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PHYSIOLOGIE – PHYSIOLOGY

Antidromic potential of the pituitary tract: Interaction with afferent stimuli

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Evoked potentials (EP) (typically 5–10 mV amplitude, 1 msec duration) were recorded in the neurohypophysial (NH) tract in response to electrical stimulation (biphasic, 1 msec, 1 mA) applied to the neurohypophysis of urethane-anaesthetized rats. EP increased with increasing stimulus strength and levelled off at ca. 0.5 mA. Repetitive stimuli (10–40 Hz, up to 120 sec duration) decreased the integrated EP (up to 15%/10 Hz) and increased the EP latency (up to 8%/10 Hz), but had little effect (± 5%/10 Hz) on the thresholds for antidromic activation of supraoptic units (0.5 mA max.). During repetitive stimulation at 2 Hz, the integrated EP decreased up to 30% and its latency increased up to 10% in response to nicotine (10–100 µg i.v.) and vaginal dilatation. Since the decreases in integrated EP were probably due to membrane depolarization and not to changes in fibre excitability, these data suggest that NH axons depolarize even during low frequency activity, and that the EP can therefore yield useful information on global NH activity in response to physiological and pharmacological stimuli.

Recruitement pattern of human muscle fibres in exercise

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Glycogen depletion and LDH-activity in isolated slow twitch and fast twitch muscle fibres in m. vastus lateralis of a trained long-distance runner were measured after different types of exercise. Needle biopsies were taken before and immediately after a 1-h continuous work period at 60% of $\dot{V}o_2max$ and again before and after 6 1-min-bouts at 150% of $\dot{V}o_2max$. After lyophiliziation at –30 °C and free-hand microdissection, a total of 186 individual muscle fibres were classified according to their ATPase activity. The resting glycogen content was equal in both fibre types. The continuous exercise produced a 25% decrease in glycogen content of the slow twitch fibres, but only a 8% glycogen utilization in the fast twitch ones. The intense intermittent exercise routine predominantly depleted the fast twitch fibres 50%, but the 32% decrease in glycogen content of the slow twitch fibres was also statistically significant. Average LDH-activity was 2.33×10^{-4} moles $\times g^{-1}$ min^{-1} in the fast twitch fibres compared with 0.86×10^{-4} moles $\times g^{-1} min^{-1}$ in the slow twitch ones.

Role of 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) in the renal handling of inorganic phosphate (Pi) in rats

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Thyroparathyroidectomy (TPTX) leads to an increase in the tubular capacity to reabsorb Pi and to a decrease in the conversion of 25-OHD_3 into 1,25-$(OH)_2D_3$. We have studied the influence of physiological doses of 1,25-$(OH)_2D_3$ on the renal handling of Pi in TPTX rats. The results show that 1,25-$(OH)_2D_3$ (26 pmoles/day i.p. for 7 days) abolishes the difference in the renal handling of Pi between sham-operated and TPTX rats fed a 1.2 g% Pi diet. Under the same conditions, 25-OHD_3 in doses of 26, 260 and 2600 pmoles/day shows no action. However, the administration of 26,000 pmoles/day of 25-OHD_3 appears to correct the renal handling of Pi in TPTX rats. A correction is also obtained by 1,24R (or S) 25-$(OH)_3D_3$ but with 260 pmoles/day, whereas the same dose of 24R-25-$(OH)_2D_3$ has no effect. In conclusion, 1,25-$(OH)_2D_3$ administered in physiological amount influences markedly the renal handling of Pi, suggesting that the chronic change in the tubular capacity to transport Pi following TPTX may be due the decreased formation of 1,25-$(OH)_2D_3$.

Sleep in the rat during extension of the photoperiod

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Light promotes sleep in the rat and also acts as a major zeitgeber of the circadian sleep rhythm. We investigated the effect of a gradual extension of the daily photoperiod on waking, slow wave sleep and paradoxical sleep (PS). These vigilance states were determined for successive 10-sec-periods by an electronic state recognition system on the basis of telemetric EEG, EMG and motor activity signals. The photoperiod was increased from 12 to 20 h by 1 h/day. This was achieved in one group of rats by delaying light-offset, and in another group by advancing light-onset. The largest circadian increase in the percentage of sleep remained time-locked to light-onset, although the effect diminished when light-onset was advanced by more than 5 h. When light-offset was delayed, sleep gradually declined towards the end of the photoperiod, while the percentage of PS relative to total sleep remained high. The results indicate that light-onset and light-offset may have phase-setting properties for the circadian sleep rhythm, and that the two sleep states may be differentially affected.

Morphometric analysis of soleus and diaphragmatic muscle in rats

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Electron micrographs of diaphragm and soleus muscle of young rats, kept for 3 weeks at 25 °C and 11 °C, were investigated morphometrically. Volume and surface densities of mitochondria and fat droplets were compared in the 2 groups: mitochondria had to be subdivided into a central group evenly distributed throughout the fibre and a peripheral group close to the plasmalemma. In diaphragm all parameters were higher than in soleus muscle, both in the controls and in the animals kept in cold. In the cold group the volume and surface density of the peripheral mitochondria, the total mitochondrial volume density and the volume and surface density of the fat droplets were higher than those in the controls. There was no difference between the 2 groups with respect to

the central mitochondria. The results indicate that diaphragmatic muscle has a higher volume density of mitochondria than soleus muscle and that cold induces an increase in mitochondria and fat in both muscle groups.

Interconnections between the oculomotor nuclei of the monkey

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The use of anterograde (radioactive amino acids) and retrograde (horseradish peroxidase – HRP) tracer substances in the brain has recently led to the 'rediscovery' of the fibre system interconnecting the oculomotor nucleus (OMN) and abducens nucleus (AbN) first suggested by Fuse in 1912. In our studies on the monkey we have been able to demonstrate some of the finer details of this system. HRP injected exclusively into AbN labels predominantly the motoneurone subgroups of OMN which innervate medial rectus and inferior rectus. The injection of radioactive amino acids into AbN labels axons terminating on these same 2 subgroups, and not on those of superior rectus and inferior oblique. Thus the pathways interconnecting abducens with the motoneurones of OMN appear to be exactly reciprocal. This supports the hypothesis that the internuclear pathway is necessary for the coordinated action of certain eye muscles, and damage to this system would explain the clinical symptoms seen in internuclear ophthalmoplegia.

Synaptic morphology of the Mauthner axon collaterals

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The electrophysiological and histological results of investigations dealing with the functions of the Mauthner axon collaterals (MAC) suggest that the short latency inhibition of the spinal motoneurones caused by the stimulation of the contralateral MA is mediated in certain teleosts by a disynaptic pathway, the mode of transmission being electrical at the first and chemical at the second synapse (H.-G. Goldscheider et al., paper submitted for publication). In the present study the ultrastructure of the synapses formed by the MAC was examined in goldfish spinal cord. Using the criteria for the identification of synapses with chemical or electrical mode of transmission, we found that all MAC make axo-dendritic synapse with the characteristic features of a chemically operating connection. Besides this excitatory pathway from MA to moto- and interneurones, some MAC in addition form axo-axonic synapses with the typical morphology of a gap-junction. The latter is assumed to be the connection providing short latency postsynaptic inhibition by exciting the axon of an inhibitory interneurone electrically.

Indirect contribution of active Na-K transport in brown fat thermogenesis

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The noradrenaline (NAdr)-induced increase in O_2 consumption (ΔMO_2) of brown adipose tissue slices from rats was found to be about twice as large at medium pH 7.8 as at pH 6.8 in Krebs-Ringer bicarbonate equilibrated with 5% CO_2 – 80% O_2 in N_2 · ΔMO_2 was inhibited by ouabain in acidic medium only. The respiratory response to NAdr was present in bicarbonate media with no Na (Li-substitution) or no Na nor K, pH 7.4, but was suppressed in the same media gassed with 20% CO_2 – 80% O_2 adjusted to pH 7.4 with bicarbonate. These qualitative results suggest that a) the active Na-K transport process is not the energy dissipator in brown fat thermogenesis, and b) the active Na-K transport is essential in the triggering of energy dissipation in mitochondria when cytoplasmic pH is low. Under this assumption, the role of the Na-K pump in NAdr-induced thermogenesis would only be to cause intracellular alkalinisation.

The retrograde axonal flow depends on neuronal activity

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In the past years horse radish peroxydase (HRP) has been widely used for tracing axons and cells by retrograde axonal flow. The following experiments have been designed in order to check if the functional state of the neurons have some influence on the transport and uptake of the HRP: Sympathetic cervical ganglia isolated from the rat have been mounted in a chamber with 3 compartments completely separated with respect to their perfusion with various solutions. The body of the ganglion is placed in the middle compartment, the pre- and postganglionic nerve being threaded in each of the 2 other compartments through a small hole that they seal up completely. Thus the postganglionic fibres can be put in contact with HRP while the neurons from which they originate are kept at rest, under stimulation, or exposed to various drugs increasing or decreasing their excitability. The results show that in every condition in which the ganglion has been stimulated, more neurons have taken up more peroxydase than the control kept at rest.

Milk-ejection: A neuroendocrine reflex

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In the nursing rat, suckling by hungry pups produces an intermittent, selective release of oxytocin, and the reflex occurs even at surgical levels of anaesthesia (Lincoln et al., J. Endocr. *57*, 459 (1973)). A sudden acceleration in neuronal firing, which reached a peak of 40–60/sec and lasted 1–3 sec, was observed in supraoptic and paraventricular neurones which otherwise fired at a low, random rate; this response preceded the rise in intramammary pressure seen at milk ejection by 10–15 sec, this time difference being similar to the latency to milk ejection following electrical stimulation of the neurohypophysis. In order to promote a release of hormone equivalent to that seen at reflexly-induced milk ejection, 1–3 sec long trains of stimuli had to be applied at frequencies > 40/sec. Longer trains or higher frequencies produced a smaller rise in intramammary pressure per stimulus pulse. The data indicate a causal role of action potential firing in neurohypophysial hormone secretion and show that the neuronal response induced by the suckling pups is optimal for milk ejection.

Efferent control of primary vestibular afferents in the cat

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In the primary vestibular afferents of the cat lateral semicircular ampulla, activation of efferent fibres causes not only a decrease of spike rate but, in units with regular spontaneous activity, transition to irregular spike trains. 'Quasi Gaussian' distribution in interspike interval histogram gives way to unimodal asymmetric distribution which is confirmed by joint interval histogram and autocorrelogram. The process has a time course over several min and is reversible and reproducible on the same unit. I. v. injection of atropine sulphate (0.16 mg/kg) abolishes the effect. These results suggest that the efferents control the flow of information in primary vestibular afferents and that acetylcholine may act as an efferent transmitter at the neuroreceptor junction.

An efferent pathway of the suprachiasmatic nucleus of the woodmouse

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Degenerating fibres have been followed using the Nauta method after coagulation of the nucleus suprachiasmaticus hypothalami. These fibres leave diffusely the laterocaudal part of the nucleus, run laterally along the ventral border of hypothalamus, turn dorsocaudally between the fornix and the ventro- and dorsomedial nuclei. They then form a more condensed fasciculus running paramedially along the 3rd ventricle up to the mesencephalic periventricular grey matter. No fibres are seen in the medial forebrain bundle. Computer controlled scanning dark field microphotometry has been used to analyse autoradiographs after injection of H3-proline in the nucleus. Diffusion of labelled material from injection site and 3rd ventricle masked the fibres. For this reason we could not confirm the results of Swanson and Cowan (J. comp. Neurol. *160* (1975)) either. The suprachiasmaticomesencephalic fibres might represent part of a link allowing the inhibition of cervical superior sympathetic neurons by electric stimulation of the suprachiasmatic nucleus (Nishino et al., Brain Res. *112* (1976)).

Evidence for brain angiotensin receptors

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Neurones in the cat subfornical organ were activated by microiontophoretically applied angiotensin II (A II) and A II (2–8) heptapeptide. Both the action of A II and A II (2–8) were blocked by saralasin (Experientia *32*, 761 (1976)). This experimental system was used for further structure-activity studies with A II fragments. Purity and identity of the fragment peptides were ascertained by thin layer chromatography, electrophoresis and amino acid analysis. A II (5–8) tetrapeptide produced a short latency excitatory action on single units which could be blocked by saralasin. In contrast A II (6–8) tripeptide failed to enhance the firing rate of the same neurones. These studies offer new insight in structure-activity relations for A II and suggest the presence of specific angiotensin receptors in the brain.

Maternal behavior differences in Roman high- and low-avoidance rats

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The effect of selection for shuttlebox avoidance on maternal behavior was determined in 3 generations of 3–4-month-old, first-litter RHA and RLA rats. 51, 74 and 71 litters were observed for the first 15 days of life, with the latter 2 generations including, respectively, 19 F_1-generation litters and 18 foster-mother litters. Multimoment analysis was used (6X/day and 30 sec/litter) in examining several behavioral traits. With sawdust bedding and torn newspaper as materials, nest quality was determined on a 0–5 point scale. Starting a few days after birth, RLA rats built significantly better, compact nests (t-test), changed their location less often, and showed a lower rate of separation of individual sucklings from the nest. The females of both strains were observed to mostly 'blanket' their young during suckling, but RHA mothers also placed themselves on their sides or backs more often than RLA mothers. Observation of the F_1 and foster-mother litters showed that the young exert no genetically-based influence of their own on maternal behavior during this period.

Evidence for functional synapses between mixed cultures of inferior olive and cerebellum

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Cultures were prepared from newborn rats by the roller tube technique. One slice of cerebellum and one of the brain region including the inferior olive were explanted to a glass coverslip and embedded in a plasma clot so that the distance between the explants was approximately 2 mm. Nerve fibres crossing from one explant to the other could be observed either in the living culture or after fixation and staining with Bodian silver stain. Analysis of the spontaneous activity of Purkinje cells revealed that some show complex spikes resembling the climbing fibre response observed in situ. Complex spikes could also be elicited in Purkinje cells by stimulation of nerve cells situated in the inferior olive explant. Exposure of the cultures to a high concentration of Mg^{2+} (4 mM) abolished these complex spikes. These results suggest that functional synaptic connections exist between cultured nerve cells of inferior olive and Purkinje cells. This preparation may therefore provide a valuable means to study the pharmacological properties of the climbing fibre response.

Comparative morphometric analysis of large mammalian lungs

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The lungs of 2 horses (b.wt 510 kg) and of 1 cow (b.wt 700 kg) were fixed in situ through the airways. The tissue was prepared for electron microscopy following standard

methods, and analyzed by morphometry. The specific maximal structural diffusion capacity of the lung, D_{Lmax}/b.wt, was estimated for the horse at 6.93 ml $O_2 \times min^{-1}$ $mmHg^{-1}$ kg^{-1} and for the cow at 2.58 ml $O_2 \times min^{-1}$ $mmHg^{-1}$ kg^{-1}. D_L is hence 2.75× larger in the horse than in the cow. Since specific O_2 consumption is likewise 2.8× higher in the horse than in the cow, this finding further supports the hypothesis that the pulmonary gas exchange apparatus can adapt to higher O_2 requirements of the body. 2 factors may accomplish this adaptation: a) the specific volume of the horse lung is twice as large as that of the cow, b) the alveolar surface density is 755 cm^2/cm^3 in the horse, compared with 635 cm^2/cm^3 in the cow. The horse lung is hence larger and contains a higher density of gas exchange surface.

