increased nitrogen excretion exhibited by VMH-lesioned rats is still undetermined.

Analysis of bile acids in serum by capillary gas-liquid chromatography

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Various liquid phases were evaluated for capillary gas-liquid chromatography (GLC) of trimethylsilylethers of methylesters of cholic, deoxycholic, chenodeoxycholic, hyodeoxycholic (internal standard), lithocholic, ursodeoxycholic, 3β-hydroxy-5-cholenoic acid and cholesterol. The results indicate that bile acid analysis is rapid (15 min) and exhibits high separation efficiency with a 20 m $\times$ 0.3 mm i.d. glass capillary column covered with a crystal layer of barium carbonate and coated with polyethyleneglycol 20 m as liquid phase according to Grob et al. (Chromatographia *10*, 181, 1977) (method of static coating with 0.2% PG 20 m). Hydrogen was used as carrier gas at a split ratio of 1:5, a flow rate of 4 ml/min and a column temperature of 230 °C. Bile acids were extracted from 2 ml of serum with XAD-2 and subjected to solvolysis, alkaline hydrolysis, methylation, silylation and capillary GLC. This procedure proved to be sufficiently specific and sensitive for quantification of the major bile acids in normal human serum.

Monolayers of a light-harvesting polypeptide and of polymethyl glutamate

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The hydrophobic LHP (light harvesting polypeptide) isolated from chromatophores of *R. rubrum* G-9 and PMG (poly-α-methyl-L-glutamate) were spread from different organic solvents at a water-air interface. Surface pressure/area isotherms of the monolayers formed were obtained with a film balance. Films of PMG spread from chloroform showed a plateau in their isotherms in contrast to those spread from pyridine. For the LHP, however, the isotherms obtained proved to be independent from the spreading solvent (chloroform/methanol or pyridine). The polypeptide films were transferred to germanium plates for attenuated total reflection IR-measurements. All films of LHP gave spectra whose amide I and II absorption bands ($\sim$1655 and $\sim$1545 cm^{-1}) indicated α-helical and random conformations. In contrast, PMG, when spread from pyridine gave a spectrum with a component characteristic of the β-conformation. Electron microscopic studies are in progress.

Enhancement of the hemolytic activity of the first component of human complement by collagen

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Clq, a subcomponent of the first component of complement (Cl) plays the dual role of binding to immune complexes and bringing about the activation of the 2 proteases in the Cl-complex, Clr and Cls. Chemical and structural studies of Clq have revealed that about 40% of the amino acid sequence of the molecule is organized in a collagen-like triple helical form. In view of the resemblance of part of the Clq molecule to collagen it is of interest to determine if mammalian collagen is capable of interfering with the biological activity of Clq. - Collagen from rat skin in soluble form was not able to replace Clq-activity. On the other hand, when collagen preparations were added to the first component of human complement (serum, euglobulin and Cl reconstituted from Clq, Clr and Cls), an enhancement of 500 to 1000 in hemolytic Cl activity was observed. A pepsin fragment of Clq containing all the collagen-like sequences present in the intact molecule was also unable to reconstitute macromolecular Cl. However it enhanced Cl hemolytic activity to the same extent as collagen. The mechanism of this unforeseen phenomenon will be discussed, taking into account previous results (interaction of Clq with collagen) and the possibility of eliminating an inhibitor of Clq by collagen.

Paracatalytic modification of ribuluse 1,5-bisphosphate carboxylase from *Pseudomonas facilis*, tobacco and spinach

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Ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBP C-O) from the 3 title sources have been found to undergo active-site-directed paracatalytic modification by $K_3Fe(CN$ and RuBP after activation with Mg^{2+} and CO_2. The bacterial, tobacco and spinach enzymes were inhibited 87%, 92% and 86%, resp., after 15 min in 80 mM Tris · SO_4 (pH 8), 20 mM Mg^{2+}, 50 mM $NaHCO_3$, 5 mM RuBP and 4 mM $K_3Fe(CN)_6$. Slight RuBP-independent inactivation was reversible by dithiothreitol. RuBP-dependent inactivation satisfied criteria that verify participation of an enzyme · substrate intermediate in the inactivation mechanism (JBC *251*, 4220, 1976). Modifications performed in borate-containing hepes buffer increased the rate of inactivation and stabilized the final inhibition complex. By analogy with known reactivities of model α-dicarbonyl compounds with proteins, it is proposed that $Fe(CN)_6^{3-}$ oxidizes a C-2 carbanion intermediate of RuBP to a 2,3-diketone and that the inhibition complex involves an arginyl-specific modification.

Binding of rabbit secretory component to dispersed mammary epithelial cells and dimeric immunoglobulin A

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Dimeric immunoglobulin A $(IgA)_2$ is specifically transported through rabbit mammary gland epithelium and appears in milk a secretory IgA (sIgA), linked with secretory component (SC). SC, a glycoprotein synthetized by epithelial cells, shows heterogeneity with respect to charge and mol.wt (68,000-83,000). Free SC purified from milk binds specifically to dispersed mammary epithelial cells as determined by competitive binding kinetics and Scatchard analysis. A Ka of 4×10^8 M^{-1} and 2000 binding sites per cell have been found. Free SC also binds to dimeric IgA with a Ka of 2×10^7 M^{-1} and a rate constant of dissociation of 8.5×10^{-5} sec^{-1}. The interaction is pH-dependent with an optimum at pH 6.0 to 6.5 which is milk pH. These results suggest that SC mediates the specific uptake and the transcellular transport of dimeric $(IgA)_2$.

Mechanistische Grundlage für die hohe Genauigkeit der Aminosäureaktivierung in der Protein-Biosynthese

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Am Beispiel der Phenylalin-spezifischen Aminoacyl-tRNS-Synthetase und tRNS liess sich zeigen, dass der als Zwischenprodukt auftretende Enzym-Aminoacyladenylat-

Komplex eine grosse Bedeutung bei der Kontrolle der Verknüpfung der «richtigen» Aminosäure mit der «richtigen» tRNS hat. Aufgrund unserer Resultate, die durch kinetische Analyse, kombiniert mit Isotopenaustausch, erhalten wurden, kommt die hohe Spezifität dadurch zustande, dass eine «falsche» Aminosäure präferentiell durch Hydrolyse aus dem Syntheseweg entfernt und die «richtige» präferentiell durch Übertragung auf tRNS im Syntheseweg belassen wird. Dieser Diskriminierungsprozess wird offenbar in starkem Masse durch tRNS gesteuert.

Amino-acid sequence of tyrosinase from *Neurospora crassa*

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The amino-acid sequence of tyrosinase from *Neurospora crassa* (EC 1.14.18.1., o-diphenol: oxygen oxidoreductase) has been determined. This copper-containing monooxygenase consists of a single polypeptide chain of 407 amino acids and has a mol.wt of 46,000. The primary structure was determined by automatic and manual sequence analysis on fragments produced by cleavage with cyanogen bromide and on peptides obtained by digestion with trypsin, thermolysin, pepsin and chymotrypsin. The amino terminus of the protein is acetylated and the single cysteine residue 96 is covalently linked via a tioether to the oxidized tyrosyl residue 94. A prediction of the secondary structure based on the amino-acid sequence of this enzyme suggests the presence of 33% α-helix, 15% β-sheet, 31% β-turn and 20% coil structure. Dye-sensitized photo-oxidation of the apoenzyme implies the involvement of histidine side chains as ligands of the active site copper.

Erythrocyte membrane structure and agglutinin-receptor binding

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Signals are transmitted through membranes by different mechanisms; one of them depends on transmembrane glycoproteins with external receptor sites and links to cytosceletal systems. Changes in erythrocyte ghost membranes induced by agglutinin-binding to the glycophorin receptor may provide a model for the initial events in the membrane in this type of triggering. Sheep and human ghosts were agglutinated by the mitogen phytohemagglutinin. From gel-electrophoresis and thin-layer chromatography it appears that the protein and lipid composition of the ghosts is unaffected by agglutination except that the agglutinin and selected plasma proteins (albumin, fibrinogen) become part of the membrane specimen. However, there is evidence that the molecular structure of native and agglutinated ghosts differs. With sheep there is a higher content of side-to-side packed transmembrane α-helices in agglutinated than in native ghosts. A not well understood rearrangement occurs in human agglutinated ghosts which involves also changes in lipid chain structure.

The interaction of actin and α-actinin with liposomes

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Recent studies have suggested that the structural protein, α-actinin may serve to anchor actin filaments to plasma membranes and secretory vesicles. In an effort to investigate this interaction we have utilized the technique of photoaffinity labeling. Unilamellar liposomes were shown to bind 14.7 µg of α-actinin and 12.5 µg of actin per mg of phospholipid. Liposomes were preincubated with the apolar photoprobe, 5-(^{125}I)-iodonaphthyl-1-azide (INA) to permit the probe to partition into the lipid bilayer. Subsequent addition of actin and α-actinin followed by irradiation of the resulting complex resulted in the covalent labeling of these proteins from within the lipid core. The results of this study show that α-actinin preferentially inserts into the lipid bilayer. The hydrophobic properties of α-actinin were corroborated using the technique of charge shift electrophoresis. These studies suggest that α-actinin may be associated with intrinsic membrane proteins, thus having the potential of functioning as part of a transmembrane regulatory system.

Segregation of glycophorin from other integral membrane proteins

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Intramembranous particles are known to aggregate when ghosts are incubated at low pH. When ghosts pretreated at pH 4.5 are washed and then transferred to phosphate buffer (pH 7.4), they segregate vesicles that contain almost exclusively glycophorin. The amount of glycophorin in these vesicles does not exceed 3% of the total glycophorin. However, the bulk of glycophorin seems to be segregated as well from other integral membrane proteins but not detached from the membranes because Triton X-100 preferentially extracts glycophorin from these membranes. The extract forms glycophorin-enriched vesicles after removal of Triton and contains up to 90% of the total glycophorin in a purity approaching that of glycophorin isolated by other techniques. The observed segregation of glycophorin implies that glycophorin is no longer associated with intramembranous particles when erythrocyte membranes have been treated at low pH.

Kinetic of oxygen consumption during phagocytosis of leukocytes: photometric method

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10^6 granulocytes are suspended in 300 µl of incubation medium containing dextran, autologous serum and purified human hemoglobin used as oxygen donor and respiration indicator (Bârzu and Borza, Analyt. Biochem., 1967). Cellular respiration provokes oxyhemoglobin dissociation and the variation of optical density is measured at 435.8 nm in a 2 mm light path-length cuvette. The respiratory burst is continuously recorded after the injection of zymosan which stimulates a cyanide-insensitive oxidase. The mean value for oxygen uptake of nonstimulated polymorphonuclear is 0.7 nmoles $O_{at} \cdot min^{-1} \cdot 10^{-6}$ PMN, while the maximum rate for stimulated cells is 11 nmoles $O_{at} \cdot min^{-1} \cdot 10^{-6}$ PMN.

Sulfatide synthesis and CNP: Glial markers for cultured gliomas?

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Sulfatide synthesis and 2',3'-Cyclic nucleotide 3'-phosphohydrolase (CNP) activity were measured in confluent cultures derived from human glial and other unrelated tumors and from normal human fibroblasts. The incorporation of

(^{35}S)sulfate into sulfatides varied over a 500fold range with no significant difference between glial tumor cultures and control cultures. Higher sulfatide synthesis was observed in rat C6-glioma, glioma line MG-118, a brain metastasis, 3 of 7 low grade gliomas and 1 of 2 neurinomas. In 14 permanent glioma lines, the CNP activity was 15.7 μmoles/h mg prot (range 7-24) as compared with 7.7 μmoles/h mg prot (range 1-26) in controls. Both sulfatide synthesis and CNP activity were lower in fibroblasts, meningiomas and glioma cultures overgrown by nontumoral cells. These results indicate that sulfatide synthesis and CNP activity are not reliable biochemical markers for cultured gliomas.

Myelin isolation of aggregating brain cultures

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Recently, we reported evidence that rotating cell aggregates of mechanically dissociated fetal rat brain myelinate in vitro (J.-M. Matthieu et al., Pediat. Res. *11*, 1011, 1977). In the present study, myelin was isolated from these cultures using density gradient centrifugation. The starting material was a pool of 20 cultures (102 mg prot.) maintained in vitro for 30 days. Purified myelin represented 1% of the total homogenate. The specific activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase showed a 5fold increase in myelin (1.0 mmole/h mg prot.) over the starting homogenate. Typical myelin proteins were identified in purified myelin after electrophoresis on polyacrylamide gels. When compared to 15-day-old in vivo rat myelin, the fraction isolated from the aggregates showed a higher content of high mol.wt proteins. Furthermore, the major myelin glycoprotein labeled with radioactive fucose was shifted toward a higher apparent mol.wt. These results indicate the presence of immature myelin.

Action of a deficiency in biotin on the activities of liver and adipose tissue pyruvate and acetyl CoA carboxylases

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Biotin-deficiency exerts a strong inhibitory effect on pyruvate carboxylase activity of liver, adipose tissue and kidney but is without effect on the incorporation of 3-^{14}C-pyruvate into blood glucose. In contrast it inhibits both acetyl CoA carboxylase activity and the synthesis of fatty acid in liver and adipose tissue. This effect is more pronounced in the last one. These results seem to indicate that pyruvate carboxylase exists in liver, in an amount much too high compared to the concentration of the substrate, the pyruvate, and even if the inhibition of liver pyruvate carboxylase is higher than that of acetyl CoA carboxylase it remains sufficient quantities of holopyruvate carboxylase to normally convert pyruvate into glucose. The similar effect of the biotin-deficiency observed in the adipose tissue acetyl CoA and pyruvate carboxylases could be explained by the presence of nearly the same quantities of both the enzymes and a preferential depletion of biotin from adipose tissue.

Temperature-dependent aggregation of bacteriorhodopsin in phosphatidylcholine vesicles

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Bacteriorhodopsin has been incorporated into large unilamellar lipid vesicles following solubilisation of the purple membrane with Triton X-100. Its aggregation behaviour was investigated using X-ray diffraction, electron microscopy, circular dichroism and rotational diffusion measurements. At temperatures below the lipid phase transition, bacteriorhodopsin crystallizes into patches with the same hexagonal lattice observed in the purple membrane. Above the phase transition, the lattice disaggregates and the protein molecules are monomeric provided the lipid:protein ratio is sufficiently high.

Labeling of human erythrocyte membranes with the triplet-probes eosin-isothiocyanate and iodacetamido-eosin: Inhibition of anion-transport

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Rotational diffusion of eosin-labeled membrane proteins may be investigated using a flash-photolysis technique. The binding of eosin-derivatives to band 3 proteins in the human red cell membrane is characterized by studying the effect of these probes on the anion-transport system associated with band 3. Up to 80% inhibition of sulphate-exchange is observed in eosin-labeled red cells, eosin-NCS being slightly more efficient than IA-eosin. The monofunctional eosin-probes are of comparable efficiency to disulfonic acid stilbene derivatives and share common binding sites with them, although other sites are labeled as well. Fluorescein-derivatives, the precursors in the synthesis of eosin-probes, are without effect on anion-transport, probably due to the higher pK_a-value of the monoanion compared to eosin. Eosin-induced inhibition is light-independent and can not be attributed to a photosensitizing action of these probes.

Factor VIII, an aggregate of subunit dimers

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Human factor VIII-related protein (F VIII), purified from cryoprecipitate by gel filtration on sepharose CL-2B, was electrophoresed on SDS - 2.0% polyacrylamide - 0.5% agarose gels. Protein staining revealed multiple aggregates with apparent mol.wts of up to 20×10^6. Treatment of void volume F VIII with cysteine resulted in a progressive, stepwise decrease of molecular size. Intervals between individual F VIII bands were estimated by calibrating electrophoretic gels with disulphide-reduced subunits of chemically cross-linked F VIII and with covalent oligomers of human γM-immunoglobulin. The difference in molecular size between F VIII oligomers was equal to a dimer of the basic F VIII subunit polypeptide chain (mol.wt ~ 250,000). Similar patterns were found in F VIII oligomers obtained from late elution fractions of cryoprecipitate. We conclude that F VIII aggregates are multimers of pairs of basic subunit chains.

Effect of phalloidin on isolated rat hepatocytes: A possible role of actin microfilaments in regulation of cell shape and triglyceride secretion

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Phalloidin, a toxin from the genus *Amanita,* produced modifications of hepatocyte shape characterized by protrusions bulging from the cytoplasm, the parenchyma being otherwise normal. At the basis of the protrusions, an accumulation of actin microfilaments was detected both by electron microscopy and immunofluorescence. Cellular water volume, ATP and potassium content were unmodified. The release of triglycerides was inhibited by phalloidin and accompanied by an increased cellular triglyceride content. The release of triglycerides was inhibited by colchicine and the inhibitory effects of colchicine and phalloidin were additive. Although colchicine inhibited total protein and albumin secretion, these were only very slightly modified by phalloidin. It is concluded that: a) microfilaments may play a role in maintaining cell shape; b) if phalloidin and colchicine effects represent, respectively, primarily changes in microfilamentous and microtubular systems, both systems may have complementary roles for the secretion of at least triglycerides.

Somatostatin: Effect on the insulinotropic and glucagonotropic activities developed by rat duodenal mucosa after arginine ingestion

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Our study has shown that intragastric arginine administration (2 g/kg b.wt) to rats was increasing the insulinotropic and glucagonotropic activities contained in duodenal mucosa, measured by an in situ rat pancreas preparation. The effect of somatostatin on these activations was investigated. A group of animals was pretreated with 500 µg/kg b.wt cyclic somatostatin. This treatment inhibited the development of the insulinotropic duodenal activity after oral load, measured through the increased release of insulin in the bioassay. In contrast, the activation of the glucagonotropic activity, measured through the increased release of glucagon in our model, was not affected. It is suggested that somatostatin might exert a differential action on the mechanisms of activation of pancreaticotropic factors of duodenum by food administration. 2 different pathways are probably involved in the activation of insulinotropic and glucagonotropic activities by arginine.

The binding sites for cytochrome c oxidase and cytochrome bc_1 on cytochrome c

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It has been conjectured that lysine residues are involved in the specific binding of cytochrome c to the inner mitochondrial membrane. We have localized these residues by differential chemical modification. Cytochrome c either alone or bound to the oxidase or to cytochrome bc_1 was modified with a trace amount of a ^3H-reagent (acetic anhydride or formaldehyde/KBH_4), fully modified with excess of unlabeled reagent and mixed with homogeneously ^{14}C-labeled cytochrome c. The reactivity of individual lysine residues could thereafter be quantitated from $^3H/^{14}C$-ratios. Some residues were remarkably less reactive in the complexes. The binding site for the oxidase was deduced from the less reactive residues and is on top and to the left of the molecule (conventional front view) involving lysine residues 13, 7-8, 86-88 and 72-73. The binding site for cytochrome bc_1 overlaps at least partially with the site for the oxidase.

Insulin-like growth factors (IGF I and II) - 2 polypeptides homologous to proinsulin

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An insulin-like activity not suppressible by antibodies against insulin (formerly known as NSILA) was isolated from human serum and identified with 2 polypeptides, IGF I and II. IGF now appears to be a growth-hormone-dependent growth factor for connective tissue cells. The complete amino acid sequences of IGF I and II have been determined. They are single-chain polypeptides with 70 (IGF II: 67) residues and with 3 disulfide bridges. Residues 1-29 (3-32) are homologous to insulin B chain, residues 43-62 (41-61) are homologous to insulin A chain. Out of these 50 (51) positions, 25 (24) are identical in human insulin. As in proinsulin, a connecting peptide bridges these 2 portions which is, however, only 12 (8) residues long. An additional feature not found in proinsulin is a C-terminal extension of 8 (6) residues. In conclusion, IGF is a new growth hormone-dependent hormone derived by gene duplication from the same ancestor molecule as proinsulin.

Hypothalamic lesions and the endocrine pancreas

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Ventromedial hypothalamus (VMH) of normal rats was destroyed by passing anodal current through stereotaxically-guided electrodes. Subsequent hyperphagia of lesioned animals was prevented by feeling VMH-lesioned rats a diet matching that of controls, using an automatic food distributor. Pancreases from VMH-lesioned rats had increased pancreatic insulin and glucagon content and, when perfused, increased glucose-induced insulin secretion without qualitative alteration of the usual biphasic secretion pattern of the hormone. Preliminary results suggest that the arginine-induced secretion of glucagon by perfused pancreases from VMH-lesioned rats was also increased when compared to controls. It is concluded that hypothalamic lesions have far-reaching consequences on the regulation of the endocrine pancreas. The origin of the latter remains to be determined.

Investigation by cross-linking of the state of aggregation of human erythrocyte membrane acetylcholinesterase observed in presence and absence of Triton X-100

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Cross-linking of purified human erythrocyte membrane acetylcholinesterase with glutaraldehyde and dialkyldiimidates of different chain length was performed in presence and absence of Triton X-100. The modified enzyme was analyzed by SDS-gel electrophoresis and sucrose density gradient centrifugation. In absence of detergent the enzyme aggregates to multiple molecular forms. These could be chemically fixed by glutaraldehyde to a much greater extent than by dialkyldiimidates. Cross-linking in presence

of detergent yielded the dimeric protomer as the only form. These results confirm the hypothesis that purified human erythrocyte membrane acetylcholinesterase is an amphipatic, dimeric protein. Removal of the detergent leads to higher mol.wt aggregates which are stabilized by hydrophobic interactions and consist of multiples of the dimer.

Identification of sulfated glycoproteins and mucopolysaccharides in lymph nodes and thymus

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The epithelial cells of the thymus have been shown to incorporate ^{35}S-sulfate. A relationship with thymocyte maturation was suggested (S.L. Clark, J. exp. Med. *128*, 927 1968). We have also detected labeling of the endothelial cells of the vascular system in the lymph nodes, which is the site of lymphocyte 'homing'. In order to identify these materials, mice were injected with $Na_2{}^{35}SO_4$. After 2-24 h, thymuses or lymph nodes were homogenized and submitted to proteolytic digestion. The labeled macromolecular material was separated by anion-exchange chromatography into 2 components. Gel filtration of the first component indicated the presence of glycopeptides. The second, more acidic component was composed of mucopolysaccharides as shown by gel filtration, electrophoresis and enzymic degradation. Since polyanionic compounds are known to be involved in various cellular interactions, these materials may play a role in cell recognition and traffic in the immune system.

Ca^{2+} redistribution and phenylephrine effects in perfused rat livers

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The absence of Ca^+ from the medium prevents phenylephrine from activating glycogen phosphorylase in hepatocytes. In perfused livers, glycogenolysis was not affected by the lack of extracellular Ca^{2+}. Livers from fed rats were perfused in a nonrecirculating system. Ca^{2+} and K^+ in the effluent were monitored by ion-specific electrodes. Since the metabolic response to phenylephrine was not affected by changing the perfusate Ca^{2+} from 1.27 to 0.01 mM the latter concentration was used to facilitate the measurement of Ca^{2+}. A transient net release of Ca^{2+} (0.2 µmoles/100 g rat weight was recorded between 50 and 260 sec after the start of an infusion of phenylephrine (0.5 µM). K^+ was first taken up, then released. An increase of glucose and lactate production reflecting an enhancement of glycogenolysis occurred already during Ca^{2+} release. 0.2 mM EGTA had no effect on Ca^{2+} release and glycogenolysis, indicating that Ca^{2+} uptake from the extracellular medium was not necessary for the triggering of the metabolic response to the hormonal signal.

Effect of fasting and of insulin on the activities of acetyl CoA carboxylase and fatty acid synthetase of liver and adipose tissue

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A short-term activating effect of insulin on the adipose tissue fatty acid synthetase in fed rats has been found, while there is no action of insulin on liver fatty acid synthetase under the same conditions. This action of insulin was accompanied by a stimulatory effect on the V_{max} for Malonyl CoA of adipose tissue fatty acid synthetase, but no modification of the K_m for this substrate. Acetyl CoA carboxylase of 100,000 × g supernatant of rat adipose tissue was found to be inhibited by a 14-h-fasting but this inhibition disappeared by administration of insulin 1 h before the execution of animals. However, when the measurement of activity of ACX was carried out with 15,000 × g supernatant no inhibitory effect of fasting was observed and insulin does not modify ACX activity. These results are in accordance with a short-term regulation of ACX and FAS of adipose tissue but not of liver by insulin and would be explained by an activating action of this hormone on the dephosphorylation of these enzymes.

Isolation, characterization and N-terminal amino acid sequence of a *Bacillus cereus* neutral proteinase

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Extracellular neutral proteinase was produced in 10-l-batches with *Bacillus cereus* x_3. The enzyme was extraced from the medium by chromatography with Amberlite XAD-7 resin. The enzyme was further purified by acetone precipitation and affinity chromatography. The mol.wt is about 35,000 daltons. The enzyme seems to represent a new, more thermostable type of neutral proteinase from mesophilic bacilli: Its thermostability is higher (60 °C) than that of the *B. subtilis* neutral proteinase (50 °C) and the amino acid sequence shows higher homology to thermolysin (68%) (produced by a strain of the species *B. stearothermophilus*) than to *B. subtilis* neutral proteinase (25%). The N-terminal amino acid sequence is:
VTGTNKVGTGKGVLGDTKSLNTTLSGSSYYLQD ...

Effect of inhibited cholesterol synthesis on the activity of a microsomal enzyme

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Cerebroside-sulfotransferase (CST), a microsomal, lipid requiring enzyme, catalyses the transfer of sulfate to cerebroside, forming sulfatide. Our previous work has shown, that the enzyme activity is modulated in vitro by the cholesterol/phospholipid ratio of microsomal subfractions. We now report data in vivo, in which the cholesterol synthesis is temporarly inhibited by estradiol. We therefore added 10 µg estradiol per ml medium to glioblastoma cells in culture. Thereby the cholesterol/phospholipid ratio of the membranes decreased from 0.32 ± 0.02 to 0.22 ± 0.03. This effect was observed from 19 to 24 h after inoculation. During this period, the CST activity was enhanced from 3200 ± 340 dpm to 6300 ± 500 dpm. After this time, the lipid ratio and the enzyme activity returned to normal values. These effects could be observed either in microsomal subfractions or homogenates of the cells. This model provides a usefull tool for the investigation of modulation effects in the living cell.

Binding of unlabeled bPTH to bovine kidney cortex plasma membranes: A method for studying hormone-receptor interactions

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This study reports differences in the binding properties of labeled and unlabeled bPTH to partially purified plasma membranes from bovine kidney cortex. Membranes with PTH-sensitive adenyl cyclase systems were used. bPTH was incubated with the membranes. Separation of the bound and the unbound hormone was achieved by centrifugation. Membrane-bound hormone was eluted by acidification, lyophilized and measured by radioimmunoassay. Maximal uptake (65%) was observed after 4 min of incubation. Inclusion of dimethylsulfoxide and Trasylol in the incupation medium improved binding. Increased binding was seen with EDTA-washed membranes. Binding was also temperature-dependent ($4 > 22 > 37$ °C). In contrast I-125 bPTH prepared by several methods did not show the binding characteristics observed with the cold hormone. The measurement of receptor bound PTH by radioimmunoassay now offers an alternative procedure for studying hormone-receptor interactions. The application of this technique for assaying bio-active hormone in body fluids is suggested.

Potential role of the glycosylation of lens crystallins in diabetic cataractogenesis

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The glycosylation of crystallins was examined in diabetic cataracts and in normal lenses from rats and humans. The lenses were homogenized, reduced with ^3H-$NaBH_4$, and fractionated into in soluble proteins, α-, β-, and γ-crystallins. Amino acid analysis of these fractions from the cataractous lenses showed a ninhydrin positive peak and tritium counts which both coeluted with N^6-1-(1-deoxyglucitolyl)-lysine. The modified lysines were not detected in comparable amounts of normal human or rat crystallins. However, glucosyllysine was found in lenses from diabetic, noncataractous animals and in normal rat lenses cultured in hyperglycemic conditions. The nonenzymatic glycosylation was studied in vitro. Solutions of crystallins incubated with ^{14}C-glucose (50 mM) or ^{14}C-glucose-6-phosphate (5 mM) slowly incorporated label into α-, β-, and γ-crystallins. This glycosylation was followed by the development of opalescence in the solutions and hyperaggregation of the crystallins. These developments were prevented or reversed with dithioerythritol.

Plasma membranes of *Candida tropicalis* involved in the oxidation of n-alkanes

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The yeast *C. tropicalis* grows on hydrocarbons. It is possible that the yeast plasma membrane, known to be involved in many solute transport functions, is responsible for the uptake and oxidation of this water-insoluble substrate. Therefore, plasma membranes were isolated from mechanically disrupted cells by differential centrifugation and aggregation of mitochondria. The isolated plasma membrane fraction was estimated to be 95% pure. Reduced-oxidized difference spectra demonstrated an increase of a b-type cytochrome in the plasma membranes of alkane assimilating cells. An increase of membrane-bound enzymes involved in the oxidation of n-alkanes was indicated, as the activity of long-chain alcohol and aldehyde dehydrogenase was 10 times higher in plasma membranes of hydrocarbon grown cells than in those grown on glucose. However, an activity of a mixed function oxygenase was not observed. Nevertheless, these results indicate that the plasma membrane represents the membrane system responsible for alkane oxidation.

Biochemical localization of hormone action within the cortical nephron of the rat

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The intrarenal site of action of several hormones was studied in the rat kidney cortex using a system of isolated tubules, enriched in distal segments (fraction A) or in proximal segments (fraction B). The basic difference in composition of both fractions was established by morphology and confirmed by differences in the alkaline phosphatase and hexokinase activities and in the protein/DNA ratio. An enrichment factor of 2–3 could be derived for the distal cell (A vs B). Hormone sensitive adenylate cyclase activation and specific cytoplasmic binding of ^3H-aldosterone were measured in vitro:

	Fraction A	Fraction B
Adenylate cyclase activation (%Δ over basal value; ±SEM)		
Parathormone 1 IU/ml	418±43	408±50
Vasopressin 20 mU/ml	293±12	100±15
Isoproterenol 10^{-6} M	167± 7	89±10
^3H-aldosterone (mol × 10^{-14}/mg protein cytosol)	7.4±0.8	3.6±0.4

These results indicate a predominently distal site of action of vasopressin, isoproterenol and aldosterone.

Carcinogenicity and bacterial mutagenicity of 47 structurally related heterocyclic compounds

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47 heteropolycyclic compounds belonging to homogeneous series were investigated for carcinogenicity by injection in s.c. tissue of XVII nc/Z mice and for mutagenicity with *Salmonella typhimurium* in the presence and absence of liver 10,000 × g supernatant from Aroclor 1254 treated rats. 21 compounds produced tumours. All but one of these carcinogens were mutagenic. Of the 26 substances which did not produce tumours 14 were mutagenic, with a potency similar to that of the carcinogens. As a possible reason for the apparently 'false positives' in the Ames test it is suggested that the complexicity of the metabolism of these compounds may lead to critical differences in metabolism in mouse skin in vivo and in liver homogenate from Aroclor treated rats.

Analysis of urinary peptides as polyaminoalcohols by gas chromatography-mass spectrometry

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Urinary peptides from a baby and his elder brother were identified by gas chromatography-mass spectrometry as Gly-Pro-Hyp-Gly; Gly-Pro-Hyp; Gly-3Hyp-4Hyp; Pro-Hyp; and Gly-Pro, analyzed as their polyaminoalcohols after reduction with $LiAlH_4$ and $LiAlD_4$.

Effect of dietary phosphate and parathyroid hormone (PTH) on phosphate uptake by isolated renal brush border membrane vesicles (BBMV)

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BBMV of rats kept for 7–8 weeks on low phosphate diet (0.15%) showed a marked increase (ca. 100%) in the sodium-dependent phosphate uptake rate compared to those isolated from animals kept on a high phosphate diet (2.0%). Phosphate uptake by BBMV isolated from normal phosphate diet (1.0%) animals decreased by 35% after PTH-administration (30 USP 1 h prior to sacrifice, i.p.). Phosphate uptake by BBMV isolated from high phosphate diet animals increased by 50% after acute parathyroidectomy. In low phosphate diet animals PTH administration decreased phosphate uptake by BBMV only if the animals had been repleted (5 ml 20 mM NaH_2PO_4 i.p. 1 h prior to sacrifice). Under all experimental conditions sodium-dependent D-glucose transport by BBMV was unaffected. The results demonstrate that dietary phosphate and PTH affect the activity of the sodium-dependent phosphate transport system. Since the dietary effects can be reversed only partially by acute changes in the PTH-system other factors must be involved in the regulation of proximal tubular phosphate transport.