Disynaptischer Weg der durch die Mauthneraxon-Erregung verursachten Hemmung spinaler Motoneurone

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Die fluoreszenzmikroskopische Untersuchung des in situ mit Procion Yellow injizierten Mauthneraxons (MA) in Serienschnitten über mehrere Rückenmarkssegmente zeigt, dass das MA keine Mittellinie kreuzenden Kollateralen abgibt. Dies bedeutet, dass die durch die MA-Erregung verursachte chemisch-postsynaptische Hemmung der kontralateral zum MA gelegenen Motoneurone durch Interneurone vermittelt wird. Die elektrophysiologischen Untersuchungen zeigen, dass die synaptische Verzögerung für die Hemmung mit 0,85 ± 0,1 msec lediglich 0,15 msec länger ist als die Verzögerung von 0,7 ± 0,1 msec bei der monosynaptischen Aktivierung derselben Motoneurone durch die ipsilaterale MA-Erregung. Die Messwerte beziehen sich auf die Zeiten zwischen Beginn des steilsten Anstieges des MA-Aktionspotentiales und Beginn des IPSPs bzw. EPSPs in demselben Rückenmarkssegment bei 10 ± 1 °C. Auf Grund dieser Befunde ist für die chemisch-postsynaptische Hemmung der Motoneurone ein disynaptischer Weg anzunehmen, bei dem die Aktivierung der hemmenden Interneurone durch das MA über eine elektrische Synapse erfolgt.

Die Kabelkonstanten des Mauthneraxons

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Die beiden Mauthneraxone (MA), die als markhaltige Riesennervenfasern im Rückenmark bestimmter Knochenfische bilateral-symmetrisch angelegt vorkommen, besitzen nach Massgabe histologischer Untersuchungen keine Ranvierschen Schnürringe. Elektrophysiologische Untersuchungen lassen erkennen, dass zur Auslösung einer MA-Erregung die simultane Aktivierung von einzeln nicht erregbaren, in jedem Axonbereich auf eine nahezu konstante Faserlänge verteilten Stellen notwendig ist, vergleichbar der «liminal length» bei marklosen Fasern (K. Greeff, Experientia *32*, 756 (1976)). Zur genaueren Bestimmung der «liminal length» wurden in dieser Arbeit die Kabelkonstanten des MA in situ an 10 Schleien mittels der Methode der Rechteckstromübertragung zwischen zwei intraaxonalen Mikroelektroden ermittelt. Der Eingangswiderstand betrug im Mittel 3,1 MΩ ± 10%, die Längskonstante 8,3 mm ± 17% und die Zeitkonstante 0,4 msec ± 20%. Daraus errechnen sich die Kabelkonstanten pro Längeneinheit: r_m = 5,1 MΩ·cm, r_i = 7,5 MΩ/cm und c_m = 80 pF/cm. Basierend auf diesen Werten dürfte die «liminal length» zwischen 3,5 und 6 mm liegen.

Effects of diamide, a thiol-oxidizing agent, on frog skin permeability

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Diamide perturbs the thiol-disulfide status of several cell systems by oxidizing intracellular glutathione. As SH-groups have also been implied in a variety of transport processes, the effects of diamide on frog skin permeability were investigated. At 5×10^{-4}M, diamide produced rather variable and inconspicuous changes in Na and H_2O transport. In contrast, the drug interfered markedly with the action of oxytocin, isoproterenol and theophylline. With diamide in the external solution, a significant reduction (50–75%) of the natriferic action of these agents was found as well as a 80% inhibition of the hydrosmotic effect of oxytocin. There was no inhibition however when the drug was present in the internal solution. These effects of diamide could be due to an interaction of the drug with SH-groups of the external membrane and/or to a shift towards oxidized glutathione in the thiol-disulfide balance of the epithelial cells. Further studies are needed to elucidate the nature of the SH-groups involved in the diamide effects on frog skin.

Denervation hypersensitivity of cortical neurones to histamine

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Histaminergic fibres ascending in the medial forebrain bundle (MFB) and projecting to the cerebral cortex have been postulated from the decline of histamine (HA) and histidine decarboxylase after MFB lesions (Garbarg et al., Science *186*, 833). We found cortical inhibitions after stimulation of the MFB as well as depressant actions of HA antagonized by metiamide (Brain Res. *122*, 269, 1977) After unilateral interruption of the MFB of guinea-pigs, leading to decreases in histidine- and DOPA-decarboxylases, a clear hypersensitivity of single neurones to microiontophoretically applied HA and noradrenaline developed ipsilaterally. The mean threshold ejecting currents for a response were + 10.5 nA (16.14 SD) on the ipsilateral and + 54.7 (25.2 SD) on the contralateral side for HA. The values for noradrenaline were + 11.8 (16.5 SD) and + 33.2 (28.0 SD) respectively. We interpret these results as a denervation hypersensitivity after lesioning of histaminergic and noradrenergic fibers.

Left ventricular diastolic pressure-dimension-relationship in the human heart

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Left ventricular (LV) diastolic properties were evaluated by relating pressure (P) to chamber dimension (D) determined simultaneously by echocardiography. P-D

relations were calculated at 20 msec intervals starting at the point of lowest P in 5 controls (group A) and 5 patients with aortic regurgitation (group B). LV ejection fraction was normal in both groups. In group A a good linear relationship between log P and D was found (r: 0.91–0.99) with a slope (k) ranging from 0.15 to 0.31. In contrast 3 patients of group B showed a biphasic relationship with a flat portion during early (k: 0.05–0.06) and a steep portion during late diastole (k: 0.44–0.97). In the remaining 2 patients the relationship was linear as in group A. Thus, in the normal left ventricle the log P-D relationship is approximately linear. In volume overload, however, a biphasic relationship is found suggesting a decreased resistance to filling in the early phase of diastolic stretching and an increased resistance during the presystolic period. Normal systolic function does not imply a linear slope in the log P-D plot.

Role of 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) in the renal handling of calcium in rats

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The relation between plasma and urinary Ca has been studied in conditions known to influence the renal production of 1,25-$(OH)_2D_3$ in rats receiving an adequate supply of vitamin D_3. a) The effect of thyroparathyroidectomy (TPTX) on the renal handling of Ca was not corrected by doses of 1,25-$(OH)_2D_3$ which normalized the intestinal Ca absorption. b) In TPTX and intact rats administration of the diphosphonate EHDP, in doses which inhibited the production of 1,25-$(OH)_2D_3$, did not alter the renal handling of Ca. c) In TPTX and intact rats the dietary phosphate-induced change in the renal handling of Ca was not altered by physiological doses of 1,25-$(OH)_2D_3$. In conclusion, the kidney does not appear to alter its tubular handling of Ca in response to endogenous variation or physiological supplementation of 1,25-$(OH)_2D_3$. This is in contrast to the marked alteration obtained by either parathyroid hormone or variation in dietary inorganic phosphate.

Transmembrane action potentials and extracellular currents after coronary occlusion

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In isolated perfused pig hearts transmembrane action potentials (TAP) were recorded from the epicardial layers with floating microelectrodes and extracellular epicardial (EPE) and intramural (IME) potentials were measured with a non polarisable electrode. After coronary occlusion (CO) the amplitude of the TAP and its upstroke velocity starts to decrease within 3–5 min. Further changes include shortening of the TAP and development of electrical alternans. These changes and the marked intraischemic conduction delay are clearly reflected in shifts of the EPE. From the comparison of EPE with IME and from determination of EPE at multiple sites extracellular currents densities (ECD) can be calculated. They are greatest (order of magnitude 2 $\mu A/mm^2$) in the border of the ischemic area and in circumscribed areas of the ischemic zone during electrical alternans. Decrease of amplitude of the EPE after 1 and 2 h of CO indicate breakdown of intercellular connections in the center of the ischemic zone. A possible relationship between ECD and the occurrence of ventricular arrhythmias is discussed.

Pulmonary stretch receptor activity in the irritant-induced hyperpnoea

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The discharge pattern of approx. 100 single stretch receptor fibres studied in the rabbit after inhalation of chemical irritants (histamine, ammonia) shows increased activity during both inspiration and expiration, effects pointing to air-trapping and hampered expiration of the affected lung units. The discharge rate during inspiration is always higher than during expiration. Unchanged, decreased or abolished activity rarely occurs. These findings differ somewhat from those described in guinea-pig, in which changes of stretch receptor activity are markedly heterogeneous and clearly point to uneven ventilation. In both species, however, delayed washout rate and deformed argon concentration curves after inhalation of irritants reveal uneven and asynchronous ventilation. – It is suggested that the differences in the discharge pattern of stretch receptor fibres during irritant-induced hyperpnoea in rabbit and guinea-pig are due to differences in the location of stretch receptors concerned. These endings in the rabbit probably lie in the bronchi, those of the guinea-pig in the peripheral airways.

Transcortical facilitation of the H-(monosynaptic)-reflex in monkeys

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In man, the recovery curve of H-reflexes is characterized by an early and a late facilitation which is superimposed on a lasting inhibition. The mechanism of late facilitation (often increased in Parkinsonian rigidity) is not known. The hypothesis was tested that a transcortical loop for low threshold muscle afferents could be responsible for late facilitation. Therefore, monkeys were trained to allow recordings of H-reflexes under standardized conditions. It was found that facilitation started at a latency of 50–60 msec and reached a maximum at about 100 msec following a weak conditioning stimulus (subthreshold for the H-reflex). We conclude that this facilitation is mediated via the motor cortex because: a) it was abolished when the motor cortex was reversibly blocked by cooling or permanently lesioned; b) the time course of late facilitation was in accordance with latencies of cortical potentials evoked by the conditioning stimulus and of motor responses elicited by electrical stimulation of the motor cortex.

Scalp EEG fields evoked from upper and lower visual half fields

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Scalp EEG fields of normal ss were evoked by checkerboard reversals shown to the upper and lower halves of the visual field and recorded simultaneously from 39

electrodes. In conventional anterior-posterior recordings, these stimuli evoke potentials of grossly similar waveform but inverted polarity between about 80 and 160 msec latency: this had given rise to the assumption that the generators are of about inverted polarity in the 2 conditions. The examination of the EEG scalp field plotted as potential distribution maps shows that in both conditions, an occipital positivity starts to develop at about 80 msec latency, but peaks for lower field stimulation at about 100 msec, and for upper field stimulation around 140 msec. The inversion of conventional records is accounted for by anterior positivities evoked by upper field stimuli. Thus, lower hemi-retina (= upper field) stimulation yields a delayed cortical response compared with upper hemi-retina stimulation.

Effects of amiloride analogues on Na transport in isolated frog skins

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Effects of amiloride analogues on Na transport were studied in isolated skins of frogs Rana ridibunda. The short-circuit current (SCC) was equivalent to net Na flux. At 10^{-4} M, 3 types of response were found: slight inhibition, no change or even a sustained stimulation of SCC; amiloride, at the same concentration, brought SCC close to zero. The stimulatory effect of the analogue LT2 (3-amino-5-dimethylamino-6-chloro-pyrazinoyl-guanidine) was already present at 10^{-6}M, reversible, dose-dependent and additive to the natriferic action of oxytocin. This increase in SCC was greatly reduced after pre-exposure of the external surface of the skin to propranolol (10^{-4}M). Amiloride inhibited totally the stimulatory effect of LT2 but its dose-response curve was modified by the analogue. Results indicate that LT2 interacts with Na entry sites also affected by Ca-displacing agents (propranolol, La^{3+}), as previously reported. Moreover, molecular structure considerations suggest that the same sites are involved in the stimulation of SCC by LT2 and in its interaction with amiloride.

The Lotka-Volterra equations as a possible model for the description of the genesis of respiratory rhythm

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This study is a further attempt to test the applicability of the nonlinear model of Lotka and Volterra with regard to the genesis of respiratory rhythm (H.-R. Lüscher et al., Experientia *32*, 758 (1976)). The 2 equations of the model, $dx/dt = ax - bxy$ and $dy/dt = -cy + dxy$, were simultaneously solved on a hybrid computer. Excitatory effect at the level of the inspiratory motoneurones was assigned to function x(t) and inhibitory to function y(t). The alternating dominance of x(t) over y(t) was assumed to be the principal output of the model. The length of phase $x(t) - y(t) > 0$ was therefore adjusted to the length of the inspiratory phase at the initial set up of the parametric conditions. The length of phase $y(t) - x(t) > 0$ itself was then equal to the length of the expiratory phase. The length of the respiratory phase taken as reference was based on that of the vagotomized rabbits. Results show that variations in a single parameter are sufficient to account for the behaviour of the inspiratory motor innervation in response to stimulation of the low-threshold, fast-conducting vagal afferent fibres.

Projection of low-threshold muscle afferents to the thalamus of monkeys

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Muscle spindle afferents are known to project to Brodmann's cortical area 3a which is transitional between precentral motor cortex and postcentral koniocortex. The thalamic relay has not been delimited in monkeys. We systematically explored the VPL-VL region. Field potentials evoked by weak electrical stimulation of the contralateral deep radial nerve were recorded in animals under Nembutal anaesthesia. The superficial (cutaneous) radial nerve was also used for comparison. The tracks were marked by passing current through steel microelectrodes (Prussian blue reaction). The recording sites could thus be correlated with cytoarchitectonic features of the monkey's thalamus. It was found that low-threshold muscle afferents of the forelimb nerve projected to a region situated mainly in the pars oralis of the VPL nucleus of the thalamus.

The delta sleep inducing peptide DSIP II. Effects of original and synthetic DSIP

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The original nonapeptide DSIP induces, besides bradypnea and bradycardia, a delta + spindle EEG increase, when infused into the ventricular system of rabbits (6 nmoles/kg). The delta increase, measured in RMS μV and time integral reaches 43% in the peptide group against control group. The synthetic DSIP induces similar bradypnea and bradycardia, with delta + spindle EEG increase, expressed by the power spectra. The difference in integrals of the delta + spindle frequency bands between peptide and control groups amounts to 53.8% for delta and 61.8% for spindles in the neocortex. A similar difference (53%) between DSIP and controls was found for a 'delta + spindle factor' revealed by factor analysis as meaningful for orthodox slow wave sleep. The specificity of original and synthetic DSIP was established by comparison with peptide analogs. DSIP might pass the blood brain barrier, since i.v. injections in free moving rabbits increases EEG delta and decreases motor activity. DSIP acts as a programming modulator a supra-transmitter level (long latency, activation of biorhythms, reversibility).

6-Channel telemetry system for animals

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This transmitter, designed for neurophysiological, in particular EEG and sleep studies in cats, is constructed as a 'plug-in' unit attachable to a chronically mounted socket on the animal's head. It has 3 single-ended-input

amplifiers for recording viz. neocortical and hippocampal EEG's and respiration and 3 true differential amplifiers for recording of geniculate waves (PGO's), neck myogram and oculogram. These amplifiers have a gain of 1000, an input impedance of 1 MOhm and a frequency response of 0.5–100 Hz. The transmitter includes a modified astable multivibrator (MV) whose switching period varies linearly with the applied input voltage. The MV drives a counter/multiplexer which controls 6 analogue switches. This allows modulation of the MV sequentially by the 6 independent voltage samples. The MV and one channel of the multiplexer modulate a VHF (100 MHz) oscillator in FM-mode. All parts are mounted in a $34 \times 20 \times 19$ mm plastic receptacle. Total weight, including 4 silveroxide batteries for 100 h operating time, is 20 g. A few examples illustrate the performance of the system.

Atropine affects ganglion cell activity in the cat retina

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Recent results from our laboratory indicated depressant effects of atropine sulfate (ATR) on ERG and optic nerve action potentials in isolated perfused cat eyes. This study was extended to single retinal ganglion cells in the same preparation. ATR was injected into the perfusate while monitoring the light evoked and spontaneous activity of the cells. 14 ganglion cells (32 applications) were tested with ATR concentrations from 0.2 to 11×10^{-3}M in the perfusate. The light response was depressed in 11 cells (4 ON-center, 7 OFF-center); 3 cells were not affected. Spontaneous activity was depressed in 8 cells. These results corroborate the ATR effects on ERG and optic nerve response (Niemeyer and Cervetto, Docum Ophthal., in press); they are concordant with data from Masland and Ames (rabbit retina, J. Neurophysiol. *39*, 1220, 1976) and with our observations on effects of cholinergic agonists and antagonists in cat retina. The data provide further physiological evidence for cholinergic mechanisms in the cat's retina.

Influence of tonicity on conduction velocity of frog single muscle fibres

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According to Hodgkin (J. Physiol. *125*, 221 (1954)) conduction velocity (θ) should depend on fibre diameter$^{1/2}$, which was 84 ± 2% in hypertonic (2T) Ringer and 114 ± 3% in hypotonic (0.5T) Ringer compared with the values in normal Ringer (1T). Average θ changed to 82 ± 1% in 2T and to 120 ± 15% in 0.5T. These changes in diameter$^{1/2}$ and θ (at konst. $[Na^+]_i/[Na^+]_0$) agree closely. Next $[Na^+]_i/[Na^+]_0$ was increased. θ in 50% $[Na^+]_0$ (1T 50% sucrose) decreased to 80 ± 2% when compared with θ in 100% $[Na^+]_0$ (1T Ringer) and to 86 ± 2% when θ in hypertonic low $[Na^+]_0$ (Ringer + sucrose to 2T) was compared with θ in high $[Na^+]_0$ (Ringer + NaCl to 2T). These results might partially be explained by assuming d^2V/dt^2, shown to depend on $[Na^+]_i/[Na^+]_0$ (Hodgkin and Katz, J. Physiol. *108*, 37 (1949)) to be the main parameter of θ, changed in these experiments. Stretching and releasing of the fibre in 2T, however, induced (in contrast to the results in 1T, see Schümperli and Oetliker; Experientia *33*, 784, 1977) marked changes in θ, suggesting that in 2T the unfolding and folding of the surface membrane, assumed for normal Ringer, is impeded.

Efficiency of muscular exercise during oxygen deficit in man: a calorimetric and thermometric approach

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Aerobic (**MR**) and anaerobic ($\mathbf{M_{an}}$) energy production at the onset of exercise was determined, from which the efficiency of **MR** and $\mathbf{M_{an}}$ for the exercise was calculated. 6 subjects performed a 20-min exercise on an ergometer at 50% of their VO_2 max. From the heat balance equation: $\mathbf{MR} + \mathbf{M_{an}} = \mathbf{H} + \mathbf{W} + \mathbf{S}$; **MR** was measured by indirect calorimetry, **H** (heat losses) by direct calorimetry, **W** (work) by ergometry and **S** (heat storage) by thermometry. **S** was estimated from a 3 compartimental thermometric model using oesophagial, skin and muscular temperatures. The major difficulty was to estimate the active muscular compartment (mc). **S** was calculated using 2 extremes of mc; $mc_1 = 20\%$ and $mc_2 = 30\%$ of the total body mass. Knowing **S**, $\mathbf{M_{an}}$ can be calculated from the above equation. During the first min of exercise, $\mathbf{M_{an}}$ was 28 ± 2% and 33 ± 3% of the total energy expenditure for mc_1 and mc_2 respectively. The anaerobic efficiency was 44 ± 1.5% (mc_1) or 35 ± 1.5% (mc_2). These results indicate that the anaerobic efficiency is greater than that of aerobic, measured during steady state work.

Continuous microspectrophotometric measurement of oxygen consumption of in vitro cultured chicken embryo

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Whole chicken blastodiscs are mounted between 2 compartments (100 µl each) of a specially designed chamber. One compartment is perfused with a nutritive medium and the other with a purified human hemoglobin used as oxygen donor as well as respiration indicator (Barzu and Borza, Analyt. Biochem., 1967). The gaz exchanges take place through a very thin transparent silicone membrane separating the embryo and the HbO_2 solution. In stop flow conditions the variation of the optical density at 435 nm corresponding to the desaturation of HbO_2 is measured. The signal recorded locally in the P_{50} domain (determined experimentally) allows the computation of the consumed oxygen quantity. The absolute oxygen consumption of the Hensen's node doubles from 38 nl $O_2 \times mm^{-2}\ h^{-1}$ (stage 4) to 73 nl $O_2 \times mm^{-2}\ h^{-1}$ (stage 8 HH). Corrected for the tissue thickness it represents a 50% increase of the oxidative metabolism during this period. Systematic scannings of the blastodiscs reveal regional variations of tissue activity and correlation to tissue protein distribution is being made.

Carbohydrate oxidation during prolonged exercise, using indirect calorimetry and mass spectrometry ($^{13}CO_2$) after ingestion of 100 g naturally labelled ^{13}C-glucose

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Using naturally labelled ^{13}C-glucose as a metabolic tracer, the utilization of exogenous glucose ingested was investigated during muscular exercise. After an oral load of 100 g ^{13}C-glucose, carbohydrate oxidation was calculated simultaneously from the non-protein respiratory quotient using indirect calorimetry and from expired $^{13}CO_2$ using mass spectrometry. 1 h after glucose ingestion, the 5 subjects pedaled for 2 h on a bicycle ergometer at 33% of their individual VO_2 max. Expired CO_2 became enriched with ^{13}C 1 h after the glucose load. The enrichment reached a peak after 90 min exercise. At this time, the utilization of exogenous glucose was 425 $\pm$ 25 mg/min representing 36% of the carbohydrate metabolism and 24% of the total energy expenditure. An average of 45 $\pm$ 2 g of the load was oxidized during 3.5 h following ingestion. However, this value is slightly underestimated due to mixing of $^{13}CO_2$ in the bicarbonate pool.

CO_2/HCO_3 buffer system and membrane potential of frog single muscle fibres

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The membrane potential of single muscle fibres was measured in the presence and the absence of CO_2/HCO_3^- buffer system at constant external pH ($\pm$ 0,1 unit). In the presence of Cl^- (87 mmoles/l; $[K^+]_o$ 2,5 mmoles/l) the introduction of CO_2/HCO_3^- (PCO_2 97 mm Hg, $[HCO_3^-]_o$ 25 mmoles/l) for 10 min induced in 7 fibres a slow depolarization reaching 11 mV in 5 min, which was not reversible on returning to the CO_2/HCO_3^- free solution. In 4 Cl^--depleted fibres (through washout for 1 h in a $SO_4^=$-Ringer solution) the same increase in PCO_2 produced a dramatic reversible depolarization (10 to 40 mV in 2 min) usually accompanied by mechanical activity. In 3 experiments at lower PCO_2 (38 mm Hg) this depolarization was also found. Several mechanisms are probably responsible for the reported depolarization. One of these might be a net efflux of HCO_3^- (or OH^-, or a net H^+ influx) if we assume that the membrane is slightly permeable for HCO_3^- (or OH^-, or H^+) ions.

Pigeon primary visual projections traced by 2 anterograde HRP labeling methods

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Primary visual projections can be anterogradely traced in the pigeon by means of axonally transported horseradish peroxidase (HRP). Depending on the mode of application of the marker 2 different patterns of labeling can be seen in the optic tectum, the main projection site of retinal ganglion cells. After injection of HRP into the vitreous body a laminar pattern corresponding to the retinal afferent layers can be observed only if a new, sensitive staining technique combining diaminobenzine (DAB) with p-cresol is applied. Direct injection of HRP into the optic nerve, however, gives a Golgi-like picture of radially oriented terminal arborizations penetrating the tectum at regular intervals; the homogeneously filled fibres and endings are detectable after normal DAB staining.

The delta sleep inducing peptide DSIP. I. Amino acid analysis, sequence, synthesis

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A nonapeptide which enhances delta and spindles EEG after intraventricular infusion was isolated from extracorporeal dialysate of rabbits in which the thalamic 'somnogenic' area of Hess was electrically stimulated. It was composed of Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu, as shown by amino acid analysis and sequence. It was synthetized, as well as 5 related peptides (1–8, 2–9, 2–8, 1–4, 5–9), 2 nonapeptide analogs and a related tripeptide (Trp-Ser-Glu). All synthetic peptides were infused intraventricularly in rabbits (6 nmoles/kg in 0.05 Ringer solution over 3.5 min) under double blind conditions. A total of 65 rabbits including controls were used. EEGs from the frontal neocortex and limbic archicortex were Fast-Fourier-transformed and analyzed by a Univac 1108 computer system. A high specificity of the synthetic Delta-EEG-Sleep-inducing Peptide (DSIP) was demonstrated and confirmed by factor analysis. A mean increase of 55% delta activity was measured in DSIP rabbits, against controls or animals receiving the other 8 peptides. A neurohumoral programming action of DSIP was suggested.

Influence of stretch on conduction velocity in frog single muscle fibres

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Conduction velocity of activation, θ, in single frog muscle fibres was determined by optical methods (see Baylor and Oetliker, J. Physiol. (1977), in press) in normal Ringers solution at sarcomere lengths (SL) from 1.9 to 5.2 µm. The 2 measurements of stretch: SL (> 3 mm away from tendon) and total fibre length correlated strongly ($r = 0.985$). Mean θ (9 fibres, 40 determinations) was 2.21 $\pm$ 0.06 m/sec. The regression: θ versus SL indicates that θ is independent of stretch ($b_{yx} = 0.6\%/\mu m$, $P_t > 0.3$), This result agrees with predictions from theoretical calculations (Hodgkin, J. Physiol. *125*, 221 (1954)) and supports the finding that folds and caveolae of the sarcolemma provide enough spare membrane for stretches of the muscle fibre exceeding the physiological range (Dulhunty and Franzini-Armstrong, J. Physiol. *250*, 513 (1975)). The measurements also add to the evidence that the birefringence changes following excitation of muscle fibres propagate with the speed of the action potential and represent a step in excitation-contraction-coupling.

The lungs of shrews, the class encompassing the smallest mammals

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The lungs of 14 shrews, ranging from 2.5 to 100 g b.wt, were fixed in situ through the airways, and prepared for electron microscopy following standard methods. By scanning electron microscopy the lung parenchyma appeared very finely structured; by morphometry this yielded unusually large surface densities of alveoli, S_{Va}, ranging from 1033 to 1923 cm^2/cm^3, and of capillaries, S_{Vc}, ranging from 823 to 1412 cm^2/cm^3; capillary volume density, V_{Vc}, was 0.092 to 0.133. These values are much larger than in human lungs, where $S_{Va} = 375$ cm^2/cm^3, $S_{Vc} = 325$ cm^2/cm^3 and $V_{Vc} = 0.055$. The harmonic mean thickness of the air-blood barrier is 0.36 μm, which is half the human value. The specific maximal structural diffusion capacity, D_{Lmax}/b.wt, reaches values up to 0.011 ml $O_2 \times min^{-1}$ mm Hg^{-1} g^{-1} in the smallest shrew, as compared to 0.0036 in human lungs. In adaptation to their high metabolism these small mammals developed a specially large gas exchange apparatus.

Direct control of the metabolic activity of brown adipocytes by sympathetic nerves

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Numerous studies on the effects of surgical denervation of the interscapular brown adipose tissue on metabolic turnover and potassium-stimulated respiration have lent support to the hypothesis that only the blood vessels are denervated by sectioning the nerve supply. To test this hypothesis, in vitro electrical stimulation was performed of the distal end of these cut nerves. Electrical stimulation was found to mimic the effects of norepinephrine on the adipocytes, i.e., it caused an increase in both NAD(P)H production and oxygen consumption of the tissue and a depolarization of the cyto-membrane. These effects were graded as a function of stimulation frequency. The temporal relationship between the 3 responses was determined using surface fluorescence microscopy and a fast responding platinum (Po_2) microcathode. It was found that depolarization started first, followed by pyridine nucleotides reduction and, lastly respiration increased. At a frequency of 4 Hz, the delays were 2, 5, 8 sec respectively. These effects were suppressed by prior reserpinization of the rat and inhibited by propranolol. In conclusion, the metabolism of brown adipocytes is under direct control of sympathetic nerves.

Increase in phosphate efflux during electrical activity in non-myelinated nerve

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Garfish olfactory nerves, or desheathed rabbit vagi, loaded with radiophosphate were mounted in an apparatus and washed with label-free solution (for details of method see Ritchie and Straub, J. Physiol. *249*, 327 (1975)). In gar nerve the resting phosphate efflux had a rate constant of 9.8×10^{-4} min^{-1} at 0.2 mM external phosphate. Stimulation for 2.5–15 min at 0.5 sec^{-1} caused an increase in phosphate efflux of 12×10^{-6} $impulse^{-1}$. The increased efflux returned to the resting level with a rate constant of 0.23 min^{-1}, somewhat more slowly than the post-tetanic hyperpolarization. The effect of stimulation decreased with decreasing extracellular Na and was slightly smaller at 2 or 0.02 and 0.002 than at 0.2 mM external phosphate. In rabbit vagus essentially the same effects were found: when both B and C fibres were stimulated, the increase was 0.83×10^{-6} $impulse^{-1}$ at normal Na and 0.2 mM phosphate. The effects of stimulation are probably due to the liberation of inorganic phosphate and the increase in intracellular Na after activity.

Synaptic localization of anterogradely transported horseradish peroxidase

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Horseradish peroxidase (HRP) is found after intraocular injection in terminal fibres and endings of retinal ganglion cells. Semithin sections viewed in the electron microscope exhibit a segmented network filled with the anterogradely transported marker substance. In thin sections the tracer is seen in tubular and vesicular profiles. Labeled dense-core and small vesicles are observed in close apposition with the presynaptic membrane. A dynamic relation between axonal and terminal smooth endoplasmic reticulum, synaptic vesicles and the synaptic junction is postulated.

Effects of tetraethylammonium on Ca movements in vascular smooth muscle

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The lanthanum method was used to measure the effects of tetraethylammonium (TEA) on Ca movements in isolated strips of rabbit main pulmonary arteries. TEA (10–70 mM) produced in a concentration-dependent manner contractions of the vascular strips, decreases in the membrane potential of the vascular smooth muscle cells as measured with intracellular glass microelectrodes and increases in La-resistant ^{45}Ca content of the arteries. The latter corresponded to a net entry of Ca into the cells. ^{45}Ca influx induced by high KCl concentrations was stimulated by TEA but the maximum response to KCl was not increased. The same held good for contractions produced by KCl. ^{45}Ca efflux from the La-resistant Ca space was increased by TEA both in the presence and in the absence of cold Ca in the medium. The results indicate that TEA increases in vascular smooth muscle cells the Ca movement in both the inward and outward directions. It remains to be determined whether this increase in Ca permeability of the cell membrane is directly related to a decrease in potassium permeability, an effect well-known for TEA.