Low molecular weight calcium-binding proteins from chicken muscle

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Parvalbumin (PV; $M_r = 12{,}600$), parvalbumin-like protein (PV1P; 12,800), an 8000-protein and 2 other components (1:12,400; 2:11,700) were purified from chicken leg muscle. 1–3 Ca^{++}/molecule were bound to all proteins. PV is distinct from PV1P, components 1 and 2 and from the 8000-protein in aminoacid composition and affinity for Ca^{++}. Antibodies against chicken PV do not corss-react with any of the other proteins. PV was only found in leg, white and red back (but not in breast) muscles and in brain whereas PV1P was detected in several muscle and nonmuscle tissues. PV could not be localized within a distinct muscle structure while PV1P could be localized within the I-band region of isolated myofibrils. In primary muscle cell cultures no accumulation of PV was detected whereas PV1P accumulated in parallel to myosin, actin and MM-creatine kinase. In vivo accumulation of PV was earliest detected in leg muscle and brain of 1-day-old chickens indicating a more specialized function for this protein.

The influence of the electrochemical potential of Na^+ ($\Delta\tilde{\mu}_{Na^+}$) on phlorizin-binding to the Na^+-dependent D-glucose translocator of intestinal brush-border membrane vesicles

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The binding of phlorizin (a competitive inhibitor of D-glucose transport, as identified by both transport kinetic and binding studies) to the Na^+-dependent glucose transporter is influenced by the electrochemical potential of the cosubstrate (Na^+) across the membrane. This was shown by imposing different ion diffusion potentials across the membrane and measuring phlorizin-binding before sizable dissipation of the $\Delta\Psi$ had occurred. For optimal phlorizin-binding the presence of both Na^+ and electrical potential difference $\Delta\Psi$ (negative inside) were found to be necessary. An inverted membrane potential decreased phlorizin-binding. The time course of phlorizin-binding during dissipation of $\Delta\tilde{\mu}_{Na^+}$ (with either $Na_i^+ = Na_0^+$ or $Na_i^+ \ll Na_0^+$) was also investigated.

Sequence studies on lactic dehydrogenases (LDHs) from thermophilic *bacilli* and comparison with LDHs of mesophilic sources

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LDHs of *B. stearothermophilus* and *B. caldotenax* are tetramers of identical polypeptide chains with mol.wts of 34,500 and 36,000, respectively. The thermophilic LDHs contain more Arg and the LDHs from mesophilic bacilli and higher organisms, correspondingly, more Lys residues. CNBr cleavage of the *B. stearothermophilus* enzyme yields 6 fragments. 75% of the primary structure was elucidated by automatic Edman degradation of the intact subunit and of the CNBr fragments. In the N-terminal region of LDH 80% sequence homology was found between the thermophilic bacilli, 65% between the thermophilic bacilli and the mesophilic bacillus B. X_1 and 30% between the thermophilic bacilli and higher organisms. A significant feature is the absence of the first 14 residues in bacterial LDHs.

Kinetic studies on NADPH-dependent aldehyde reductase from human liver

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Double reciprocal plots of initial velocities with respect to the substrates of the reaction NADPH + H^+ + D-glucuronate $\rightleftarrows$ L-gulonate + $NADP^+$ yielded intersecting patterns indicating a sequential kinetic mechanism. At pH 7.4 the K_m-values for NADPH, $NADP^+$, D-glucuronate and L-gulonate were 1.6 µM, 5 µM, 3 mM and 5 mM, respectively. The ratio of V_1/V_2 was 200 and the K_{eq} 10^{10} M^{-1}. Product inhibition studies in the forward direction gave noncompetitive patterns with the exception for the nucleotides which were mutually exclusive. In the back reaction competitive inhibition patterns were obtained with NADPH for $NADP^+$ as well as L-gulonate as variable substrates. At pH 7.4 initial velocities of the glucuronate inhibited reaction could not be determined due to immediate nonlinearity of the progress curves. Noncompetitive inhibitions were, however, obtained at pH 8.8. These findings are best explained by an ordered reaction mechanism with the reduced coenzyme binding first and the oxidized coenzyme leaving last.

Polymorphism in sarcoplasmic Ca-binding proteins from crustaceans

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Earlier studies from this laboratory have revealed the existence of sarcoplasmic Ca-binding proteins, SCP's, monomer mol.wt ca. 20,000, in various invertebrate species (arthropod, annelids, molluscs) as well as in protochordates. In crustaceans, these proteins exist as dimers that dissociate in the presence of SDS or urea in subunits of mol.wt 22,000, possessing each 3 high affinity Ca-sites ($K_D < 10^{-6}$ M. Upon DE-52 cellulose chromatography, the SCP from crayfish emerges in 3 peaks in the proportion of

14:1.5:1. The iso-SCP's corresponding to these peaks do not differ in Ca-binding properties. Gel electrophoresis and isoelectrofocusing experiments, in the absence and in the presence of urea, lead to the conclusion that the 3 iso-SCP's have a subunit composition of α_2, $\alpha\beta$ and β_2 with respective pI's of 4.70, 4.55 and 4.40 for crayfish. Similar results have been obtained in the instance of lobster.

Homology between myosin light chains and invertebrate sarcoplasmic Ca-binding proteins

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Invertebrate sarcoplasmic Ca-binding proteins (SCP's) bind strongly 1 to 3 g atoms of Ca per monomer (mol.wt 17,000–22,000). The amino acid composition of SCP's from 6 invertebrate species has been determined and compared with the values published for muscular Ca-binding proteins such as myosin light chains, troponin C and parvalbumins. To this effect, the Cornish-Bowden index of composition divergence (J. theor. Biol. *65*, 735, 1977) was used. Significant sequence homology was found between *Amphioxus*, clam and sandworm SCP's and myosin light chains from rabbit and scallop. Although the analysis used here gave no indication as to whether a sequence homology can be postulated between crayfish, lobster and earthworm SCP's and the above myosin light chains, it suggests nevertheless a possible ancestral relationship between invertebrate SCP's and myosin light chains, as well as with troponin C and parvalbumins, the sequence homology of which is well documented (Collins, Nature *259*, 699, 1976).

Evolution of the Ca-binding properties of troponin C

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Our latest studies on troponin C (TN-C) from crayfish tail and carp skeletal muscle show that a) both proteins possess 1-2 sites that bind exclusively Mg^{2+}; b) crayfish TN-C has a single site, specific for Ca^{2+}; c) carp TN-C possesses 4 sites that accommodate Ca^{2+}, 2 of which can also bind Mg^{2+}. Striated muscles from other vertebrates examined so far (e.g. rabbit skeletal and bovine heart) also have a TN-C with 3-4 calcium sites. Only actin-linked control is found in these muscles. In contrast, invertebrate muscles (lobster and *Limulus*, beside crayfish) possess a TN-C with a single site for Ca^{2+}, but are doubly regulated by both actin- and myosin-linked controls (Lehman, Biochem. J. *163*, 291, 1977). It appears that in the course of evolution, TN-C's have acquired multiple Ca-sites which provide a cooperative response of myofibrils to Ca-binding, allowing a more sensitive tuning of contractile activity by variations in calcium concentration. This might explain why the myosin-linked control is no longer found in vertebrate striated muscles.

The oxygen cuvette: A new concept for the measurement of the oxygen concentration in gases and fluids

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A simple and precise photometric method for the determination of the oxygen concentration in gases and fluids was developed, which is based on the colour reaction between oxygen and alkaline catechol solution containing Fe(2+)-ions. The cuvette is produced by filling the reaction solution (2.5 ml of 25 mM catechol + 5 mM $Fe(NH_4)_2(SO_4)_2$ in 0.1 N NaOH) into a cylindrical cuvette (10 mm light path) in an oxygen-free atmosphere. It is closed by an airtight membrane suitable for sample injection. At 490 nm, the absorbance is proportional to the oxygen content of the sample up to an absorbance of 2.5. The cuvette is calibrated by injection of 10 µl of air or of a suitable $NaJO_3$-solution by a precision syringe through the membrane. By use of a portable (battery-operated) photometer, the oxygen concentration can be easily determined in 10–100 µl samples of gases and fluids with an accuracy of 1-2% within 2 min for a wide clinical, environmental and laboratory application.

NMR-studies of the solution conformation of glucagon

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Dilute aqueous solutions of monomeric glucagon were investigated by high resolution ^1H NMR-techniques. Essential structural information was obtained from studies of selected synthetic partial sequences and analogue peptides. While glucagon is predominantly in an extended flexible form, the solution conformation includes a quite rigidly structured region extending from residues 22 to 25, for which a detailed description was obtained. In contrast to the general experience so far with globular proteins, the solution conformation of this polypeptide hormone is of a different type than the crystal structure. In particular, the occurrence of a C-terminal α-helix can be excluded on the basis of the NMR-data. It remains to be seen whether the all important polypeptide conformation on the receptor site is related more closely to the solution conformation or the crystal structure.

Evidence for a cAMP-independent stimulation of lipolysis in rat adipose tissue (AT)

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Digestion of normal and diabetic AT with collagenase results in a 6- and 15fold decrease in the sensitivity of fat cells (FC) to the lipolytic action of epinephrine (epi), but in a 4- and 20fold increase in their sensitivity to the lipolytic action of ACTH. The kinetics of the dose-response curves of epi- and ACTH-induced lipolysis are completely different in AT and FC. The dose-response curves of the epi-stimulated increase in cAMP and protein kinase (PK) activity are, however, similar in AT and FC. In contrast, half-maximal stimulation of cAMP and PK occurs at 100fold lower ACTH concentrations in FC than in AT. At epi-and ACTH-concentrations, which stimulate lipolysis nearly half-maximally, no significant increase in cAMP- and PK-activity is detectable. Our results seem compatible with the hypothesis that low epi- and ACTH-concentrations mediate lipolysis in AT mainly by a cAMP-independent mechanism, which is partly lost after preparation of FC.

Increased sensitivity to epinephrine (epi) of lipolysis and of the adenylate cyclase-protein kinase (PK) system in diabetic rat adipose tissue (AT) and fat cells (FC)

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Epi - but not ACTH - stimulates glycerol release from diabetic rat AT half-maximally at 8fold lower concentrations than in normal AT. Lipolysis in diabetic FC is 3 times more sensitive to both epi and ACTH than normal FC. Epi - but not ACTH - causes half-maximal cAMP and PK responses in both diabetic AT and FC at 3fold lower concentrations than in normal AT and FC. In accordance with these findings is a 3fold increase in epi-sensitivity of the adenylate cyclase in FC-ghosts from diabetic AT. Basal cAMP-levels and PK-activities are not significantly different in AT of normal and diabetic rats if expressed per μg of protein. However, per g fresh weight, cAMP and PK activity are elevated in diabetic AT. Increased sensitivity to catecholamines of diabetic AT provides an additional explanation for elevated plasma-free fatty acids in the diabetic state. Furthermore, insulin seems to play a role in modifying the catecholamine response of AT.

Purification of phosphatase activity of human crythrocytes

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In order to find out any relationship between high-affinity Ca^{2+}-ATPase (EC 3.6.1.3) and Ca^{2+}-dependent p-nitrophenylphosphatase (EC 3.1.3.1) of human erythrocytes, we tried to isolate and characterize the phosphatase activity. - Human blood (group 0, Rh^+) was washed 3 times with isotone KCl-solution. The packed erythrocytes were diluted 1:1 with 10 mM $CaCl_2$ and 40 mM morpholinopropanesulfonic acid (MOPS), pH 7.5, and hemolyzed by freezing at −80 °C. After thawing, the hemolyzed erythrocytes were diluted 1:1 with 10 mM MOPS, pH 7.5, and the membranes were sedimented at 37,000 × g for 40 min. Soluble phosphatase activity of the supernatant was purified on DEAE-sepharose CL-6B using a linear gradient of 20–250 mM KCl. After precipitation of the most active fractions with 80% $(NH_4)_2SO_4$, further purification was carried out on sephadex G-100 at 150 mM K^+, 2 mM Mg^{2+}, 0.2% mercaptoethanol and 20 mM MOPS, pH 6.8. The phosphatase activity could be purified 2100fold up to 11.6 U/mg protein. As shown by SDS-gel-electrophoresis, the mol. wt of the active material is ≤ 30,000 daltons.

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Dopamine currents in cell R15 of *Aplysia*

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Aplysia californica abdominal ganglia are isolated, bathed in culture medium and desheathed. Cell R15 is penetrated with 2 electrodes for voltage clamping. Dopamine and dopamine antagonists are perfused over the preparation. Hyperpolarizing currents elicited by step increases in dopamine concentration reach their final levels in 10–20 min. Complete washout requires 20–40 min. Dopamine currents can be elicited by concentrations as small as 10^{-6} M; saturating currents require concentrations in excess of 10^{-4} M. Hyperpolarizing synaptic currents elicited by stimulation of the branchial nerve are depressed by bath application of dopamine in proportion to the current elicited directly by dopamine. Both the dopamine current and the synaptic current are inhibited stereospecifically by LSD in concentrations of 10^{-6} M or less. The results suggest that dopamine is an agonist at postsynaptic receptors and possibly at nonsynaptic receptors that have characteristics similar to the synaptic receptors.

Serum-free hepatocyte culture: Evidence for de novo synthesis of multiple forms of cytochrome P450

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Chick embryo hepatocytes in primary monolayer culture maintain highly differentiated drug-metabolizing functions comparable to those of mammalian liver in vivo. Induction of at least 3 forms of P450 hemoproteins was observed when cells were exposed to appropriate inducer compounds. Induction was shown by a) analysis of CO-binding spectra, b) relative increases in activities of aminopyrine demethylase and arylhydrocarbon hydroxylase, c) increase in particular protein bands of mol. wt 49,000–57,000 daltons on SDS-polyacrylamide gel electrophoresis. De novo synthesis was confirmed by increased incorporation of ^{35}S-methionine into drug-induced protein bands relative to ^{3}H-methionine into controls. Addition of insulin (10^{-7} M) was required for induction. This chick embryo liver cell system provides a new tool for the study of the regulation of hepatic metabolism of drugs and carcinogens.

'Permissive' role of the beta-adrenergic system in drinking induced by circulating renin

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In uninephrectomized rats, constriction of the artery to the remaining kidney produced a 5fold increase in plasma renin level and a 50% decrease in renal renin concentration. This renin release stimulated water intake (28 ml/kg 6 h verus 9 ml for sham constriction). Pretreatment with 1 or 3 mg/kg 1-propranolol s.c. 30 min before constriction did not block renin release, but diminished the drinking response (20 and 10 ml/kg 6 h, respectively). We suggest that drinking induced by the increase in circulating renin levels (provoked by renin release following constriction) is in some way mediated by the beta-adrenergic system.

Effects of ions, analogs and drugs on high-affinity uptake of taurine by glial and neuronal cells

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High affinity uptake of taurine hy cultured glial (clones NN and I6) and neuronal (neuroblastoma clones M1 and M1NN) cells was dependent on the external $[K^+]$, $[Na^+]$ and $[Ca^{2+}]$. Kinetic analysis suggested that 2 Na^+ were required for uptake of 1 molecule of taurine. The uptake was less efficient without K^+ as well as at $[K^+]$ higher than 10 mM. In the absence of Ca^{2+} the uptake by glia but not neurones was reduced by 50%. The Ca^{2+}-independent component was not affected by high $[K^+]$ (up to 50 mM). β-Alanine and hypotaurine inhibited uptake of taurine in all 4 clones while GABA and glycine were much weaker inhibitors. Several other analogs had only marginal effects, but certain drugs (strychnine and p-chloromercuriphenyl sulphonate but not picrotoxin and bicuculline) were strong inhibitors. Comparison of glial and neuronal uptake showed no significant difference in structural specificity or sensitivity to drugs.

The effects of the blockade of presynaptic α-receptors by yohimbine and mianserine on the levels of 4-hydroxy-3-methoxyphenethyleneglycol sulphate (MOPEG-SO_4) in different brain regions of the rat: Influence of mianserine on daily rhythm in MOPEG-SO_4 level

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Yohimbine, a selective blocker of presynaptic noradrenergic α-receptors, and mianserine, an antidepressant drug which also blocks these receptors, were investigated with respect to their effects on MOPEG-SO_4 levels in different brain regions. Yohimbine (2 mg/kg i.p.) caused similar increases in MOPEG-SO_4 content in all the brain regions tested (50-60%), with the exception of the cerebellum (22%). These effects were not altered after inhibition of catecholamine synthesis by α-methyl-p-tyrosine, suggesting that measurement of MOPEG-SO_4 gives a valid index of noradrenaline release. In contrast to the effects of yohimbine, mianserine (30 mg/kg s.c.) preferentially increased the MOPEG-SO_4 level of the cortex. The mianserine-induced increase faded after 8 h, but a second increase was observed in the brain stem during the following night.

The tumor promoting action of diterpene esters in vivo correlates with their potency to release prostaglandins from macrophages in vitro

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Some diterpene esters of different plant origin have been found to exert considerable cocarcinogenic in terms of initiation promoting and irritant effects in mouse epidermis in vivo. Both effects may be related to perturbation of cell membranes. Prostaglandin (PG) release may also be regarded as the result of membrane perturbation. We, therefore, measured the potency of a variety of diterpenes (initiation promoters) to release PG's from macrophages. We found that there is a close correlation between both effects. The most potent promoters, e.g. TPA, were effective inducers of PG release at 10^{-8} M. This effect was paralleled by vacuole formation. Many chemically related compounds having little or no promoting activity likewise have no PG releasing potency. Solitary carcinogens (initiators and irritants) did not induce PG release from macrophages. The implications of these observations are discussed.

Influence of sex hormones on oral consumption of, tolerance to and dependence on morphine under 'crowded' and 'uncrowded' housing conditions in the rat

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Male and female rats were castrated or sham-castrated. 3 weeks later castrated animals were split into 2 groups, one receiving i.m. injection of opposite sex hormones, i.e. testosterone (as isobutyrate: 50 mg/kg once) for ex-females and oestradiol (as benzoate + 3benzoate-17acetate = Ovocyclin Depot®: 5 mg/kg on 7 consecutive days) for ex-males, the other receiving vehicle. Sham-operated received vehicle. Rats were then housed 2 or 10 per cage, 'uncrowded' or 'crowded', and given 0.5 mg/ml morphine-HCl in 10% v/w sucrose as only drinking fluid. After 2 weeks tolerance and dependence were assessed. Endogenous and exogenous male hormones reduced morphine consumption, b.wt and survival, tolerance and dependence. Female hormones had opposite effects. Castrated rats without hormone treatment showed intermediate results. Crowded housing depressed consumption and survival in both hormonal males and females but not in nonsubstituted castrates.

Specificity of histamine-sensitive adenylate cyclase and its blockade by H_2-receptor antagonists

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Adenylate cyclase from gastric mucosa of guinea-pigs was measured in the presence of KCl, NaCl, Tris pH = 7.5, theophylline 10 mM each, EDTA 1 mM and ATP 0.5 mM. The enzyme was activated 2- to 4fold by histamine, 4-methylhistamine, 4-, N-dimethylhistamine, the phosphonate analogue of GTP, GMPPNP and a thiourea derivative, dimaprit, at 10^{-4} M. Dopamine and isoproterenol (10 mM) were inactive. NaF (10 mM) increased the cyclic AMP formation about 20fold. Cimetidine, a H_2-receptor blocking agent, antagonized the action of 4-methylhistamine (10 mM) by about 50% at 22 µM (= IC_{50}), similar to metiamide (70 µM). However, compounds of different classes such as imipramine (2.5 µM), amitriptyline (1.7 µM), chlorimipramine (1.3 µM), Ro 11-2456 (0.3 µM), an inhibitor of 5-HT uptake, phenoxybenzamine (8.5 µM), clemastine (04 µM) or mepyramine (50 µM) (both H_1-receptor blocking agents) antagonized the action of 4-methylhistamine. Therefore, this enzyme preparation is not specific enough to characterize a H_2-receptor blocking agent.

Effect of general anesthetics on plasma catecholamine concentrations

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A chronic intrajugular cannulation technique permitted sampling of blood from freely moving rats in absence of stress. Determination of the sample plasma levels of adrenaline (A), noradrenaline (NA) and dopamine (DA) was performed by means of a new specific and sensitive radioenzymatic method (Da Prada et al., Life Sci. *19*, 1161, 1976). The values obtained (A = 175±31, NA = 509±46, DA = 84±9 pg/ml) were about 1/10 of those from decapitated or handled rats. A 2-h-lasting increase of A and NA was measured in ether anesthesia whereas with halothane only NA was affected. Whilst ketamine (60 mg/kg i.p.) induced a transient increase in NA only (peak at 20 min), urethane (1.2 g/kg i.p.) caused a long-lasting elevation of all 3 amines. In contrast to thiogenal (100 mg/kg i.p.) which induced a 2.5fold increase of NA, nembutal (45 mg/kg i.p.) was inactive on A-, NA- and DA-levels. Chlorisondamine pretreatment (15 mg/kg i.p.) prevented the ether-induced increase of both A and NA plasma levels.

Tolerance and cross-tolerance to the effects of nicotine and amphetamine on food intake and body weight

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Rats were habituated to a schedule of 4 h daily food access and water ad lib. Following 1 week of baseline measurements (100%), 10 rats were injected s.c. with 0.4 mg/kg nicotine (NIC), another 10 rats with 1.5 mg/kg d,l-amphetamine (AMPH) shortly before and after daily food access. Over 4 weeks of treatment the food intake, which was reduced drastically at the beginning, recovered partially for the first-h- (NIC: 75%, AMPH: 60%) and more so for the 4-h-period (NIC: 90%, AMPH: 115%). Only AMPH-rats lost b.wt (p 0.01). Substituting NIC for AMPH immediately increased first-h-intake to 108% (p 0.01); 4-h-intake remained stable (113%). When AMPH was substituted for NIC first-h-intake decreased comparable to first-wk anorexia (22%; p 0.01); 4-h-intake was reduced to 83%. In the second week of treatment with substitute drugs, each drug produced its pattern of intake as seen prior to cross-tolerance testing. These results suggest that with the development of tolerance to AMPH, tolerance also develops to NIC - but not vice versa.

Changes in the social behaviour of mice after antidepressants and neuroleptics

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The social activity shown by pairs of caged adult male OLAC mice in a 6-min encounter was determined ethologically after the dominant mice had received single oral doses of drugs 1 h beforehand. 6 behavioural activities were measured: Nonsocial, social-investigation, aggression, defensive-ambivalence, flight and sex. In dominant mice, aggression was increased by imipramine (1 and 5 mg/kg), dibenzepin (0.5, 1, 2.5 and 5 mg/kg) and phenelzine (5 and 10 mg/kg), higher doses being more inhibitory. D-amphetamine (0.1, 0.5, 1.5 and 10 mg/kg) progressively inhibited aggression but increased flight. Clozapine (0.5, 1 and 3 mg/kg) and chlorpromazine (0.5 and 1 mg/kg) reduced aggression but tended to increase sex and defensive-ambivalence. Social behaviour studies thus provide a means of characterizing different classes of psychoactive drugs even when very low doses are used.

^{3}H-LSD labels, a novel dopamine receptor in molluscan ganglia

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^{3}H-LSD binds to both dopamine and serotonin (5HT) receptors in a particulate fraction derived from the central nervous system of *Helix pomatia*. In the presence of 100 µM 5HT, binding of ^{3}H-LSD is only to dopamine receptors (> 90% pure). This dopamine-sensitive LSD-binding is inhibited stereospecifically by d-LSD and d-butaclamol, but not by cis-flupenthixol. Also, while ergot alkaloids are potent inhibitors of binding ($K_d \leq 1$ nM), neuroleptics are generally weak ($K_d > 0.1$ µM). Dopamine and analogues such as epinine and (3,4-dihydroxyphenylamino)-2-imidazoline (DPI) are inhibitors in the micromolar range, as are tryptamine derivatives such as bufotenine, N-methyltryptamine and 6,7-dihydroxytryptamine. This spectrum of pharmacological action is similar to that reported for inhibitory dopaminergic synapses on *Helix* neurones, but is different from that found at vertebrate dopaminergic receptors.

Cardiovascular effects of calcium antagonists in chronically instrumented, conscious dogs

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The cardiovascular effects of verapamil (V), nifedipine (N) and Ro 11-1781/001 (Ro) were studied in 4 conscious dog models. Mean arterial blood pressure, measured at the modified carotid loop, decreased after oral administration of 30 mg/kg of V by 15%, but not after N and Ro. Coronary blood flow increased by 91% after V (30 mg/kg p.o.), by 110% after N (100 mg/kg p.o.) and 170% after Ro (30 mg/kg p.o.). The abdominal aortic blood flow increased after 30 mg/kg p.o. of V by 50%, of N by 90% and of Ro by 178%. Left ventricular pressure and dp/dt were measured with a telemetric device. 30 mg/kg p.o. of V decreased LVP and dp/dt, N was inactive and Ro produced a weak positive inotropic effect. V caused tachycardia followed by bradycardia, N and Ro increased heart rate. The structurally related V and Ro markedly differ in their vasodilator properties and in their effects on myocardial contractility. It is concluded that calcium antagonists may display a variable degree of selectivity towards cardiac and vascular smooth muscle.

Chronic application of serotoninergic drugs does not modify serotonin receptor binding

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Chronic application of substances acting on the noradrenaline system has been found to cause sub- or supersensitivity of β-adrenergic receptors. We were interested to see if similar changes occurred in the serotoninergic system. ^{3}H-5HT binding was measured in rat cortex membrane preparations after daily i.p. injection of the precursor amino acid l-5HTP (100 mg/kg day for 5 weeks), the 5HT uptake inhibitor clomipramine (10 mg/kg day for 4 weeks), and the 5HT receptor antagonist metergoline (2 mg/kg day for

3 weeks). There was no difference in 5HT-binding between untreated controls, acute administration to chronically treated saline controls or to drug-treated animals, in any group. These results indicate that neither sub- nor supersensitivity can be induced in 5HT receptors using this methodology.

The measurement of some cardiovascular parameters in chronically instrumented dogs

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The experiments were carried out on 22 trained female Alsatian dogs with a mean b.wt of 24.5±0.6 kg. For blood flow studies, electromagnetic flow-probes of appropriate size and a pneumatic occluding cuff for establishing zero flow were implanted and cables and catheters exteriorized on the dogs' back. Measurements were made on, nonsedated dogs with a Micron flowmeter. In coronary flow studies (n=8) mean control flow in the circumflex branch of the left coronary artery was 63.6±7.9 ml/min, heart rate (HR) 89.4±5.7 bpm and mean duration of successful use of the dogs (DSU) 32.4±10.2 days. For measurements of abdominal aortic blood flow (n=8) probes and cuffs were placed rostral to the aortic bifurcation. Mean control flow was 330.0±31.8 ml/min, HR 75.0±3.5 bpm and DSU 143.4±13.2 days. Peak left ventricular pressure (LVP) was recorded with a telemetric device (n=6) and the following control values were obtained: $dLVP_{(max)}$ 109.5±1.6 mm Hg, dLVP/dt 2800±129 mm Hg · sec^{-1}, dLVP/dt · P^{-1} 68.3±4.9 sec^{-1} and HR 99.2±5.7 bpm.

Interference of rat liver microsomal UDP-glucuronyltransferase (GT) and β-glucuronidase (βG)

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Microsomal fraction contains the whole of hepatic GT as well as part of βG. The activities of the 2 enzymes were assayed under identical conditions using untreated rat liver microsomes at pH 7.5. With p-nitrophenol and UDP-glucuronic acid, a net glucuronide formation of 0.38 µmoles/h · g liver was measured. In the presence of saccharolactone at concentrations selectively blocking βG, a glucuronidation rate of 0.53 µmoles/h · g liver was determined. With p-nitrophenylglucuronide in the absence of UDP-glucuronic acid, the activity of βG and its kinetic parameters were determined (K_m=0.06 mM, V_m=0.075 µmoles/h · g liver). Using these data to correct for βG's interference with the glucuronidation process, a calculated glucuronidation rate of 0.48 µmoles/h · g liver was obtained. Thus, according to the 2 methods, only 72 and 79% of the glucuronide formed by GT escape the site of origin due to the action of microsomal βG.

Pharmacological characterization of the 5-hydroxytryptamine (5HT) receptor of blood platelets

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5HT causes a reversible shape change of platelets in plasma, manifested by a decrease in light transmission. In the present work, the pharmacological specificity of the 5HT receptor of platelets and possible analogies with those of the CNS were investigated using suspensions of rabbit platelets in an artificial medium. Among a great variety of drugs, tryptamine and derivatives (especially 5HT), quipazine (5HT receptor agonist) and ADP caused the most pronouced shape change (ED_{50} 5×10^{-6} to 2×10^{-7} M). Mezcaline was weaker (ED_{50} 3×10^{-5} M). The most potent antagonists of the shape change induced by 10^{-6} M 5HT were ergoline derivatives, cinanserine and neuroleptic drugs (IC_{50} 10^{-8} to 10^{-9} M). In contrast, methysergide was a weak inhibitor of the ADP-induced shape change (IC_{50} $>10^{-4}$ M). D-LSD was a mixed 5HT antagonist/agonist. Rabbit platelets contain receptors which are relatively specific for tryptamine derivatives and whose pharmacological properties seem to be partly similar to those of some 5HT receptors in the CNS.

Intracellular recording from cortical slices of rat and man in vitro: Action of diazepines

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Hippocampal slices, incubated in a perfusion chamber, retain electrophysiological properties similar to those found in vivo. Using intracellular recording from CA1 and CA3 pyramidal cells, antidromic and orthodromic action potentials, EPSPs and IPSPs after stimulation of alveus or fimbria, as well as spontaneous firing were routinely observed. Diazepines (Diazepam, Flurazepam, Ro 11-7800) were applied to the bath or by microdrops (1-5 µg) on the recording site. A hyperpolarization by up to 5 mV, sometimes accompanied by a conductance increase, was observed in about half of the cells. The recurrent IPSP was increased in some cells but was usually decreased, an effect which can be explained by the potent blocking action (independent of a hyperpolarization) of the diazepines on the evoked spike. Similar effects of the diazepines on the resting potential and a depression of spontaneous activity were observed on slices from human neocortex.

The nucleus of the solitary tract, a site of action of clonidine

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In cats anaesthetized with urethane, a field potential - evoked by electrical stimulation of the ipsilateral carotid sinus nerve - was recorded with glass microelectrodes from the nucleus of the solitary tract (NTS). Previous experiments had shown that this field potential may reflect transmission through the first synapse of the baroreceptor reflex. Clonidine (10, 30 and 100 µg/kg i.v.) diminished the amplitude of the field potential in a dose-dependent manner, an effect which was virtually completely antagonized by the α-adrenoceptor blocking agent piperoxan. Antidromic potentials evoked by electrical stimulation of the NTS and recorded from the carotid sinus nerve were not affected by clonidine. This finding is not in favour of an effect of clonidine on the endings of the baroreceptor afferent fibres. The intensity of electrical stimulation of the NTS necessary to produce a threshold hypotensive response was reduced by clonidine. The results of the experiments suggest that clonidine stimulates the second order neurones of the baroreceptor reflex.

Beta-dependent-alpha-stimulated adenyl cyclase in rat cerebral cortex

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Adenyl cyclase activity was measured in prisms (0.26×0.26×1 mm) of rat cerebral cortex by prelabeling with ^{3}H-adenine, according to the method of H. Shimizu et al., J. Neurochem. *16*, 1609, 1969. The enzyme can be

stimulated by both noradrenaline (NA) and isoproterenol (ISO). The maximum stimulation induced by NA was about twice that found with ISO. In contrast to published reports (J. Schultz and J.W. Daly, J. Neurochem. *21*, 1319, 1973) complete blockade of the activity stimulated by both agonists was obtained with low concentrations of the specific β-blockers propranolol and oxprenolol. At the same time, 50-60% of the activity stimulated by NA was inhibited by the α-blockers phentolamine and WB-4101. These compounds had no effect on ISO-stimulated activity. It appears that both α- and β-agonists can activate adenyl cyclase, but the α-agonist activity can be expressed only if a β-agonist has previously reacted with a β-receptor.