Effect of propranolol on reflex milk-ejection and on paraventricular neurones

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Most lactating rats suckled by a litter of pups display reflex milk-ejections at intervals of 3–15 min. The β-adrenergic antagonists propranolol and oxprenolol promote a normal pattern of reflex milk-ejections when given to animals which are not milk-ejecting or have ceased to milk-eject in response to the suckling stimulus. In an attempt to locate the site of action of β-antagonists in facilitating the reflex, extracellular recordings were obtained from paraventricular neurones during suckling in urethane-anaesthetized rats. Oxytocin-neurones showed a characteristic burst of high frequency firing at the time of each reflex milk-ejection. Propranolol, 300 µg/kg i.v., had no effect on the resting firing rate of oxytocin-neurones; in refractory animals, reflex milk-ejections occurred within a few min and were accompanied by a high frequency burst of neuronal firing. These results indicate that propranolol facilitates the milk-ejection reflex by acting on its afferent limb, but not onto the neurosecretory cells.

Neuronal mechanisms of stereopsis: Sensitivity to orientation disparity

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Stereoscopically viewed contours of slightly different orientations in the 2 eyes can produce the sensation of slant in depth. Searching for the underlying neuronal mechanism, binocular units in the cat striate cortex were investigated. Sensitivity to orientation disparity could not always be predicted from monocular orientation tuning: Unexpectedly, some units with very broad tuning in either eye were most sensitive to orientation disparity. When correction was made for rolling of the eyes in paralysis, nearly all of about 50 units tested so far responded best to parallel binocular stimuli. In many units, responses varied asymmetrically with orientation disparity, showing a steep falling off at negative disparities, corresponding to forward slant, and a more gradual decrease towards positive disparities (backward slant). This is interpreted as a consequence of an environmental bias: Normally, horizontal planes are more frequent in the lower half of visual space, and, in such a plane, contours always produce positive disparities.

Demonstration of a hormone sensitive 'shunt-path' in the frog skin epithelium

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Further investigations about the role of the mitochondria rich cell (MR cell) in hormone mediated transport regulation in the epithelium of frog skin brought the following results: Unlike toad bladder (Ludens), in frog skin the spontaneous potential difference cannot be reversed when Na transport is blocked. A similar situation is obtained when, in addition to transport-blockade, one applies a chemical gradient for chloride to the epithelium. Under these conditions we found that in the intact preparation as well as in the separated epithelium: a) the reversed current (RC) is linearly related to the number of MR cells; b) RC is mainly carried by a passive, transcellular chloride flux inwards (Kristensen and Voûte); c) RC is sensitive to noradrenaline (10^{-7}M): The hormone reduces RC in winter skins, to do the opposed in most summer preparations. The beta-blocker propranolol abolishes this effect. We propose that noradrenaline mediated transport regulation in frog skin is (parallel to aldosterone) directly or indirectly guided by the beta-receptor, the mitochondria rich cell.

Phase locked responses to low frequency tones in the medial geniculate body

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In the auditory nerve, neuronal discharge evoked by low frequency tones are time-locked to the phase of the stimulus. How is this temporal information preserved in the higher auditory centers? Single unit activity was studied in the medial geniculate body of nitrous oxyde anesthetized cats. Only about 5% of the units showed sustained responses to frequencies below 5000 Hz. 30 of these units were analyzed by means of high resolution interval and 'period' histograms. One third of them had their spikes locked to the phase of the stimulus for frequencies below 2000 Hz. Out of 9 cells responding to frequencies below 500 Hz, 6 had a phase-locked activity. There is not a simple overall integration of spikes through the different synapses of the auditory pathway, but the timing of individual events is maintained with a precision in the range of the millisecond up to the MGB. Coincidence detectors based on the temporal structure of spike trains are conceivable even in the higher auditory centers.

Inspiratorische Nachwirkung afferenter Vagusreizung

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An vagotomierten, beatmeten Kaninchen wurden die niederschwelligen Vagusfasern mit 100–200 Hz afferent gereizt und die für die Erhaltung exspiratorischer Atmungsreaktionen (EAR) notwendige Reizdauer in Abhängigkeit von der zeitlichen Beziehung zwischen Reiz- und Atmungsphasen anhand des Phrenicogramms bestimmt. Die Versuche zeigen: 1. Die Dauer der EAR ist in Abhängigkeit von der Dauer der vorangehenden Inspirationsphase zeitlich limitiert, wobei die EAR um so länger aufrechterhalten werden kann, je später die vorangehende Inspirationsphase abgebrochen wird. 2. Dem exspiratorischen Reizeffekt folgt keine gleichsinnige Nachwirkung, sondern eine verlängerte Inspirationsphase, die um so länger dauert, je länger die vorangehende EAR aufrechterhalten werden kann. Eine Interpretation der Befunde ergibt sich aus der Annahme, dass dem exspiratorischen System in der die inspiratorisch-motorische Innervation periodisch hemmenden Funktion eine sich selbst inaktivierende Tendenz innewohnt, die durch die exspiratorisch wirksamen Vagusafferenzen zwar vermindert, aber nicht aufgehoben wird.

BIOCHEMIE – BIOCHIMIE – BIOCHEMISTRY

Proteolytic fragmentation of Clr

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The proenzyme form of Clr is a molecule of 200,000 daltons comprising 2 apparently identical noncovalently linked 100,000 dalton polypeptides. Incubation of the precursor molecule at 37 °C in the absence of Ca^{2+} results in proteolytic cleavage of both chains. After 10 min at 37 °C Clr is converted to activated $\overline{Clr}$ by cleavage of both chains into disulfide-linked subunits of 65,000 (heavy chain) and 35,000 (light chain) daltons, respectively. Smaller split products appear by further incubation at 37 °C and result after 18 h in a fragment of 54,000 daltons. At 0 °C Clr is slowly converted to $\overline{Clr}$ which is stable at this temperature. Ca^{2+} ions strongly delay proteolysis of Clr. The 54,000 daltons fragment generated by proteolysis of Clr has been isolated and shown to comprise 2 disulfide-linked subunits of 35,000 and 18,000 daltons. This fragment is still able to cleave Cls, but cannot reconstitute Cl upon addition of Clq and Cls. As in $\overline{Clr}$, it is the 35,000 dalton subunit that bears the active site of the fragment, as shown by incorporation of labelled diisopropylfluorophosphate. Thus, proteolytic fragmentation of Clr involves degradation of the heavy chain of the molecule, while the light chain remains intact. This is consistent with an autocatalytic mechanism of Clr cleavage.

Effects of anthracycline antibiotics on mitochondrial functions in rats

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Adriamycin, daunorubicin and rubidazone are widely used in cancer therapy. Cardiotoxicity is their most dreaded side-effect. Correlation of ECG changes in rats with changes in heart mitochondrial metabolism, both cumulative in nature, was possible. Rubidazone and daunorubicin are uncouplers of oxidative phosphorylation, adriamycin inhibits electron transfer reactions in vitro and in vivo. Calcium transport into heart mitochondria was investigated, since cellular calcium levels are essential for muscle contraction and relaxation. Calcium uptake in mitochondria can be energized by electron transfer or ATP-splitting. In vitro adriamycin and daunorubicin inhibited the former in conc. above 1×10^{-5} M, rubidazone above 5×10^{-9} M. Adriamycin and daunorubicin did not inhibit calcium transport energized by ATP, but rubidazone did so at conc. above 1×10^{-7} M. Adriamycin (5×4 mg/kg) and rubidazone (8×8 mg/kg) were tested in vivo (i.p.). Heart mitochondria isolated from these animals showed normal calcium uptake with ATP as energy source; energized by substrate oxidation, both compounds were inhibitory (adriamycin > rubidazone).

Immunofluorescent subcellular localization of muscle proteins

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The role played by parvalbumins in vertebrate muscle and by calcium-binding proteins in invertebrate muscle is still unclear. The possible relationship of these proteins with enzymes or structural proteins has not been established. Antibodies against carp and perch parvalbumins, crayfish arginine kinase, glycogen phosphorylase and calcium-binding protein have been used in conjunction with antiactin autoantibodies for indirect immunofluorescent localization of their corresponding antigens in muscle sections and myofibrils. This technique shows that parvalbumins are practically not retained on myofibrils, which confirms their even distribution previously observed on muscle sections; moreover incubation of myofibrils with parvalbumin solution does not show any fixation of the latter protein on these structures. All the other crayfish proteins tested appear slightly fixed on myofibrils and located at the same place as actin. The differences observed between crayfish muscle sections and myofibrils reveal new properties of crayfish actin.

Distinction of 2 galactosyltransferase-activities in human serum

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Human serum galactosyltransferase activity (GT) catalyzed the transfer of galactose from UDP-Gal to N-acetylglucosamine (Glc-Nac)- and N-acetylgalactosamine (Gal-Nac)-containing glycoprotein acceptors. Sialic-acid-galactose-free-α_1-acid-glycoprotein (SGf-AG) and sialic-acid-free ovine submaxillary gland mucin (Sf-OSM) were used respectively. Removal of a Glc-Nac specific GT by affinity chromatography on Glc-Nac-derivatized beads yielded 2 different GT activities: a) the unretained enzyme (GT-A) and b) the enzyme eluted with competing Glc-Nac (GT-B). The SGf-AG/Sf-OSM activity ratio for the native GT was approx. 20, for GT-A: 1, for GT-B: 50. Incubation of equal amounts of enzyme activity as measured towards Sf-OSM with free glucose and α-lactalbumine produced lactose only with GT-B, none with GT-A. With temperature, GT-A-activity increased steadily from 4 °C to 45 °C; in contrast, GT-B had a broad temperature optimum between 20 °C and 37 °C and significant activity at 4 °C. Both enzymes depended equally on Mn^{++} concentrations.

Human red cell membrane sialoglycopeptides: Electrophoretic resolution and immunochemical comparison

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Purified glycophorin can be dissociated into 10 different bands as revealed by PAS staining or by autoradiography of labeled protein on PAGE-SDS using a discontinuous buffer system. The major components are PAS-1 (Rf 0.22; MW 110,000) and its monomeric form PAS-2 which is resolved into 2 distinct bands (Rf 0.52; MW 46,000 – Rf 0.54; MW 42,000). A 3rd form is represented by PAS-4 (Rf 0.31; MW 85,000). Finally PAS-3 (Rf 0.74; MW 24,000) is always co-purified with glycophorin. In addition, PAS-2 dissociates into 2 bands (Rf 0.68; MW 29,000 – Rf 0.90; MW 15,000) whereas PAS-1, PAS-4 and PAS-3 can form aggregates of apparent MW respectively 165,000, 150,000 and 55,000. When tested in a 2-dimensional immunodiffusion assay using antibodies to PAS-1 or PAS-3, all subcomponents can be precipitated with both antisera thus suggesting the presence in all of them of constituants with common antigens. These results indicate that glycophorin is composed of at least 2 different glycopeptides PAS-2 and PAS-3 which can form various aggregates yielding the complex electrophoretic pattern observed.

A contribution to the characterization of Na-dependent transport systems in brush border membranes

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The addition of the Cu^{2+}/O-phenantroline complex (1:2 mol ratio) to freshly prepared brush border membrane vesicles from rabbit small intestine inhibits sodium dependent transport functions, e.g. D-glucose, L-alanine, L-methionine uptake, but has no effect on the sodium independent D-fructose transport. The interaction with the Cu^{2+}/O-phenantroline complex is accompanied by oxygen consumption; it is only partly reversible by the addition of reducing agents such as dithiothreitol, and it does not render the membrane leaky. Furthermore the interaction is competitively prevented by those substrates which are transported in the presence of sodium. This indicates that the oxidation catalyzed by Cu^{2+}/O-phenantroline involves SH-groups located at the transport sites.

Inhibitors of 3′,5′, cAMP-phosphodiesterase also lower respiration and ATP-content of human blood platelets

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Inhibitors of 3′,5′-cAMP-phosphodiesterase diminish O_2-uptake, ATP-content and ADP-induced aggregation in the $\leq$ millimolar range. However, other inhibitors of aggregation (e.g. local anesthetics or Antazoline) induce similar changes without affecting [cAMP]. The exceptionally strong depression of ATP-content by papaverine and congeners is not fully explained by their known site-I respiratory inhibition.

Turnover of exchangeable arterial cholesterol in intact arteries of rats and rabbits

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The turnover (TO) of exchangeable arterial cholesterol (EAC) was measured in 15 rats and 9 rabbits by a new method (submitted for publication) which assumes that plasma cholesterol and EAC are in metabolic equilibrium. 3H- and 14C-cholesterol are injected into each animal i.v. at different times. From the plasma time course of the 2 tracers and the double label of the a. thoracica at the end of the experiment the influx of plasma cholesterol into the artery (= TO of EAC), t/2 and pool size of EAC are calculated. Values for rats (rabbits in brackets): TO of EAC: 0.74 $\pm$ 0.06 γ/mg d.w./day (0.19 $\pm$ 0.02); t/2 of EAC: 0.81 $\pm$ 0.06 days (4.0 $\pm$ 1.5); pool size of EAC: 0.80 $\pm$ 0.03 γ/mg d.w. (0.92 $\pm$ 0.14). EAC represents 20–25% of total arterial cholesterol (TAC): 4.52 $\pm$ 0.24 γ/mg d.w. (3.93 $\pm$ 0.14). Conclusion: Pool sizes of EAC and TAC are comparable in rats and rabbits. TO of EAC is about 4 times larger in rats than in rabbits. This might be related to the known resistance of rats to atherosclerosis, to differences in lipoprotein metabolism and/or body size.

A carboxylate residue at the active center of external yeast β-fructosidase

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External yeast β-fructosidase is irreversibly inactivated by conduritol-B-epoxide, an active site-directed inhibitor of this enzyme. The inactivation is prevented by the presence of substrates. During the inactivation approximately one mol of [^{3}H] conduritol-B-epoxide is bound covalently per mol of enzyme. The release of the label by treatment with hydroxylamine and the apparent pK_a value of the reactive group suggest that a carboxylate group is present in the active center of the enzyme.

On the hydrophobic segment of sucrase isomaltase

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Sucrase-isomaltase from rabbit small intestine is bound to the brush border membrane and lecithin bilayers through its isomaltase subunit. The hydrophobic portion of the enzyme interacting with the lipid bilayer is a low mol. wt polypeptide at the N-terminal end of the isomaltase subunit. The isolation and characterization of that peptide is described.

Different activation of striatal adenylate cyclase by dopamine and GTP-analogues

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Adenylate cyclase (AC) of rat striatal homogenates was activated by dopamine (DA), whereas GTP-analogues, which are known activators in other organs, were inactive or even slightly inhibitory. However, after preincubation up to 30 min without the substrate, GMPPCP, GMPPNP

or NaF increased AC severalfold, while the basal or DA-stimulated activity was decreased. After a 10-min preincubation, the basal activity, as well as the action of GMPPNP or NaF was increased by Mg^{++}. Conversely, DA stimulation decreased with increasing Mg^{++} concentrations. GMPPNP-induced activation was irreversible, whereas the effect of DA could be reversed by dialysis. GMPPNP 10^{-6}M together with DA 3×10^{-5}M showed synergistic activation. However, the effect of NaF 10^{-2}M plus DA was less than additive. Haloperidol antagonized DA- but not GMPPNP-induced activation; in contrast GMPPNP was strongly inhibited by GTP. Therefore AC has, beside the specific DA sensitive site, at least one other site for activation, which reacts differently towards activators and inhibitors.

Sulfatide metabolism of mouse cerebrum and cerebellum during development

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The in vivo synthesis and degradation of sulfatide, a myelin lipid, and the activities of cerebrosidesulfotransferase (CST) and cerebrosidesulfate sulfatase (CSS) are compared during postnatal myelination. The gross synthesis was measured by the incorporation of $^{35}SO_4$ into sulfatide. The net synthesis was estimated by the increment of sulfatide. The in vivo degradation was calculated as the difference between gross and net sulfatide synthesis. Gross and net synthesis in the cerebellum were 2–3 times higher than in the cerebrum. The in vivo degradation was higher in the cerebellum. Its developmental pattern was comparable to the CSS activity (arylsulfatase A). The developmental pattern of the CST activity was similar to the gross synthesis of sulfatide up to 17 days. Later, CST activity decreased, whereas gross synthesis remained high. The net sulfatide synthesis, regulated by CST and CSS, is the result of the functional activity of these enzymes in vivo being only partially identical with the activities, measured in vitro.

The mitochondrial locus OXI I codes for cytochrome oxidase subunit II in yeast

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The mitochondrially-translated polypeptides were analyzed in yeast mutants which specifically lacked cytochrome oxidase as a result of mutations in the mitochondrial loci OXI 1, OXI 2 and OXI 3. The OXI 1 locus was shown to code for the mitochondrially-translated cytochrome oxidase subunit II by the following criteria: a) in 2 OXI-1-mutants cytochrome oxidase subunit II is replaced by a smaller fragment which cross-reacts weakly, but significantly with an antiserum against subunit II; b) in mitochondrial revertants of these 2 OXI-1-mutants subunit II has regained its strong interaction with anti-subunit II serum, but has a mol. wt smaller than wild-type subunit II and larger or smaller than the fragment; c) in a nuclear revertant of an OXI-1-mutant, neither the immunological cross-reaction nor the mol. wt of the subunit II fragment are altered. We conclude that the OXI 1 locus is the structural gene for cytochrome oxidase subunit II and suggest that the 2 OXI-1-mutants studied here have suffered deletions in this gene.

Isolation of the precursor form of Cl and studies on its activation

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A modification of an affinity chromatography technique for the isolation of Cl from serum is described. After application of the serum, the Sepharose-IgG is rapidly washed strictly at 0 °C. The Ca^{2+}-dependent Cl subcomponents can then be eluted with EDTA in their proenzyme form. Fractionation of this eluate with strong anion and cation exchangers yields 3 proteins in pure form, which were identified by their antigenic and molecular properties as Cls, Clr and Clt. The precursor nature of Cls and Clr was ascertained by SDS-PAGE of the reduced proteins. Both reduced Cls and Clr were found to have an apparent molecular weight of 100,000 daltons. Thus Cl may be exposed to aggregated IgG for periods up to 2 h at 0 °C without cleavage of Cls and Clr. This observation, together with the fact that $EAC\overline{14}$ may be generated by exposing EA at 0 °C to serum (containing precursor Cl), suggests that cleavage of Cls and Clr is not indispensable for Cl activity. This has been investigated using labelled active site-directed reagents for serine esterases.

The glycosyl-portion of J-chain in immunoglobulin polymers

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In IgM and IgA an additional polypeptide chain, the J-chain, is responsible for polymerization. The J-chain has a particular elongated shape, and carbohydrate units might stabilize it. In order to analyze the possible role of carbohydrates, J-chain was obtained from a murine myeloma protein of the IgA_2 class (MOPC-315 from BALB/c mice) and was separated by submitting the reductively cleaved (dithiothreitol 20 mM, urea 10 M) and alkylated (iodoacetic acid 60 mM) protein to polyacrylamide gel electrophoresis. J-chain was identified as the most anodic migrating band and was isolated by elution from the gel. As measured by gas chromatography, the sugar content of the J-chain was about 8%, which is as high as for the complete IgA, but the proportion of the various monosaccharides was different comparent to the α-chain. The presence of arabinose and xylose, as well as an excess of galactose, suggest a series of short-chain saccharides which indeed might serve to stabilize an elongated shape and thus facilitate polymerization.

Magnesium-induced cooperativity in the binding of calcium to a monomeric protein

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A sarcoplasmic calcium binding protein (SCP) with a mol. wt of 17,000 and a pI of 4.3 has been isolated from the worm Nereis virens. The protein has a high Met and Phe, and a low Tyr, Trp and His content. Hence it seems to be related to the parvalbumin-troponin C family, particularly to TNC from dogfish. Nereis SCP binds 3 g atoms of either Ca or Mg per 17,000 mol. wt. The titration of SCP by Ca in the absence of Mg, using EDTA-buffered

equilibrium dialysis, indicates that the 3 sites are indistinguishable with pK = 8.2, and show no cooperativity. Under physiological conditions (1 mM Mg, pH 7.4, 0.1 I), magnesium affects the calcium binding by a) decreasing the apparent pK according to the rule of calcium-magnesium competition, and b) inducing interaction between the calcium binding sites. Indeed, in the presence of Mg, the Hill's coefficient becomes larger than 2, which indicates strong cooperativity, although SCP is monomeric. Cooperativity in calcium binding would confer to SCP the ability to regulate calcium levels in a more sophisticated way than do parvalbumins in vertebrate muscle.

Characterization of a protein associated with bacteriochlorophyll and lipid from Rhodospirillum rubrum

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By using a chloroform/methanol mixture we have been able to extract the protein component of a light-harvesting bacteriochlorophyll(BChl)-lipid-protein complex from R. rubrum G-9. The protein dissolved in the organic solvent is separated from BChl and phospholipids by gel filtration on Sephadex LH-60. The polypeptide isolated yields a single band in analytical SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and a linear sedimentation equilibrium plot. By using the same organic solvent treatment on chromatophores from R. rubrum G-9, together with SDS-PAGE and amino acid analysis, we have been able to show that the same polypeptide dissolves from both chromatophores and light-harvesting complex. The purified polypeptide shows a high content of nonpolar residues, and no tyrosine or cysteine. Minimum molecular weights were estimated at 9–12 kd by SDS-PAGE, 12.5 kd by sedimentation equilibrium analysis and 13 kd by amino acid analysis. Similar results are obtained for the organic solvent soluble polypeptide from chromatophores of the wild-type R. rubrum.

The Friend cell 15 S globin mRNA contains only β-globin sequences

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The 15 S globin RNA, a putative precursor of 10 S globin mRNA, identified in pulse-labeled RNA from induced Friend cells (Curtis and Weissmann) has been purified by the poly(I)-Sephadex method and characterized by T_1 RNAase fingerprinting. A number of oligonucleotides in the fingerprint could be assigned to β-globin RNA but none to α-globin RNA. Several distinct oligonucleotides absent in α- and β-globin mRNA were found in a region characteristic for U-rich oligonucleotides. For further identification of the 15 S globin RNA in regard to α and β sequences we have constructed plasmids containing α- and β-mouse globin cDNA. The globin-specific sequences were excised by the procedure of Hofstetter et al. and characterized with respect to size. Millipore filters with bound α- and β-globin plasmid DNAs were used to assay for α- and β-globin RNA in 20 min pulse-labeled RNA fractionated by sucrose gradient centrifugation. The ratio of β- to α-specific sequences in the 15 S peak was greater than 20:1, and in the 10 S region about 1:1.

Localization of heme in cytochrome *c* oxidase as measured by fluorescence energy transfer

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Purified yeast cytochrome *c* oxidase was covalently labelled with 2 fluorescent thiol reagents, N-iodoacetyl-N′-(5-sulfo-1-naphthyl) ethylenediamine (1,5-I-AEDANS) and its 2,6 isomer (2,6-I-AEDANS). By preparing the radiolabelled 1,5-I-AEDANS, it was shown that the reagent binds to a sulfhydryl group on subunit II of the oxidase. Up to 0.5 mole of AEDANS was bound per mole of heme *a* indicating a single reactive group per 140,000 daltons of cytochrome *c* oxidase. The specific activity of the oxidase was not affected by the labelling procedure. Nsec fluorescence decay measurements were performed on the labelled oxidase to determine if the intrinsic heme *a* of the enzyme was close enough to the probe site for fluorescence quenching to occur through singlet-singlet resonance energy transfer. No such quenching was seen. The distance between the reactive sulfhydryl group on subunit II and the enzyme-bound heme *a* is thus greater than 50 Å.

Aurovertin-resistant mitochondrial ATPase

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In order to identify the genes coding for the individual polypeptide subunits of the mitochondrial ATPase F_1, yeast mutants resistant to aurovertin were isolated. This antibiotic specifically binds to F_1, thereby inhibiting its function. A small fraction of the aurovertin-resistant yeast mutants isolated contained altered F_1. The altered protein was still active in oxidative phosphorylation, but no longer bound aurovertin as shown by fluorescence measurements. The mutant phenotype was caused by the mutation of a single nuclear gene. Tryptic fingerprints of each of the 3 major F_1 subunits suggest that the largest of these subunits is altered by the mutation. This opens the way for mapping one of the structural genes for mitochondrial ATPase.

Relationship between phosphorylase activation and adenosine 3′,5′-monophosphate accumulation in rat C-6 glioma cells

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C-6 glioma cells respond to β-adrenergic agents with a rise in cAMP (A. G. Gilman and M. Nirenberg, Proc. nat. Acad. Sci. USA *68*, 2165 (1971)) as well as with a conversion of phosphorylase b to a (E. T. Browning et al., Molec. Pharmac. *10*, 162 (1974)). We have found that phosphorylase conversion occurs at very low agonist concentrations, e.g. 10 pM isoproterenol is required for 50% activation, whereas the EC 50 for cAMP accumulation is 1.2 nM. The effects of the agonists on the 2 parameters were blocked stereospecifically by 1-propranolol, although much higher concentrations were required to block phosphorylase conversion. Results on cAMP accumulation with a range of β-agonists indicate that only 1–2% of the cells maximal ability to form cAMP is required for full phosphorylase activation. A number of β-blockers were found to be full or partial agonists on

phosphorylase activation. The results suggest that homogeneous cell systems are useful in evaluating the partial agonist activity of β-antagonists and to evaluate the role of 'spare receptors'.

Inhibition of horse liver alcohol dehydrogenase by salicylate

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From cristallographic data salicylate derivatives are known to bind in the hydrophobic adenosine-binding region of horse liver alcohol dehydrogenase. Salicylic acid (SA) yields noncompetitive inhibitions versus NAD^+ and NADH. The replots are 2/1-functions curved upwards except for the intercept with NADH, which shows 2/1-downward curvature. The inhibition patterns versus ethanol, acetaldehyde and propionaldehyde are noncompetitive and the replots curved upwards. At saturating NADH and propionaldehyde concentrations SA-inhibition exhibits an intermediary plateau in the Dixon-plot. This kinetic behaviour suggests the formation of a kinetically significant mixed complex with SA on one subunit and coenzyme on the other, whereby SA increases the K_m for NADH by negative cooperativity.

Characterization of a cAMP-insensitive protein kinase isolated from nuclei of calf ovaries and GH_3-cells

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Nuclei from calf ovaries were purified through 2.0 M sucrose whereas the nuclei from GH_3-cells were prepared with 0.5% Triton X-100. Both nuclear preparations were extracted with 0.3 M NaCl and subsequently fractionated on phosphocellulose. The chromatography of these nuclear extracts revealed 2 protein kinases of different substrate preference: a protamine-sensitive protein kinase (PK-I) was eluted at 0.3 M NaCl and a hposviiin-specific protein kinase (PK-IV) between 0.5 and 0.6 M NaCl. The specific activity of PK-IV was approximately 10,000 pmoles ^{32}P/incorp./min mg prot. (ca. 20–50fold purification). The apparent K_m values for phosvitin were 150 μg/ml for PK-IV of the GH_3-cells and for the enzyme of the calf ovaries. The pH and NaCl vs. activity curve of PK-IV is monophasic with optima at pH 6.5 and 220 mM NaCl respectively. The PK-IV enzyme is cAMP-insensitive. The K_m values for ATP of PK-IV is 6 μM for both enzymes.

Lactic acid production by rat calvaria cells in culture

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Lactic acid production probably plays an important role in the mechanism of bone resorption. The milieu inside the bone differs from the one outside. A bone 'membrane', separating the 2 sites seems to be responsible for this phenomenon. It is not known if factors influencing bone metabolism act on this membrane or if they act directly on the bone cells. To study this question, cells from rat calvaria are cultured and the effect of several factors on lactate production is determined. These cells drastically decrease the lactate production when the pH in the culture medium is lowered, changing from 100% to 20% for a pH shift from 7.4 to 6.75. L-lactate inhibits its own formation, by 40% at 20 mM, PTH (820 units/mg) at a concentration ranging from 0.2 to 5.0 units/ml stimulates slightly the lactate production in a log-linear response, the ratio treated over control changing from 1.1 to 1.3. The maximal stimulation is observed between 6 and 9 h PTH treatment. The largest stimulation of PTH is observed at pH 7.0. Isolated cells do not seem to respond differently from the intact calvaria.

Glycosylated Hb in whole blood. Rapid quantitation and functional properties

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Hb A_{1c} is the major glycosylated hemoglobin: Hb A_{1a} and A_{1b} are minor ones. They increase when blood glucose is high. 45 blood samples were tested for Hb A_{1c}-content by CM-Sephadex chromatography. Hb A_{1c} and fasting blood glucose correlated well ($c = 0.88$). 8 cases, not fitting this pattern, had a recent abrupt change in diabetic condition. Thus, glycosylated Hbs represent a time integral of blood glucose. In view of their clinical importance a simpler assay for these Hbs was developed. 5-Hydroxymethylfurfural (5-HMF) or its derivatives liberated from the glycosylated Hbs were measured after the original N-glycosidic bond had undergone Amadori rearrangement (yield: 80–90% of theoretical value). Hb A_{1c} levels and 5-HMF determinations correlated linearly. Normally occurring, diabetic and synthetic Hb A_{1c} were functionally identical. They differed from Hb A in O_2 affinity in the stripped form (log $p_{O_2}50$ of 0.67 for Hb A_{1c} and 0.54 for Hb A) and susceptibility to 2,3-DPG at saturation (log $p_{O_2}50$ of Hb A_{1c} 1.03 and 1.21 for Hb A). The values of n and the Bohr factor were the same for Hb A_{1c} and Hb A.