A simple microfiltration device for binding studies

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Vacuum filtration using glass-microfibre-filters has recently been introduced as a method for the rapid separation of bound from free radioligands, notably for the determination of binding capacities and affinities of specific receptor sites (e.g. T.K. Harden et al., Molec. Pharmac. *12*, 1, 1976). A simple apparatus is presented which consists of a flow-through microfiltration module, a dispenser for washing solution and a vacuum regulator. The device allows the individual filtration steps, such as prewashing, sample filtration and washing, to be carried out under controlled conditions and at optimum flow rates. The major advantages of this device are a high reproducibility ($SD \leq 5\%$) and a low retention of unbound ligand in the filter. After filtration the filterdiscs can be dried and introduced into a scintillation vial for determination of radioactivity. Technical details are presented and preliminary binding experiments discussed.

The effect of glutamate on the membrane potential of cat caudate neurons

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L-monosodium-glutamate (GLU) was applied iontophoretically onto intracellularly recorded caudate neurons. It depolarized 14 cells and increased their otherwise very low firing rate. Sometimes different spike amplitudes appeared during GLU-ejection. The effect of this compound on the cortically evoked EPSP-IPSP sequence was to decrease the EPSP-amplitude and to increase the IPSP-amplitude. On 3 cells short pulses of GLU (-130 nA to -155 nA) were given. 200 msec long pulses at a frequency of 0.3/sec caused depolarizations of approximately 5.5 mV and lasting 1.5 sec. With 300 msec long pulses the depolarizations were still 1.5 sec long but 7 mV high. Firing threshold was passed at 500 msec pulse duration where bursts of 5-10 spikes appeared on each depolarization of 8-mV-amplitude. Cl^--pulses of equal duration and intensity were without effect. Thus a dose-dependent action of glutamate on cat caudate neurons was shown.

Comparative studies on the covalent binding of the carcinogen benzo(a)pyrene (BP) to DNA in various model systems

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Measuring the binding of a chemical or one of its metabolites to DNA could serve as a screening for potentially carcinogenic compounds. In order to find the most sensitive assay we have measured the covalent binding of tritiated BP to DNA in various model systems. Per 10^7 dpm administered or incubated, the following radioactivity (dpm) was recovered as BP bound to the total DNA isolated: Rat liver, in vivo: 16; rat liver perfusion in situ: 40; incubation of BP with liver single cells: 100, with liver homogenate: 36, with fibroblasts from a rat granuloma pouch: 17, with a rabbit cornea cell line: 150, with a monkey kidney cell line: 100; DNA incubated with BP in the presence of rat liver microsomes: 520. The systems differ from each other by a factor of about 30 but the choice cannot be based only upon these figures but must take into account the biological significance which is highest for the intact mammalian organism.

Aldosterone antagonists: Comparison of biological activity as potassium-sparing diuretics with affinity to receptors in the kidney of adrenalectomized male rats

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It is generally accepted that aldosterone antagonists interfere with biological effects of aldosterone by competitive inhibition at the mineralocorticoid receptor level. Surprisingly the activity of different antagonists shows poor correlation with their relative binding affinity as determined in vitro. If, however, the interference of the antagonists with the specific binding of ^3H-aldosterone is measured after injection of the compounds in effective doses, relative binding affinities of the antagonists correlate well with their effect on electrolyte excretion. The differences between relative affinities of aldosterone antagonists for mineralocorticoid receptors under in vivo and in vitro conditions can be explained in terms of their metabolic fate in vivo.

The nature of beta-adrenergic receptors involved in isoprenaline-induced drinking

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To investigate the type of beta-adrenergic receptor responsible for inducing drinking to peripheral isoprenaline (β_1 and β_2 receptor), log dose-response curves were established for s.c. D,L-isoprenaline (as sulfate) and 2 more selective β_2 agonists: salbutamol (as free alcohol), considered as highly β_2-selective and hexoprenaline (as dihydrochloride) thought to be somewhat less β_2-selective. Hexoprenaline was found to be ~ 5 times more potent than isoprenaline, to be expected if the affinity of hexoprenaline for the same receptor is twice that of L-isoprenaline. Salbutamol was ~ 5 times less potent than isoprenaline: its maximal effect was smaller than that of isoprenaline = hexoprenaline. Atenolol, a beta-receptor blocker with some selectivity for β_1, significantly depressed drinking to equimolar doses of isoprenaline and salbutamol, but surprisingly not of hexoprenaline. The receptors involved in isoprenaline-induced drinking, thus appear to show predominantly β_1, but also some β_2 characteristics.

'Permissive' role of plasma renin in beta-adrenergic drinking

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In rats the drinking responses to supramaximal doses of s.c. isoprenaline (0.12–0.94 mg/kg = 0.57–4.45 μM/kg) was confirmed to be abolished 20 h after bilateral nephrectomy. I.v. injection of 0.125 Goldblatt U/kg hog renin 5–20 min before s.c. isoprenaline in the nephrectomized rats resulted in a significant (~ 10 ml/kg · 2 h) drinking response to isoprenaline (0.12 mg/kg), which was smaller than that to isoprenaline in sham-operated rats (~ 30 ml/kg · 2 h). 0.125 U/kg hog renin i.v. neither induced drinking in nephrectomized rats nor enhanced drinking in response to isoprenaline in intact rats. – These results suggest that the presence of renin (and angiotensins) in circulating blood rather than beta-adrenergic renin release, is the prerequisite for the dipsogenic effect of isoprenaline. The basis of the 'permissive' role of the renin-angiotensin system for beta-adrenergic drinking could be an enhancement of the dipsogenic effect of isoprenaline by angiotensin II, rather than vice-versa.

Lisuride-induced mounting behaviour and changes in cerebral monoamine turnover in the rat depend on the route of drug administration

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Lisuride (LIS, an ergoline known to stimulate cerebral DA and 5-HT receptors) when injected i.c.v. or i.p. to rats caused a rapid reduction in HVA and 5-HIAA in the brain lasting for 2–3 h whereas the reduction seen after LIS p.o. showed a delay in onset and lasted for at least 8 h. The reduction after LIS i.p. and p.o. was increased and prolonged by proadifen (PRO, a substance inhibiting microsomal enzymes). Mounting behaviour (thought to depend on the stimulation of DA-receptors and 5-HT-autoreceptors) was induced for 2 h by LIS i.p. After LIS p.o., mounting occurred only at much higher doses than those required i.p.; pretreatment with PRO lowered the dose of LIS p.o. necessary to induce mounting. It is suggested that these effects in the rat are brought about by LIS itself. The delayed reduction of HVA and 5-HIAA and the lack of mounting after lower doses of LIS p.o. may be due to slow absorption and/or inactivation by microsomal enzymes.

Demonstration of an in vivo d-LSD-binding method

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A reproducible in vivo d-LSD binding method in rat brain has been developed, with high affinity (Kd of 5 pmoles/g ww), stereospecificity (d- vs l-LSD), and regional selectivity. It may be a useful adjunct to in vitro methods for measuring changes in turnover at the synaptic level related to the intact receptor. We tried to apply this method to differentiate serotoninergic and dopaminergic components of the LSD-binding site by different agonist/antagonist drugs.

Lisuride and LSD decrease spontaneous firing of neurons in the raphe

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Multiunit activity was recorded from the raphe dorsalis by means of platinum-iridium wires (50 μm in diameter) in curarized rats. The impulses, sifted through a window discriminator were counted every 4 min for statistical evaluation (t-test). Lisuride maleate (10 μg/kg i.v.) induces a 50% decrease (4 rats; $p < 0.001$) and recovery is almost complete 1 h later. In the pontine reticular formation no major modification occurs (3 animals) with the same dosage. LSD bitartrate (10 μg/kg i.v.) induces a 20% decrease (5 rats; $p < 0.001$ of the multiunit activity; 1 h thereafter the baseline level has not yet been reached. The effect of lisuride has been qualitatively confirmed by single unit analysis of neurons in the raphe medialis under urethane anaesthesia. In conclusion, lisuride, a structural analog of LSD, is 2–3 times more potent than LSD in decreasing firing of raphe neurons. These results are in accordance with the hypothesis that lisuride can in part act through serotoninergic receptors on raphe neurons. On the other hand it should be emphasized that, despite its greater potency than LSD on raphe neurons, lisuride exhibits no hallucinogenic effects in man.

Clonidine (CL) reduces noradrenaline utilization in nucleus tractus solitarii (NTS), locus coeruleus (LC) and sympathetic lateral column (SLC)

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CL is believed to exert its hypotensive action mainly by stimulation of central α-adrenoceptors, since i.a. it reduces the L-α-methyl-p-tyrosine methyl ester · HCl (MPT)-induced decrease of noradrenaline (NA) in whole rat brain and spinal cord (Andén et al., Archs Pharm. *292*, 43, 1976). Our data show that CL reduces the NA-utilization also in 3 small CNS-nuclei influencing blood pressure, i.e. NTS, LC and SLC. Rats were injected i.p. with MPT 1–4 × 400 mg/kg 8 to 2 h before sacrifice. NTS, LC and SLC were semiquantitatively evaluated by fluorescence microscopy for formaldehyde-induced catecholamine fluorescence and punched out from tissue sections for the radioenzymatic assay of NA. Both methods demonstrated that CL · HCL, 1–2 × 0.3 mg/kg p.o. 8.5 and 4.5 h before sacrifice, reduced the MPT-induced NA decrease thus indicating an α-adrenoceptor stimulation in all 3 nuclei involved in cardiovascular regulation.

The metabolism of (^{14}C)-aflatoxin B_1 in pigs

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Excretion of radioactive aflatoxin B_1 and its metabolites during 2 weeks after peroral administration of ring-labeled (^{14}C) aflatoxin B_1 were investigated in pigs. The major excretory route was found to be via faeces. Most of the via faeces and urine excreted radioactivity was not extractable with methylenechloride. Radio-thin-layer chromatography of the methylenechloride-soluble fractions indicated the presence of aflatoxin B_1 and M_1, the latter being quantitatively the most important CH_2Cl_2-soluble, radioactive compound in the urine. A small part of the radioactivity showed the same chromatographic behaviour as aflatoxicol. The distribution of radioactivity in the edible tissues of pigs at different times after administration of a single dose (^{14}C)-labeled aflatoxin B_1 was determined.

In vivo covalent binding of chemicals to DNA as a short-term test for carcinogenicity

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Binding of a chemical to DNA appears to be the first step in the chemical induction of a tumor. With the use of radioactive compounds the limit of detection of a binding is given by the amount of radioactivity administered, the yield of DNA from the target organ and the criteria for a significant radioactivity. An incorporation of radioactivity into DNA due to a biosynthetic uptake of metabolites or degradation products must either be accounted for or excluded by appropriate choice of the label. Repair mechanisms which excise DNA-bound molecules must be included in the evaluation of the carcinogenic properties of a compound as well as the mutagenic potency of a specific type of damage. Differences of species and organ are important with respect to the metabolic pathways and the repair capabilities. The data presented show that even weak carcinogens can quickly be detected but that a refined assessment requires much more work.

Pharmacological and binding studies of retinal dopamine receptors

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Intact rabbit retinae in vitro were used to investigate the effects of thioxanthene isomers or benzamide derivatives such as sulpiride or clebopride on the cAMP accumulation induced by 10^{-4} M dopamine. Only cis-isomers of thioxanthenes which are clinical neuroleptics were potent antagonists, whereas trans-isomers were totally inactive at 10^{-4} M. Benzamides were either inactive (sulpiride) or slightly active (10^{-4} M clebopride) and their clinical or potential antipsychotic activity might be not related to the inhibition of dopamine-sensitive adenylate cyclase. Dopamine receptors of rabbit retinae were also characterized in binding experiments with 1-50 nM ^{3}H-dopamine in the presence or absence of 10^{-6} M (+)-butaclamol. Affinity for H_3-dopamine binding was about 11 nM dopamine with saturability of binding sites at around 20 nM. Attempts to correlate effects of drugs on cAMP system and on dopamine binding sites are currently under investigation.

Detection of chemically induced point mutations in vivo in somatic cells of rats (Granuloma Pouch Assay)

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Growth of granulation tissue was initiated with croton oil at the inside of a s.c. air pocket in rats (Granuloma Pouch Assay). 36 h later 0.05, 0.2, 0.6, or 1.8 mg N-methyl-N′-nitro-nitrosoguanidine (MNNG) were injected into the pouch. After a 36-h-expression time in vivo, the granulation tissue was excised, and single cells isolated by enzymatic digestion. Cells able to form clones were tested in vitro for the presence of autosomal point mutations leading to ouabain resistance. Mutation frequencies increased with dose. The maximal response was reached with 0.6 mg/pouch. Alternative routes of application were explored. Highest tolerated doses (50 mg/kg) were injected i.p. or given by gavage. No mutant cells forming clones were recorded. This in accordance with the known rapid degradation of the test compound.

Binding of the carcinogens benzo(a)pyrene (BP) and 7,12-dimethylbenz(a)anthracene (DMBA) to *Salmonella* DNA as compared to the corresponding mutagenicity

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DMBA is a more potent carcinogen than BP and is bound to a higher extent to DNA in mammalian organs. In contrast to this correlation, the mutagenic activity of DMBA in Ames' *Salmonella*/microsome test is only about 1/6 that of BP. This decrepancy could be explained if the binding of DMBA to the *Salmonella* DNA is lower than that of BP. We have incubated bacteria with tritiated DMBA or BP in the presence of rat liver microsomes. Aliquots were assayed on petri plates for mutants, and DNA was isolated from the bulk incubation mixture. The mutagenic activity of DMBA was lower than that of BP, and the same order was found with the binding to DNA. The low mutagenic potency of DMBA is therefore due to low DNA-binding, probably because the metabolic pathways in the mutagenicity test system are different from those in a mammalian organ. A quantitative evaluation of carcinogens from bacterial mutagenicity data seems to be difficult.

Pharmacokinetic basis of 1,3-butanediol (BD) administration to man

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BD, a food additive and calorie source, may counteract manifestations of acute ethanol withdrawal (AEW) in rats. Before applying BD to man its pharmacokinetics were studied in 5 dogs. After i.v. administration (5.5 mmoles · kg^{-1}) plasma concentrations were measured enzymatically. The apparent zero order disappearance rate constant (k_0) was 18.8±SD 1.6 nmoles · $ml^{-1}min^{-1}$, the volume of distribution 970±140 ml · kg^{-1} and the elimination rate (ratio of dose to extrapolated time to reach zero concentration, ER) 18.1±1.4 µmoles $min^{-1}kg^{-1}$. Oral administration resulted in an ER of 16.2±0.8 ($p > 0.05$). With this background, the same dose was given orally to 4 human volunteers. No pharmacological effect was experienced, k_0 amounted to 21.8±3.5 and ER to 19.0±1.5. It is concluded that the fate of BD is similar to the one of ethanol. Since therapy of AEW is expected to require the doses of BD investigated, clinical trials should account for the consequences of cumulation of a drug exhibiting zero order kinetics.

Activation of an inhibitory noradrenergic pathway projecting from the Locus coeruleus to the cingulate cortex

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Stimulation of the rat Locus coeruleus evokes inhibition of about 50% and excites about 9% of all cells recorded in cingulate cortex. Pretreatment of the rats with reserpine and α-methyl-p-tyrosine drastically reduces the percentage of cells inhibited by Locus coeruleus stimulation. The cells inhibited as well as those not inhibited by Locus coeruleus stimulation are depressed by microiontophoretically applied norepinephrine (NE). This inhibitory effect of NE is observed in rats anaesthetized either with urethane, chloral-

hydrate or with nembutal. The NE as well as the transsynaptically evoked depression of the cell's firing rate is blocked by microiontophoretically applied β-receptor blocking drug MJ 1999. Our results suggest that the inhibitory action on cingulate cortical cells elicited by Locus coeruleus stimulation is mediated by the dorsal ascending noradrenergic pathway.

Behavioural and biochemical effects of the tropane derivative Ro 12-7982

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The methyl-3-(p-chlorophenyl)tropane-2-carboxylate Ro 12-7982 provoked signs of central stimulation in rats and mice. Therefore, we studied the effects of this drug in 4 behavioural models as well as its effects on the uptake and turnover of the biogenic monoamines, 5-HT, DA and NA. Ro 12-7982 caused a dose-dependent hypermotility in rats and mice, antagonized the prochlorperazine-induced catalepsy and produced turning towards the lesioned side in rats with unilateral 6-OH-DA-lesions of the MFB. Ro 12-7982 most potently inhibited the uptake of DA and 5-HT into striatal and forebrain synaptosomes, respectively. In rat whole brain, the drug elevated the level of HVA and reduced that of 5-HIAA in a dose-dependent manner. In α-MT pretreated rats, Ro 12-7982 enhanced the disappearance of DA from brain. We conclude that this tropane derivative strongly interferes with dopaminergic and serotoninergic neurotransmission.

Effects of a synthetic enkephalin analogue on the cat spinal cord

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In spinal cats, a small dose (1 mg kg^{-1} i.v.) of a synthetic enkephalin analogue (FK 33-824, Roemer et al., Nature *268*, 547, 1977) depressed segmental polysynaptic reflexes, spontaneous γ-motoneurone activity and slightly increased the excitability of primary afferent (PA) endings. FK 33-824 was $1/3$ as potent as morphine. Naloxone (0.1 mg kg^{-1} i.v.), not bicuculline (0.5 mg kg^{-1} i.v.), reversibly antagonized these effects. Given after naloxone, a higher dose of FK 33-824 (3 mg kg^{-1} i.v.) clearly augmented PA excitability and prolonged dorsal root potentials. Since naloxone did not antagonize the effects of diazepam (0.3 mg kg^{-1} i.v.) which enhances GABA-ergic transmission, FK 33-824 seems to activate a non-GABA-mediated presynaptic inhibitory mechanism. This study supports the notion, based on neurochemical and anatomical data (Jessell and Iversen, Nature *268*, 549, 1977; Hökfelt et al., Psychopharmacology *39*, 1978), that spinal enkephalinergic neurones may presynaptically modulate afferent inputs at a spinal cord level.

Delta sleep-inducing peptide (DSIP) and the sleep-wakefulness cycle of cats

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The effects of i.v. injected synthetic DSIP on the sleep-wakefulness cycle were studied in cats using EEG- and EMG-telemetry. Within recording sessions of 6 h after the injection, a low dose of DSIP (30 nM kg^{-1}) shortened the sleep latency, reduced the waking time, enhanced the NREM- and in particular the REM-sleep, as compared with control saline injections. The enhancement of REM-sleep was due to an increased number of REM-sleep episodes, In contrast, a higher dose of DSIP (300 nM kg^{-1}) had no significant effects. There were no apparent qualitative alterations in EEG and behaviour after eigher the low or the high dose of DSIP. Present results suggest that i.v. administered DSIP induces sleep in cats within a narrow dose range, partly in agreement with a previous observation in rabbits (Monnier et al, Neurosci. Lett. *6*, 9, 1977). In addition, they show a strong REM-sleep enhancing effect of DSIP in cats.

Storage site and concentration of catecholamines in blood platelets

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Adrenaline (A), noradrenaline (NA) and dopamine (DA) have been determined in human and animal blood platelets (P) by a very sensitive and specific radioenzymatic method which allows the simultaneous measurement of the 3 catecholamines (CA) (Da Prada et al., Life Sci. *19*, 1161, 1976). Human and animal (cat, cow, guinea-pig, mice, pig, rabbit and rat) P contain the 3 CA, NA prevailing on A and DA. The concentration of the CA in guinea-pig platelets ($A = 0.22 \pm 0.04$; $NA = 1.56 \pm 0.14$; $DA = 0.23 \pm 0.06$ pmoles/mg protein) is about 500 times less than that of 5-HT. In rabbit platelets 5-HT (40.10 ± 4.68 nmoles/mg protein) exceeded several 1000 times the CA-level ($A = 1.61 \pm 0.31$; $NA = 4.20 \pm 0.53$; $DA = 1.26 \pm 0.24$ pmoles/mg protein). CA are probably stored together with 5-HT, since a) reserpine released both 5-HT and CA from rabbit P, b) P of Fawn-Hooded rats with storage-pool deficiency contain only minute amounts of both 5-HT and CA; c) subcellular fractionation studies show that CA are mainly localized in the fraction rich in 5-HT.

Simultaneous radioenzymatic assay of adrenaline (A), noradrenaline (NA) and dopamine (DA): Recent improvements

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Modification of the original radioenzymatic assay for the simultaneous measurement of femtomole concentrations of A, NA and DA (Da Prada et al., Life Sci. *19*, 1161, 1976) permits to speed up the procedure without loss of sensitivity, specificity and reproducibility. The residue of the high vacuum evaporation once dissolved in methanol/HCl is spotted by an unique, time-saving application on 3×20 cm, preadsorbant TLC plates (Type LQF, Quantum Ind.). The plates are developed in 60 min with chloroform/dimethylamine (4:1). The elution of the ^{3}H-methoxytyramine by methanol (containing 10% glacial acetic acid and 2% Triton X-100) increased counting efficiency. ^{3}H-Metanephrine and ^{3}H-normetanephrine spots are also scraped directly into a scintillation vial and converted to ^{3}H-vanillin. ^{3}H-Vanillin is then extracted into a toluene-based scintillation solution without transferral. The improved method allows the differential assay of the 3 catecholamines in duplicate in 30 plasma or tissue samples within 1 day.

Antitryptaminergic effects of l-propranolol, l-oxprenolol and their enantiomers in the rat

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The effects of l-propranolol, l-oxprenolol and their enantiomers were studied in 2 models assumed to reflect stimulation of central 5-HT neurones: excitation produced by tryptamine (15 mg/kg i.p.) in MAOI-pretreated rats and disruption of conditioned behaviour by N,N-dimethyltryptamine (DMT, 30 mg/kg i.p.). The tryptamine-induced excitation was inhibited by l- and d-forms of propranolol and oxprenolol. However, differences in the efficacy were observed: 30 mg/kg i.p. of l-propranolol suppressed all symptoms by 90%, whereas at the same dose l-oxprenolol showed only a partial antagonism. The d-forms were in general less active. DMT-induced impairment of active avoidance and discrimination responses was significantly decreased only by l-propranolol (10 mg/kg i.p.). These results are in accordance with the postulated central 5-HT blockade by propranolol (Green and Grahame-Smith, Nature *262*, 594, 1976) and may have a bearing on its 'antipsychotic' action.

Effects of bromocriptine (I), α-ergocriptine (II) and DH-α-ergocriptine (III) on cAMP- and cGMP-phosphodiesterases (PDE) from rat brain striatum

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The effects of I, II and III on the hydrolysis of cyclic-AMP and cyclic-GMP in crude homogenate from rat brain striatum has been investigated using a modification of the method described by G. Pöch, Naunyn-Schmiedebergs Arch. Pharmak. *268*, 272, 1971. At high substrate concentrations (cAMP = 30 μM), cAMP-PDE was inhibited uncompetitively by (I) (K_i = 18.5 μM) and (II) (K_i = 21 μM) and noncompetitively by (III) (K_i = 250 μM). At lower substrate concentrations (cAMP ≤ 3 μM), (III) inhibited cAMP-PDE competitively (K_i = 80 μM), whereas the other 2 compounds were much less potent. Cyclic-GMP hydrolysis was inhibited noncompetitively by all 3 substances tested, with dissociation constants (K_i) of 18 μM (III), 42 μM (II) and 65 μM (III). On the basis of these in vitro results potential drug concentrations in vivo are considered to be too low to influence cAMP- and cGMP-PDE activities.

Release of newly synthetized ^{3}H-5-hydroxytryptamine from the rat spinal trigeminal nucleus

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The stimulation-evoked release of ^{3}H-5-hydroxytryptamine (^{3}H-5HT) was investigated by mean of an in vitro perfusion system on slices from the subnucleus caudalis of the rat spinal trigeminal nucleus (substantia gelatinosa) preincubated with ^{3}H-tryptophan. ^{3}H-5HT was separated from tryptophan and indoleamines by mean of a sephadex $_3$G-10 column. A pulse of 47 mM K^+ elicited a significant release of newly synthetized ^{3}H-5HT which was abolished in a calcium-free medium. The effect of 2 pain-related substances playing a role in the synaptic transmission of the trigeminal nucleus was studied on this ^{3}H-5HT release; morphine (10 μM) showed no effect whereas substance P (10–50 μM), putative transmitter of primary nociceptive afferents, stimulated the spontaneous release of ^{3}H-5HT. 50 μM carbamazepine (Tegretol®), potent drug against trigeminus neuralgia, did not affect the ^{3}H-5HT release.

Thyroid hormone inhibition of the late aldosterone response in the urinary bladder of the toad *Bufo marinus*

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Aldosterone (A) increased the net Na^+-flux (measured as I_{scc}), within 1-3 h (early response) by raising the apical membrane conductance (λ) (measured as amiloride inhibition of I_{scc}), within 3-8 h (late response) beyond the rise of λ, presumably by improving Na^+-pumping. Triiodothyronine (T_3, 60 nM) added in vitro 2 h before A (80 nM), or thyroxine (T_4) given for 6 days (20 μg/kg day) before sacrifice did not affect the early response to A, but depressed I_{scc} between 6 and 8 h after A by -85 ± 17 μA ($p < 0.001$), without significantly depressing λ ($-48\pm23\times10^{-4}$ mho, $p > 0.1$). These data suggest that thyroid hormone inhibits the action of (A) at the level of the energetic factors and not of the apical Na^+-conductance.

Effect of reserpine-like drugs on 5-hydroxytryptamine (5HT) synthesis in rat brain

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Monoamine-depleting drugs enhance the cerebral dopa accumulation due to decarboxylase inhibition, indicating an increase of dopamine synthesis. A similar effect on 5-hydroxytryptophan (5HTP) accumulation has not been demonstrated up to now. Therefore, the effect of monoamine depletors with a long (reserpine) and a short (benzoquinolizine Ro 4-1284) duration of action on the increase of 5HTP caused by the decarboxylase inhibitor 3-hydroxybenzylhydrazine was investigated. Both drugs (5 mg/kg i.p.) markedly enhanced the 5HTP rise for 5 and 3 h, respectively after their administration. With Ro 4-1284 the duration of this enhancement corresponded to that of the 5HT decrease and of the increase of 5-hydroxyindoleacetic acid (5HIAA). In contrast, with reserpine the change in the 5HT and the 5HIAA content lasted much longer (> 16 h) than that of the 5HTP increase. The monoamine-depleting drugs probably enhanced 5HT synthesis as a consequence of a positive feedback mechanism. Diazepam decreased their effect.

Uptake of allantoin by rabbit renal cortical slices

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Rabbit renal cortical slices incubated under 100% O_2, at 25 °C for 2 h, in Cross and Taggart medium, concentrated ^{14}C-allantoin to a tissue water/medium (T/M) ratio of 1.38 ± 0.03 (mean±SEM) (n = 22). Replacement of O_2 by pure N_2 inhibited the concentrating mechanism: T/M fell to 0.90 ± 0.02 (n = 14): the inflow, thus, appears to depend on energy, or else, the outflow could be inhibited by O_2. Probenecid (10^{-3} M), a competitive inhibitor of the renal tubular organic anion transport system, induced a smaller depression of the T/M-ratio to 1.02 ± 0.01 (n = 10). Similarly quinine sulfate (10^{-3} M), an inhibitor of the tubular organic cation transport system, depressed T/M to 1.07 ± 0.01 (n = 5). The simultaneous addition of probenecid (10^{-3} M) and quinine sulfate (10^{-3} M) depressed the T/M-ratio to 0.96 ± 0.01 (n = 4), i.e. a value close to that in anaerobiosis, under N_2, which may represent the equilibrium ratio. These data suggest that allantoin, an amphoteric substance like creatinine, may be transported into cells of the renal cortex by both the organic anion and the organic cation transport system.

Differential drug effects on rat strains and activity measures

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The effects of nicotine (0.2 mg/kg s.c.) and amphetamine (0.4 mg/kg s.c.) on exploratory efficiency and locomotor activity were simultaneously evaluated using 2 distinct maze configurations which were presented on alternate days. Female rats of the Roman High Avoidance and Roman Low Avoidance strains were used. Nicotine improved exploratory efficiency and stimulated locomotor activity in the RHA-strain but was ineffective in the RLA-strain. Amphetamine was found to stimulate locomotor activity in both strains. In the RHA-rats, the amphetamine treatment stimulated locomotor activity less than did the nicotine treatment. Amphetamine impaired exploratory efficiency in the RHA-rats but had no effect on exploratory efficiency in the RLA-strain. These results differentiated the effects of nicotine and amphetamine on the 2 categories of behavior evaluated and the 2 strains of rats tested.

Unscheduled DNA synthesis (UDS) in rabbit germ cells induced by methyl methanesulfonate (MMS)

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Rabbits were treated with single i.v. injections of 2.8, 5.6, 11.25, 22.5 and 45 mg/kg MMS. Immediately thereafter (^{3}H)thymidine (^{3}H-T, 8 μCi/rabbit) was injected into both testicles. Sperms were collected by serial ejaculation. ^{3}H-T incorporation into sperm DNA was measured by LSC. Significant radioactivity was measured in sperms which were in meiotic and postmeiotic stages of spermatogenesis at the time of labeling and which normally do not synthesize DNA. This is good evidence for DNA repair following MMS-induced damage (UDS). A does-related increase in UDS was observed from 2.8 to 11.25 mg/kg MMS. No further increase occurred with higher doses. UDS in rabbit germ cells may be demonstrated with MMS at doses that are 5-10fold lower than those effective in other in vivo mammalian mutagenicity tests, i.e. specific locus test, dominant lethal test, and cytogenetic studies in meiotic cells.

Central actions of valproate sodium

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Systemically administered valproate sodium (VPA) at a dose of 200 mg/kg i.p. strongly antagonizes electroshock-induced seizures in rats within 3-5 min. The same dose of i.p. applied VPA reduces the firing-rate of spontaneously active neocortical neurons after a latency of from 3 to 7 min. The inhibitory action of iontophoretically administered GABA on neocortical cells is significantly potentiated by iontophoretically applied VPA, whereas the inhibitory effect of glycine remains unaffected. Transsynaptic inhibition of nigral cells induced by activation of the striato-nigral GABAergic fibre tract is potentiated in only 4 out of 15 cells by iontophoretically administered VPA. I.p. applied VPA is slightly effective in potentiating this transsynaptic inhibition in only 1 out 8 cells. These results are discussed in view of the proposed GABA-mediated anticonvulsive mode of action of VPA.

Effect of p-chlorophenylalanine on specific serotonin-binding and serotonin level in rat brain

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Administration of p-chlorophenylalanine (pCPA 300 mg/kg i.p.) increased specific ^{3}H-serotonin (5HT)-binding in rat forebrain within 1 day to 226% of the control level. Scatchard analysis performed 2 days after drug administration revealed no change in the number of binding sites, but an increase in affinity. The 5HT-level showed a minimum (24% of control) on day 3 and was still at 62% on day 10 when the specific binding value (106%) did no longer differ significantly from the control level. When a second injection of pCPA was given on day 8, specific binding was elevated 2 days later to only 162% of control whereas 5HT-level was reduced to 29%. The time-course of pCPA-induced behavioral changes may be reflected more closely by changes in specific 5HT-binding than by alterations in 5HT-level.

Effects of some vasodilators on Ca-fluxes in vascular smooth muscle

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The lanthanum method was used to measure the effects of vasodilators on the ^{45}Ca influx produced in isolated strips of rabbit main pulmonary artery after replacement of 50 mM NaCl by KCl in the bathing solution. 2 Ca-antagonists, verapamil and Ro 11-1781 produced dose-dependent inhibitions of ^{45}Ca-influx with IC_{50} of 4.6×10^{-7} and 3.8×10^{-6} M, respectively. Papaverine caused inhibitions comparable to those induced by Ro 11-1781 ($IC_{50} = 1.8 \times 10^{-6}$ M). High concentration of diazoxide (10^{-4} to 10^{-3} M) and nitroglycerin (10^{-3} M) were necessary to cause inhibitions which were not greater than 40%. While prazosin 3×10^{-4} M slightly stimulated ^{45}Ca-influx, nitrite and sodium nitroprusside had no effect. The present results obtained with a direct method confirm that Ca-antagonists block Ca-influx into vascular smooth muscle and suggest that of the other drugs tested only papaverine may cause relaxation by interfering with Ca-fluxes.

Cerebral ventricular infusion of artificial CSF and excess calcium: Effects on sleep in the rat

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The vigilance states of the rat were recorded during infusion of artificial cerebrospinal fluid (aCSF) containing regular or excess concentrations of calcium, into the lateral or 4th ventricle. 2 types of aCSF that have been previously used by Myers and coworkers, were infused: a) Na 150.0 mM, K 5.1 mM, Ca 1.82 mM, Cl 158.7 mM in aqua dest.; or b) Na 127.6 mM, K 2.5 mM, Ca 1.3 mM, Mg 1.0 mM, Cl 134.7 mM in aqua dest. During a 1-h-infusion of either type of aCSF in the sleeping animal, paradoxical sleep (PS) was reduced by 30-70% as compared to the uninfused control value while total sleep was little affected. Suppression of PS was counteracted by increasing the level of Ca in aCSF by a factor of 2-3. The results indicate that ions in the CSF my exert selective effects on sleep states.

Attempt to correlate effects of ergot derivatives with central dopaminergic stimulant properties in functional tests

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The actions of 7 ergot derivatives (bromocriptine and the ergoline derivatives CF 25-397, CM 29-712, CH 29-717, 32-084, lergotril, lysenyl) were compared in 5 functional tests (Johnson et al, Br. J. Pharmac. *56*, 59, 1976; M.C. Shelesnyak, Proc. Ist Int. Cong. Endocr. Clbh. *677*, 1960). A significant correlation in potency was found between turning induced in 6-OHDA nigrostriatal lesioned rats and inhibition of reserpine-akinesia (corrected Speerman test: $r = 0.8$) or tetrabenazine-akinesia ($r = 0.7$). Potencies between turning induced in 6-OHDA lesioned rats and apomorphine stereotypy induced in intact rats were also correlated ($r = 0.8$); however no significant correlations were found between inhibition of nidation in rats and turning induced in 6-OHDA lesioned rats ($r = 0.3$) or stereotypy in intact rats ($r = 0.4$). This study confirms that ergot derivatives possess different affinities to DA substrates in nigrostriatal and pituitary-hypothalamic systems.

Modulation of the in vivo binding of the carcinogen benzo(a)pyrene (BP) to rat liver DNA by selective induction of microsomal or nuclear aryl hydrocarbon hydroxylase activity (AHH)

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The binding of reactive BP-metabolites to DNA seems to be the first step in the tumor induction. AHH is necessary for the metabolism of BP. It is located in the endoplasmic reticulum (ER) and in the nuclear envelope. Since the latter is closer to the DNA than the ER, an unstable metabolite could have a better chance to react with DNA if it was formed by the nuclear enzymes. To check this hypothesis we selectively induced the microsomal or nuclear AHH by choosing the appropriate dosage schedule with dieldrin and phenobarbital. 20 h before sacrifice, we administered tritiated BP, determined for each rat the liver microsomal and nuclear AHH-activity and counted the radioactivity on liver DNA as a measure of bound BP. A doubling of the microsomal AHH-activity roughly doubled the binding whereas an increased nuclear AHH-activity reduced it. These unexpected results are discussed in terms of more complicated metabolic pathways.

Attenuation by scopolamine of the increase in dopamine turnover by neuroleptics: Quantitation of their anticholinergic properties

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Dose-response curves (DRC) of the effects of haloperidol (HAL), chlorpromazine (CPZ), clozapine (CLZ), thioridazine (THI), sulpiride (SLP) and GP 50302 on DOPA accumulation after central decarboxylase inhibition by Ro-4-4602 (RO) in striatum (CS) and mesolimbic area (MA) were compared with and without concomitant treatment with scopolamine (SCO). Treatment schedule was as follows: RO (800 mg/kg i.p.) was given 45 min, neuroleptics 2 h and SCO (10 mg/kg i.p.) twice, 4 and 1¾ h before decapitation. SCO antagonized the effects of these neuroleptics to various extents. The shift in DRC was estimated by the displacement of the ED_{200}. In CS, SCO shifted the DRC towards higher doses by the following factors: HAL 7.4; CPZ 6.8; THI 2.2; SLP 2.2; GP 50302 2.0; CLZ 1.5. In MA, the order was somewhat different; CPZ 6.3; HAL 5.0; THI 3.2; CLZ 2.7; GP 50302 2.0; SLP 1.4. It is suggested that SLP and GP 50302 possess indirect anticholinergic properties.

The 'psychological refractory period' in the rat: A measure of central information processing

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The response to a stimulus is delayed and reduced if a prestimulus occurs shortly before. The psychological refractory period in man is the interstimulus-interval (ISI) over which this occurs. It is thought to result from processing of the prestimulus. It was studied in rat through the effects of mild acoustic prestimuli on acoustic startle responses. A 2-phase-effect was seen. Short ISI's increased responses and reduced latencies. Longer ISI's led to response inhibition and delay. The maximal effects and their ISI's were determined. Multiple regression of these data derived from various conditions showed that the parameters were well described by power-curve functions of prestimulus intensity and background level. Clearly, only minor changes in stimulus conditions could profoundly change the behavioural response. The method may provide a model of information processing and a means of examining biochemical, physiological, anatomical, behavioural and pathological influences on it.

Chronic hepatotoxicity of bromobenzene (Bb) in starved and ad libitum fed rats

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Bb is a classic hepatotoxin that is activated in the liver and excreted manily as mercapturic acid conjugate. The glutathione (GSH) level of the liver is, therefore, crucial for the detoxification of Bb. 48 h starvation lowers total liver GSH in rats by more than 40%. Weekly oral doses of 1 and 2 mmoles/kg Bb for 16 weeks to ad libitum fed rats caused minor liver fibrosis and no functional changes. In rats starved for 48 h prior to Bb treatment 1 mmole/kg caused moderate fibrosis and significant increase in serum alkaline phosphatase; 2 mmoles/kg induced severe micronodular liver cirrhosis and abnormalities of liver function.

Potentiation of analgesia in the mouse by baclofen

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In various test systems baclofen displays an intrinsic analgesic effect, and under certain conditions it potentiates the action of narcotic analgesics. This latter effect of the compound was assessed in relation to its possible influence on motor activity in 2 different models of analgesia in mice treated orally or parenterally with baclofen and, either simultaneously or 60 min later, morphine, pentazocine or nalorphine. In the hot-plate test, baclofen potentiated the effects of the analgesics under all conditions, without exerting any significant influence on the animals' behaviour. In the tail-flick test, on the other hand, the same simultaneously given doses proved virtually inactive. The antinociceptive effects of morphine, pentazocine and nalorphine were, however, markedly intensified in animals given baclofen 60 min before. These studies indicate that baclofen has an unusual spectrum of activity, as regards both its intrinsic analgesic effect and its ability to potentiate analgesia.

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

Giant polyoma RNAs are tandemly repeated transcripts of the entire polyoma genome

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Polyoma DNA is transcribed into 'giant' RNA molecules up to several times the DNA length (5400 b.p.), during the late phase of productive infection of mouse cells. Giant RNA was isolated by preparative hybridization, followed by sedimentation on DMSO-sucrose gradients. RNA was then hybridized to a 90% fragment ($Hind_{II}$-1) of polyoma DNA, and the hybrids were examined by electron microscopy. Hybrid molecules containing a short single-stranded region bounded by 2 double-stranded regions were photographed and measured. Analysis of 38 such molecules showed that most contained RNA larger than 1 genome length (up to 3.5 genome lengths). The absence of deletion or substitution loops in hybrid regions showed that all DNA sequences in a given strand are transcribed and no nonviral sequences are present in the RNA. Several hybrid molecules showed a regular repeat of 2 or 3 alternating 10% genome length single-stranded and 90% genome length double-stranded regions showing that these RNAs contain tandem repeats of the base sequence of the entire viral DNA.

Nucleotide sequences at the ends of phage Mu DNA

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The ends of Mu DNA are involved in the integration and transposition of the phage DNA within the host genome. In this and other respects Mu resembles the IS elements which appear at various sites without sequence permutations. Plasmid or λpMu hybrids particles were used to establish the nucleotide sequences at the 2 ends. A small stretch of homology was found to exist at the very end parts of the phage genome. Particular sequence features, different at the 2 ends, will be presented and discussed.

4 *Drosophila* heat shock proteins are associated with chromatin fractions

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We have studied the localization of heat-shock induced proteins in *Drosophila melanogaster* tissue culture cells. The proteins of high mol.wt (84, 70, 68 Kdaltons) remain in the cytoplasm, particularly in the postmitochondrial supernatant. Heat shock proteins are not found associated with mitochondria. The small mol.wt proteins (26, 27, 22, 23 Kdaltons) are concentrated in the nucleus. These proteins are enriched in chromatin preparations and behave like previously defined nonhistone chromosomal proteins.

Nerve growth factor: Correlation between bio- and radioimmunoassay

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NGF is a protein essential for the development of peripheral sympathetic neurons. High concentrations occur in the male mouse salivary gland. In order to determine the NGF-distribution in other organs and species, radioimmunoassays (RIA) have been developed using mouse NGF as antigen. These RIA are usually based on a competition principle: ^{125}I-NGF is reacted with a limiting number of specific binding sites (the antibodies). When a sample is tested, any decrease in bound radioactivity is interpreted as evidence for the presence of NGF. However, NGF-levels predicted in this manner could not be confirmed by bioassay. Taking mouse and rat serum as examples, it is shown that binding of ^{125}I-NGF to serum macromolecules is responsible for the erroneously high values determined by the competition RIA. A 2-site-RIA based on a different principle has been developed. It gives results which are consistent with those of the bioassay.

Gel electrophoresis of viral nucleoprotein complexes

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Viral nucleoprotein complexes containing SV40 or polyoma DNA, host cell histones and other proteins, are extracted either from the nuclei of infected cells or from virions. The complexes are analyzed by electrophoresis on low percentage agarose gels. Their migration depends on length, shape and composition, and differs from that of the corresponding naked DNAs. The usefullness of this method to isolate nucleoprotein complexes in the process of transcription or replication is being examined.

Mechanism of action of micrococcal nuclease on SV40 DNA and SV40 chromatin

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Micrococcal nuclease has been widely used to investigate chromatin structure. It is claimed that the enzyme introduces double strand breaks in DNA in chromatin. We have characterized the mode of action of this enzyme on SV40 DNA and SV40 chromatin. Superhelical SV40 DNA was incubated (60 sec, 0 °C) with increasing concentrations of nuclease and the products analyzed by electrophoresis on 1.4% agarose gels. With increasing enzyme the proportion of superhelical DNA decreases, while the proportion of the nicked circular form increases. This is followed by an increase in the amount of the linear form. 2 dimensional electrophoresis of nuclease digests using neutral and alkaline buffers shows that micrococcal nuclease cleaves double stranded DNA through 2 successive single strand breaks on opposite strands. The same mechanism applies to micrococcal nuclease digestion of SV40 chromatin.

Evidence of cAMP-dependent protein kinase translocation to chromatin-binding site

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Partially purified cAMP-dependent protein kinase (mol.wt 230,000) and its catalytic subunit (mol.wt 40,000) were incubated with sheared calf ovarian chromatin in the presence of increasing amounts of salt concentrations (0.05–1.0 M NaCl). Maximal binding of the holoenzyme and the catalytic subunit to chromatin was obtained at 0.6–0.8 M NaCl. This binding was followed with an increase of cAMP-dependency of the enzyme activity. Such a binding

gives evidence for the presence of a specific cAMP-binding protein capable of recombining with the free catalytic subunit. The addition of BSA, ovalbumin did not affect the above binding of the enzymes to chromatin. – Digestion of the chromatin with trypsin prior to the addition of the enzymes prevented the binding of the holoenzyme and catalytic subunit to chromatin, whereas native chromatin revealed a specific cAMP-binding protein which could be extracted by Triton and physically demonstrated by polyacrylamide electrophoresis.

The effects of calcium on the depolarized rat uterus

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The contractile response of an isolated, K^+-depolarized rat uterus to a sudden increase in calcium concentration in an originally Ca-free medium displays 2 phases; an initial rapid increase in tension (about 10 min) is followed by a slow decrease in tension (considerably longer than the first phase) down to a steady state level. Experiments under either isotonic or isometric conditions show a similar response pattern. Relaxation to the basic level was achieved by a washout with Ca-free medium. The contractile response depends not only on the Ca-concentration used for stimulation, but also on the duration and intensity of previous stimulations. Preincubation of the uterus with Ca has a positive effect on contractility, whereas previous exposure to tension diminishes the response to Ca. One can hypothesise that at least 2 different Ca compartments, which participate in generation of the tension response to extracellular Ca, are linked to the contractile system of the uterus.

A temperature-sensitive mutation in the initiation codon of the rIIB gene of phage T4

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HD263 is a temperature-sensitive rIIB mutation which affects the translation of the rIIB-mRNA. The ribosome binding sites of wild type and HD263 rIIB-mRNA have been sequenced and the coding sequence determined by identification of the site altered by an amber mutation in the second codon of the gene. The only difference between wild type and HD263 is a change in the initiation codon from AUG to AUA. Since the wild type and HD263 proteins have the same fmet peptide, this result shows that AUA, which normally codes for isoleucine, can be recognized by the fmet-tRNA and used to initiate translation. It has been proposed that the recognition of initiation sites by ribosomes involves a transient pairing between the 3′ end of the 16S RNA and a sequence located 5′ to the initiation codon. In the ribosome binding site of the rIIB-mRNA there is a stretch of 6 bases which are homologous to the 16S RNA. The possible role of this sequence in the temperature-sensitive translation of the mutant rIIB-mRNA will be discussed.

Biochemical and ultrastructural study of Gunn rat thyroid

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Gunn rats disclosing alterations both of thyroid hormones secretion and thyroglobulin composition (Gomba et al., Virchows Arch. *B20,* 41, 1976), the proteolytic action of pronase on thyroid has been studied on the 105,000×g supernatant fraction and at the ultrastructural level. After in vivo ^{125}I-isotopic equilibration, a greater, nonhydrolysable, soluble iodine fraction was found in thyroids of Gunn rats, compared with Wistar rats (9.1%±1 vs 5.2%±0.5). Thyroid rat iodine distribution showed a larger proportion of iodotyrosines (MIT, DIT) and a lower one of iodothyronines (T_4, T_3) than in normal rats. Electron microscopy examination showed the pigment granules characterizing the thyroid gland of Gunn rats to be more resistant to pronase hydrolysis than the other protidic components (colloid, apical vesicules). The study of the nature of pronase-resistant material is now in progress.

Immunocytochemistry of enzymes in normal and diabetic rat exocrine pancreas

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On the basis of morphological and biochemical differences, the exocrine pancreatic tissue has been divided in peri- and tele-insular regions. The enzymatic profile of these regions has been investigated in normal and diabetic (streptozotocin treated) rats with the immunofluorescence technique using antibodies against 9 major pancreatic enzymes (α-amylase, lipase, elastase, chymotrypsinogen A, trypsinogen, carboxypeptidase A and B, RNAse and DNAse). All acinar cells of the normal rat were positive to the 9 different enzymes, but those located in the peri-insular regions of most of the islets gave a brighter fluorescence. In the diabetic rat, amylase was virtually absent in the peri-insular region while chymotrypsinogen was normally distributed. The tele-insular tissue showed both positive and negative cells for amylase. These data confirm the inhomogeneity of the exocrine pancreas and show in addition that peri- and tele-insular tissues react differently to a diabetic state as far as amylase and chymotrypsinogen contents are concerned.

DNA-polymerases of mouse P815 cells: Subcellular distribution and partial purification

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All 3 commonly distinguished DNA-polymerases α, β and γ are found in exponentially growing mouse mastocytoma cells (P815) irrespective of the subcellular origin (cytoplasm, nucleoplasm or high salt extracts of chromatin) and are separated by ion exchange chromatography. Further purification of DNA-polymerase α on affinity columns [poly(rA)- or poly(dT)-CL-sepharose] resolves 2 forms of activity: I and II. By rechromatography under identical conditions form II yields again forms I and II whereas form I remains I. The absence of striking differences between these 2 forms in a) catalytic properties in vitro, b) sedimentation properties in velocity gradients and c) SDS gel analysis, suggest that the observed molecular heterogeneity (due neither to subcellular origin nor to proteolysis) might result from molecules co-purifying with the enzyme; the role of such factors for structure and/or function of DNA polymerase α are currently under investigation.

A protease system associated with the major cell surface glycoprotein of the ciliates *Pseudomicrothorax dubius* and *Paramecium primaurelia*

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The salt alcohol method of Preer extracts from the cells a set of proteins, among them a large (260,000 daltons) glycoprotein (i-Ag). These proteins are mainly located on the external surface of the cell as shown by SDS-PAGE of ^{125}I, peroxidase-labeled cells. The i-Ag is quickly degraded after denaturation of the extracts. The degradative activity can be separated from the i-Ag by sephadex or affinity chromatography. A protease, mol.wt 25,000, with a high activity on the i-Ag is responsible for this degradation. The extracts contain another unidentified molecule which can inactivate the protease by a PMSF-sensitive mechanism. We suggest that this system plays a role in the in vivo regulation of the i-Ag.

Continued in vitro growth of human T-lymphocytes with retention of their function

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Human cultured T-cells (CTC) were grown using conditioned medium prepared from the supernatant of PHA-stimulated, fresh peripheral blood leukocytes (PBL). These CTC have characteristics of polyclonal activated T-cells: 1. They were cytotoxic only for allogeneic target cells, which was much enhanced by the addition of lectins. 2. They responded with accelerated kinetics to alloantigens in mixed lymphocyte culture (MLC). Effector cells from MLC reactions were grown for an additional 14 days and retained considerable levels of allospecific cytotoxicity. Actively growing CTC lacked Fc-receptors for IgG or IgM and were devoid of natural killer (NK) or killer (ADCC) activitites. CTC could also suppress a number of PBL proliferative responses. Overall, CTC will allow crucial studies of the functional and biochemical properties of human T-cells.

Mapping of the repetitious regions within the nontranscribed spacer of *Xenopus laevis* rDNA by restriction and DNA-sequencing

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Detailed restriction analysis reveals 3 distinct repetitious regions within the nontranscribed spacer. Region 1 is cleaved frequently by GC-sequence specific restriction enzymes to reveal ~ 100 b.p. units. This region differs from regions 2 and 3 which are cut by enzyme Alu I to give alternating ~ 80 b.p. and 60 b.p. units. Region 3 differs from 2 in having Sma I sites within its 80 b.p. units. The similarity of regions 2 and 3 indicates a common ancestry which, owing to the presence of Sma I sites, must have diverged early in its evolution. Regions 1 and 2 are separated from each other by an area of DNA centred around a Bam HI site. A similar Bam HI area is found to separate regions 2 and 3 and analysis reveals an identical sequence for over 150 b.p. between these 2 Bam HI cuts.

Histone genes in HeLa-cells

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Restriction analysis of DNA of HeLa-cells suggests that human histone genes are included in DNA-fragments of high mol.wt (Melli et al., Experientia *33*, 824, 1977). We have now carried out a detailed study of the structural unit, hybridizing restricted DNA of HeLa-cells with pure single histone genes of the sea urchin *Psammechinus miliaris*. The 'single gene' analysis has been carried out with single and double DNA digests, using mainly the restriction enzymes EcoRI and Hind III. The results suggest that human histone genes are considerably heterogeneous, possibly in their spacer sequences. The genes coding for histones H_{2a}, H_3, H_{2b}, H_4, H_1 are clustered in units of mol.wt $> 15 \times 10^6$ daltons. The 5 genes seem to be distributed within a $\sim 7 \times 10^6$ dalton DNA-fragment, which has on both sides long 'spacer' regions. The gene units are tandemly repeated and the order of the genes within each unit is identical to that of sea urchin.

Subcellular localization of plasminogen activator in human neutrophils

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Plasminogen activator (PA) is released from transformed cells, activated macrophages and PMA-stimulated neutrophils. Human neutrophils (> 95% pure), were homogenized by cavitation and fractionated by zonal sedimentation at 10,500 rpm (J. Cell Biol. *63*, 251). The fractions were centrifuged at 3×10^6 g-min and plasminogen-dependent and plasminogen-independent fibrinolysis was assayed in the resuspended pellets using ^{125}I-fibrin plates. PA was recovered in a broad slow-sedimenting peak in the upper half of the gradient which was resolved from both alkaline phosphatase (membranes) and lysozyme (specific granules). Fibrinolysis was high in azurophil granule fractions mainly due to the presence of elastase, but no increase in activity was observed upon addition of plasminogen. We conclude that PA is preformed in neutrophils and is presumably localized in the C-particles of these cells.

Characterization and in vitro translation of Rous Sarcoma virus genomic and virus-specific RNA's

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We have analyzed the products of in vitro translation of RSV genomic RNA in the rabbit reticulocyte system. In addition to the major product (the Pr76 precursor to virus Gs-antigen) a complex but reproducible pattern of polypeptide synthesis is observed. This was found not to be a result of random nicking of RSV RNA nor to be dependent on the tertiary structure of this RNA. Surprisingly, an identical pattern is observed on translation of 30-40S RSV RNA present in infected cell cytoplasm. We have analyzed the nature of polypeptide synthesis with regard to multiple initiation or termination or to subsequent processing events. In addition we have improved the procedure for the isolation of subgenomic RSV RNA from infected cells using hybridization to mercurated c-DNA; the characterization of these RNA-species will be discussed.

Rod-shaped particles in the plasma membrane of the mitochondria-rich (MR) flask cell of amphibian epidermis

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MR-cells are specialized cells found in epithelia with a high ionic and/or water flux. They have been identified in toad bladder epithelium and mammalian kidney collecting tubule. A cell type resembling MR-cells occurs in the epidermis of amphibian skin (the flask cell). By freeze-fracture, MR-cells have previously been shown to contain elongated particles on the P-face of their plasma membrane in addition to the usual globular particles. Freeze-fracture of *Xenopus laevis* and *Rana ridibunda* skin now reveals similar rod-shaped particles on the P-face of the apical, lateral and basal plasma membrane of the flask cells. The E-face of the membrane has complementary elongated pits. Elongated particles also exist on the P-face of some intracellular vesicles. The presence of these peculiar particles in the flask cells of the epidermis as well as in the MR-cells from the bladder and the kidney, suggests that these cells have a functional similarity at the level of the cell membrane, in which elongated particles are implicated.

Activation of macrophages by soluble factors: II

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Mouse peritoneal macrophages can be activated to kill intracellular microorganisms in vitro by incubation with activating factor(s) (AF) released by Con A-stimulated spleen cells (Experientia *33*, 814, 1977). AF appears to induce activation by interacting with macrophage membrane, because a) when AF-containing media were preincubated with macrophages for 1 h at 37 °C, they lost their capacity to activate a second macrophage culture, suggesting removal of AF by macrophages during the first incubation, b) when macrophages were treated with inhibitors of serine-esterases prior to activation, they displayed an increased sensitivity to AF, suggesting the presence on macrophage membrane of esterases which tend to decrease the reactivity to AF. Activation of macrophages was accompanied by an elevation in acid phosphatase excretion and by a 2- to 3fold increase in the activity of the pentose phosphate shunt. Both parameters may reflect the increased requirements for enzymes and oxygen metabolites necessary for killing and digestion of the intracellular organisms.

Cell wall of *Actinomyces viscosus* Ny 1: composition and pathogenic role in periodontal disease

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Cell wall was prepared from *Actinomyces viscosus* Ny 1 (A.v.), serotype 1. Its amino acid and amino sugar content was analyzed. The molar ratio for NacGlN, NAcMuramic acid, and the major amino acids in the sample Ala, Glu, and Lys was approx. 2:2:3:3:1.5. Cell wall was agglutinated by hyperimmune sera. We then investigated whether gnotobiotic RIC-rats, orally associated with A.v. to produce periodontal disease, formed antibody and/or sensitized T-lymphocytes against epitopes of A.v. cell wall. 29 sera of 32 were negative, 3 agglutinated cell wall to $\log_2$ titers of 2 and 3 but all sera agglutinated A.v. to titres from 8 to 16. Spleenic T-cells showed an anamnestic in vitro response using a soluble antigen fraction from A.v. but none with cell wall. In conclusion, cell wall of A.v. does not contribute via specific immune mechanisms to the pathogenesis of periodontal disease.

Specific antibodies against ACTH and α-MSH for radioimmunoassay and for studies of hormone-protein-interactions

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Radioimmunochemical determination of structurally related polypeptide hormones, such as ACTH and α-MSH, at very low concentrations requires antisera of high specificity and sensitivity. Prolonged immunization of goats with chemically defined complex-antigens (HSA containing a rather low number of covalently bound ACTH or α-MSH molecules) resulted in antisera which had high titers (best titers: 1:220,000 and 1:10,000,000 for ACTH and α-MSH, respectively), low cross-reactions (anti-ACTH serum: $< 0.005\%$ with α-MSH; anti-α-MSH serum: $< 0.05\%$ with ACTH) and high sensitivity (detection limit for ACTH: < 1 pg; for α-MSH: < 0.5 pg). These antibodies, which may be useful receptor models for studying the physical and chemical interactions with the hormone (Kopp et al., Eur. J. Biochem. *75*, 417, 1977), are being purified by affinity chromatography using a new polyacrylamide resin with a high content of covalently bound hormone (up to 10%).

On the determination of optimal conditions for binding studies

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The danger of wrong or over-interpretation of binding data from ligand-biopolymer interactions (e.g. hormone/receptor, antigen/antibody, enzyme/substrate) is very large if an unsuitable titration procedure is compounded with a poor choice of concentrations. Using computer simulations we have looked at the influence of stochastic and systematic errors on the determination of dissociation constants and other binding parameters as a function of concentrations of binding species. The results are interpreted on the basis of information theory. In addition, we compared various evaluation techniques (Scatchard analysis, etc.) and considered the influence of approximations and false reaction models on the reliability of the derived binding parameters.

The developmental potential in culture of muscle cells homozygous for motor end plate disease

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Motor end plate disease (MED), a lethal recessive neuromuscular disorder, was investigated by tissue culture. Med/med myoblasts form contracting myotubes, a fraction of which developed cross striations and survived for at least 30 days longer than a med/med animal. In combination with wild type embryonal spinal cord, med/med myotubes like their +/? counterparts were contacted by nerve fibres and responded by accumulating acetylcholine esterase at the nerve-muscle contact site. Binding of ^{125}I-α-bungarotoxin to med/med myotubes revealed concentrations of acetylcholine receptor ('hot spots') like in wild type myotubes. However, the density of α-bungarotoxin binding sites is lower on med/med myotubes. When preparing myoblasts, on the average only 60% of the yield of cells was obtained

from med/med leg muscles as compared to +/? muscles. Since 2-day-old animals do not show any clinical abnormalities, these differences may hint to the primary cause of the MED phenotype.

Expression of mutagen-induced damage to sperm and spermatids during early mouse embryogenesis

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After treatment of postmeiotic stages of spermatogenesis of the mouse with the chemical mutagen TEM (triethylenemelamine), dose and stage of spermatogenesis-dependent disturbances of early embryonic development can be observed both in-vivo and in-vitro cultures of embryos. With the doses used (0.2 and 0.4 mg/kg), TEM-treatment of sperm and spermatids did not affect the rate of fertilization nor the number of cleaving eggs, but it severely disturbed further development of embryos up to implantation, expressing maximum effect in morulae. The results of cytological analysis suggest that lesions induced by TEM in postmeiotic male germ cells of the mouse are expressed during early embryogenesis in 2 ways: a) as aberrations of the chromosome type already detectable in first cleavage metaphases, b) as long lasting changes in chromatin, not detectable in very early cleavage divisions, but leading to disturbance of the postimplantation development of embryos.

Observations on a nu/nu ↔ +/+ mouse chimaera

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A nu/nu ↔ +/+ mouse chimaera has been obtained by in-vitro aggregation of early embryos from the strains nu/nu (BALB/c) and CBA/T6T6 and subsequent transfer to a pseudopregnant foster mother. One chimaeric male has been analyzed when 7 months old. Nude and hairy skin were arranged in alternating patches. The pattern of coat colour was independent of the hair distribution, thus demonstrating the autonomous migration of melanocytes to the skin during fetal development. Analysis of 90 offsprings of the allophenic male indicated that sperm carrying the recessive nude gene had been exclusively produced. The relatively long life span of the chimaera maintained under conventional housing conditions suggests a functional immune system. Histological and functional experiments to test this possibility are in progress.

Mass isolation of germinal vesicles and nucleoli from vitellogenic oocytes of *Xenopus laevis*

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We have developed a novel technique which allows the isolation of large amounts of germinal vesicles from vitellogenic oocytes of *Xenopus laevis* (F. Scalenghe et al., Chromosoma, in press, 1978). The technique is rapide and simple and avoids centrifugation or filtration steps. It involves essentially a Pronase treatment and a lysis of the oocytes by means of a nonionic detergent (NP-40). Isolated nuclei are able to incorporate radioactive precursors into RNA products and transcription is stimulated by addition of exogenous DNA. Pure nucleoli can be easily obtained from isolated germinal vesicles. These nucleoli are transcriptionally active and are one of the subjects of our present investigation.

Biosynthesis of creatine kinases in differentiating chicken myogenic cells

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Synthesis of M-CK and B-CK subunits was measured by highly specific and sensitive immunoprecipitation methods after exposing cells to pulses of ^{3}H-leucine. The incorporated radioactivity was normalized to DNA-content. During the first 3 days of culture a sharp increase, later a decline in B-CK-synthesis was observed. M-CK-synthesis was not detectable in 24-h-cultures and increased dramatically during the 3rd day. The relative rate of M-CK-synthesis increased while that of B-CK decreased. Depletion of Ca^{++} in cultures (by means of EGTA) blocked fusion and synthesis was reduced as compared to standard cultures 2fold for B-CK and 3.5fold for M-CK. If differentiation was inhibited with BrdUrd, incorporation into B-CK was 2.5 times lower, but was undetectable into M-CK even after prolonged cultivation. CK-synthesis in BrdUrd-cells resembled that of subcultured fibroblasts. Synthetic rates seem to be most important for intracellular CK-concentration, differences of degradation seem not to be crucial.

Binding, internalization and lysosomal association of ^{125}I-insulin in isolated rat hepatocytes

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The fate of ^{125}I-insulin after its binding to isolated rat hepatocytes was studied by a quantitative EM autoradiographic analysis. Isolated rat hepatocytes were incubated with ^{125}I-insulin at 20 °C and 37 °C. Internalization of label is evident by 2 min of incubation at 37 °C and continues as a constant function of binding at both temperatures. At steady state approximately 30–40% of the label has shifted intracellularly. When cell associated radioactivity is extracted and filtered on G-50 sephadex from 75 to 90% of radioactivity co-elutes with native insulin. When internalized radioactivity is analyzed further at 2–5 min of incubation at 37 °C there is a 5fold preferential association with lysosomes and by 30–60 min a 10fold association. These studies demonstrate directly that insulin initially localizes to the plasma membrane of isolated hepatocytes, is internalized and the hormone or receptor complex associates preferentially with lysosomes.

In vitro transformation of T4-head-related particles produced by mutants in gene 17, to final T4 capsids

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The particles accumulated in cells infected with T4 mutants in gene 17 are composed of cleaved major head protein (gp23*) arranged in an unexpanded lattice. These particles can be transformed in vitro into structures that are expanded and share common physical characteristics with the final T4 capsid.