The transcobalamin II isoprotein pattern

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Vitamin B_{12} is transported in blood by specific carrier proteins, the transcobalamins (= TC). 3 TC have been observed. TC I and III have been separated by isoelectric focusing and were reported to belong to 1 group of acidic isoproteins. TC II is immunologically different from TC I and III. Chemically, TC II lacks sialic acid which is a part of TC I and III. TC I and III can be degraded by Neuraminidase, whereas TC II is unaffected. The polyacrylamid electrophoretic system reported here utilizes this degradation to obtain an effective separation of TC II from TC I and III. Co^{57}-B_{12} labelling of unsaturated and neuraminidase-treated serum proteins, with subsequent autoradiographic evaluation, exhibited TC II fractions containing 2 or 3 or rarely 4 isoproteins. Individual TC II patterns were demonstrated in a family where both father and daughter possessed 4 TC II isoproteins. Serum from a case of congenital TC II deficiency was used as one of the controls in this study.

Myasthenia gravis and acetylcholine receptor

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Recent evidence favors the idea that acetylcholine receptor might be involved in myasthenia gravis as an autoantigen. For example, antibodies directed against the receptor have been demonstrated in the serum of myasthenic patients. A test that allows to detect anti-acetylcholine receptor antibodies in minute amounts of human serum will be described. Several animal models of myasthenia gravis will be discussed according to the most recent findings. Hypotheses concerning the pathogeny of the disease will be presented.

Reversible dissociation of factor VIII subunits by Rhizopus lipase

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Highly purified human factor VIII (antihaemophilic factor) does not enter 3% polyacrylamide gel (extrapolated exclusion limit $> 5 \times 10^6$ daltons). Triglyceride lipase from Rhizopus arrhyzus dissociated this macromolecular aggregate into smaller subunits. The dissociated subunits retained full procoagulant and ristocetin cofactor (von Willebrand factor) activity and reaggregated spontaneously within 6 h at 37 °C. The resulting reconstituted macromolecular complex remained functionally fully active and was not dissociated in 6 M urea-0.2% SDS. It could, however, be repeatedly decomposed by addition of fresh lipase. The reaggregation reaction was inhibited at low temperature, but was markedly accelerated in the presence of PMSF (phenylmethylsulfonyl fluoride) or EDTA. It is proposed that the subunits of factor VIII are linked together by non-covalent interactions with unidentified lipid species. These lipids might reversibly bind to the active lipase (enzyme-substrate complex) and thus lead to the dissociation of the factor VIII.

Control of mutagenic benzo(a)pyrene metabolites by epoxide hydratase and glutathione

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Benzo(a)pyrene (BP) was activated by liver microsomes to mutagens for Salmonella typhimurium, and 2 potential inactivating systems were studied: Epoxide hydratase (EH) and conjugation with glutathione. The role of EH depended on the pattern of primary metabolites which depends on the monooxygenase forms present: EH protected during activation by control microsomes (containing control cytochrome P-450). In contrast EH had a potentiating action during activation of BP by microsomes from methylcholanthrene treated mice (i.e. in presence of cytochrome P-448), but this potentiation occurred only at low BP concentrations favoring further oxidation of BP metabolites. Using different Salmonella strains, it could be shown that in the former situation monofunctional arene oxides and in the latter dihydrodiol epoxides were mainly responsible for the mutagenicity. Glutathione and glutathione transferases reduced the mutagenicity, but only to a limited extent. The limitation seemed to be due to their location in the cytoplasmic fraction and the preference of the mutagenic BP metabolites for membranes.

The effect of two diphosphonates on the collagen metabolism of mouse and rat bone and cartilage

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The diphosphonates, compounds related structurally to pyrophosphate, characterized by a P-C-P instead of a P-O-P bond are known to interfer with Ca metabolism. The classical explanation is found in its effects on apatite formation and dissolution. Recently some evidence has indicated that these compounds act also on a cellular level. The effects of various diphosphonates on the metabolism of collagen from bone and cartilage were now studied. The compounds were injected s.c. in various concentrations into newborn rats daily for 12 days. The animals were subsequently sacrificed and their calvariae and tibiae cultured for 24 h in MEM containing 10% foetal calf serum, 20 μCi/ml ^{3}H-proline and 1 mM of BAPN but no diphosphonates. In addition isolated bone (rats) and cartilage cells (rabbit) were cultured for 8 days in the above mentioned medium but in the presence of different diphosphonates. The incorporation of ^{3}H-proline into collagen as well as the possible changes in the molecular structure were determined.

The conformation of the lecithin polar group

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The conformation of the polar group of lecithin and lysolecithin has been investigated in fully hydrated single bilayer vesicles and micelles, respectively, using paramagnetic probes (lanthanides). With both lipids 2:1 lipid to metal ion complexes are formed. In the presence of lanthanides the conformations of the glycerylphosphorylcholine group of both phospholipids are similar as derived from perturbations in the ^{1}H, ^{13}C and ^{31}P NMR spectra of the lipids. The computer analysis of the perturbations gives an average preferred conformation. A rather limited range of population of conformation contribute to that average, the details of which are described.

Comparative studies and sequence investigations of several biliproteins from the thermophilic blue-green alga Mastigocladus laminosus Cohn

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Allophycocyanin I and II, phycoerythrin and C-phycocyanin are the primary absorbers of light used in photosynthesis by blue-green algae. Each of these biliproteins contains a bile pigment prosthetic group which is firmly bound to the apoprotein. These chromoproteins were characterized with respect to homogeneity, absorption spectra, isoelectric point, molecular weight, amino acid and subunit composition. Each of the biliproteins consisted of two subunits, designated α and β, present in

equivalent molar proportions. The stability of the phycobiliproteins was studied with respect to light, heat and pH. Amino acid compositions of the biliproteins as well as amino terminal sequences were compared. Cyanogen bromide cleavage of the individual subunits was partially followed by tryptic and chymotryptic digestions. The resulting peptides were purified and investigated with respect to their amino acid sequences.

UDP-gal 4′-epimerase of human red cells: Affinity chromatography on substrate

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UDP-galactose 4′-epimerase of human red cells was found to bind its substrates by their uridine moieties. Therefore, the design of substrate-matrix complexes for affinity chromatography had to avoid the uridine part of the substrate molecule. The second carbon of the substrate hexose was suitable for linkage to a solid matrix. Such linkage did not impair binding of the enzyme to the immobilized substrate. Affinity chromatography on this medium yielded epimerase of high purity. Characteristics of holoenzyme and apoenzyme binding to immobilized substrate at different temperatures were studied. Relative insensitivity to changes in temperature were noted.

Incorporation of human erythrocyte acetylcholinesterase into liposomes

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The acetylcholinesterase (AChE) from human erythrocytes is a membrane bound enzyme. In order to study its kinetics in an environment similar to that found in vivo, the enzyme was incorporated into liposomes of different phospholipid composition. Several methods for preparing small (preferably unilamellar) liposomes with incorporated AChE were investigated. The size of the liposomes was established by gel filtration and AChE incorporation was monitored by density gradient analysis. Mere incubation of preformed liposomes with AChE resulted in poor enzyme incorporation. Formation of liposomes by sonication in the presence of AChE resulted in complete incorporation (70% of the activity associated with the outerside). This procedure, however, led to a loss of enzyme activity up to 60%. The use of DOC in conjunction with dialysis or gel filtration also led to the formation of liposomes with complete incorporation of AChE. Despite a certain heterogeneity in the size of the liposomes, these systems were the most favourable and resulted in the separation of 99.96% of the DOC from the liposomes.

Structure of the M-line in chicken muscle

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Characterization of the proteins comprising the M-line will be necessary for elucidating its structure and function. 3 M-line proteins are generally recognized, with subunit MW's of 1.6, 1.0 and 0.4×10^5. The smallest is MM-creatine kinase, the next largest, glycogen phosphorylase b (Ph-b). We have presented evidence that the largest is glycogen debranching enzyme (D-E). To obtain material for further studies, both Ph-b and D-E were purified to homogeneity from chick muscle. Ph-b isolated in 2 steps by $(NH_4)_2SO_4$ precipitation (0–41%) followed by chromatography on DEAE-cellulose is a dimer (MW 200,000) as determined by high speed sedimentation. It is converted to the tetrameric a form by incubation with rabbit phosphorylase kinase, Mg^{+2}, and ATP. Purification of D-E involved $(NH_4)_2SO_4$ precipitation, chromatography on DEAE-cellulose and 'hydrophobic' chromatography. The 160,000-MW putative M-line protein extracted from myofibrils was indistinguishable from isolated D-E in specific enzymic activity and mobility in SDS gels.

β-Actinin from chicken muscle

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β-actinin was purified to homogeneity from water extracts of chick leg muscle by chromatography on DEAE-cellulose and gel filtration through Sephadex G-200. The mol. wt estimated from polyacrylamide gel electrophoresis in the presence of SDS was 65,000. Sedimentation equilibrium experiments gave a mol. wt of 67,000. The results show that β-actinin is a monomeric protein. Double diffusion using anti-β-actinin serum indicated that an immunologically indistinguishable forms of this protein is present in muscle as well as in non-muscle tissues of the chick. In situ localization of β-actinin using indirect immunofluorescence gave a regular, intense cross-striation pattern within the I-band of isolated myofibrils. It could be demonstrated in an EM investigation that β-actinin does not polymerize to filamentous structures under conditions where G-actin is transformed to F-actin but is able to inhibit polymerization of G-actin and promote depolymerization of F-act in invitro. The immunological, physico-chemical and EM data prove β-actinin to be a protein quite distinct from actin.

High affinity uptake of glycine, serine and alanine in pigeon optic tectum

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After superficial application of tritiated glycine, serine or alanine to the pigeon optic tectum, the label is selectively accumulated in the soma of cells in the nucleus isthmi, pars parvocellularis. In an attempt to understand the underlying mechanism of this observation, the uptake of these aminoacids was studied in crude mitochondrial fractions. High affinity uptake systems were found for glycine, serine and alanine. The K_m (μM) was: gly 50, ser 21, ala 25. The uptake was Na^+ and temperature dependent and localized in the synaptosomal fraction. Whereas a similar glycine uptake was measured in the spinal cord (K_m 32 μM) only a low affinity uptake was found in the hemisphere. Serine and alanine uptake was competitively inhibited by glycine but the inhibition of glycine uptake by serine and alanine was only 55% and 1/uptake vs [inhibitor] plots were hyperbolic. This might indicate the presence of two high affinity uptake systems, one for glycine only and one for glycine, serine and alanine. This observation supports the hypothesis that glycine is a neurotransmitter in the tectum.

Calcitonin binding and stimulation of cyclic AMP in cultured human lymphoid cells

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Salmon calcitonin (sCT) binding and stimulation of cyclic AMP (cAMP) formation were studied in cultured lymphoid cells. The plateau of the specific binding of [^{125}I] sCT was reached at 240 min, whereas maximal cAMP responses to sCT occurred after 5 min. sCT and CT analogs inhibited competitively the binding of [^{125}I] sCT and stimulate cAMP levels. Their binding inhibition or cAMP stimulating potency was comparable to their relative activity in the hypocalcemic rat assay. The addition of EGTA or calcium did not affect cAMP formation, whereas the calcium ionophores A 23187 and Br-X-537 suppressed basal and sCT stimulated cAMP levels. In conclusion, cultured lymphoid cells bound [^{125}I] sCT with high affinity. A small fraction of the total number of binding sites was required for the mediation of the cAMP response, suggesting the presence of spare receptors. Calcium ionophores antagonized the stimulation of cAMP by sCT.

The influence of aging on glycolytic enzymes in human and rat brain

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The activities of 14 glycolytic enzymes in human cortex and rat brains homogenates were determined. The human material was obtained from local pathological institutes (n = 25, age 19–91 years; time after death 4–24 h). Rat brain were removed from animals of 4 age-groups (20, 60, 100, 120 weeks) (n = 6 each group) after freezing in liquid nitrogen. No significant correlation between either human or rat enzyme activities and age could be shown. However some enzymes such as: fructose-6-phosphatekinase (FPK), hexokinase (HK), aldolase and phosphoglycerate-mutase in human, but only FPK and HK in rat brain tissue, show slight age dependent differences of their activities determined at usual test conditions (Bergmeyer). FPK and HK are believed to be glycolytic regulatory enzymes. Further studies under reduced substrate concentrations could give us more detailed aspects about the influence of aging on glycolytic enzymes.

Purification of a bacterial elongation factor by affinity chromatography

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Protein synthesis elongation factors Tu and G respectively transfer aminoacyl tRNA to, and function in the translocation of, ribosomes in E. coli. Both factors tightly bind guanine nucleotides in the absence of ribosomes. This fact suggested to us a one-step purification procedure by the use of Sepharose-linked GDP as affinity matrix. The dialdehyde derivative was obtained by periodate oxidation of the vicinal hydroxyl groups in the ribose moiety of GDP, and covalent attachment to AH-Sepharose was achieved by reduction. To 1 ml of settled matrix, an ammonium sulfate fraction (37–64%) obtained from 150 mg of wet cells was added. After extensive dialysis and subsequent washing in a column, bound protein was eluted by the addition of free ligand to the elution buffer. This procedure yields quantitative retention and release of EF-Tu, whereas the weaker affinity of EF-G to the modified ligand limits the usefulness of this system for its purification.

Identification of bile acids in human amniotic fluid by gas chromatography-mass spectrometry

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Studies of bile acid composition of human meconium suggest that a foetal pathway of hepatic bile acid synthesis exists which leads to formation of 3β-hydroxy-5-cholenoic acid, a bile acid not found in serum of normal pregnant women. To obtain additional support for this hypothesis, bile acids in human amniotic fluid were identified by combined gas chromatography-mass spectrometry. A glass capillary column (25 m × 0.24 mm) Silar 10 precoated with barium carbonate (prepared by K. and G. Grob, ETH Zürich) and a Finnigan Model 1015 D GC-MS apparatus with a Model 6000 Interactive Data System were used. Besides cholic, chenodeoxycholic and deoxycholic acid, the major bile acids of adult humans, 3β-hydroxy-5-cholenoic acid was identified in human amniotic fluid by mass chromatography. The presence of this bile acid was further ascertained by comparing the mass spectrum corresponding to its peak in the total ion current chromatogram with the mass spectrum of authentic 3β-hydroxy-5-cholenoic acid. GC-MS proved to be a powerful method for identification of uncommon bile acids in biological fluids.

^{1}H NMR studies at 360 MHz of cytochrome c-552 from Euglena gracilis

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Earlier NMR studies had shown essential features of the tertiary structure of cytochrome c-552 from Euglena gracilis to be quite similar to those of mammalian cytochromes c [R. M. Keller, K. Wüthrich and A. Schejter, Biochim. biophys. Acta, in press]. Here, we describe ^{1}H NMR studies of the molecular dynamics. The relatively slow exchange of a number of amide protons against deuterium of D_2O implies that the protein contains a compact core, which is more rigid in the ferrous than in the ferric state. The aromatic proton resonances revealed the occurrence for 4 of the 6 Phe and Tyr residues of rapid 180° flips about the C_β-C_γ bond at ambient temperature. Cytochrome c-552 thus exemplifies typical features of protein conformations in solution: the overall rather compact and rigid molecular structure, as evidenced in the slow exchange of interior labile protons, is traversed by vibrations of sizeable amplitude which cause the temporary local flexibility required for intramolecular rotational motions of aromatic rings.