Repressor and inhibitory activities associated with masked globin messenger ribonucleoprotein complex

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Duck erythroblast polyribosomal globin mRNA actively translated in polyribosomes is associated with characteristic proteins which are different to those of the free cytoplasmic

form of the same mRNA. The latter ribonucleoprotein complex (mRNP) is not only untranslatable in a wheat germ protein synthetizing cell-free system, although its deproteinized mRNA is fully active, but furthermore inhibits the co-translation of any other mRNA present. Tests in a (RNase-treated) reticulocyte lysate show that the free mRNP is equally repressed but, in this system, it is devoid of the inhibitory activity towards other mRNAs. Salt-dissociated 'core' particles retain these same characteristics. Work is in progress to characterize the masking and inhibitory factor(s) linked to mRNA.

rDNA spacer transcrips in normal and cold-treated *Xenopus* cells

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The different fragments obtained from *Xenopus* rDNA by restriction with Eco R1 and Bam H1 enzymes have been inserted into bacterial plasmids (vector pBR313). The DNA of strain HM5, containing a 1100 b.p. fragment of nontranscribed spacer sequences, was used as a probe to identified spacer transcripts in sucrose gradients of RNA from normal and cold treated *Xenopus* cells. The results show that, in normal cells, RNA-molecules (5-23 S) are transcribed from the so-called nontranscribed spacer. Upon cold treatment, which inhibits maturation cleavages of rRNA, small spacer transcripts are still present and, in addition, large RNA-molecules (45 S) are found containing both spacer sequences and the sequences of the first stable pre-rRNA (40 S).

The intervening sequence of a mouse β-globin gene is transcribed into the 15 S β-globin mRNA precursor

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The mouse β-globin gene is interrupted by a 550 base pair intervening sequence of DNA, as indicated by the R-loop formed between cloned β-globin genomic DNA and globin mRNA (Tilghman et al., Proc. nat. Acad. Sci. USA, in press, 1978). Kinetic and structural evidence has led to the conclusion that the mature 10 S β-globin mRNA is synthesized via a 15 S precursor (Curtis et al., Cold Spring Harbor Symp. Quant. Biol., in press, 1977), the length of which roughly equals or slightly exceeds the total length of the coding and intervening sequences of the β-globin gene. Electron microscopy of R-loop hybrids between this gene and the purified 15 S β-globin mRNA precursor showed that the intervening sequence is present in the 15 S precursor. This suggests that processing of the precursor involves removal of the intervening sequence and precise repairing within the gene sequence.

The ribosome-binding site on Rous Sarcoma virus RNA for the 'gag' gene translation

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In various cell free extracts RSV RNA directs the synthesis of a major polypeptide of mol.wt 76,000 daltons; this protein has been shown by fingerprint to be identical to the precursor (Pr76) to virus group specific antigens previously identified in pulse labeled-infected cells. We have used the reticulocyte lysate protein synthesis system to promote the binding of ribosomes to RSV RNA in vitro and we have characterized the RNA protected from nuclease digestion. This RNA fragment has been located at the 5′ end of the genome and part of its sequence has been established by direct analysis. The sequence of the whole protected fragment has been inferred from the sequence of the DNA complementary to the 5′ terminal 100 nucleotides of RSV RNA. 6 initiation codons are present in this nucleotide sequence but only one is functional for in vitro translation of the Pr76 protein.

A unifying model to explain segmentation, compartmentalization and regeneration in *Drosophila* development

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3 important aspects of insect development, namely the appearance of segments in the embryo, compartmentalization in imaginal discs during development and regeneration of discs after experimental interference, have been the object of extensive investigation. To account for each phenomenon, an internally-consistent hypothesis has been formulated during the last few years. As yet, however, little attempt has been made to relate these 3 fields to each other. A model, based on the sequential establishment of a series of gradients whose function is to provide positional information to the cells and cause them to initiate defined developmental programmes, has been developed to account for the clonal restrictions that occur throughout development, for the way in which cells behave during regeneration and for the action of a number of mutations.

Membrane association of the superoxide-forming enzyme in human neutrophils

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Superoxide which is formed by neutrophils during phagocytosis is a major microbicidal product of these cells. Neutrophils were activated with opsonized zymosan or phorbol myristate acetate and the subcellular distribution of the superoxide-forming enzyme (SFE) was studied by zonal sedimentation. Under conditions where specific and azurophil granules are wellresolved (13,500 rpm), 50–60% of SFE was associated with the membrane marker alkaline phosphatase while 30–40% was recovered together with the azurophil granules. Little activity was found in fractions containing the specific granules. Sedimentation at lower speed (6500 rpm) resulted in complete resolution of SFE from the azurophil granules. Over $^2/_3$ of the SFE-activity coincided with alkaline phosphatase. The rest was associated with fast-sedimenting aggregated material. These results demonstrate that SFE is localized in membrane fragments presumably derived from the plasma membrane.

Culture of haploid and monosomic corn cells

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Cultures of haploid and monosomic corn cells are needed for in vitro selection of auxotrophs. In the absence of successful anther and microspore culture in corn, certain high haploidy-inducing genetic stocks in combination with suitable genetic markers can be used to screen haploids at the seed or seedling stages. Callus derived from such tissues, however, fails to regenerate plants. A system involv-

ing *ig*- and *adh*°-mutants may be used to screen immature haploid embryos from which morphogenetic callus is obtained. Monosomics may be more stable than haploids in culture. A system involving a nondisjunction inducing strain and a multiple chromosome tester can be used to screen for aneuploid (2n±?) tissues, including monosomics (2n−1).

Transcription of the vitellogenin gene from chick liver chromatin in vitro

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Chromatin, defined by size, nucleosome structure and endogenous RNA polymerase content, from estrogen-treated chicks and from controls was transcribed in vitro. Specific transcription of the vitellogenin gene (determined by hybridization to $cDNA_{vit}$) could be achieved when a crude estradiol-receptor complex preparation was added to control chromatin, but not when chromatin from estrogen-treated chicks alone was used.

Bacteriophage T4 head assembly

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The head of bacteriophage T4 is a complex multicomponent structure. Its size and elongated shape are determined during the formation of a precursor, the prehead. At least 3 structural proteins of the prehead are indispensable for correct assembly. We have purified several T4 prehead proteins. In vitro assembly of a mixture of all structural proteins yields prehead-like structures. Assembly properties of the individual proteins and mixtures thereof shows what structural information is carried by the different structural components of the prehead.

Differentiation in vitro of larval and adult muscles from embryonic cells of *Drosophila*

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The differentiation of muscles in primary cultures of cells from *Drosophila melanogaster* embryos of the late gastrulation stage (6–8 h) was investigated. In early cultures, and in the absence of exogenous ecdysone, 2 main classes of contractile, striated muscle were found, namely flattened 'sheet muscles' and 'myotubes'. Comparison, by light- and electron microscopy, of multinucleated myotubes with muscles from 3rd instar larvae shows that this class corresponds to the muscles of the body wall of the larva. When α- or β-ecdysone is added to the cultures, they undergo a number of metamorphic changes and 2 new types of muscle form. Ultrastructural and light microscopic examination of these 2 types indicates that they correspond to the 2 classes of skeletal muscle (fibrillar and tubular) found in adult flies.

Retrograde axonal transport of macromolecules as a tool to obtain information on membrane properties of nerve terminals

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The retrograde axonal transport of macromolecules from adrenergic nerve terminals in the rat iris to the superior cervical ganglion exhibits great selectivity, not dependent on the general physicochemical properties of the molecules but on a specific binding to constituents of the nerve terminal membrane. Since nerve growth factor, tetanus and cholera toxins and several lectins, all of which are transported retrogradely, are known to bind selectively to different membrane glycoproteins and glycolipids, the relationship between the amount of ligand injected and the amount transported gives both qualitative and quantitative (affinity, number of binding sites) information on the composition of the terminal membrane. For the majority of the ligands the transport is saturable, wheat germ agglutinin exhibits the highest capacity and NGF the highest affinity. Independence of NGF receptors and various cross-reactivities for other ligands were shown in competition experiments.

Biological activity of synthetic dogfish α-MSH

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2 types of α-melanocyte-stimulating hormone (α-MSH) have been isolated from the pituitary of the elasmobranch *Squalus acanthias:* the nonacetylated tridecapeptide H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · NH_2, and its analogue with a carboxylic C-terminal end (Lowry et al., Biochem. J. *141*, 427, 1974). We have now synthesized these 2 peptides and some additional derivatives by a classical approach using the fragment condensation method. The determination of the biological activity with the frog skin assay (Eberle and Schwyzer, Helv. Chim. Acta *58*, 1828, 1975) revealed that both dogfish α-MSH's exhibit $2 \cdot 10^9$ U/mmole, i.e. 5% of that of mammalian α-MSH. Thus, the melanotropic potency is scarcely affected by the presence of a charged C-terminus, at least in the case of the nonacetylated dogfish α-MSH. The activity is, however, lowered to $< 10^9$ U/mmole if Met^{13} is oxidized to its sulfoxide. The N^a-acetylated form of dogfish α-MSH, [Met^{13}] α-MSH, exhibits the same biological activity as mammalian α-MSH, namely $4 \cdot 10^{10}$ U/mmole.

Separation of *Drosophila* tRNA by 2dimensional polyacrylamide gel electrophoresis

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A 2dimensional gel electrophoresis system was adapted for separation of *Drosophila* tRNA. The first dimension - 10% polyacrylamide, 4 M urea, pH 8.3, with a spacer gel of 5% polyacrylamide, 4 M urea, pH 6.7 - separates crude tRNA into 16 bands. In the second dimension - 20% polyacrylamide, 4 M urea, pH 8.3 - the tRNA is resolved into 50–70 distinct spots, most of them representing pure tRNA species. Number and intensity of the spots are in agreement with the isoacceptor patterns of RPC-5 chromatography. Good separation is also achieved in the precursor region between 4S and 5S RNA. These gels represent a helpful tool for the isolation and characterization of tRNA isoacceptors, as well as for the identification of tRNA plasmids.

Gap junctions and pattern formation in the development of *Drosophila*

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Pattern formation in multicellular organisms requires cell communication, which is probably achieved by exchange of molecules through gap junctions. We have studied time of appearance and frequency of this type of junction during

development and in various cell types of *Drosophila*. In cellular blastoderms, close membrane appositions are found which might represent incipient gap junctions. The first discrete gap junctions appear in early gastrulae and become very frequent in the embryo. A similar increase in frequency is found in wing discs between 22 and 120 h after egg deposition. Imaginal disc cells of a long-term culture, which does not differentiate normal adult patterns, show a reduced frequency of gap junctions. The same is observed in imaginal disc cells derived from embryonic primary cultures which do not produce any specific patterns. Our results suggest that the presence of gap junctions alone is not sufficient for normal pattern formation, although their frequency may be important.

The chromatin repeat length of cortical neurons shortens during early postnatal development

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We have previously shown that the DNA content of rat cortical neurons increases from 2c at all fetal stages to 3c at 7 days postnatal age. We therefore investigated whether this DNA increase might be accompanied by structural alterations of the chromatin from neuronal nuclei of the rat forebrain cortex. Electrophoretic analysis of the DNA fragments resulting from partial digestion of the nuclei with micrococcal nuclease revealed an average DNA repeat length of 195±8 base pairs for fetal and of 174±3 base pairs for 7-, 30- and 60-day-old neuronal chromatin. Thus, a close temporal correspondance exists between the DNA increase and the shortening of the chromatin repeat length. In contrast, the DNA repeat length of liver chromatin increases during postnatal development from 185±3 base pairs in fetuses to 207±4 base pairs in adults. In both cell types theses structural alterations are confined to the linker between nucleosomes.

Ultrastructural visualization of RNA transcription complexes in mouse cells

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Cultured P815 cells were lysed using the detergent NP40 and then spread according to Miller and Bakken (1972). Some cultures were spread after 3 or 5 min labeling with ^{3}H-uridine and processed for high resolution autoradiography. The spread material was, in some cases, treated with RNase. Radioactive labeling allows to reveal easily transcription complexes of mainly 2 types. They are represented by gradients of lateral RNP fibrils sometimes appearing as tandemly arranged complexes on the DNA-axis on one hand and mostly individual fibrils of various length on the other. The fibrils are RNase-sensitive and would correspond respectively to pre-rRNA and pre-mRNA growing chains.

Localization of isoenzymes of O-acetyl-L-serine sulfhydrylase in *Spinacea oleracea* L.

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O-acetyl-L-serine sulfhydrylase (OASSase; E.C. 4.2.99.9) catalyzes the formation of cysteine from O-acetyl-L-serine and H_2S. In spinach, the enzyme can be detected in leaves and roots. In 11-day-old seedlings with fully developed cotyledons, 30–35% of the total OASSase activity was measured in the nongreen parts. OASSase present in the roots is partially localized in the proplastids. About 20% of the total OASSase activity in leaves is localized in the chloroplasts. The chloroplast isoenzyme has a lower mobility than the extrachloroplastic isoenzyme in disc gel electrophoresis with 0.1 M tris-glycine buffer at pH 8.3. It can be further distinguished from the extrachloroplastic isoenzyme by its lower stability.

Estrogen-induced secretion of vitellogenin and accumulation of its mRNA in organ culture of *Xenopus* liver

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Liver cubes of male *Xenopus* were cultivated in serum-free medium, and assayed for secretion of vitellogenin and accumulation of mRNA after differential estrogen stimulation. To monitor vitellogenin secretion, cultures were labeled with $^{32}PO_4^{3-}$. The concentrated medium was electrophoretically separated, and the ^{32}P radioactivity of vitellogenin determined. To estimate accumulation of mRNA, total cytoplasmic RNA was hybridized with cDNA synthesized on vitellogenin mRNA. In each type of estrogen stimulation the appearance of vitellogenin strictly coincided with significant levels of mRNA in the cytoplasm of hepatocytes. The tissue response was delayed at primary stimulation, but faster and more intense at secondary stimulation of livers, prestimulated in vivo 41 days earlier. An adaptation period prior to estrogen stimulation not only reduced the lag in vitellogenin secretion and mRNA accumulation but also variation in response, but always the same linear relationship between mRNA accumulation and vitellogenin secretion was observed.

The surface morphology of the cat subfornical organ (SFO)

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On the basis of a study using the scanning electron microscope, the cat SFO can be divided in 3 distinct regions: 1. A central zone consisting of hexagonal ependymal cells without specialized surface structures. 2. An intermediate zone with heterogeneous appearance, characterized by the cobble stone pattern of ependymal cells. These cells may have a solitary cilium and are very often encircled by a line of microvilli. The surface is considerably enlarged by funnel like openings and canaliculi entering the ependymal layer. 3. A ciliated zone with evenly distributed kinocilia and microvilli resembling the surface covering the ventricular wall. The fact that the SFO has intimate contact with the cerebrospinal fluid and its location outside the blood brain barrier renders the surface morphology of this organ increased importance.

Proteolytic enzymes in wheat ears during maturation

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Activities of aminopeptidase (hydrolysis rate of L-leucine-p-nitroanilide), carboxypeptidase (hydrolysis rate of N-CBZ-phenylalanine-alanine) and endopeptidase (hydrolysis rate of azocasein at pH 7.5) were assayed in glumes (glume, lemma and palea) and in kernels of field grown wheat during maturation. The kernels were also subdivided into the outer chlorophyll free part of the pericarp, the chlorophyll containing cross cells, the endosperm and the embryo. Aminopeptidase showed highest activities in the embryo, in developing and in physiologically active tissues (young glumes, photosynthesizing cross cells). Carboxypeptidase activity was high in fully developed tissues and remained high, when senescence started. Endopeptidase activity at neutral pH was high in senescing tissues during nitrogen mobilization (ageing, glumes, pericarp), but in the embryo and in the endosperm this activity was extremely low.

The primary structure of C-phycocyanin

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C-Phycocyanin, the main photosynthetic light harvesting biliprotein from the thermophilic cyanobacterium *Mastigocladus laminosus* consists of 2 subunits with mol.wts of 18,300 and 16,100. Because of the advanced methods for automated Edman degradation now available, our strategy was to produce large peptides which behave well on the Sequenator. Thus, specific cleavage was carried out at methionine, tryptophane and arginine which are present in suitably small amounts. Separation methods for these fragments had to be well-adapted to their extreme properties which are due to the bulky and hydrophobic chromophore. By this strategy, we determined 85% of the α-chain-sequence and 94% of the β-chain-sequence with automated degradations. The remaining sequences were determined by manual Edman degradation, carboxypeptidase digestion and hydrazinolysis.

Cytoskeletal proteins in normal, infected and virus-transformed cells

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Normal, virus-infected and virus-transformed cell cultures have been studied by means of immunofluorescence using purified anti-actin and antitubulin antibodies. Actin positive stress lines are visible in normal flattened cells slightly before and at confluence. They are also present in transformed cells at confluence, however, they appear later in transformed than in normal cells. Infection with SV40 or polyoma virus, with development of T-antigen, does not change the pattern of actin distribution in cells of confluent cultures. Tubulin distribution is not significantly altered during different stages of growth in normal cells or after infection and transformation.

2 chromatographically separable forms of *E. coli* protein biosynthesis elongation factor Tu

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2 forms of the protein biosynthesis elongation factor Tu have been separated by ion exchange chromatography on DEAE-sephadex (PNAS, in press). They are distinguishable and unique molecular species as shown by a rechromatography experiment since either separately gives the component peaks and a mixture gives a double peak of activity. Other possible chromatographic artifacts have been ruled out. The 2 factors have as yet similar biological activities, but some evidences for a functional difference have been deduced from the apparent higher affinity of 1 factor for the ribosomal pellet.

The growth of fibres from transplanted eyes in amphibian larvae

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Factors regulating the directional growth of nerve fibres were studied in *Xenopus laevis*. Embryonic optic vesicles, transplanted into the dorsal region at the tail bud stage, developed into morphologically normal eyes. Results were studied at late larval stages after enucleation and reduced silver staining for degenerated fibres. The axons of retinal ganglion cells had entered the spinal cord: most of them grew rostrally, some grew caudally. Extensive terminal degeneration was found only in the nucleus tractus solitarii (NTS), suggesting that ectopic fibres terminated in the medulla oblangata without reaching the optic tectum. However, clear terminal degeneration *was* found in the tectum (as well as in NTS) when the same grafting experiment was followed by removal of the normal optic vesicles. The presence of normal optic fibres in the tectum seems to interfere with the ability of the ectopic fibres to reach this target organ.

Structural gene identification: Blocking the in vitro translation of mRNA by hybridization to cloned DNA

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In the isolation of recombinant molecules carrying specific genes, it is useful to be able to identify sequences coding for a given polypeptide. We have used in vitro translation as a specific assay for messenger mRNA-DNA hybridization. A mixture of mRNA is hybridized to excess dna, and translated in vitro; the mRNA in hybrid form is blocked for translation (S.C. Inglis, D.J. McGeoch and B.W.J. Mahy, Virology *78*, 522, 1977), so that the corresponding polypeptide is missing in the analysis of the translation products. We have applied this technique in the study of cloned *Drosophila melanogaster* genes.

Detection of prolactin receptors: Problems raised by use of radioiodinated hormones

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Jodinated peptidic hormones do not behave as unlabeled ones with specific receptors. ^{125}I-ovine PRL, prepared by lactoperoxidation, was incubated at 20°C with female rat liver membranes; specific binding (SB) gradually increased to 7% after 24 h. In contrast, with cold oPRL preincubated 1-180 min with the membranes, maximal occupancy of

available sites occurred within 30 min; washing and ^{125}I-oPRL 24-h-incubation showed no further exchange, revealing a different affinity of tracer for receptor. Loss of binding activity by iodination also varied with hormone and tissue tested: rat PRL displaced ^{125}I-human PRL better than ^{125}I-rPRL from rat liver (SB: 16% vs 4%), mammary gland and kidney; ^{125}I-oPRL showed significant SB to rat liver (7%), but none to kidney. Tissue receptors have distinct selectivity for different tracers; receptor occupancy by endogenous hormone might influence it. Washing off endogenous PRL from receptors by in vitro incubation with specific antiserum doubles ^{125}I-rPRL SB to rat kidney.

Stimulation of RNA-synthesis in dissociated glia-rich rat brain cultures

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RNA-synthesis in dissociated glia-rich rat brain cultures can be stimulated by various drugs including noradrenaline. The effect of noradrenaline, which is both dose- and time-dependent, is preceded by an increase in the intracellular level of cAMP. However, addition of cAMP or its derivatives does not provoke a stimulation of RNA-synthesis in these cells. Noradrenaline-induced stimulation can be partially inhibited by either α- or β-blocking agents and the combination of both blockers produces complete inhibition. Incorporation of ^{3}H-uridine or ^{32}P-orthophosphate into RNA reaches a maximum between 15 and 60 min following drug addition and decreases again. ^{3}H-thymidine incorporation is not altered by noradrenaline after 7 h of incubation. Analysis of newly synthesized RNA on sucrose gradients and by gel electrophoresis reveals an increase of both poly(A)$^{+}$RNA and poly(A)$^{-}$RNA and points to prominent increase in the high molecular range as well as in the 4S-, 5S-region.

Intracytoplasmic immunoglobulin classes in individual *Xenopus* lymphoid cells

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Splenocytes of normal and immunized *Xenopus* were examined for cytoplasmic Igs by immunofluorescence microscopy and simultaneous double staining by fluorescence conjugated class-specific anti-195 Ig-TRITC and anti-75 Ig-FITC. The cytoplasm of 70% of the Ig-containing cells (Igcc) showed positive reaction for both Igs. Tumoral cells from one available spontaneous lymphoid tumour bearing *Xenopus*, with an increased serum level of 195 Ig, reacted with anti-19S Ig-TRITC antiserum only. This result and the lack of cross-reactivity in Ouchterlony tests confirm the class-specificity of the rabbit antisera. The simultaneous production of 19S Ig and 7S Ig by the majority of Igcc is in agreement with a parallel activity of the 2 classes of antibodies observed in the serum of immunized *Xenopus*. It is not known yet if the 2 classes of Igs in these cells share the same antibody specificity. Phylogenetically, the expression of more than one gene coding for the C-regions of H-chains in a single secreting plasma cell might reflect a primitive character.

Replication and metabolic stability of ribosomal DNA in *Physarum polycephalum*

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The rRNA-genes of the slime mould *Physarum polycephalum* are located on free linear, palindromic DNA-molecules of a discrete size, 38×10^{6} daltons. Their replication proceeds by means of bidirectional fork displacement and is – in contrast to the situation with bulk DNA – unscheduled. During active, balanced growth, the proportion of DNA coding for rRNA is shown to stay constant at the time of early prophase over several consecutive mitotic cycles. Under the same conditions, these rDNA-molecules do not undergo any measurable turnover. In this respect, they behave in the same way as chromosomal DNA.

Protoplast fusion and enrichment of heterokaryons using an isoosmotic density gradient procedure

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Mesophyll protoplasts of various Gramineae species have been fused with protoplasts from maize suspension cultures to establish hybrid cell lines and to study problems of somatic hybrid formation between species of decreasing phylogenetic relationship. Heterokaryon formation and cell wall regeneration around fusion products has been observed in all combinations tested. First divisions occurred in some cases. We are now attempting to establish the culture conditions required for sustained divisions of the somatic hybrids. As long as physiological selection systems of general applicability for the selective recovery of somatic hybrids are lacking, systems based on physical characters could be applied. We therefore developed an isoosmotic discontinuous density gradient system which allows the fractionation of various types of protoplasts. When applied to the fractionation of protoplasts after fusion treatment, enriched fractions of hybrid protoplasts can be recovered because of their intermediate density.

Phosphorylation of ribosomal protein S6 during transition of resting fibroblasts into the cell cycle

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We searched for phosphorylated cellular proteins of chick embryo fibroblasts after stimulation by mitogens from the resting state into G_1 phase. Synchronized cells were incubated with $^{32}PO_4$ for 30 min with serum, insulin or insulin-like growth factor (IGF). Within this time period the only difference between control and mitogen-treated cultures occurred in the 40S ribosomal fraction. Analysis of these proteins revealed a phosphorylated protein of 31,000 mol.wt, shown to be S6 and its derivatives. The extent of phosphorylation and the electrophoretic shift of S6 derivatives were the same irrespective of the mitogen used. Ribosomes from stimulated cells showed 20–30 times the level of radiophosphate as compared to controls. It is highest within the first 30 min after releasing cultures from the resting state and decreases as cells progress through G_1 phase. We propose that phosphorylation of S6 is related to an early translational event immediately following stimulation of growth.

Rous sarcoma viral protein p15 induces intracellular processing of viral precursor pr76

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The protein precursor, pr76, to the group specific antigen (gag) proteins of Rous sarcoma virus (RSV), synthesized in a cell free system is processed in vitro upon addition of purified viral gag protein p15 (v.d. Helm, Proc. nat. Acad. Sci. *74*, 911, 1977). When viral protein p15 was introduced by fusion-injection via erythrocytes into RSV-transformed hamster cells, which do not produce virus progeny but contain small amounts of viral protein precursor pr76, this precursor was intracellularly processed. When p15 was injected into *Xenopus* oocytes which synthesize protein precursor pr76 (upon injection of RSV-RNA) this precursor was cleaved in a significantly higher rate than without injected p15.

The plasma membranes of the cellular slime mold *Polysphondylium pallidum*

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Plasma membranes were isolated by the concanavalin A-Triton method. Proteins and glycoproteins were separated by SDS-gel electrophoresis. 2 of the major components of the membranes are actin and myosin heavy chains. A protein kinase was found on the cell surface. Labeling of whole cells and isolated membranes with [γ-^{32}P]ATP, lactoperoxidase/^{125}I, 1-fluoro-2,4-dinitro[U-^{14}C]benzene and [^{125}I]iodonaphthylazide was used to determine the orientation of proteins and glycoproteins in the plasma membrane.

Modifications induced in plant cell wall ultrastructure by hormonal treatment

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Changes in cell wall surfaces occurring by axial elongation of *Triticum vulgare* coleoptiles treated with indolyl-3-acetic acid (IAA) and abscisic acid (ABA) where shown by SEM (Hofer, Schlienger and Pilet, 1977). As exogenous IAA produces a growth stimulation, the longitudinal stress increasing within the walls during cell extension may promote the formation of 'cracks'. In contrast, these were never found on the controls or on segments treated with ABA at a concentration for which significant inhibition of elongation was obtained. Cryoultramicrotomy and cytochemistry on the polylamellate cell wall are used to study the steps of 'crack' induction and the possible chemical changes occurring in the microfibril structure when promoting or inhibiting growth by hormonal treatment.

Cell-free system for the coupled transcription and translation of the influenza messenger RNAs

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The negative-strand influenza A viruses have a segmented single-stranded genome consisting of 8 pieces of RNA coding for 8 viral proteins, and contain a virus-specific RNA-dependent RNA-polymerase which is responsible for the primary transcription of the genome upon infection of a cell. We have developed an in vitro assay for the influenza polymerase which allows the synthesis of RNA complementary to the genome (shown by base composition) and distributed in 8 size classes (shown by gel electrophoresis under denaturing conditions). In a cell-free system consisting of disrupted virus and a reticulocyte lysate, transcription and translation were coupled resulting in the synthesis of 6 viral polypeptides (characterized by size and peptide map). The synthesis of the 2 viral glycoproteins, haemagglutinin and neuraminidase, has not yet been detected in our system.

Ultrastructural localization of β-D-galactan in the nuclei of the myxomycete *Physarum polycephalum*

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The acellular slime mold *Physarum polycephalum* produces an extracellular sulfated and phosphorylated β-D-galactan which was recently isolated from the nuclei of this organism (D.R. Farr and M. Horisberger, Biochim. biophys. Acta, in press). This polysaccharide has now been localized on thin sections of *P. polycephalum* by the following method: Thin sections of microplasmodia were incubated with RCA_I, a lectin specific for galactose. The bound lectin was then marked with gold granules labeled with desialylated ceruloplasmin, a galactose-terminated glycoprotein. The galactan was found in the nuclei mainly associated with chromatin and also, but to a smaller extent, in the cytoplasm and in some vacuoles. The specificity of this method was examined by marking under the same conditions the galactomannan present in the cell wall of *Schizosaccharomyces pombe*, a yeast dividing by fission. The polysaccharide was found only in the cell wall and the septum.

Simultaneous localization of an hepatic binding protein specific for galactose and of galactose-containing receptors on rat hepatocytes

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The hepatic binding protein specific for asialoglycoproteins (galactose-terminated proteins) and the galactose-containing receptors have been simultaneously localized on isolated rat hepatocytes using gold markers of different sizes. The binding protein was marked with gold granules (5 nm in size) labeled with desialylated ceruloplasmin, the galactose-containing receptors with granules (17 nm in size) labeled with RCA_I, the *Ricinus communis* lectin specific for galactose. It was established that both markers did not interact with each other. Examination of stereo-electron micrographs of thin sections of hepatocytes marked successively with the desialylated ceruloplasmin and the RCA_I

markers indicated that the binding protein was distributed over the whole plasmamembrane, sometimes in clusters. Part of the RCA_I receptors were found in the proximity of the binding protein. If it can be demonstrated that the RCA_I receptors (probably mainly glycoproteins) interact with the binding protein of an adjacent cell, this would increase cell to cell interaction.

DNA-polymerase-γ from rat forebrain cortical nuclei is identical with DNA-polymerase-mt from synaptosomes

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DNA-polymerase-γ and mitochondrial DNA-polymerase were isolated from brain nuclei and synaptosomes, respectively. The presence of a single DNA-polymerase in synaptosomal mitochondria was established by chromatography on DEAE-cellulose, phosphocellulose and DNA-cellulose as well as by sedimentation analysis and isoelectric focusing. A great similarity between the purified nuclear DNA-polymerase-γ and the mitochondrial enzyme was found by the following criteria: chromatographic behaviour in 3 column systems; essentially complete inhibition by NEM; optimal requirements for Mn^{++}, Mg^{++} and pH; template preferences; lack of activity on single-stranded polynucleotides and $(dT)_{12}$-primed mRNA; mol.wt; sedimentation and isoelectric point. We therefore conclude that brain nuclear DNA-polymerase-γ and synaptosomal mitochondrial DNA-polymerase are closely related and may even be identical.

Involvement of IS1 in the formation of hybrids between phage P1 and the R-plasmid NR1

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IS1 elements occur directly repeated at the 2 junctions between the r-determinant and the RTF DNA and 1 copy is present in P1 DNA at map unit 20. We have isolated a large number of P1-R hybrid phages from P1 and NR1. Restriction cleavage analysis reveals: 1. The r-determinant of our NR1 is a transposon with directly repeated IS1 at boths ends. 2. Only one of the P1Cm phages carries a Cm-transposon equal in size to Tn9, the others are smaller or larger. These different Cm-transposons could be derived from the r-determinant by IS1-mediated deletion formation. 3. A co-integrated plasmid is found in which recombination occurs between the IS1 of P1 and one of the IS1 of NR1. This co-integrated plasmid could be a precursor to the P1 carrying an r-determinant at map unit 20.

Effect of photoperiod on larval development and chromosomal puffing in *Chironomus*

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Long-day (18 h light/6 h dark) results in subitan development of *C. tentans* (duration of L4 at 18 °C: < 30 days); short-day (6 h/18 h) induces oligopause in mid L4 which may be maintained for > 150 days. A precocious switch to long-day causes oligopausing larvae (OL) to resume subitan development into prepupae (PP). OL in contrast to PP exhibit an extremely low morphogenetic activity; in addition, puff frequency and size are generally depressed in their polytene salivary chromosomes. Particularly, the ecdysone-inducible regions BR1 and I-18-C are (at least in young OL) completely unpuffed, unlike BR2 and the juvenile hormone-inducible puff I-19-A_1. In PP BR1 and I-18-C are maximally puffed. Impalement of glands from OL and subsequent in-vitro-culture causes I-18-C and I-19-A_2, but not I-19-A_1, to puff; with PP the opposite is found. The relationship between photoperiodic control of development and gene activities needs further elucidation.

Characterization of mRNA coding for P-enolpyruvate carboxykinase(GTP)

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Messenger RNA coding for the cytosolic form of the gluconeogenic enzyme P-enolpyruvate carboxykinase(GTP) (PEPCK) can be translated in a wheat germ protein synthesis system. Using this system, we showed that hormone-dependent changes in PEPCK-synthesis in rat tissues result from changes in functional PEPCK mRNA level. We have now partially purified and characterized the message from rat liver. As judged from its retention on oligo(dT)-cellulose, 80% of the mRNA is polyadenylated. Size estimates based on sucrose gradient centrifugation and gel electrophoresis indicate that considerable noncoding sequence(s) are present. Inhibition of PEPCK mRNA cell-free translation by 7-methylGMP suggests the presence of a 5′ cap structure. Thus, PEPCK mRNA has all features typical of most eukaryotic mRNAs.

Enrichment and restriction analysis of the gene coding for vitellogenin in *Xenopus*

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Isolation and characterization of the gene coding for vitellogenin is essential for studying the mechanism of its activation by estrogen. In a first step we enriched *Xenopus* DNA 30fold for the DNA coding for vitellogenin by CsCl-centrifugation of the R-loops formed between sheared DNA and vitellogenin mRNA. Digestion of this enriched DNA with the restriction enzyme Hind III revealed in agarose gels 7 DNA-fragments of 3.7 to 14 kb hybridizing to ^{125}I-labeled vitellogenin mRNA. The same DNA-fragments were also found in DNA not enriched for vitellogenin DNA. Only 2 Hind III fragments of 6.8 and 8.7 kb reacted with the specific mRNA-fragment containing the oligo(A) sequence. The poly(A)-containing mRNA-fragment hybridized to the DNA-fragments of 4.3, 6.8 and 8.7 kb. Different and more complex patterns of DNA-fragments were obtained after restriction of the enriched DNA with Bgl II and Bam HI. All available data are consistent with a model suggesting that the DNA-sequences coding for vitellogenin are not contiguous in the chromosomal DNA of *Xenopus*.

The effect of an antisuppressor on tRNA in the yeast *Schizosaccharomyces pombe*

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Antisuppressor (sin) mutations abolish the expression of suppressor genes. 1 class of antisuppressor mutations could lead to a defect in a tRNA modifying enzyme. Such altered base modification can change the chromatographic behavior of tRNAs. We screened tRNAs from wild type and antisuppressor strains charged with radioactive amino acids

on high pressure reversed phase chromatography (RPC-5) columns. tRNA-Tyr, tRNA-Trp and tRNA-Ser from the antisuppressor strain sin 1 show markedly differing elution profiles. Sequence analysis revealed that a base modification is missing adjacent to the 3'-side of the anticodon in tRNA-Tyr isolated from the sin 1 strain. We conclude that the antisuppressor mutation sin 1 affects a modification in the tRNA-Tyr, tRNA-Trp and tRNA-Ser.

Mapping of rRNA genes on the circular *Euglena gracilis* chloroplast DNA

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Out of the 135 kilobasepairs (kb) of circular *Euglena* chloroplast DNA 16.8 kb are organized in 3 contiguous 5.6 kb repeats each carrying a rRNA gene set. The overall repetitious character is deduced from a repetitive pattern of cleavage sites for various restrictions enzymes and hybridization studies with chloroplast rRNA. An additional DNA region outside of the 3 repeat units hybridizes to rRNA. Its significance is discussed.

Sequence studies of heat shock genes from *Drosophila melanogaster*

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Plasmid 132E3 contains 2 copies of the gene coding for the major heat shock protein (Schedl et al., 1978, Moran et al., 1978). In order to sequence these genes and the small spacer between them we have subcloned various restriction fragments and constructed a detailed map of the restriction sites in the regions of interest. Sequencing of the small fragments is in progress and preliminary results will be presented.

Induction of cereal cell lines: Role of 2,4-dichlorophenoxyacetic acid

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The induction of cereal cell lines is difficult. Dicot cell lines are obtained using 2,4-dichlorophenoxyacetic acid (24-D). Why does 24-D not operate on cereal tissues? 24-D is more readily conjugated with sugars in cereals than in dicots. 24-D glucosides are thought to be inactive. 2 amino-acid-24-D conjugates (considered active) were prepared but found less active than the free acid in both monocots and dicots. We examined the competitive inhibition of the glucosylation process using inactive compounds like benzoic acid, TIBA and 25-D. We attempted to replace 24-D by hydroxylamine derivatives as very effective inhibitors of phenylalanine ammonia lyase. The responses of monocot, dicot and 24-D-independent cells were very different.

Processing of the glycoprotein precursor of Rous sarcoma virus, a late extracellular event in virus maturation

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The precursor (pr 92) of the 2 glycoproteins of RSV, gp 85 and gp 35, can be immunoprecipitated from pulse-labeled infected cells using monospecific anti gp 85 or anti gp 35 sera. After chase-periods of between 1.5 and 6 h, pr 92 can no longer be detected, but instead a glycoprotein of lower mol.wt (gp 90) is immunoprecipitable by both antisera. During the 6 h following a pulse labeling no gp 85 or gp 35 was detected intracellularly, though, by that time, the majority of the labeled glycoprotein was found in released virus particles. We therefore conclude that the precursor is cleaved outside the cell. Virus which is collected not later than 2 min after release from cells previously labeled with mannose doesn't contain gp 85 nor gp 35, but a glycoprotein which migrates identically as gp 90 on SDS PAGE. This agrees with the idea that the glycoprotein precursor is cleaved after budding from the plasma membrane.

Molecular cloning of the *E. gracilis* chloroplast genes coding for ribosomal RNA

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E. gracilis is a unicellular, green algae. Its chloroplasts contain a circular DNA with a size of about 92×10^6 daltons (Manning and Richards, BBA *259*, 285, 1972) containing 3 genes coding for rRNA, which are clustered on the linked Bam H 1 fragments E and D (Jenni and Stutz, EJB, in press, 1978). These fragments were cloned separately using the plasmid pBR 322 (Bolivar et al., Gene *2*, 95, 1978) and *E. coli* as host. The newly constructed recombinant plasmids EgcKS8 and EgcKS11 containing the Bam H 1 fragments E and D, respectively were analyzed and characterized by gel electrophoresis, analytical ultracentrifugation and electron-microscopy.

Attempts to induce embryogenesis from single isolated callus protoplasts of citrus

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Although over the past few years great progress has been made in inducing embryogenesis from cultured cells, in all cases embryo production occurs only from cells which are part of a multicellular mass. It has not been demonstrated that an isolated cell is able to embark upon embryogenesis without passing through an intervening callus phase. Citrus calluses derived from the nucellus are highly embryogeneic and further they can give rise to protoplasts capable of sustained divisions. We are therefore testing various factors on isolated protoplasts in attempt to determine whether an isolated cell can undergo direct embryogenesis. Success and control of these aims will be of importance in solving many of the problems currently inherent in plant tissue culture.

Biological activities of cloned eukaryotic genes inserted into frog oocytes and eggs

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Using a simplified injection procedure, cloned fragments of sea urchin histone DNA and of *Xenopus laevis* tDNA have been inserted into the oocyte nucleus of *X. laevis*. Both kinds of DNA are transcribed at very high rates after injection. The transcription of tDNA appears to be faithful whereas most of the histone DNA transcripts, normally produced by a different RNA-polymerase, are polydisperse and arise from symmetrical transcription. Nevertheless bona fide sea urchin histone proteins appear to be made in injected oocytes. A 'surrogate genetics' approach is being used to try to define the tDNA and histone DNA transcriptional and translational units. A similar approach, but with *X. laevis* eggs, may be useful in defining the origin of replication of cloned eukaryotic genes.

Localization of $tRNA^{Asp}_{2\delta}$ genes from *Drosophila melanogaster* by in situ hybridization

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RPC-5 chromatography resolves $tRNA^{Asp}$ of *Drosophila melanogaster* in 2 major and 3 minor isoacceptors. One of the major forms contains a modified base Q linked to mannose in the first position of the anticodon. This isoacceptor can therefore be isolated with Concanavalin A-sepharose chromatography. In such a manner purified $tRNA^{Asp}_{2\delta}$ was iodinated with ^{125}I in vitro and hybridized in situ to salivary gland chromosomes according to standard procedures. Label was found in the regions 29 D and E. Sometimes grains were also found in the region 25 D. Charging experiments with aspartic acid gave values of 900 pM/A_{260}, iodinated $tRNA^{Asp}_{2\delta}$ yields 1 spot after 2 dim. PAA-electrophoresis. From this and since the hybridization was done at very low R_0t-values (30) we conclude, that the genes for $tRNA^{Asp}_{2\delta}$ are localized in the regions 29 D+E and possibly 25 D.

The inner surface of denuded rabbit aorta exposed to flowing blood: A comparative transmission- and scanning-electron microscopical study

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The aorta denuded of its endothelium is used in thrombosis and atherosclerosis research to study the interaction of blood platelets with the vessel wall. The topographical arrangement of the structural elements of the subendothelial surface, characterized by TEM as amorphous material, elastin, collagen- and microfibrils, was investigated by SEM. For exposure to blood and to reduce the structural damage during subsequent processing, denuded aorta segments were mounted - inside out - on brass cylinders of the approximate vessel diameter. At high magnification SEM revealed a flat meshwork of fibrils of various diameters (probably collagen- and microfibrils) embedded in a matrix (amorphous material and elastin). After exposure to flowing blood, typical patterns of adherent and aggregated platelets were observed.

Studies on a possible physiological role for endogenous virus in the immune system

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Previous work form our laboratory demonstrated that several B-lymphocyte mitogens are efficient inducers of endogenous C-type viruses in mouse spleen cultures. Recently antisera raised against these viruses were found to suppress the humoral immune response of mouse lymphocytes. The antisera react specifically with the major virus constituents plus an unidentified component shown by gel analysis of the immunoprecipitants. Examining the molecular mechanism of virus induction, we have found cellular DNA-synthesis a prerequisite for virus production. With a cDNA probe to the induced virus we demonstrated that mitogen-stimulation of spleen cells causes de novo synthesis of viral-specific RNA. However, unstimulated cells also contain measurable amounts of viral RNA consistent with our hypothesis that virally coded sequences play a role in the immune system.

Separation of 2 migration factors from tumour cell line

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SV28 cells are hamster cells transformed by SV40 virus. When injected into hamsters they give rise to invasive metastasizing tumours. These cells produce factors that induce cell migration on wounded 3T3 mouse cell monolayers. The serum-free medium from SV28 cell cultures can be concentrated and fractionated on Dowex 50W. The active fraction is concentrated and applied on a sephadex G-100 superfine column. There are 2 peaks of activity which when run on a sephadex G-75 superfine column are clearly separated. These 2 components appear to have different biological activities. The active fraction with higher mol. wt (ca. 40,000 daltons) induces only migration. The fraction with lower mol. wt (ca. 25,000 daltons) induces migration and also morphological modifications of the cells and a formation of multilayered clumps.

Difference in nuclear Na^+ and K^+ between 2 developmental stages of *Chironomus tentans*

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Salivary gland nuclei were isolated in the lyophilized state, weighed, and analyzed for their total Na^+- and K^+-content by flameless atomic absorption spectrometry. Nuclei of prepupae contain only ½ as much Na^+ but 1½ times as much K^+ (per dry wt) as nuclei from oligopausing (diapausing) larvae. Both these differences are statistically significant. The decrease in the ratio of total Na^+ to total K^+ towards pupation confirms previous predictions based on the observations of naturally occurring and ion-induced puffing changes in salivary gland chromosomes. These results are a necessary complement to measurements of ion-activities (see abstract by Wuhrmann), in order to better understand the effective ionic environment of chromosomes at different developmental stages.

Analysis of SV40 T-antigen tryptic peptides by ion exchange column chromatography

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We analyzed SV40 Tumor (T)-antigen extracted from various SV40 transformed cell lines. In SDS-polyacrylamide gels the main band of T-antigen had an apparent mol. wt of 88 K; in addition we always observed a band at 20 K ('small' T-antigen). Some mouse cell lines exhibited one or more additional immunoprecipitable minor bands with mol. wts between 80 and 130 K. The pattern of the ^{35}S-labeled tryptic peptides obtained by ion exchange column chromatography of 88 K T-antigen extracted from the transformed cells and from cultures undergoing lytic or abortive infection were virtually the same. By the same technique ^{35}S-labeled tryptic peptides from minor bands present in transformed mouse cells, from 'small' T-antigen as well as from the 42 and 56 K proteins coded by adeno-SV40 hybrid $Ad2^+ND2$ were analyzed. The results obtained allowed us to establish a preliminary map of the ^{35}S-methionine labeled peptides.

Cytochemistry of pericanalicular ectoplasm in liver of rats following experimental cholestasis induced by phalloidin and Na-taurolithocholate

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Marked ultrastructural alterations of the pericanalicular ectoplasm have been reported in rat liver following production of cholestasis by sodium-taurolithocholate and/or phalloidin. In order to study the importance of these changes with respect to the hepatocyte membrane at the biliary pole and to the ectoplasmic microfilaments, we have compared the effects of several histo- and cytochemical techniques (ruthenium hexamine trichloride, tannic acid, digitonin, filimarisin) in control and cholestatic rats. For each reaction, penetration procedures and technical conditions were compared in order to obtain the best results.

Solanaceae as 'model plants' in protoplast technology

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Plant protoplasts are well suited for approaching genetic modification of plant cells. The general applicability, however, is drastically reduced by the problems involved in the culture and regeneration of isolated protoplasts. The most advanced protoplast technology exists at present for certain members of the family Solanaceae. In vitro shoot cultures have been established from different species, varieties and mutants of *Atropa, Hyoscyamus, Nicotiana* and *Petunia.* This material guarantees an optimal source for the production of protoplast cultures. By variation and improvement of the culture conditions protoplasts from the above genera have been successfully cultured and regenerated. The established protoplast culture procedures now enable the use of several in vitro selection systems for performing genetic modification experiments with protoplasts.

Immunological fibre characterization of cross-striated rabbit skeletal muscles

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Myosin extracted from fast and slow rabbit muscles and purified to a high degree (99%) was used for antibody (=AB) induction in guinea-pigs. By a new technique, the gel-electrophoresis-derived enzyme-linked immunosorbent assay (GEDELISA) it could be shown that AB are directed against the heavy and light chains of the myosin and to a certain degree against the 1% of contaminating protein. The so characterized AB were used in indirect immunofluorescence for the identification of fast and slow myosin containing fibres of 5 different rabbit skeletal muscles. After incubation with antifast myosin AB these muscles showed the following percentage of fast (fluorescent) fibres: M. longissimus dorsi: 95%, M. soleus: 15%, M. tib. ant.: 90%, M. psoas maj.: 95%, M. psoas min.: 92%.

RNA-synthesis of isolated *Chironomus* salivary gland nuclei

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Mass isolated nuclei from *Chironomus* salivary glands incorporate ^{3}H-UTP into RNA for several hours. RNA-polymerase I and II activities were characterized by different ionic strength optima, differential sensitivities to α-amanitin and selective activation by divalent cations. Labeled RNA synthezised by nuclei in vitro under low (150 mM Na^+ plus K^+) or high salt (600 mM Na^+ plus K^+) conditions was extracted and analyzed for mol.wt by electrophoresis on agarose-acrylamid gels. If nuclei were incubated under low salt conditions RNA with mol.wts of 38, 23, 16 and 4S were found, values also observed in RNA from whole glands (B. Meyer et al.). Addition of α-amanitin did not affect this synthesis. The incorporation of γ-^{32}P-ATP and γ-^{32}P-GTP suggests, that new RNA-chains were initiated. RNA from nuclei incubated under high salt conditions was of heterogeneous size (between 18S and 4S). The synthesis of this polydisperse RNA was inhibited by low concentrations of α-amanitin, known to inhibit polymerase II. 5-7% of the radioactive RNA bound to polyU-Sepharose indicating its polyA content.

In vitro synthesis of chloroplast and cytoplasm polypeptides of *Chlamydomonas reinhardi*

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RNA extracted from *Chlamydomonas reinhardi* cells has been separated into 2 fractions, 1 containing polyA$^+$ and 1 polyA$^-$ mRNA. These fractions have been translated in vitro in the reticulocyte system. The polyA$^+$ mRNA stimulates the system 90fold above the endogenous level while the polyA$^-$ mRNA gives a 5fold stimulation. The products of the translation have been immunoprecipitated with antiserums (prepared by N.H. Chua, Rockefeller Univ., USA) against carboxylase and chloroplast membrane proteins. The polyA$^+$ mRNA directs the synthesis of the small subunit of carboxylase and of over 20 membrane polypeptides. The polyA$^-$ mRNA directs the synthesis of the large subunit of carboxylase and of at least 6 membrane polypeptides. Experiments in progress in order to identify specific chloroplast genes will also be discussed.

Organization of the nuclear ribosomal DNA of *Chlamydomonas reinhardi*

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Southern hybridization of labeled cytoplasmic ribosomal RNA to a Bam digest of nuclear DNA of *Chlamydomonas reinhardi* reveals the presence of 2 major ribosomal DNA fragments of mol.wts 2.9 and 2.3×10^6 daltons. Similarly, in the case of a SALI digest, the major hybridization occurs with 2 fragments with mol.wt 3.7 and 1.5×10^6 daltons. These 4 fragments have been inserted into the pBR 3130 plasmid. A map of the ribosomal region has been established by double digestion of the ribosomal fragments with Bam, Sal, BglI, HindIII and SmaI and by hybridizing nick-translated ribosomal fragments to Bam and Sal digests of the nuclear DNA. The results indicate that the size of the

ribosomal unit is 5.2×10^6 daltons. Hybridizations of the P^{32}-labeled purified ribosomal RNAs show that the 5S gene is located between the 25S and 18S ribosomal genes and on the same strand as these genes. There appears to be little heterogeneity in the size of the ribosomal unit.

Cellular RNAs-synthesis in presence of low doses of actinomycin D in SV40 or polyoma infected cells

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Confluent primary monkey kidney and mouse kidney cell cultures were infected with SV40 or polyoma virus, respectively. Appearance of viral T-antigen was rapidly followed by stimulation of overall cellular RNA-synthesis (mainly ribosomal RNA), this was paralleled by an increase in nucleolar size and was followed by host chromatin replication (S-phase) and viral DNA-synthesis. We wanted to investigate the role of stimulated rRNA-synthesis for subsequent chromatin replication using actinomycin D at doses that inhibit preferentially synthesis of rRNA. Before appearance of T-antigen, infected mouse and monkey cell cultures exhibited the same sensitivity toward actinomycin D as did uninfected control cultures. However, shortly after appearance of T-antigen and the begin of virus-induced stimulation of cellular RNA-synthesis, the latter was no longer inhibited by the same concentration of actinomycin D. Parallel studies revealed apparently a similar resistance of RNA-synthesis in uninfected actively proliferating cells. We presently try to determine the reason for this decreased sensitivity towards actinomycin D.

Concanavalin-A-Markierung hexagonal dichtestgepackter Intramembranpartikeln in stationärer Bäckerhefe

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Die Anordnung der Intramembranpartikeln im Plasmalemma von Bäckerhefe *(Saccharomyces cerevisiae)* ist vom Wachstumszustand abhängig. In der stationären Phase ist ein Teil der Membranpartikeln zu parakristallinen Aggregaten vereinigt, in exponentiell wachsenden Zellen dagegen sind sie statistisch verteilt. Markierungsversuche zur Abklärung von Eigenschaften und Zusammensetzung dieser regelmässigen Partikelmuster scheiterten bisher daran, dass die üblichen Methoden zur Protoplastenherstellung nur an exponentiell wachsenden Zellen zum Erfolg führt. Wir haben eine Methode entwickelt, die es erlaubt, Protoplasten mit den ultrastrukturellen Merkmalen stationärer Hefezellen in hoher Ausbeute herzustellen. Markierungsversuche mit Concanavalin A an solchen quasistationären Protoplasten zeigen, dass das hauptsächliche Glycoprotein der Hefeplasmamembran am Aufbau der Partikeln beteiligt ist.

Isolation and determination of size distribution of RNA from postmitochondrial fractions of early chick blastoderms

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Cytoplasmic postmitochondrial RNA from early chick blastoderms were isolated. During development, not only does the amount of RNA/blastoderm increase but also the proportion of RNA/blastoderm recovered from the postmitochondrial fraction increases. However, the amount of postmitochondrial RNA/cell first reaches a maximum at full primitive streak and the decreases gradually. The size distribution of native and glyoxal-denatured RNA was determined by electrophoresis on polyacrylamide-agarose composite slab gels as described (McMaster and Carmichael, Proc. nat. Acad. Sci. USA *74*, 4835, 1977). Both native and glyoxal-denatured RNA-samples from chick blastula (Hamburger Hamilton, stage 1) contain significantly smaller amount of stable 28s rRNA than that from gastrula (stage 4) and neurala (stages 8 and 13). At all developmental stages examined, major cleavage products of 28s RNA are found. The mol. wts of major chick embryo RNAs were estimated after denaturation and found to be: 28s rRNA (1.61×10^6 daltons), 18s rRNA (0.68×10^6 daltons), 5s RNA (0.042×10^6 daltons) and 4s RNA (0.028×10^6 daltons).

Stimulation of insulin release increases gap junctions in pancreatic islet cells

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Gap junctions (g.j.) have been described between the endocrine cells of the islets of Langerhans and in view of their possible role in the regulation (via intercellular coupling) of the integrated secretory activity of the islet, their development has been studied in different conditions of insulin release. Islets isolated from control and from glibenclamide treated rats were freeze-fractured and the surfaces of g.j. on islet cell membranes were measured by planimetry. In resting condition, g.j. of islet cells occupy only $0.023 \pm 0.008\%$ of the exposed plasma membrane, being both small (0.0037 ± 0.0004 μm^2) and scarce (5.76 ± 1.61 g.j./100 μm^2). In islets stimulated by glibenclamide, g.j. increase in both size (0.0056 ± 0.0003 μm^2; $p < 0.005$) and number (10.08 ± 1.59 g.j./100 μm^2; NS), representing $0.056 \pm 0.010\%$ of the exposed membranes ($p < 0.02$). The fact that g.j. are modified upon stimulation of insulin release is thus in favor of the hypothesis that g.j. (and coupling) may influence the secretory activity of islet cells.

Characterization of plasmids containing sequences of bacteriophage Qβ

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Plasmids containing cDNA sequences transcribed from Qβ RNA fragments have been prepared (Mekler et al., Experientia *33*, 824, 1977). We have determined which part of Qβ RNA is represented in several of these clones. ^{32}P-Qβ RNA was hybridized to Qβ plasmid DNA fixed to Millipore, treated with RNase T_1 and the protected RNA analyzed by T_1 fingerprinting to identify the large T_1 oligonucleotides previously mapped within the Qβ RNA. All Qβ RNA sequences, except possibly for the very 5′ terminus, were represented in the set of plasmids analyzed. Preliminary results from restriction analysis and Maxam-Gilbert sequence determination show that the same Qβ DNA segments in independent plasmids differ in single nucleotide positions, suggesting that the Qβ RNA used for reverse transcription was heterogeneous or that preparation of the cloned DNA involved a step of low fidelity.

Variations in the amount of polysomes in mature oocytes of *Drosophila melanogaster*

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Quantitative measurements of polysomes and ribosomes of *Drosophila melanogaster* egg chambers, mature oocytes and embryos were done using sucrose gradient analysis. The amount of polysomes per egg chamber increases about 20 times from stages 5 to 13, and then remains constant up to the end of embryogenesis. The percentage of ribosomes in polysomes is fairly constant during oogenesis and embryogenesis (56%±7). Depending on the fly population, the percentage of ribosomes in polysomes of mature oocytes varies from 10 to 70%. It is shown that the percentage of polysomes in mature oocytes decreases with the time of retention of the mature oocytes in the ovary. 24-36-h-old flies kept in optimal conditions retain their mature oocytes 2-3 h. These mature oocytes still contain 40-60% ribosomes in polysomes. Conditions are given which allow to obtain reproducibly high amounts of polysomes from mature oocytes of *Drosophila*.

Gel electrophoretic characterization of rRNA synthesized in vitro by *Chironomus* salivary glands

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Mass isolated salivary glands of *C. thummi* were incubated with ^{3}H-uridine (200 µC/ml) for 2 h. Extracted RNA was electrophoresed on agarose-acrylamide gels which were scanned for optical density (OD) at 254 nm, sliced and counted. The scan shows OD-peaks at the positions of 38, 28, 23, 18, 16 and 4 S. The 28-S-peak has a shoulder on its heavy side. When the RNA was treated with 50% formamide (10 min/50 °C) before electrophoresis, the 38-S-peak decreased and a new peak appeared around 18 S. In the radioactivity profile of untreated RNA only the 38-, 23-and 16-S-peaks were ^{3}H-labeled (^{14}C-RNA from *Drosophila* was used as internal marker). Formamide treatment caused the breakdown of 38-S RNA leaving the 23-S- and 16-S-peaks unchanged. The same OD and radioactivity profiles were obtained with RNA of manually isolated glands. Our results can not be explained by the current model of rRNA processing in *Chironomus*.

Occurrence of deletions among the progeny of plasmids treated with restriction enzymes

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Treatment of plasmid PβG, which contains a single EcoRI site, with excess EcoRI endonuclease resulted in a reduction of infectivity to about 8×10^{-4}. 9 out of 122 clones obtained from transfection with EcoRI-cleaved PβG contained a plasmid resistant to EcoRI. Restriction analysis showed that all had a deletion in the region of the EcoRI site which was probably removed entirely or in part. Although the clones are independent isolates only 2 sizes of deletions were found, one spanning about 170, the other about 190 bp. We are currently investigating whether the deletions occur between short regions of identical sequence known to exist in the relevant region of PβG.

Amplification of IS1-mediated Cm-transposons carried by coliphage P1

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The DNA of several P1Cm hybrid phages derived from phage P1 and the R-plasmid NR1 was analyzed by restriction enzyme cleavage and electron microscopy in order to determine size and location of the Cm-insertion. The Cm-insertions differed in size and were flanked by directly repeated IS1 elements, thus having a structure similar to transposon Tn*9*. 2 independent Cm-insertions were found to be tandem duplications of the structure −IS1−Cm−IS1−Cm−IS1−. This structure had considerable stability even in the absence of chloramphenicol. We studied the gene dosage effect in P1Cm lysogens carrying a single or duplicated Cm-transposon and grown in the absence of chloramphenicol and in its presence. Although the level of resistance also depends on the individual transposon, a direct relationship is demonstrated between the degree of oligomerization of the transposon and the resistance level. Monomeric transposons oligomerize less readily than tandem duplicates.

Fusion in the eye-antennal discs of *Drosophila melanogaster* during differentiation in vitro

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Pairs of eye-antennal discs, attached to the cephalic ganglia, were cultured in vitro with a concentration of β-ecdysone optimal for imaginal differentiation. The eye-antennal discs fused to form a vesicle inside which the antennae were partially everted and on the inner surface of which imaginal structures differentiated. Examination of the imaginal cuticle revealed that the 2 eye discs had fused to form an integrated pattern of bristles and hairs in vitro. In particular, the 3 ocelli, 1½ of which are derived from each disc, differentiate normally. This indicates that the processes of pattern regulation which presumably occur when discs fuse prior to the formation of the adult integument, can take place in vitro.

Zytogenetische Untersuchungen an der Chinesischen-Hamster-Zellinie 19/1 nach Kombinationsbehandlung mit 8-MOP und UV-A

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8-MOP wird in Kombination mit UV-A (320-380 nm) in der Dermatologie zur Behandlung von Psoriasis angewandt. Es fragt sich, ob diese Kombinationsbehandlung unter Umständen krebsfördernd wirkt und in Körperzellen zusätzlich Mutationen auslöst. Zur quantitativen Erfassung der Chromosomenschäden pro Einzelzelle wurde die Häufigkeit der Chromatidenaberrationen, induziert hauptsächlich in der G_2-Phase, der fibroblastenähnlichen Zellinie des Chinesischen Hamsters 19/1, untersucht. Es wurden zwei Endkonzentrationen von 8-MOP (Xanthotoxin, Fluka) verwendet: 1,62 und 16,2 µg/ml in PBS und 0,03%

Alkohol, in Kombination mit 0,05; 0,2 und 0,5 Joules/cm^2 UV-A (0,004 Joules/cm^2/sec). Am Ende einer weiteren 120-min-Kultivierungszeit wurden die mit Colchicin angereicherten Metaphasen fixiert und analysiert. Es zeigte sich gegenüber den Einzelbehandlungen ein erhöhter Effekt nach 0,2 und 0,5 Joules/cm^2 in beiden verwendeten Kombinationen.

2 dimensional electrophoresis of native and denatured DNA from chromatin digests

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A system is described for electrophoretic determination of single- and double-strand mol.wt of the same DNA-sample in a single resolving gel using neutral and alkaline buffers for the first and the second dimensional electrophoresis, respectively. During the course of digestion of duck embryo nuclei with micrococcal nuclease, the enzyme introduces single strand breaks in DNA in the internucleosomal regions and, subsequently, some of these are converted to double-strand breaks giving rise to the well-known series of low mol.wt DNA-repeats. In terminally differentiating chick lens fibre cells, nuclei degenerate and disappear, and low mol.wt DNA-fragments which are multimers of the smallest size class appear (Appleby and Modak, Proc. nat. Acad. Sci. USA *74* (12), 1977). 2 dimensional electrophoresis of fibre DNA show that the chromatin-degradation occurs in 2 kinetically independent steps: In the first, single-strand breaks affect high mol.wt DNA from young fibres and, in the second some of the s.s. breaks are converted to double strand breaks giving rise to unnicked monomer to penta/hexamer size DNA repeats in terminally differentiated fibres.

Digestion in vivo of chromatin from terminally differenting chick lens fibre cells

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Early during their differentiation, lens fibre nuclear DNA accumulate free 3'-OH ends (Modak and Bollum, 1970, 1972). Towards the end of their differentiation, fibre cell nuclei degemerate and disappear (Modak and Perdue, 1970) with a concommittant breakdown of DNA (Modak et al., 1969; Modak and Bollum, 1970, 1972). We have isolated central fibres (CF) and examined the size distribution of DNA by gel-electrophoresis. Beginning 15 days of embryogenesis, low mol.wt DNA-fragments which are multimers of 180 base pairs appear in CF and, progressively, the repeat size decreases to 170 (17 days) and 160 (19 days) base pairs. Homogenates of CF were fractionated by differential centrifugation at 1000×g (10 min), 10,000×g (15 min) and 260,000×g (16 h, through 15% w/w sucrose). Both high- and low mol.wt DNA were present in 10,000×g pellets, while the supernatant of this step, as well as the 260,000×g pellet, contained only low mol.wt monomer-penta/hexameric DNA-fragments. Electrophoresis in SDS-polyacrylamide gels of H_2SO_4-extracts of 260,000×g pellets revealed all 5 major histones which were absent in the supernatants of this step. Thus, in terminally differentiated lens fibres, the chromatin-DNA is degraded at sites between nucleosomes.

Chromatin structure in ageing mouse liver

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Mouse liver nuclei were isolated from F_1-hybrids (C57B1 ♀ × A ♂) of ages, in months, 0.4, 1.75, 3.5, 6, 11, 18, 22, 28 and 33. Between 3.5 and 18 months, the average amount of DNA/nucleus increased due to polyploidization and remained at a constant value thereafter. Nuclei were digested with micrococcal nuclease and the size-distribution of DNA-products was analyzed by gel electrophoresis. Regardless of the age, the average size of the DNA-repeat was found to be 207±3 base pairs. At ages, 3.5, 11 and 18 months, 47–50% of chromatin-DNA was found to be resistant to micrococcal nuclease and this fraction increased to 60% at 28 and 33 months. Thus, the proportion of liver DNA bound to nucleosomes is estimated to increase from 70 to 74% at 3.5–18 months to 90% at 28–33 months, while the absolute amount of DNA free of nucleosomes is estimated first to increase 2.5 times between 3.5 and 18 months and then to decrease to the original value in very old tissues. From the data, we postulate that in 'old' liver chromatin the accessibility of damaged sites in DNA to repair enzymes decreases.

Polyoma virus-specific mRNAs on different polysomal fractions of productively-infected mouse cells

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As part of a study on RNA processing and transport, we have studied the distribution of polyoma-specific RNA in the nucleus and in various cytoplasmic fractions. Polyoma-specific RNA either associated with free and membrane-bound polysomes (prepared by sedimentation from the postnuclear supernatant of dounce homogenized cells) or present on polysomes from bulk cytoplasm (postnuclear supernatant from NP40 lysed cells) and in perinuclear cytoplasm (NP-40-deoxycholate nuclear wash) showed similar sedimentation profiles. Mebrane-bound polysomes may be heavily contaminated by free polysomes which represent 80% of total polysomes. 80% of polyoma-specific RNA is in free polysomes and 20% in membrane-bound polysomes. 90% of polyoma-specific polysomal RNA is present in bulk cytoplasm and 10% in perinuclear cytoplasm. These data do not rule out the possibility that a minor polyoma-specific RNA may be present exclusively on one polysomal fraction.