Primary structure of human liver and equine kidney metallothioneins

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Metallothioneins (MT) are proteins of extremely high metal (Zn, Cd, Cu)- and cysteine-content which occur in parenchymatous tissues of many species and are thought to play a regulatory function in heavy metal metabolism and/or detoxication. The unusual amino acid sequence determined on a variant (MT-1B) from equine kidney (Kojima et al., PNAS *73*, 3413 (1976)) suggests that the metallothioneins belong to a novel, as yet unknown superfamily of proteins. To explore their structural features further we have now established the sequence of another variant of equine renal MT (MT-1A) as well as of one of the principal forms of human liver MT. The sequences reveal striking homology among the three proteins, especially with respect to the metal chelating structures. While there are amino acid substitutions, the positions of almost all Cys and of the Ser and basic amino acid residues located adjacent to the Cys are invariant. The selective conservation of these sequences in evolution implies remarkably rigid structural requirements for metal binding to these proteins.

A photometric assay for monoamine oxidase

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A new photometric assay for the determination of monoamine oxidase activity was devised. In this simple and sensitive assay the monoamine oxidase dependent production of hydrogen peroxide is measured using a coupled indicator reaction. In this reaction the hydrogen peroxide dependent oxidation of leuco-2′,7′-dichlorofluorescein to 2′,7′-dichlorofluorescein is catalysed by horse radish peroxidase and followed at 502 nm. The reduced dye does not absorb at 502 nm, whereas the oxidized form has a molar extinction coefficient of 91,000. Linear calibration curves with varying enzyme concentrations were obtained with benzylamine, phenylethylamine, tyramine, octopamine, normethanephrine, tryptamine, 5-hydroxytryptamine and 5-methoxytryptamine as substrates. The assay is especially suitable for determining substrate specificities for physiological amines as well as inhibitor studies with pargyline or the monoamine oxidase A and B form specific inhibitors clorgyline and deprenyl.

Characterization of a Ca-binding protein from the primitive chordate Amphioxus

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The acidic soluble Ca-binding proteins (SCP) from muscle present slightly different properties in vertebrates and invertebrates. SCP's of the latter have a mol. wt of 17,000–22,000, bind 1–3 g atoms Ca per mole (pK′ = 7.2 in 1 mM Mg) and their UV spectra give 280/260 ratios from 1.3 (annelids and molluscs) to 1.7 (crustacea). Vertebrate SCP's form a more homogeneous group (parvalbumin) with a mol. wt of 12,000, 2 g atoms Ca per mole (pK′ = 6.7) and an unusual UV spectrum (280/260 = 0.1). It was of interest to find out which type of Ca-binding protein exists at the junction between vertebrates and invertebrates. The SCP from the cephalochordate Amphioxus has a mol. wt of 21,000, binds 3 Ca (pK′ = 6.7) and its spectrum gives the 280/260 ratio 1.8. In Amphioxus as in crustacea (crayfish) only one of the 3 Ca can be replaced by Mg whereas in parvalbumin both Ca are exchangeable for Mg. Furthermore, SCP in Amphioxus as in crayfish has 2 reactive –SH and a masked one that is revealed by EDTA. To conclude, the protein from chordate is more related to the invertebrate SCP's than to parvalbumin.

Membrane-associated secretory component of parenchymal cells from rabbit mammary gland

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Secretory IgA is processed by the mammary gland and secreted into milk. Dimeric IgA is produced by local plasma cells, taken up specifically and transported into the milk space by the parenchymal cells, where it is associated with secretory component (SC). SC is heterogeneous when analyzed by ion exchange chromatography and immunoelectrophoresis. SC is resolved into 6 bands on SDS-PAGE (mol. wt 70,000 to 80,000). An antiserum raised against one of the band cross reacts with all bands. At the EM level SC is detected in the Golgi elements, in apical vacuoles and on the plasma membrane of the parenchymal cells. To further investigate the role of membrane associated SC in the specific uptake of IgA, the gland is dissociated into dispersed cells and parechymal cells are enriched by isopycnic centrifugation, thus allowing full accessibility to the plasma membrane. Dispersed cells exhibit positive fluorescence for SC. The surface nature of the labeling is confirmed by immunocytochemistry using ferritin conjugates and by a complement dependent cytotoxicity test which parallels morphological results.

The interaction of melittin with a lipid-water interface

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Melittin, the major peptide of bee venom, functions in membrane systems as a direct lytic agent and as synergistic activator for phospholipases. To investigate relationships between structure and function, the interactions of melittin with detergents, e.g. N,N-dimethylalkyl-N-amine oxides, in monomeric and micellar forms were studied by fluorescence and NMR techniques. ^{1}H NMR studies at 360 MHz showed that melittin strongly self-associates in aqueous solution. Binding to detergent micelles caused changes of both fluorescence and NMR spectra of the single tryptophan in melittin. The ^{1}H NMR of melittin bound to detergent micelles showed that self-aggregation of melittin was prevented by the detergent. Well resolved peptide and detergent ^{1}H NMR lines in the micelle-melittin complexes provide a basis for further detailed studies of the interactions of melittin with detergents and the molecular conformations of the peptide which prevail at the lipid-water interface.

Glycine receptor in the pigeon optic tectum estimated by strychnine binding

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The existence of a glycinergic pathway in pigeon optic tectum has been suggested by the presence of stimulation induced release of glycine and by histochemical observations. This is supported by the facts that glycine is taken up by a high affinity system into tectal synaptosomes and that its microiontophoretic application depresses the firing of tectal neurons. The presence of postsynaptic receptor sites has been determined by the method of strychnine binding as described by Young and Snyder (PNAS *70*, 2832 (1973)). In intact animals a K_D of 4,6 nM and a B_{max} of 285 fmoles/mg protein were found. Unspecific binding was determined with 10^{-3} M glycine and was approx. 55% of total binding at 2 nM. Binding was pH + Na^+ dependent and temperature sensitive.

Paracatalytic modification of fructose-1,6-P_2 aldolase: Covalent incorporation of substrate

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Carbanion intermediates of certain enzymes may branch off the normal catalytic pathway via oxidation by suitable electron acceptors. Unusually reactive intermediates generated in such paracatalytic reactions may in their turn react with groups at or near the active site of the enzyme. Thus, 5 different enzymes have been found to date to be progressively inactivated during the oxidative trapping of their carbanionic reaction intermediates (Meth. Enzymol., 1977, in press). As an example, muscle aldolase loses 95% of its enzymatic activity within 60 min in the simultaneous presence of 2 mM fructose-1,6-P_2 (or dihydroxyacetone P) and 0.5 mM hexacyanoferrate (III) (pH 7.6, 25 °C). With U-[^{14}C]fructose-1,6-P_2 as substrate approximately 1 radioactive 3-carbon fragment and 1 phosphate group per subunit are found to be covalently incorporated. Both inactivation of the enzyme and incorporation of the substrate are strictly linked to the paracatalytic oxidation of the substrate. Peptide fractionations indicate that the substrate is incorporated into a specific position of the primary structure of the enzyme.

Pepsin-catalyzed coupling between aromatic amino-acid residues

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Our group has recently issued some papers dealing with peptide synthesis catalyzed by proteolytic enzymes. For example, α-chymotrypsin has been used to prepare peptide-esters (Luisi et al., J. mol. Catalysis, in press) and peptide-amides (Saltman et al., Biopolymers, in press) which contain the sequence -Al-Ar- (Al, aliphatic and Ar aromatic residue). As an extension of this work, we have now found conditions by which pepsin can catalyze the coupling between aromatic residues. For example, several Z-Phe-Ar-OMe, Z-Al-Phe-Ar-OMe, and the corresponding amides, have been prepared with yields ranging between 70% and 90%. Reactions take place in water or water-methanol solution at 40 °C (by simply mixing the 2 reaction partners in the presence of ~ 1 mg/ml enzyme), yielding products with the maximal optical purity and practically free from side-products. However, the syntheses are effective only for products which are insoluble under the used experimental conditions. Attempts to overcome this limiting factor, as well as attempts to find conditions for the enzymatic polymerization of amino-acids, are in progress in our laboratory.

Release of spectrin-free vesicles from human erythrocytes during ATP-depletion

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Human erythrocytes incubated without glucose at 37 °C release spectrin-free vesicles. The release of vesicles is dependent on ATP-depletion. If the endogenous level of ATP is maintained, vesicle release is completely inhibited up to 54 h. Vesicle release is independent of hemolysis because in vitro aged cells and cells that maintain their ATP level lose identical amounts of hemoglobin up to 45 h. Of all the membrane particles released 93% constitute a uniform population of spheres with a diameter of 185 ± 23 nm. Vesicles contain hemoglobin and half the amount of membrane protein per phospholipid phosphorus that is found in ghosts. However, their content of protein component III, glycophorin, and cholesterol remains unchanged when referred to phospholipid phosphorus and compared to that of erythrocyte ghosts. On the contrary, spectrin is almost completely absent. The phospholipid composition is representative of the intact membrane. It is suggested that this release of spectrin-free vesicles from cells aged in vitro represents an acceleration of the physiological aging process (in press, JCB).

Effect of fatty acid replacement

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The membrane fatty acyl composition of lymphocytes was altered by growth in lipid-depleted serum containing fatty acid supplements, as well as avidin to block endogenous synthesis of fatty acids. Under these growth conditions the supplied fatty acid represents over 50% of the total fatty acid in membrane phospholipid. Enrichment of lymphocyte membranes with oleate (cis 18:1) or elaidate (trans 18:1) shifted the optimum temperature for mitogenic stimulation by Con A as measured by [^{3}H]-thymidine uptake. These results suggest that the fluidity of the lipid phase plays a role in the process of lymphocyte triggering by lectins.

Characterization of myelin and axolemma-enriched fractions from rabbit brain

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Myelin and axolemma-enriched fractions were isolated using a slightly modified procedure for bovine CNS (De Vries, Neurosci. Letters *3*, 117 (1976)). There was a 9fold enrichment of Na^+, K^+-ATPase in the axolemma fraction over the starting homogenate. CNPase specific activity in axolemma was 30% of that myelin, contrasting with the AChE value which was 642% of that found in myelin. Cytochrome c oxidase and NADPH-cytochrome c reductase activities suggested 15–20% contaminants in axolemma but only 1–5% in myelin. The protein content was 78% and 28% for axolemma and myelin, respectively. Polyacrylamide gel electrophoresis showed the presence of many high molecular weight proteins and glycoproteins in axolemma with practically no contamination by myelin components. Electron microscopy of the axolemma fraction showed mostly paired membranes with few mitochondrial fragments. The axolemma was enriched in phospholipids but had only half the galactolipids of myelin. Myelin and axolemma fractions from rabbit CNS seem suitable to study of the glial-axonal complex at the molecular level.

Spectroscopic evidence for adenine tryptophan interaction in creatine kinase

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Binding of ADP and ATP to creatine kinase (CPK) induces anomalous ORD near 260 nm which was tentatively ascribed to an interaction of the coenzyme with an aromatic residue (Kägi et al., Biochemistry *10*, 1007 (1971)). We have now compared difference CD-spectra of complexes of CPK with several purine nucleoside phosphates. ATP, ADP, AMP and their Mg-complexes show spectra with a large positive maximum at 262.5 nm as well as a shoulder at 275 nm and a small negative maximum at 295 nm attributable to tryptophan (Trp) perturbation. The amplitudes vary in function of phosphate chain length and $[Mg^{2+}]$, ranging in $[\theta]_{262\cdot5}$ from $+19{,}000°$ for MgAMP to $+58{,}000°$ for MgADP. Evidence for purine-Trp interaction comes also from fluorescence studies of CPK-complexes: all nucleoside phosphates quench Trp-emission in function of binding site occupation. Quenching by MgADP, MgIDP and MgGDP parallels the overlap of their absorption spectra with the Trp-emission thus supporting a Förster-transfer interaction between the purine and a Trp near the active site.

Dehydroxylation and rearrangement reactions in the tyrosine metabolism

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After loading with deuterated tyrosine (Tyr), patients with irregularities in the Tyr metabolism, excreted in urine benzoic acid (BeA), a dehydroxylation product, and 3-hydroxyphenylhydracrylic acid (3OHPhHAcrA), a rearrangement product. When neomycin was given to the patients, Tyr was no longer converted to BeA and 3OHPhHAcrA. Analogous reactions were found in vitro by anaerobic incubations of faecal specimens with deuterated Tyr metabolites. The aromatic acids were analyzed by gas chromatography-mass spectrometry. In analogy to BeA we found phenylpropionic acid to be a dehydroxylation product of 4-hydroxyphenylpropionic acid. 3-hydroxyphenylpropionic acid was a rearrangement product of 4-hydroxyphenylpropionic acid and 4-hydroxyphenyllactic acid. A hydroxylation by tyrosine-3-hydroxylase, followed by para-dehydroxylation is not probable, because the incubations were done under anaerobic conditions. Our first results indicate a shift of the side chain, caused by an enzyme of the intestinal flora.

Radioimmunological measurement of antibodies to human acetylcholine receptor

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A radioimmunoassay was developed for the quantitative evaluation of antibodies to the acetylcholine receptor in the serum of myasthenic patients. AcChR was extracted from human muscle. A detailed preparation of the ^{125}I-α-Bgt-AcChR complex used as antigen is reported. Usually, an average of 20 pmoles were obtained from 100 g muscle. This preparation is stable for 1 month in presence of an inhibitor of proteolysis and sufficient for performing about 15 assays. The labelled complex was incubated with increasing amounts of sera and precipitated with anti-human IgG serum. Titers were expressed in pmole ^{125}I-α-Bgt-AcChR complex precipitated per ml serum. Out of 39 sera tested, 36 had positive titers ranging from 0.1 to 46 pmoles/ml. No anti-AcChR were detected in the sera from 27 patients used as controls.

Regulation of glycogen synthesis by some amino acids in rat liver hepatocytes

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It is known from studies with perfused livers and hepatocytes from fasted rats that only little glycogen synthesis occurs at glucose concentration below 10 mM. We used hepatocytes incubated in the presence of 10 mM glucose. Addition of 9 mM lactate/1 mM pyruvate caused a glucose increase and an average 3fold stimulation of glycogen deposition whereas 10 mM alanine caused no glucose increase and an average 4fold increase in glyc. dep.; addition of lac/pyr and ala resulted in an average 8fold increase in glyc. dep. These C-3 compounds stimulated glyc. dep. also in the presence of up to 50 mM glucose. Glutamine, proline and to a lesser extend serine gave similar effects as alanine. α-Cyano-4-hydroxycinnamate, an inhibitor of mitochondrial pyruvate transport, inhibited gluconeogenesis from serine to more than 90% but did not prevent the increase in glyc. dep. showing that the conversion of serine to carbohydrate is not required for the stimulation of glyc. dep. The hypothesis is put forward that glycogen synthesis may be regulated by an inhibition of glucose-6-phosphatase by carbamylphosphate.