A method to facilitate the base sequencing of highly repetitive DNA

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When a DNA-molecule, enzymatically extended with poly dA at one end, is partially digested with a restriction enzyme and the products fractionated by affinity chromatography on poly dT-cellulose, an overlapping series of DNA-fragments with a common poly dA 'plus' terminus may be obtained. These fragments may then be ^{32}P-labeled, fractionated by gel-electrophoresis and their base sequences analyzed. Using this approach it is possible to base sequence large segments of highly repetitive DNA, for which up to now no method has been available. The technique has been used to sequence a highly repetitive DNA-segment of about 700 bases, existing in the nontranscribed spacer of *Xenopus* rDNA (ref). It is believed that

the usefulness of this technique is not confined to the sequencing of repetitive DNA, but it may also be of use when the sequencing of large DNA-fragments is undertaken or when an overlapping set of DNA-fragments is required from in vitro analyses of transcription or replication.

The sequence of 800 nucleotides of *Xenopus laevis* DNA containing 2 $tRNA_I^{met}$ genes

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800 bp of a cloned tRNA gene fragment from *X. laevis* have been completely sequenced. This DNA contains 2 $tRNA_I^{met}$ genes about 350 bp apart on the same strand and presumably sequences necessary for their correct transcription. Both genes contain the nucleotides predicted from the $tRNA_I^{met}$ sequence but not the triplet coding for the 3′-terminal C-C-A. Neither gene contains an insertion sequence. The spacers lack short repetitive sequences and they possess few common sequences except for the presence in the transcribed strand of an A-rich and then T-rich tract beyond the 3′-end, and a pyrimidine-rich tract about 140 bp away from the 5′-end of both genes. Comparison of these sequences with those flanking other genes transcribed by RNA polymerase III suggests that transcription occurs between these 2 regions. If correct, this implies a length for the initial $tRNA_I^{met}$ precursor of about 220 nucleotides.

Length heterogeneity in the terminal region of the vaccinia virus genome

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The terminal *Hind*III and *Sst*I restriction fragments of the DNA of uncloned vaccinia virus (VV), strain Elstree, migrate as diffused bands in gel-electrophoresis but form distinct bands after cloning of the virus. Cleavage of the DNA with *Sst*I, *Eco*RI, *Xho*I and *Kpn*I produces end-fragments of identical length of 7.4, 7.0, 5.3 and 4.3×10^6 daltons, respectively, from both ends of the genome. This data suggests that repeated sequences found by analysis of reassociation kinetics (L. J. Grady and E. Paoletti, Virology *79*, 337, 1977; G. Pedrali-Noy and A. Weissbach, J. Virol. *24*, 406, 1977) are located in the terminal regions of the VV-genome. Length heterogeneity in the vaccinia virus DNA-molecule population is possibly due to variability in the number of reiterated sequences in the terminal restriction fragments.

X-ray microanalysis of the wall of a phytopathogene ascomycete

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In an ultrastructural study of *Monilia fructigena,* elemental composition of the cell wall at different stages of development of this ascomycete was determined by X-ray microanalysis. These fungi were cultivated on malt-agar added with casamino-acid; preparations were freeze dried without wet chemical fixation in order to avoid any deplacement of diffusible ions. Hyphae, conidia and interhyphal-cement of sclerotiae were analyzed separately. P, K, S and Cl were found major constituents, whereas Ca and Mg were observed only as traces. The relative concentration of S/Cl is higher in the sclerotiae than in the hyphae and conidia, which is interpreted as revealing the presence of melanin-like pigments.

Genetic control of somatic sex determination in *Drosophila*

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In *Drosophila,* the genetic information of males (X;AA) and females (XX;AA) is qualitatively identical. The difference of XX vs X is purely quantitative, yet it leads to all the qualitative differences that exist between male and female. - A number of autosomal mutants change the sexual differentiation determined by the number of X-chromosomes. For example, the mutant *tra* produces males even if 2 X's are present, and *dsx* allows the differentiation of female genitalia in the presence of a single X. *tra* or dsx^D flies with 3 X's still develop male genitalia, showing that even 3 X's cannot restore femaleness. We therefore propose that male and female characteristics result from differences in the state of activity at a few genes which serve as control genes for sexual differentiation. This differential gene activity is established in an as yet unknown way by the difference between XX and X. Our observations suggest that the classical theory of gene balance in which X-chromosomes carry female and autosomes carry male determining genes, has to be modified.

Growth parameters of hemopoietic cells in vitro at the level of whole colonies

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Surface adhesion of hemopoietic mouse bone marrow cells in methyl-cellulose-containing media has been used for recovery of colonies as whole plaques. Morphological definition of cell types, their spatial relationships combined with cytokinetic parameters determined by in-situ autoradiography following exposure to ^3H-TdR, open new insights concerning their growth characteristics. Recovered colonies are essentially different from fibroblast plaque-forming units described by others.

Mouse Ss-protein: Molecular identification and characterization of the antigen

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The Ss-substance has been partially purified from EDTA plasma of adult DBA 2J male mice using chromatography on SP-sephadex and QAE-sephadex. The antigenically reactive material was radiolabeled with I-125. Upon reaction with rabbit anti-mouse Ss-serum a molecule with apparent mobility of 150,000 daltons in SDS-polyacrylamide gel-electrophoresis is specifically precipitated. This molecule is also specifically precipitated with goat antiserum against the purified human complement component C4, a characteristic property of the mouse Ss-substance (Meo et al., Proc. nat. Acad. Sci. USA *72*, 4536, 1975). The subunit composition of the precipitated molecule and its relationship to the mouse Slp-protein are currently investigated.

Appearance of vitellogenin mRNA in immature chick liver following primary and secondary stimulation by estradiol

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Vitellogenin mRNA present in total liver RNA was determined by hybridization with labeled DNA complementary to vitellogenin mRNA. In the liver of control chicks there are less than 9 molecules of vitellogenin mRNA per diploid genome. 6 h after primary stimulation vitellogenin mRNA increases at an initial rate of about 2 molecules per min per gene giving after 48 h a total of 7000 mRNA-molecules per diploid genome. 3 weeks later, when the concentration of vitellogenin mRNA had returned to the base line, secondary stimulation gave an immediate increase in vitellogenin mRNA sequences. A total of 14,000 mRNA-molecules per diploid genome was reached after 48 h.

Ornithine decarboxylase (ODC) induction: A characteristic biochemical response of target cells to nerve growth factor (NGF)

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NGF is a protein that is necessary for the normal development of sympathetic and sensory neurons. So far no common specific biochemical response is known to occur in both adrenergic and sensory neurons. A clonal cell-line (PC12) of rat pheochromocytoma which exhibits a high degree of differentiation and responds to NGF by a characteristic neuronal fibre outgrowth was used for corresponding studies. NGF elicits within 4 h a 50- to 80fold increase in ODC-activity, the rate-limiting enzyme in polyamine biosynthesis. The NGF-mediated increase in ODC-activity is a cyclic AMP-independent response, which can be abolished by cycloheximide and actinomycin D. The effect of NGF is specific, since other polypeptide hormones such as insulin or epidermal growth factor and proteins with similar physicochemical properties such as cytochrome C were ineffective. The fact that NGF also produces an increase in ODC in sympathetic and dorsal root ganglia suggests that ODC-induction is a specific biochemical response to NGF of the known target tissues.

External ATP, calcium and chemotaxis in the cellular slime mold *Dictyostelium discoideum*

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D. discoideum amoebae are positively chemotactic towards folic acid and cAMP. We propose that these substances bind to specific sites on the cell surface and increase the regional uptake of calcium into the cells. Calcium uptake leads to a localized contraction of microfilaments attached to the cytoplasmic surface of the plasma membrane and results in pseudopodia formation. Folic acid and cAMP may increase calcium uptake either: a) indirectly by inducing the release or extracellular synthesis of ATP which is utilized by a surface protein kinase to activate the calcium pump; b) directly by increasing the efficiency of the calcium pump. We present evidence in support of this hypothesis.

Structural and chemical differentiation within the epiplasm of the ciliated protozoan *Pseudomicrothorax dubius*

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The cytoplasmic surface of the cortex is lined by the epiplasm which contains attachment sites of basal bodies (the 'terminal plates') and trichocysts. Attachment sites can be selectively dissolved from the isolated epiplasm, permitting their identification by SDS-PAGE as 5–6 protein or mucopolysaccharide-positive bands of $35–75 \times 10^3$ daltons. The isolated epiplasm contains a protease active on the major, 80×10^3 - dalton structural protein of the epiplasm. This proteolytic activity is abolished following dissolution of the terminal plates. The potential significance of this protease for insertion of new basal bodies into the cortex will be discussed.

The M-line of chicken skeletal muscle

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The M-line traverses the myofibril in the centre of the A-band. According to models of Knappeis and Carlsen (1968) and Luther and Squire (1977) the M-line consists of longitudinal M-filaments and 1 or 2 transversal elements (M-bridges). One of the M-line proteins (M_r 88,000) was identified as MM-creatine kinase and immunofluorescence studies demonstrated a direct binding to the H-zone, where the M-line is located. A more detailed electron microscopic investigation on myofibrils incubated with monospecific anti-MM-CK IgG showed an exclusive binding of the antibody to the M-line structure. Fab'-fragments were prepared to achieve better antibody penetration and resolution of binding site. Incubation with Fab'-fragments resulted however in the removal of the M-line. Mainly M-CK was extracted, as demonstrated by gel-electrophoresis, immunreplication, indirect immunofluorescence and activity measurement. These results are further proof that M-CK contributes to the organization of the M-line.

mRNA for creatine kinases in differentiating myogenic cells from chicken

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RNA from myogenic cell cultures was used to prime a mRNA-dependent cell free protein synthesizing system from reticulocytes. Relative concentrations of mRNA for the creatine kinases M-CK and B-CK could be estimated by the amount of radioactivity incorporated into CK-peptides. Their authenticity was verified by: a) immunoprecipitability with specific antibody against CK; b) comigration of synthesized peptides with purified CK in SDS-gels; c) interaction of synthesized CK with purified CK to yield dimeric enzyme molecules. mRNA for CK was determined in polysomal RNA isolated from developing myogenic cell cultures. The relative concentrations of mRNA for CK was in accordance with CK-biosynthesis. The occurrence of nonpolysomal forms of mRNA for CK was investigated during early phases of differentiation. Experiments with BrdUrd demonstrated inhibition of accumulation of mRNA for M-CK but not for B-CK. Subculture into standard medium resulted in the reappearance of mRNA for M-CK.

Role of the host in the dissociation of the R-plasmid P111

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The transferable R-factor P111-ApCmSm and its derivatives P111-Ap, -ApSm, -ApCm, and -Sm have been characterized by electron microscopy and gel-electrophoresis, using different *E. coli* strains as hosts. Dissociation patterns are independent of the host; some of the derivatives were found only in a cointegrate form whereas others were also present in an aggregate form. A population of small molecules were found with the P111-ApCmSm, -ApSm and -Sm derivatives. In the *E. coli* K12 wild type host harbouring P111-ApCmSm and -ApSm, the small determinants have a mol.wt of 6.4×10^6 daltons. However, in 3 other different *E. coli* strains, these molecules have a mol.wt of 9.6×10^6 daltons. P111-Sm shared the same mol.wt of 6.4×10^6 independent of the host. Transformation assays with the purified determinants showed that these molecules are nontransferable replicons, the larger ones determining ApSm-resistance and the smaller ones Sm-resistance. These results suggest that host functions could be implicated in plasmid dissociation phenomena. Genetic experiments are in progress to determine these functions.

Analysis of a variant histone DNA clone of the sea urchin

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Stage specific histone variants are found during sea urchin development. Histone DNA was isolated from a pool of *Psammechinus miliaris* sperm by restriction enzymes and cloning using a λ-vector phage. 80% of these clones show a similar restriction pattern (h22 type), whilst the other 20% differ strongly. One of these clones, h19, is 10% longer and has been investigated more accurately by hybridization, restriction mapping and sequencing. h19 DNA hybridizes poorly to labeled histone mRNA's prepared from cleavage stage. $^2/_3$ of the H2A gene are now sequenced near the C-terminal end. Comparison with the H2A sequence of the clone h22 shows 13% divergence, mainly in the 3rd position of the codons with only 1 amino acid exchanged. Sequencing of a part of the prelude region of H2A shows that 30 bp are identical in both clones 140 nucleotides away from the start of the H2A gene. Interestingly this shows that highly conservative sequences exist outside the coding region of the gene.

Culture of cereal protoplasts

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Isolated protoplasts are promising experimental systems for studying genetic modification of plant cells. One major prerequisite is their culture and their regeneration to plants. Although this is possible for other plant species, this has not been achieved for a single cereal. We have now shown for the first time that protoplasts from intact corn *(Zea mays)* plants can undergo sustained divisions (Potrykus et al., MGG *156*, 347, 1977). From these protoplast derived cultures rapidly dividing suspension cultures have been established. The optimized culture conditions will be presented. Protoplasts isolated from these cultures again divide and yield fast growing cultures. The techniques used to optimize division response together with the optimized culture conditions will be presented. This corn protoplast system is now being used for combining genetic information of several cereal species with that of corn.

Identification of bacterial strains by restriction endonuclease digestion of their DNA

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Due to the so far absolute sequence specificity of restriction endonuclease, any uniform population of DNA-molecules will give rise to a unique set of fragments upon treatment with these enzymes. Therefore, bacterial clones can be characterized and identified by isolating the total bacterial DNA and treating it with one or several restriction endonucleases. Bacteria for which only few taxonomic criteria exist, as in the case of several thermophilic bacilli, have been rapidly characterized by first determining their restriction pattern and then comparising the pattern to already characterized bacterial strains.

Fusion and differentiation of chick myogenic cells in suspension

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When cultured in serum-free LH medium (which contains Mg^{2+} and Ca^{2+}) chick myogenic cells detach from the dish and remain viable for at least 2 weeks. In LH supplemented with horse serum (HS) or cell attachment protein (CAP) from HS, the suspended cells attach to a gelatinized substrate and differentiate into contractile myotubes. HS treated with antibodies against CAP does not mediate cell-substratum attachment. In LH or in LH supplemented with anti-CAP (which also precipitates chicken fibronectin), the suspended cells aggregate and fuse, yielding round multinucleate cells. This suggests that neither exogenous nor endogenous CAP-like proteins are required for cell-cell adhesion and fusion. The ionic requirements are like those for attached cells: with Ca^{2+} as sole divalent cation the fused cells are not viable; with Mg^{2+} alone the cells aggregate but do not fuse. Muscle differentiation proceeds in nonattached cells: they contain MM-creatine kinase, as well as myofibrils detectable in electron micrographs.

Isolation and characterization of cell lines derived from pyrimidine auxotrophic 'rudimentary' mutants of *Drosophila melanogaster*

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Mutations in the *rudimentary* (r) locus of *Drosophila melanogaster* lead to abnormal wings, female sterility and pyrimidine auxotrophy. The mutant phenotype is the result of enzyme deficiencies in the pyrimidine biosynthetic pathway. In r-mutants the first 3 enzymes of pyrimidine biosynthesis have been shown to be affected: Carbamylphosphate synthetase (CPSase), aspartate transcarbamylase (ATCase), dihydroorotase (DHOase). 5 independent cell lines have been established from embryos homozygous for the allele r^1 (DHOase deficient) and 1 line was obtained from embryos homozygous for the allele r^{36} (ATCase deficient). Cultures were initiated from 4- to 20-h-old embryos in Shield's and Sang's medium. A pyrimidine depleted modification of the culture medium has been developed in order to investigate the pyrimidine dependence of the 2 mutant cell types in vitro.

Daily rhythm of protein synthesis in rat visual cells

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The rhythmic occurrence of 2 degradative pathways in rat visual cells has been recently demonstrated. LaVail (Science *194*, 1976) has shown that the shedding of packets of disks from rat visual cell outer segments followed a circadian rhythm. Remé et al. (Albrecht v. Graefes Arch. Ophthal. *203*, 1977) have demonstrated that autophagy, the intracellular degradation of the cell's own organelles, followed a daily rhythm in rat visual cell inner segments. We have now evidence that the uptake of labeled protein precursor into rat visual cell inner segments follows a 24-h-rhythm as well. Rats were exposed for 2 weeks to a constant light-dark-cycle. 1 h before sacrifice of the animals, radioactive leucine was injected intravenously. The rats were killed at 7, 12, 17, 21 and 1 h. 5 animals at each time point were sacrificed in 3 series of experiments. Quantitative analysis of the material indicated that the uptake of radioactive leucine into visual cell inner segments was significantly higher during the dark phase than during the light phase. It appeared that an overall protein synthesis in rat visual cell inner segments took place at night, whereas degradation occurred at day time. These data indicate a metabolic balance between mainly synthetic and mainly degradative activities in rat visual cells within a 24-h-period.

Localization of polyoma tumor-antigen related polypeptides in subcellular fractions

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In lytic infection of mouse kidney cells and in polyoma-transformed rat cells 3 polypeptides are regularly complexed by anti polyoma T-serum. In polyacrylamide gel-electrophoresis they migrate at 98 K ('large T'), 57 K and 23 K ('small t'). To learn more about their mode of formation and biological function we subfractionated infected and transformed cells into a nuclear, a cytoplasmic and a crude membrane fraction using hypotonic buffers (pH 6 and pH 7.4) without detergent. As was already observed earlier by immunofluorescence assays, the bulk of large T (98 K) is present in the nucleus, while small t (23 K) is mainly detected in the cytoplasmic fraction. In fact, small t can be preferentially extracted without disruption of the cells by washing the cultures with hypotonic buffer (pH 6) containing 0.1% NP 40. The 57 K polypeptide seems to be associated with membranes, although part of it was also found in the nuclear and the cytoplasmic fraction. We are currently trying to detect to 57 K polypeptide in a cleaner plasma membrane preparation obtained with a polyethylene-glycol-dextrane 2-phase-system.

Superoxide dismutase activity in Friend cells and hepatoma cells

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Cells with a high hemoglobin or cytochrome content, such as erythrocytes or liver cells, display elevated levels of superoxide dismutase (SOD). We examined the activity of this enzyme in Friend erythroleukemia cells and 1 mouse hepatoma cell line to see whether SOD could be added to the list of specialized functions that these cells maintain in vitro and, thus, further confirm the suitability of using these transformed models for studying cell differentiation. We found that the hepatoma line had an activity corresponding to 5.3 µg SOD per mg protein of crude extract, while 7 near-diploid Friend cell lines had activities ranging from 2.6 to 5.6 µg SOD per mg. 1 near-diploid Friend line had no detectable activity and 3 highly heteroploid lines had lower activities ranging from 0.2 to 1.9 µg SOD per mg. Control cell lines, including mouse transformed fibroblasts, myeloma cells, and Friend virus-producing, but non-erythroid, cells had either undetectable or low activities (at most 1.5 µg SOD per mg). While somatic hybrids between Friend cells and hepatoma cells retained, as expected, a high level of SOD expression, hybrids between a high activity Friend cell line and transformed fibroblasts, which were known to have lost expression of erythroid functions, showed extinction of the enzyme as well.

Isolation of an unstable R'1ac derivative of the antibiotic resistance factor R100-1

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It was found that cultures of an R'1ac (pAR1) host strains rapidly accumulate lac^- segregants. Chemostat experiments and fluctuation tests showed that this is the result of the selection of lac^- derivatives of pAR1 (deletions of varying parts of the episome adjacent to the 1ac insertion) and also complete loss of the episome which occurs at a higher level than in R100-1 host strains. In some strains, UV-irradiation increases the instability of pAR1. Measurements of the copy number of pAR1 (DNA-DNA hybridization on filters) suggest that the loss of pAR1 is more likely due to a partition defect of the episomes in daughter cells than to a replication defect. Induction of the lac operon of pAR1 results in a greatly reduced ability of the cells to form colonies on MacConkey lac plates. This allows the selection of lac deletion derivatives, particularly RTF-TCs which have been shown to originate from the loss of the r-determinant of R100-1. The lac insertion is very close to an IS1 which is one of the boundaries of the r-determinant.

Gene localization on the chloroplast DNA of *Chlamydomonas*

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A physical map of the chloroplast DNA of *Chlamydomonas* has been constructed. The map is circular and corresponds to a genome size of 126×10^6 daltons. The map was established by comparing the double digests of individual DNA-restriction fragments and by hybridizing knick-translated fragments to digests of chloroplast DNA obtained with the restriction endonucleases EcoRI, BamI, BglII and SalI. The genes coding for the chloroplast ribosomal RNAs are repeated twice and they are located on opposite sides on the map in an inverted orientation relative to each other. The order of the ribosomal RNA genes is 16S, 2S, 23S and 5S. The ribosomal RNAs are all transcribed from the same strand. The 2S RNA originates from the large subunit of the 70S ribosome and its gene is located close or adjacent to the 5'-end of the 23S RNA gene. Hybridization of ^{32}P-labeled 4S RNA to EcoRI digested chloroplast DNA according to the Southern procedure shows that at least 10 EcoRI-fragments hybridize to 4S RNA and that the 4S RNA genes are intersperced throughout the chloroplast genome.

Effects of adenosine-3′ 5′-phosphorothioate (cAMP-S) on the development of *Dictyostelium discoideum*

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CAMP-S is an agonist of the cyclic-AMP receptors on the cell surface which are involved in chemotaxis and in the control of development of *D. discoideum* (Gerisch and Malchow, Adv. Cyclic Nucleotide Res. 7, 49, 1976). Because of its slow hydrolysis it can be used in studies on the role of cAMP during the life cycle. In agar plate cultures, development of the strain Ax-2 is blocked prior to the aggregation stage by 5×10^{-7} M cAMP-S. The analogue can be used also to select cAMP-S resistant mutants with disorders in the expression of biochemical markers of development. Studies using washed cells in liquid suspension have shown that cell differentiation from the growth phase to the aggregation competent stage as well as the aggregation process are sensitive to cAMP-S. The analogue strongly stimulates extracellular phosphodiesterase activity and inhibits the production of the inhibitor of this enzyme. Cellular, membrane-bound phosphodiesterase is not stimulated. cAMP-S inhibits only slightly the development of the cell adhesion system.

Isolation of epithelial cells from the urinary bladder of the toad

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The bladder epithelium is composed of 5 cell types whose physiological role in the hormone-dependent transport of electrolytes has not been determined with certainty. Receptor binding assays on homogeneous cell populations should provide a first attempt to correlate function with a given cell type. The epithelium was dissociated by collagenase and EGTA treatment and recoveries were based on cell counts and DNA content. From 1 g of fresh tissue $54 \pm 5 \times 10^6$ single cells were recovered. Fractionation by isopycnic centrifugation yielded 4 fractions: a) very light ($\bar{\rho}$ 1.025) composed of 30% mitochondria-rich cells (MR) and 50% filament-rich cells (F), b) light ($\bar{\rho} = 1.045$): 75% vacuolated granular cells (G), 12% MR. 19% F, c) havey ($\bar{\rho} = 1.065$): 82% G, d) pellet ($\bar{\rho} = 1.085$): erythrocytes, 18% goblet cells and 26% basal cells. Viability of the cells was assessed by trypan blue exclusion (>95%), reaggregation ability including junctional complex formation, steady level of oxygen consumption and physiological monovalent cation distribution.

Viral nucleoprotein complex in cells infected by Herpes virus

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50% of Herpes DNA in infected cell nuclei remains of high mol.wt after Staphilococcal nuclease digestion, while host chromatin is digested into small oligonucleosomes. SDS polyacrylamide gel analysis of acid extracted proteins from chromatin of HSV-1 infected cells shows the presence of 1 capsid protein and of 3 main virus induced polypeptides which are not constituents of the virion. These results suggest that the intranuclear viral DNA may differ from cellular DNA in its association with proteins.

Early and late viral proteins synthesized in *Xenopus* oocytes injected with SV40 DNA

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Purified SV40 DNA injected into the nucleus of *Xenopus* oocytes, is transcribed and translated into virus specific proteins. All viral genes are expressed resulting in the formation of both small and large tumour antigens as well as capsid protein. The proteins induced by the injection of DNA were characterized by immunoprecipitation and electrophoresis on SDS-gels. They were further identified by the analysis of their tryptic digests. Since the sequences coding for the large tumour antigen are split in the SV40-genome, the formation of T-antigen implies correct splicing of its mRNA in the oocyte.

Localization of RNA-polymerase B and histones on the Y-chromosome of *Drosophila hydei*

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In primary spermatocytes of *Drosophila hydei*, the Y-chromosome is organized in loop-like structures. This allows us to study the distribution of RNA-polymerase and histones in morphologically distinct sections along the axis of a single chromosome. Indirect immunofluorescence microscopy using antibodies directed against RNA-polymerase B, histone H1 of *Drosophila melanogaster*, and H2B and H3 of calf thymus, revealed the simultaneous presence of both, RNA-polymerase B and histones, on the main loop structures, whereas the nucleolus does not stain for RNA-polymerase B. Various mutants deficient for some of the loops were used to identify the individual chromosomal structures which were stained in wildtype nuclei. The results suggest that, in *Drosophila hydei*, all loops of the Y-chromosome are transcribed by RNA-polymerase B.

In vitro synthesis of polyoma tumor antigens

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In polyoma virus infected mouse kidney cells (lytic infection) and in polyoma-transformed rat cells, 3 polypeptides related to polyoma tumor (T)-antigen are regularly observed in SDS-polyacrylamide gel-electrophoresis: 98 K (large T), 57 K and 23 K (small t). To investigate whether the smaller polypeptides (57 K and 23 K) might be post-translational cleavage products, we translated polyadenylated RNAs extracted from the cytoplasm of polyoma-infected mouse kidney cells or transformed rat cells in a mRNA-dependent translation system derived from rabbit reticulocytes. We found that all 3 polypeptides are synthesized in vitro and that they have the same electrophoretic mobility as the T-antigens synthesized in infected or transformed cells. While large T-antigen and small t-antigen are probably translated from differently spliced mRNAs (M. Fried, personal communication), the mechanism of formation of 57 K-polypeptide which shares common tryptic peptides with large T and small t is not known yet.

Isolation of a minichromosome containing the ribosomal genes from *Physarum polycephalum*

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The ribosomal genes of *Physarum* are located on linear DNA-molecules of 38×10^6 daltons (rDNA) present in each nucleolus in 100–200 copies. These molecules have now been solubilized from nucleoli as active transcription complexes. This nucleolar chromatin sediments in sucrose gradients as a homogeneous fraction with a sedimentation coefficient of 150 S, containing rDNA as major DNA-component and RNA-polymerase I, but not II. Buoyant density analysis indicates the presence of significant amounts of protein associated with rDNA in addition to RNA-polymerase. RNA synthetized in vitro by the endogenous RNA-polymerase is complementary to rDNA and is transcribed mainly from the sequences coding for 19 S and 26 S ribosomal RNA.

Study of serotype transformation in *Paramecium primaurelia*

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Simultaneous transformation of 90% of the cells in a culture of *P. primaurelia* was induced from serotype G (at 20 °C) to serotype D (at 30 °C) or vice-versa after a 7-h-incubation in the specific antibody ($1/_{20}$ of the immobilizing concentration) or with both the antibody and puromycin. SDS + ME PAGE of the i-AG of the 2 serotypes shows a major band (mol.wt 260,000 daltons) which is common to both and one supplementary band with a slightly heavier mol.wt in serotype G only. This heavier band dissappears if cells are transformed from G to D. Immunodiffusion tests have confirmed that a distinct protein exists in the serotype G. Changing from type D to type G may involve synthesis of a new protein, a hypothesis we are attemptive to confirm. Cells incubated in specific antiserum are immobilized after 2 h but they recover their mobility after 36 h. Following this treatment, they are resistant to the antiserum. This may be explained by a defense mechanism similar to that of Tetrahymena. We are performing tests to check this hypothesis.

Proteolytic activity, lactic acid production, N-acetyl-D-glucosamine fermentation and plasmids in *Lactobacillus helveticus* subsp. *jugurti*

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2 strains of *L. helveticus* subsp. *jugurti* S13-8 and S36-2 a low and high lactic acid producer, respectively, originally isolated from Parmesan cheese were studied as a model system, to determine why certain strains were capable of producing more lactic acid than others. Following acridine treatment of strain S36-2, approx. 60% of isolates had lost N-acetyl-D-glucosamine fermenting ability. Upon culturing in milk these isolates exhibited reduced proteolytic activity and as strain S13-8 reduced lactic acid production. Following density gradient centrifugation and electronmicroscopy these isolates were found to have lost a single plasmid species of 13×17 kb. That there was no variation in the proportions of lactic acid (D or L) after curing, indicates that this plasmid probably contains a positive effector of the chromosomal lactic acid producing determinant which results in its increased production. 3 plasmid species were observed in strain S13-8, their function is so far undetermined.

Synthesis of histone messenger RNA during embryonic development of the sea urchin *Paracentrotus lividus*

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Pulse labeled high mol.wt RNA from sea urchin early blastulae was denatured in 99% formamide gradients. Hybridization of the RNA to histone DNA of *Psammechinus miliaris* shows the existence of high mol.wt RNA containing histone messenger. Denatured hn RNA of approximately 2×10^6 daltons was hybridized with the pure H4 gene of *Psammechinus miliaris*. A similar hybridization was carried out between total histone mRNA and H4 DNA. The RNA from both hybrids was eluted and hybridized to the other genes. The eluted hn RNA hybridizes with the 5 histone genes whereas the eluted mRNA only with H4. This is consistant with the existence in sea urchin of high mol.wt RNA which might be a precursor of hs mRNA.

Prolactin receptors on dispersed cells from rabbit mammary gland

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Receptor sites for lactogenic hormones on dispersed mammary cells obtained from virgin, pregnant or lactating rabbits has been established by binding studies and autoradiography. Homogeneous epithelial cell populations were obtained by isopycnic centrifugation after enzyme digestion. Association and dissociation of ^{125}I-oPRL or ^{125}I-hGH are time- and temperature-dependent. The binding sites involved exhibit complete specificity for PRL. At 23 °C, equilibrium is reached by 30 h and 4 °C by 120 h (resversible second order kinetics). This slow rate of association is similar for plasma membrane receptor preparations obtained from fresh tissue but is in contrast to the much faster binding of ^{125}I insulin. Scatchard plots suggest a single class of binding sites (Ka: 10^{10} M^{-1}) with ~ 1750 PRL sites per cell in mature virgin, ~ 600 in midpregnant and ~ 1900 in lactating animals. Half receptor sites are located on the plasma membrane and half are intracellular as evidenced by EM-autoradiography.

Denervation of the posterior pituitary by implantation under the kidney capsule

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The pituicytes are the glial cells of the posterior pituitary. In order to get some proof of their so far unknown function we have tried to separate them from the neurosecretory nerve terminals, which come down from the hypothalamus. One possible approach is to let the neurosecretory axons degenerate, after dissection of the gland. Therefore, posterior pituitaries, either from normal or from Brattleboro rats, were implanted under the kidney capsule of another rat. After 4, 8 or 15 days the rats were decapitated and the implanted pituitaries were taken out. EM thin sections were

checked for the presence of neurosecretory granules. The amounts of vasopressin and oxytocin were measured by radioimmunoassay and fluorescamine assay (Gruber et al., Proc. nat. Acad. Sci. *73*, 1314, 1976). The decreasing neurophysin content was followed by polyacrylamide gel-electrophoresis. After about 6 days it seems that almost all nerve terminals with their neurosecretory granules have disappeared.

Membrane channel formation by the matrix protein from *E. coli*

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A novel approach of incorporating proteins into bimolecular lipid membranes (BLM) has been developed. Vesicles consisting of lipids and proteins in defined ratios are used to prepare monolayers whose properties have been studied in a Langmuir balance. 2 monolayers, formed in separate chambers, are combined to give a bilayer. Incorporation of matrix protein into BLM reveals conductance changes which occur in steps. These are due to the formation of channels about 1 nm in diameter. Multiples of the smallest steps probably represent the simultaneous activation of different pore assemblies. The step height distribution is sensitive to the lipid, pH and voltage used; the number of open channels is roughly proportional to the protein concentration and strongly dependent on pH and voltage. Below pH 8, the equilibrium current voltage curve shows a sharp maximum. Pronounced inactivation occurs in small steps upon voltage jumps into the region of negative resistance. Above pH 9, voltage dependence is lost, and the conductance increases cooperatively by orders of magnitude. The size of the channels has been confirmed by radiotracer methods.

Target size analysis of adenylate cyclase

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By irradiation inactivation analysis (12 MeV electrons) the functional molecular size(s) of adenylate cyclase in liver plasma membranes has been measured in situ. Under noncyclase conditions (nontreated membranes), the enzyme displays 2 characteristic sizes, a large size (about 1.4×10^6) and a smaller size (3×10^5). Pretreatment of the enzyme under assay conditions with Gpp(NH)p or GTP leads to 'dispersion' of the large size to the small size of the enzyme system. Pretreatment with glucagon (+GTP) also leads to dispersion but to a distinctly bigger size (5×10^5). The glucagon receptor in its unoccupied state has a mol.wt of 6×10^5, which is higher than that of the enzyme system activated by the nucleotides and/or hormone. The data suggests an important role for aggregation and dispersion mechanisms in the regulation of adenylate cyclase activity by hormones and guanine nucleotides.

Localization of $tRNA_4^{Glu}$ genes from *Drosophila melanogaster* by in situ hybridization

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$tRNA_4^{Glu}$ was isolated from *Drosophila melanogaster* by means of anticodon-anticodon affinity chromatography. The $tRNA_4^{Glu}$ was further purified on a RPC-5 column, iodinated in vitro with ^{125}I and hybridized in situ to salivary gland chromosomes of the mutant giant (gt, 1-0.9). The slides were coated with Kodak NTB emulsion and exposed for 55 days at 4 °C. Label was found on the 3 regions 52 F, 56 EF and 62 A. Competition with a 300fold excess of cold 5s rRNA does not reduce the number of grains at 56 EF, the site of the 5s rRNA genes. The label at this region is therefore not due to contaminating 5s rRNA. Hence we conclude, that the genes for $tRNA_4^{Glu}$ are localized in the regions 52 F, 56 EF and 62 A.

Peptide mapping of large and small SV 40 T-antigens

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The A-gene of SV 40 specifies large T-antigen (mol.wt 88,000) and small T-antigen (mol.wt 19,000). A method to isolate large T-antigen from extracts of SV 40-infected CV-1 cells by immunoaffinity chromatography on staphylococcal protein A-sepharose has been described previously (M. Schwyzer, Coll. INSERM, in press, 1977). We now show that the method also yields small T-antigen from the same extracts. To investigate the structural relationship between large and small T-antigen, tryptic peptide maps of the proteins - labeled with (^{35}S)methionine, (^3H)arginine, (^3H)leucine, or (^3H)tyrosine - were prepared according to M. Lichaa and E. Niesor, Coll. INSERM, in press, 1977. Part but not all of the peptides from small T-antigen correspond to peptides from large T-antigen with regard to chromatographic mobility and distribution of label. Thus, small T-antigen seems to share part of its primary structure with large T-antigen.

Subcellular distribution of plasminogen activator in human fibrosarcoma cells

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Enhanced production of plasminogen activator (PA) is a characteristic of many transformed cells. The subcellular distribution of PA in cell line 1080A derived from a human fibrosarcoma was studied by means of rate sedimentation and isopycnic equilibration. In both systems PA was resolved from lysosomal N-acetyl-β-glucosaminidase, from catalase and from α-naphthyl acetate esterase. It showed similar but not identical distribution as 2 plasma membrane markers, alkaline phosphodiesterase and leucyl-β-naphthylamidase. Resolution between PA and these 2 markers was further demonstrated by a difference in the equilibrium density shifting which is induced by treatment of the particles with digitonin. These results suggest that PA is associated with specialized segments of the plasma membrane or with cytoplasmic carrier vesicles.

Energy barriers and surface potentials of asymmetric lipid bilayers

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A simple method for the determination of asymmetry of energy barriers in lipid bilayers is described. The method is based on the dependence of bilayer capacitance (C_m) on transmembrane voltage. The capacitance was measured at 1000 Hz by rectifying the 90° component of the current to give a direct measurement of C_m. A superimposed triangular wave (100 mv/s) results in a hysteresis-like time-course of capacitance. The centre of the hysteresis figure is shifted along the voltage axis by an amount related to the differ-

ence of the dipole and/or surface-charge potentials of the 2 sides of the bilayer. The results with this capacity dependent method for asymmetrically shielded phosphatidyl serine bilayers were compared with a) the value predicted by the Gouy-Chapman equation and b) the value from the current-voltage curve (nonactin-K^+ as permeant) using a theoretical expression derived from the integrated Nernst-Planck equation for a trapezoidal energy barrier. All 3 approaches gave consistent results.

Rabbit poxvirus stimulates 2-deoxy-D-glucose uptake by HeLa cells

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A significant enhancement of the uptake of 2-deoxy-D-[1-^{3}H]glucose (2-DG) by HeLa cell monolayers can be observed as early as 0.5 h post-infection (p.i.) with rabbit poxvirus and at all subsequent time points investigated (until 12 h p.i.). Time course experiments performed at 3 h p.i. show a similarity in the uptake by infected and mock-infected monolayers with higher values of the former throughout the reaction. The enhancement is observed over a wide range of 2-DG concentrations (5×10^{-2} to 2.5×10^{-7} M). It seems from double-reciprocal plots that both V_{max} and K_m-values are altered in infected cultures. As both heat- and UV-inactivated virus fail to stimulate 2-DG uptake, it is suggested that a functional viral genome is necessary for inducing the described change.

Interconversion of 2 forms of AMV reverse transcriptase separated by poly(rC)-CL-sepharose

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Reverse transcriptase from avian myeloblastosis virus is separated into 2 forms by chromatography on poly(rC)-CL-sepharose: a low affinity (I, eluting at ~ 0.15 M KCl) and a high affinity (II, ~ 0.32 M KCl) peak of activity. Following SDS-PAGE both peaks show the 2 characteristic protein subunits: α and β; enzyme preprarations containing only subunit α or dissociated with DMSO lead to 2 peaks as well. Rechromatography of peak I yields both forms, whereas peak II gives form II only. Such interconversion of form I into form II can also be induced by treatment of the enzyme with micrococcal nuclease (10 γ/ml) or by addition of Mg^{2+} (10 mM) or spermidine (10 mM) to the column buffer. The hypothesis is advanced that binding of enzyme to nucleic acids may be the structural basis for the separation of these 2 forms (e.g. peak I bound, peak II not bound) and is further tested. Since the murine viral enzymes tested (MoMuLV, FMuLV) yielded a single peak, this interconversion seems to be unique to avian reverse transcriptase.

Evidence for an in vitro differentiation of glial factor producing cells in rat brain primary cultures

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Rat brain primary cultures initiated at early developmental stages remain unable to produce glial factor-like activity even if kept up to 25 days. However, it becomes possible to detect this activity in secondary and tertiary cultures derived from such unproductive primary cultures. The amount of glial factor-like activity produced under these conditions is a function of the age of the primary culture at the initiation of the secondary culture. Primary cultures initiated at very early developmental stages require larger incubation periods in order to give rise to productive secondary and tertiary cultures. Since this in vitro incubation period correlates with the timing of the in vivo brain development, this system provides evidence for a parallelism between the in vitro and the in vivo cellular differentiation.

Tetanus toxin (TT) coupled to colloidal gold: EM evidence for selective binding and uptake by nerve terminals and retrograde axonal transport

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TT, like cholera toxin, nerve growth factor and certain lectins, is selectively and efficiently transported retrogradely by various neurons. Using colloidal gold coupled to TT we found TT selectively associated with cell membranes of nerve terminals in the rat iris. Subsequently the TT-gold complexes were taken up and transported retrogradely in smooth membrane compartments. In contrast, albumin-gold complexes were neither associated with nervous membranes nor taken up and transported. We conclude that binding to specific membrane components is the prerequisite for selective uptake and retrograde transport of TT.

Degradation of ribosomal genes by nucleases in *Physarum polycephalum*

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The genes coding for rRNA in *Physarum polycephalum* are extrachromosomal and located in the nucleolus. Incubation of nuclei with micrococcal nuclease leads to similar amounts of acid soluble digestion products for both ribosomal DNA and bulk chromosomal DNA. The sequences coding for 19S and 26S rRNA are not preferentially degraded by micrococcal nuclease as judged by hybridizations of nuclease fragmented DNA and unfragmented control DNA to rRNA. Micrococcal nuclease, however, cleaves rRNA coding sequences to fragments heterogenous in length and differing from those obtained with bulk chromatin. In addition, ribosomal genes are preferentially digested by pancreatic DNAse (DNAse I). This is also the case when the nuclei, which are used for the digestion studies are in mitosis and no RNA is being transcribed. Therefore ribosomal genes in *Physarum* behave like active genes in terms of their accessibility to nucleases.

A novel conformation of duplex DNA: form IV

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Under hybridization conditions, complementary circular single strands from plasmid PβG or bacteriophage PM2 DNA associate, giving rise to a duplex DNA, form IV, with an electrophoretic mobility between that of form I and denatured DNA. Form IV has less thermal hyperchromicity (about 22% in 1×SSC) than forms I and II. However, melting is noncooperative: absorbance increases gradually between 50 and 95 °C. In the electron microscope this DNA appears as highly folded duplex molecules indistinguishable from form I. Increasing concentrations of ethidium bromide lead to relaxation and recoiling of form I but not of form IV DNA. A large excess of ethidium bromide

reduces the electrophoretic mobility of form IV to that of form I. We postulate that form IV contains a similar (but not necessarily identical) number of right- and left-handed helices; a model such as that of Rodley et al. (Proc. nat. Acad. Sci. USA *73*, 2959, 1976), could account for our findings.

Relationship of cleavage with structural transformation in giant T4 capsids

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We have analyzed the surface lattices of giant pro-capsids derived from canavanine-treated cultures infected with T4.21⁻ and T4.17⁻ mutants. These particles exemplify respectively the 'uncleaved, unexpanded' and 'cleaved, unexpanded' states of the pro-capsid shell. The results correlate cleavage of gp23, prior to the following surface lattice expansion, with a subtle change in unit cell morphology. The most significant aspect is a change in the capsomer orientation angle from 9.5° to 3.5°. This change can be accounted for by the removal of 6 stain-excluding portions from the uncleaved hexamer. These portions may possibly represent the pieces of the gp23-molecules excised by the T4 prohead protease. Study of the rarely observed 'intermediate' giants, different zones of whose surface lattices are in different structural states, give clues as to the transformation dynamics. Both the cleavage pattern and the expansion process appear to be polar, initiating in 1 cap and propagating along the particles in sharp transition zones.

Migration and morphological changes induced in normal cells treated with purified extracts of conditioned medium from a transformed cell line

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Normal 3T3 cells grown in culture form a confluent, regular and nonoverlapping monolayer. When this monolayer is wounded, cells do not migrate into the wound. Tumor cells, or virally transformed cells, in contrast show less of such growth and migration restraints. We have found substances that elicit part of the transformed behaviour in normal cells in conditioned medium from SV28, a malignantly transformed hamster cell line. There are 2 distinct fractions that stimulate migration of 3T3 cells into a wound (migration factors β and γ). In addition to migration, MFγ has a marked effect on the morphological appearance of the monolayer. The effects of MFβ and MFγ and the differences between them are documented photographically and analyzed by statistical means. A comparison is made with the effects of known growth stimulating substances.

Selective killing of dividing plant cells in culture using nucleoside analogs

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To construct a method for auxotroph enrichment in plant cell cultures we are studying the effect of nucleoside analogs on a model system. Auxotrophy is simulated in *Rosa* cell cultures by nitrogen starvation of the wild-type strain. Nitrogen withdrawal stops division immediately but the resting cells remain viable for at least 14 days. Bromodeoxyuridine (BUdR) at concentrations above 5×10^{-6} M kills dividing cells without strongly affecting viability of nitrogen-starved cells. At 3×10^{-5} M BUdR the viability of dividing cells rapidly falls within 1 day, a time equal to the mean generation time of exponentially-dividing *Rosa* cells. From our experiments a theoretical enrichment factor of $\times 4000$ has been calculated for the resting cells. The effectiveness of BUdR in *Zea* cell cultures is now being investigated.

Stimulation of hydra head regeneration by Substance P

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To test a possible action of Substance P (SP) on hydra head regeneration a method slightly modified from that of H. C. Schaller (J. Embryol. exp. Morph. *29*, 27, 1973) was used. It was shown that SP, at concentrations ranging from 10^{-12} to 10^{-5} M, was able to stimulate hydra head regeneration. The measurement was performed by counting the number of regenerated tentacles and expressed in percentage of activation which rose from 33% (10^{-12} M SP) to 160% (10^{-5} M SP). The dose-response curve was established for 8 progressive concentrations of SP. The differences between stimulated hydrae and controls were significant ($p = 0.001$). These results indicate a possible role of SP in the nerve control over regeneration which might be complementary to that of catecholamines and cyclic AMP recently found in newt forelimb regeneration (Taban et al., Nature *271*, 470, 1978).

Macromolecular 35S-labeled secretion product of murine macrophages

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We have labeled peritoneal macrophages of mice (both before and after in vivo activation with thioglycollate broth) with Na_2SO_4 (^{35}S). The isotope is incorporated into material which is excluded from sephadex G-25 and fails to enter SDS-gels known to allow entry of proteins of mol. wt beyond 200,000. This material is therefore provisionally considered to be a proteoglycan. After pulse-labeling for 1 h an efficient discharge of the material can be demonstrated; the kinetics of this secretion are similar to the kinetics of secretion of macrophage secretory *proteins* labeled with ^{3}H-leucine. The mol. wt of the secreted material has been estimated at 25,000 by gel-filtration. When the secretory product is reincubated with macrophages (but not with cell supernatants derived from macrophage cultures) an appreciable portion is degraded to low mol. wt products, presumably following upon pinocytosis or phagocytosis.

The ionic strength dependence of chromatin folding

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The fixation of chromatin by glutaraldehyde was standardized and the BAC spreading method (Vollenweider et al., P.N.A.S. USA *72*, 83, 1975) was modified with the aim of reducing structural changes due to adsorption of the chromatin to the carbon support films. Chromatin samples were fixed in different buffer solutions with ionic strengths up to about 100 mM. At very low ionic strength chromatin appeared in the electron microscope as a filament with a diameter of single nucleosomes comparable to the 100 Å nucleofilament (Finch and Klug, P.N.A.S. USA *73*, 1897, 1976). At increasing ionic strength a folding of this fibre can be observed. One typical appearance occurred at around 10 mM NaCl with a fibre diameter of about 250 Å

similar to our earlier findings (Thoma and Koller, Cell *12*, 101, 1977). At around 100 mM NaCl solenoid-like chromatin forms were observed with a diameter of about 300 Å comparable to a model proposed by Finch and Klug, P.N.A.S. USA *73*, 1897, 1976.

Problems in plant regeneration from cultured cells of crop plants

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The successful regeneration of plants from single cells is a necessary prerequisite for the successful genetic modification of plants. Such regeneration can be achieved in *Brassica napus* through the use of leaf protoplasts and stem embryogenesis, but the techniques are not always reproducible. In graminaceous crop plants e.g. *Sorghum bicolor* and *Zea mays* plant regeneration can only be achieved from complex explants such as proliferating immature embryos. No success has yet been obtained in inducing plant regeneration from protoplast-derived cultures from more mature parts of these species. Are existing cell lines so genetically abnormal that they never regenerate plants or are cells of mature plant parts completely nontotipotent as a result of normal differentiation processes? Alternatively, there may be growth compounds as yet unknown which may be required for plant regeneration from more recalcitrant species.

Composition of xylem sap and leaf nitrate reductase activity in beans

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14-day-old, NO_3^- grown plants were transferred to N-free, NH_4^+, NH_4NO_3 and NO_3^- nutrient solutions (total N: 3.55 mM) and after 40 h xylem sap and leaf N-compounds were measured. The total sap N-content (Kjeldahl-N + NO_3^--N) of N-starved plants decreased by approx. 50%, remained constant with NH_4^+- or NO_3^--treatment, and increased in NH_4NO_3-treated plants. Compared with the sap from 14-day-old plants, sap from N-starved plants contained 39% less Kjeldahl-N and 72% less NO_3^--N. With NH_4^+, Kjeldahl-N increased by 83% whilst NO_3^--N decreased by 65%. There was no change in the relative composition of the sap from NO_3^--treated plants but with NH_4NO_3 treatment, Kjeldahl-N increased by 68% whilst NO_3^--N decreased by 31%.

Modification of polyoma tumor antigen during lytic infection

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Polyoma tumor (T)-antigen is the product of the early viral gene and is thought to be a plurifunctional protein. The main band of polyoma T-antigen present early in lytic infection (mouse kidney cells) and in transformed cells, migrates in gel-electrophoresis at 98 K. In lytic infection, after induction of cellular and viral DNA-replication, a second slightly slower moving band (101 K) is observed. Our results obtained in time course experiments at 37 °C and 27 °C, in temperature shift experiments and with inhibitors of protein synthesis suggest that 98 K T-antigen is modified by the host cell to 101 K T-antigen. We propose that 98 K T-antigen is capable of inducing the mitogenic response of the host cell (S-phase) and that the formation of 101 K T-antigen by the permissive host cell is required for initiation of viral DNA-replication. In addition to the main band of T-antigen, 2 smaller polypeptides, 57 K and 23 K (small t) are regularly observed. Analyses of the ^{35}S-methionine-labeled tryptic peptides of 98 K, 57 K and 23 K polyoma T-antigens will be presented.

Characterization of histone mRNA and histone genes in mouse

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Histone mRNA isolated from a synchronized culture of L-cells was characterized by translation in a wheat germ cell-free system and cross-hybridized with histone DNA of sea urchin. The single mRNAs were identified by analysis of their product of translation. A pure histone mRNA-fraction, obtained by hybridization with sea urchin histone DNA, was labeled with the Kinase reaction at the 5′-end. This was used as a high specific activity probe, for the detection of the mouse histone genes. EcoRI restriction of total mouse DNA followed by gel-electrophoresis, Southern transfer to millipore filter and hybridization with hs mRNA, shows a complex pattern of restriction with a prominent band approximately 6.5 kb long. In collaboration with Dr Steinmetz (Munich) we are now isolating from embryonic mouse DNA an enriched histone DNA-fraction containing the 6.5-kb-fragment which we intend to clone under P3EK2-conditions. The enrichment is obtained by RPC-column chromatography and preparative gel-electrophoresis.

Histones in ageing mouse liver

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Mouse liver nuclei from F_1-hybrids (C57B1 ♀ × A ♂) of ages in months, 1.75, 3.5, 6, 11, 18, 28 and 33, were edtracted with H_2SO_4 to isolate histones. Throughout the life-span, the ratio histone:DNA remained constant. Histones were characterized by electrophoresis either in one-dimensional polyacrylamide gels containing sodium dodecyl sulfate (SDS), or acetic acid-urea, or triton-X-100-acid-urea, or in 2dimensional gels using combinations of either acetic acid-urea or triton-X-100-acid-urea in the first dimension and SDS in the second. In acid-urea gels, we find 3 classes of H1 and of these the band of highest electrophoretic mobility increased in amount at 28 and 33 months. In triton-X-100-acid-urea gels, histone H3 migrates as 3 bands and of these, about 83% are in the most hydrophobic class migrating the fastest, while the slightly less-hydrophobic H3 constitute 14% at age 1.75 months and then decreases to about 5% by 11 months to remain constant thereafter. Thus, the tertiary structure of H3 changes by mid-age thereby increasing the number of hydrophobic groups accessible for histone:histone and histone:DNA interactions. Increased amount of highly charged H1 in late age also suggests increased chromatin inactivation.

Histones in lens epithelium, lens fibres, liver, brain and erythrocytes from chick embryos

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From the isolated nuclei of 19-day chick embryo lens epithelium, lens fibres, liver, brain and erythrocytes, histones were extracted with H_2SO_4 as well as $HClO_4$, and analyzed in polyacrylamide gels containing either sodium dodecyl sulfate (SDS), or acetic acid-urea, or triton-X-100-acid-urea. Histones were also analyzed by electrophoresis in 2 dimensions using either acid-urea or triton-X1100-acid-urea in the first dimension and SDS in the second. Excepting erythrocytes, all chick tissues examined contained different histones in the same proportions and these shown the same distribution regardless of the gel system used for the analysis. In all systems, we observed 2 bands for H1 and 1 each for histones H2B, H3 and H4; erythrocytes contain an additional band of H5-histone. In gels containing either SDS or acid-urea, H2A was present as a single band, while it was resolved into 2 separate classes of molecules in triton-X-100-acid-urea gels.

Localization of α-galactomannan in the yeast *Schizosaccharomyces pombe*

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Schizosaccharomyces pombe cells were saturated with the *Ricinus communis* lectin (RCA_I) specific for galactose. The lectin bound to galactomannan was then marked with gold granules (50 nm in size) labeled with a glactose-terminated glycoprotein (desialylated ceruloplasmin). When the cells were examined by scanning electron microscopy, galactomannan was found on the cell surface and at the ends beginning to grow but not on the wall established by division. Thin sections were marked by the same procedure using smaller granules (5 nm in size). When the sections were examined by transmission electron microscopy, galactomannan was found deposited in 2 layers in the cell wall, near the periphery of the wall and near the plasmalemma. The septum was also marked but mainly near the plasmalemma. Galactomannan was not found in the cytoplasm. These results confirmed that galactomannan is elaborated onto the outside of the wall during extension but not during septum formation in *S. pombe*.

Characterization of RNA synthesized by nuclei isolated from concanavalin-A-stimulated lymphocytes

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Mouse lymphocytes were stimulated with concanavalin A for 50 h. Nuclei were isolated with 0.25% NP-40 and purified by sucrose density gradient centrifugation. The incorporation of ^{3}H-UTP into TCA-precipitable material was determined and shown to be sensitive to RNase. RNA-synthesis was 50% inhibited by 1 μg/ml α-amanitin and over 90% by concentrations above 10 μg/ml, indicating primarily polymerase II-activity. With aurin tricarboxylic acid (ATA) a 2fold increase in RNA-synthesis is observed. The significance of this stimulation could be questioned, because of the known effect of ATA to induce nuclear swelling. However, this swelling is inhibited by Mg^{2+} at concentrations far below the concentration used in our system. Moreover, pulse-chase experiments showed that ATA inhibited the endogenous nucleases present in the nuclear preparation, indicating that the increasing RNA-synthesis is due to decreased degradation.

Heterogenicity of the epithelium at the bifurcations of the tracheo-bronchial tree: A scanning- and transmission electron microscopical study in *Macaca fascicularis*

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The distribution of the various cell types lining the tracheo-bronchial tree at the bifurcations was studied in the primate *Macaca fascicularis*. Crest-like projections at the bifurcations were found to display areas of variable extension, which lacked ciliated and secretory cells. They were covered by cells, the apical surface of which was enlarged by varying numbers of microvilli. In these areas, the pseudostratified tracheo-bronchial epithelium was found to be reduced to a simple squamous type which, occasionally, contained neutrophilic granulocytes. It is speculated that these bifurcational areas lacking ciliated cells might, on the one hand, facilitate airflow, on the other, however, represent sites were inhaled particulate matter is retained for prolonged periods of time.

Purification and characterization of DNA-polymerase-β from rat forebrain cortical neurons

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DNA-polymerase-β (E.C. 2.7.7.7) has been purified from 10-day-old rat cortical neurons. The purification procedure consisted of 5 successive chromatographic steps. These were performed on 2 columns of DEAE-cellulose followed by chromatographies on phosphocellulose, sephadex G-100 and double-stranded DNA-cellulose resulting in a highly purified preparation. The biochemical and physicochemical characteristics of the purified enzyme are in good agreement with those of DNA-polymerase-β from other tissues.

Site-directed mutagenesis in the β-globin DNA insert of plasmid PβG

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The principle of site-directed mutagenesis, previously applied to phage Qβ RNA (Flavell et al., J. molec. Biol. *89*, 255, 1974), was used to generate point transitions in a predetermined region of the β-globin DNA insert of plasmid PβG. The plasmid was nicked at the EcoRI site, which is located within the globin gene. Substrate-limited nick translation using DNA-polymerase I and N^4-hydroxy dCTP, dCTP, and dATP led to the replacement of dTMP by the nucleotide analog in the immediate vicinity of the nicks. The substituted DNA was amplified in vivo, treated with EcoRI and retransfected. 1.9% of the progeny clones contained EcoRI-resistant plasmid DNA. Sequence analysis on 6 EcoRI-resistant isolates revealed that 2 plasmids had 1, 3

had 2 and λ had 3 AT → GC transitions, all located within the substituted region. The codon changes are glu[121] to gly, and phe[122] to leu, pro or ser. No point mutations ($< 3 \times 10^{-3}$%) were found in control preparations.

Haploid production via anther and microspore culture

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It is possible, but still only with difficulty, to induce the formation of haploid plantlets from microspores cultured within anthers in a limited number of species including some of our crop plants, e.g. *Secale cereale* and *Brassica napus*. However, even in such successful cases problems arise such as low anther and microspore response, no response at all in other varieties of the same species and albinoism amongst regenerated plants. It is therefore necessary to learn more about the factors controlling androgenetic development. To this end experiments have been undertaken to culture isolated microspores in model systems such as *Nicotiana* and *Hyoscyamus*. In *Nicotiana tabacum* it has been shown that the yield can be increased using the isolated microspore technique instead of anther culture, whereas in *Hyoscyamus albus* to date the success is lower. Analysis of the essential factors may lead to more general application of this in vitro method even in our most recalcitrant crop plants.

Nuclear development of growing murine oocytes in vitro

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Oocytes from all the mammalian species studied sofar are able to resume meiosis from the dictyate stage to metaphase II in vitro when collected from preovulatory follicles and cultured in a suitable medium. In this study it was found that a certain number of oocytes can continue nuclear development in vitro also at different stages of their growth. Living cells were isolated by enzymatic digestion of small pieces of ovaries from infant mice aged 1, 2 or 3 weeks, and were rapidly collected by means of a micropipette. The position of the oocyte nuclei in the meiotic division was determined on the basis of chromosome preparations. In addition, morphological characteristics of the oocytes tested were analyzed by light or scanning and transmission electron microscopy.

Endogenous in vitro protein phosphorylation in nuclei isolated from differentiating *Dictyostelium discoideum* cells

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Nuclei isolated from various stages of the differentiation cycle of *D. discoideum* were incubated with (γ-^{32}P)ATP and the phosphorylated proteins detected by SDS-gel electrophoresis and autoradiography. Protein kinase activity was detected at all stages. The labeling pattern of nonhistone proteins (NHP) changed dramatically during differentiation. During the starvation stage ca. 25 NHP are phosphorylated. The number drops to ca. 10 phosphoproteins during aggregation and increases again to ca. 20 phosphoproteins following tip formation. 4 of the 8 histone bands are phosphorylated during differentiation. Bands 1a and 1b (H1) are phosphorylated during the early stages, bands 2b and 2c during the entire differentiation time and 3b first following aggregation. The endogenous protein kinases are not affected by cAMP or cGMP. The possibility of ^{32}P incorporation into nucleic acids was excluded by incubation with actinomycin D or RNase A and treatment of the gels with hot 5% TCA.

Terminal repetition in rabbitpox virus DNA

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Cross-hybridization of the terminal restriction fragments of rabbitpox virus (strain Utrecht) DNA has suggested the presence of homologous DNA-sequences at both ends of the genome. The precise location and size of these sequences was investigated by restriction enzyme analysis. The terminal restriction fragments produced by cleavage of the rabbitpox virus DNA with *Eco*RI (fragment $G = 4.75 \times 10^6$ daltons, fragment $H = 4.6 \times 10^6$ daltons) were isolated from agarose gels and the *Hinf* sites present in these fragments were located by the partial mapping procedure. Identical maps for approx. 12 *Hinf* sites, comprising a region of 3.4×10^6 daltons from each end of the rabbitpox virus genome were obtained. The symmetrical distribution of the *Hinf* sites shows that this terminal repetition is inverted.

Does the K^+-activity measured by microelectrodes in salivary gland nuclei of *Chironomus* change during development?

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To what extent ionic balances in the nuclear sap influence genetic activities is still open. One unsolved problem is: Does the nuclear ion-activity change under physiological conditions? We therefore measured the nuclear K^+-activity (a_K) in explanted salivary glands of *C. tentans* at 2 different developmental stages with K-sensitive microelectrodes. 2 nuclei per gland were impaled in succession, giving the following a_K-values of the first and second impalement, respectively (in mM): oligopausing larva (OL), 25±10 and 49±9; prepupa (PP), 64±15 and 62±14 (mean of 15 glands per stage ±99% confidence limits). Thus, a drastic change of a_K between OL and PP is found. The in vitro effect of successive impalements of OL glands needs further investigation. Electrodes: 5% valinomycin and 2% K-tetra-phenyl-p-chlorophenylborate dissolved in 25% 2,3-dimethyl-nitrobenzene and 68% dibutylsebacate as ion-selective liquid; slope: 58.4 mV; selectivity ($-\log K^{Pot}$): Na, 3.2; Mg, 5.0; Ca, 4.5; acetylcholine, 2.5.

Morphological and proliferative response of Schneider's *Drosophila* cell line 3 to ecdysterone

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Schneider's *Drosophila* cell line 3 (S3) was grown in a modified ZH medium (Wyss, J. Insect Physiol. *23*, 739, 1977) with 10% fetal calf serum and varying concentrations of ecdysone. Qualitatively the growth response of S3 is the same as that of a clone of Echalier's and Ohanessian's Kc-line, KcC7 (Wyss, Experientia *32*, 1272, 1976), but the sensitivity of S3 appears to be higher. At ecdysone concentrations above 1 ng/ml most cells detach from the plastic

substratum and become spindle shaped even in suspension. At early stages of this development colcemide (1 μg/ml) but not cytochalasin B (100 μg/ml) causes the cells to round up again. Later, colcemide has no effect on cell morphology and retraction of cell processes can only be achieved by enzymatic treatment with helicase or (crude) chitinase but not with trypsin, pronase, subtilisin, hyaluronidase or collagenase.

Quantitative studies of [^{125}I]-tetanus toxin binding in neuroblastoma cells in tissue culture

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We have previously demonstrated the binding of tetanus toxin in neuroblastoma cells using immunofluoroescent techniques (Zimmerman and Piffaretti, Naunyn-Schmiedebergs Arch. Pharmak. *296*, 271, 1977). Quantitative studies using [^{125}I]-tetanus toxin have confirmed and further characterized the binding. The specific binding in cultures induced to differentiate by depriving them of serum shows a very high affinity ($K_D = 10^{-14}$ M), whereas in control cultures in presence of serum $K_D = 10^{-13}$ M. Using glial cell conditioned medium, dibutyrylcyclic AMP and aminopterin as inducers the K_D-values are 10^{-11} M, 10^{-12} M and 10^{-12} M, respectively. Since very little is known about the exact mechanisms of these inducers, the changes in value of K_D possibly reflect the differences in the action of the respective agents. Part of the toxin bound is dependent upon sites sensitive to neuraminidase, and another part sensitive to β-galactosidase. Although neuroblastoma are tumor cells, these results have served as useful guidelines for further binding studies in other systems.

Influence of cyproheptadine on the elimination of myeloid bodies from cultured rat pancreatic islets

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β-cells of cultured rat pancreatic islets contain myeloid bodies. Ultrastructure and acid phosphatase (AP) cytochemistry suggest that they originate from GERL. Many such bodies seen extracellularly appear to be released by exocytosis. High numbers of myeloid bodies are seen in cultures exposed to 10^{-5} M cyproheptadine (CPH) for 8 days. Accumulation probably results from reduced elimination rather than increased production since both the number of extracellular myeloid bodies and the AP-activity of the medium are significantly decreased compared with controls. Concomitantly, intracellular Ca^{2+} is lowered and insulin secretion inhibited. The observation that CPH inhibits the exocytotic release of a variety of hormones by reducing Ca^{2+}-influx suggests that the elimination of myeloid bodies normally occurs by Ca^{2+}-dependent exocytosis (defecation). These CPH-effects are fully reversible when islets are cultured for a further 8 days in CPH-free medium.

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