A hereditary defect of the transformation of p-hydroxyphenylpyruvate into homogentisic acid

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Previously, two of us (D.M.D. and P.T.) described a new form of prolonged transient tyrosinemia in a young baby, biochemically characterized by a severe metabolic acidosis and striking p-hydroxyphenyllactic- and p-hydroxyphenylpyruvic aciduria with only mild tyrosinemia (Acta paediatr. scand. *64*, 209 (1975)). In addition, the patient and her mother excreted an unknown compound in the urine. This compound was identified by gas chromatography–mass spectrometry, chemical degradation and comparison with the synthesized reference compounds, as being (2-cystein-S-yl-1, 4-dihydroxycyclohex-5-en-1-yl)-acetic acid, and was named hawkinsin. It probably originates from 4-quinolacetic acid- or an epoxide-intermediate in the 4-hydroxyphenylpyruvate hydroxylase reaction (EC 1.13.11.27 formerly 1.14.2.2) due to a defective rearrangement system. The conjugation with cystein (resp. glutathione) followed by reduction may be a kind of detoxification of the accumulating unstable intermediate.

Regulatory effects on metabolism of lymphocytes by cytochalasin B. Antibody synthesis

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Exposure of eucaryotic cells to cytochalasin B (CB) causes nuclear extrusions, disrupts microfilaments, inhibits capping and is a potent inhibitor for the membrane transport of various substances. Con A and LPS stimulated T or B cells respectively isolated from mouse spleen by sedimentation in a serum density gradient were fixed with poly-L-lysine to plastic discs and enucleated by centrifugation (En-cells). A fluorescein diacetate-test (FDA-test) demonstrated enzymatic activity in most of the En-cells. En-cells prepared by Ludox HS-40 density gradient centrifugation were also positive in the FDA-test and were synthesizing protein. In intact lymphocytes CB reversibly inhibited DNA- and protein-synthesis and reduced the amount of antibodies secreted per cell. The number of plaque forming cells and the size of plaques was decreased after removal of antibodies at the surface by washing the cells in presence of CB. This suggests that plaque formation is independent from synthesis of new antibodies which was inhibited by CB.

Plasma catecholamines (pCA) in man and animals: Assay by a new radiometric method

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Plasma adrenaline (A), noradrenaline (NA) and dopamine (DA) have been measured by a new highly specific and sensitive radiometric method allowing their simultaneous determination by enzymatic labelling with ^{3}H-S-adenosylmethionine in the presence of catechol-O-methyltransferase. In man, dog, cat, rabbit, cow as well as in 4 different strains of rat the levels of Na were at least twice as high as those of A, when blood collection was performed under minimal stress by means of an implanted catheter; also DA was consistently detected. In contrast in rat after decapitation, stress contention or even simple handling the levels of pCA, including DA, increased to several times the previous values. In catheterized Wistar rats (SPF) A and Na were higher in carotid then in jugular plasma (454 $\pm$ 95 and 1141 $\pm$ 223 versus 177 $\pm$ 88 and 510 $\pm$ 70 pg/ml). Our results clearly underline the necessity to be very cautious in interpreting data on pCA in man and animals since the procedure for blood collection critically affects their levels.

Enzyme activity changes in the aging human brain

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Several enzyme activities, representative of different brain functions, were investigated in human brains with respect to aging (n = 36, 19–92 years old, 4–24 h after death). All cases were free of neurological diseases. Protein kinase demonstrated a gradual decline of cAMP-dependent activity with increasing age, which was more pronounced in cortex than in putamen. The diminished activation in old age was seen at all cAMP-concentrations studied. At all ages, maximal protein kinase activation occurred at cAMP = 5 µM. Acetylcholinesterase activity was found to decrease only in putamen, whereas carbonic anhydrase showed a loss of activity in both cortex and putamen. No correlation with age could be established for alkaline and acid phosphatase. The results demonstrate that aging affects enzyme activities in a non-uniform manner. Post mortem changes of these enzymes were examined in rat brains. They seem to be of minor influence as to the evaluation of possible effects due to aging (McGeer et al., J. Neurochem. *26*, 65 (1976)).

Structure-specificity relationships in phosphorylcholine binding immunoglobulins

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Monoclonal immunoglobulins with specificity for phosphorylcholine from man and mice were compared with respect to the primary structure of their variable domains and to their affinity for phosphorylcholine and other choline derivatives. All proteins had similar affinities for phosphorylcholine, but differed extensively in their binding affinity for choline. While a considerable similarity was observed in the sequence of the 40 N-terminal amino acid residues of the heavy chains studied so far (Riesen, Braun and Jaton, Proc. nat. Acad. Sci. *73*, 2096 (1976)), an extensive sequence diversity was noted among their light chains. Phosphorylcholine binding immunoglobulins with high affinity for choline, however, shared important similarities also within their light chains, suggesting a close correlation between primary structure and specificity also for antibody molecules.

Cyclic AMP increase in vagus nerve by β-adrenergic drugs, adenosine, NaF and ATP

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Desheathed rabbit vagus nerve accumulates cyclic AMP under various conditions (Ph. Roch and A. Salamin, Experientia *32*, 1419 (1976); J. Neurochem., in press (1977)). 1-Isoproterenol 2.5 μM and epinephrine 50 μM cause a maximal increase of about 300% of controls. d,1-Propranolol 5 μM completely inhibits these effects. α-Agonists as phenylephrine or dopamine have no influence on the cyclic AMP level. Adenosine promotes a dose-dependent increase of cyclic AMP reaching a maximum of 300% of controls. The cyclic AMP level is increased twice by ATP 10 μM and 3.5 times by ATP 2 mM. Fluoride 50 mM raises the cyclic AMP level to 250% of controls. Depolarizing agents are inactive in this preparation. Theophylline 10 mM inhibits the effect of adenosine. It has no influence on control values nor on β-stimulated ones. But theophylline is able to double the effect of fluoride reaching levels of cyclic AMP six times greater than control ones. These observations suggest that cyclic AMP plays a rather complex role in non-synaptic nerve tissue.

'Aged' thyroxine, an extremely potent inhibitor of carboxypeptidase A

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Thyroxine dissolved in dimethyl sulfoxide on storage is transformed into a mixture containing 1 (or several) inhibitor(s) of carboxypeptidase A, the inhibitory activity increasing with time, and at the limit exceeding that of L-benzylsuccinic acid (L. D. Byers and R. Wolfenden, Biochemistry *12*, 2070 (1973)). 'Aged' thyroxine was shown to be heterogeneous by thin-layer and column chromatography; however, the active component(s) has (have) not yet been isolated. A modified acetylated casein has proved to be a more suitable polypeptide substrate than that devised by Y. Lin, G. E. Means and R. E. Feeney, J. biol. Chem. *244*, 789 (1969).

Preparative polyacrylamide electrophoresis of calf ovarian protein kinases

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3 cAMP-dependent protein kinases (PK-I, PK-II and PK-III) have been isolated from calf ovarian homogenate. The isolation procedure consisted of fractionation on DEAE-cellulose and gel filtration on agarose acrylamide. The final purification of kinases was performed on polyacrylamide gels (pH 10.2 and 11.0, 18 mm diameter gel tubes). Preparative PAGE fractionation yielded homogeneous preparations of all 3 protein kinases. Recovery of the protein kinase activity on PAGE averaged 60–70% and the overall purification of PK-I was 2000fold. The mol. wts were estimated to be 223,000 (PK-I), 120,000 (PK-II) and 87,000 (PK-III). PK-I is the major protein kinase in cytosol. Experiments with tissue slices indicate that a conversion of PK-I to PK-II and PK-III can be induced in vitro. A close structural relationship of these 3 enzymes is suggested by the observation that all 3 protein kinases were composed of similar subunits.

Separation of brain proteins by two-dimensional gel electrophoresis

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The proteins of the pigeon optic tectum have been analyzed using the two-dimensional system of O'Farrell (J. biol. Chem. *250*, 4007 (1975)) with several modifications. The polypeptides were first separated by isoelectric focusing (IEF) in tube gels in the presence of pH 3.5–10 ampholines, followed by electrophoresis in a SDS slab gel of 13,5% acrylamide. The size of the slab gel (400 × 180 × 2 mm) was such that 3 IEF gels of 12 cm length could be processed simultaneously. The polypeptides were located by coomassie blue staining. The following tissue fractions were analyzed: whole homogenate; 100,000 g supernatant and pellet after hypotonic extraction; the pellet of the osmotically shocked crude mitochondrial fraction (P_2) and the microsomes (P_3). Each one gave a characteristic 'fingerprint'. On photographs of the gels over 200 spots could be identified in the whole homogenate and over 100 spots in each of the 4 other fractions.

Biochemical properties of plasma membranes from Candida tropicalis

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Plasma membrane vesicles were isolated from mechanically disrupted Candida tropicalis cells by filtration, differential centrifugation and aggregation of the mitochondrial vesicles at pH 4.3. As judged by biochemical, cell-electrophoretic and electron microscopic criteria, a pure plasma membrane vesicle preparation was obtained. For chemical analysis the contents of the vesicles were released by cytolysis. Electron micrographs of thin sections showed empty vesicles and there were no proteins detectable in the supernatant after a second lysis. The protein pattern of the plasma membranes by SDS polyacrylamide gel electrophoresis demonstrated about 18 bands ranging from 10,000 to 200,000 Daltons. A comparison was made among the protein patterns of the plasma membranes from hexadecane grown C. tropicalis, glucose grown C. tropicalis, and glucose grown S. cerevisiae. The protein, lipid and fatty acid composition of the membranes were determined and compared. Incubation of the plasma membranes with gamma-32 P ATP and $MgCl_2$ resulted in the incorporation of 32 P into protein bands.

Regulation of fructose 1,6-bis-phosphatase and sedoheptulose 1,7-bis-phosphatase in chloroplasts

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Fructose 1,6-bis-phosphatase is a key regulatory enzyme of carbon assimilation in chloroplasts. Evidence is now increasing that this enzyme, as well as the sedoheptulose 1,7-bis-phosphatase are activated by ferredoxin reduced during the light reactions of photosyntnesis. This activation by reduced ferredoxin necessitates the presence of

two additional proteins indigenous to chloroplasts and provisionally named 'assimilation regulary protein a' (ARP_a) and 'assimilation regulatory protein b' (ARP_b). The 2 proteins have been isolated, separated and purified. The highly purified ARP_b is a chromophore-free protein with an approximate mol. wt of 20,000. Dithiothreitol, the non-physiological sulfhydryl reagent can, in the presence of ARP_b only, replace reduced ferredoxin and ARP_a in the activation of the 2 enzymes. The results suggest that reduced ferredoxin, produced photochemically during the light reactions of photosynthesis controls via ARP_a and ARP_b the 2 enzymes of the photosynthetic CO_2 assimilation.

Production, large scale isolation procedure and N-terminal sequence studies of extracellular thermostable, neutral proteases from B. stearothermophilus and B. caldolyticus

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Proteinases from B. stearothermophilus and caldolyticus were isolated. The temperature at which they are still thermostable varies: 60 °C, 65 °C, 70 °C and 80 °C. Without calcium these enzymes are thermolabile. Phosphate buffer in the culture medium affects formation of thermostable neutral proteases. Ammonium sulfate or phosphate buffer applied during the purification cause very low yields of enzyme. Cultivation- and isolation methods are described. A cultivation procedure at 55 °C was developed and the optimal calcium ion concentrations in the medium for the production of extracellular protease by the 4 different strains were established.
The large scale purification includes as main steps: Flash evaporation in order to reduce the volume of the medium, extraction of the protease from the culture medium by adsorption on Amberlite XAD-7 resin and purification by affinity chromatography. The homology of the partial N-terminal amino acid sequences is considerably higher to thermolysin than the sequence homology of B. subtilis neutral protease and thermolysin.

Modulation of microsomal enzyme activity by lipids extracted from the microsomal membrane

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Microsomal cerebroside-sulfotransferase (CST), is one of the enzymes regulating myelination. If microsomes are extracted from developing mouse brain and delipidated with acetone, the age dependent CST activity pattern is lost. This pattern can be restored by readdition of the extracted lipids, which results in a recombination as could be shown by gradient centrifugation. If delipidated microsomes of different ages are recombined with acetone extracted microsomal lipids of different ages, the resulting reactivation depends on the age of the lipid source and not on that of the enzyme source. This is true for every age of microsomes during the myelination period. In order to find the critical factor of the added microsomal lipids, single phospholipids, cholesterol and mixtures of both were used for recombination experiments. The results show, that the age dependent CST activity pattern depends on the age dependent phospholipid/cholesterol ratio.

Aggregation of the Triton-solubilized small-intestinal sucrase-isomaltase

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The aggregation properties of small-intestinal sucrase-isomaltase, solubilized by either papain or Triton X-100 (H. Sigrist, P. Ronner and G. Semenza, Biochim. biophys. Acta *406*, 433 (1975)) were comparatively analyzed by electron microscopy and Sepharose 4B chromatography. In aqueous media the detergent solubilized enzyme complex aggregates regularly in particles with an average diameter of 24 nm. Under identical conditions the protease solubilized enzyme appears as monodisperse particles of uniform size (4.5 nm diameter). The observed difference in aggregation is confirmed by distinct separation of the 2 forms in Sepharose 4B chromatography. The particular aggregation phenomena indicates further the existence of an amphipathic monomer form of the Triton-solubilized sucrase-isomaltase.

Labeling of membrane proteins in the apolar phase

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[^{125}I]Iodonaphthylazide, an apolar, nitrene-generating compound, has been used to covalently label proteins of the intestinal microvillus membrane which are embedded in the lipid bilayer. The hydrophobic azide is allowed to dissolve into the membranes in the absence of light. Light is then flashed, converting the azido groups into nitrenes which react with proteins within the lipid core. High incorporation of the iodonaphthylazide occurred in proteins of 99,000, 86,000, 65,000, 54,000 and 30,000 daltons. Minimal labeling occurred in proteins of 300,000, 135,000 and 125,000 daltons. Iodonaphthylazide-labeled proteins were not removed by papain digestion. Neither enzymatic nor transport activities were inhibited by the presence of the label or the irradiation process.

Mitochondrial aspartate aminotransferase evolved more conservatively than the cytosolic isoenzyme

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2 distinct isoenzymes of aspartate aminotransferase (AAT) occur in eucaryotic cells, one being located in the mitochondria and the other in the cytosol. The 2 isoenzymes are homologous proteins, e.g., those from pig heart have an amino acid sequence identity of $\sim$ 50%, immunologically, however, they do not cross-react. The degree of structural similarity of the cytosolic isoenzymes from different vertebrate species was compared with the degree of similarity of the mitochondrial isoenzymes from the same species by means of quantitative microcomplement fixation. Using antiporcine AAT antisera the following ratios of the average immunological distances for the cytosolic and the mitochondrial isoenzymes were determined: other mammals 54/29, birds 200/100, reptiles 220/110, frog 240/125, fishes 210/140. The data indicate that during vertebrate phylogenesis the mitochondrial

isoenzyme changed at only half the rate of the cytosolic isoenzyme. Apparently, additional evolutionary constraints are operative on the structure of this organelle-confined isoenzyme.

Preparation and characterization of leaky and sealed vesicles from bovine myelin

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A central feature in a membrane's organization is the asymmetrical distribution of functions between its 2 surfaces. A prerequisite to investigate these properties in the myelin membrane is the preparation of well-characterized vesicles. Vesicles are generated from purified bovine brain myelin by adapting vesiculating techniques used for erythrocytes membranes. Myelin membranes incubated under these conditions show a density change in Ficoll gradients following loading with albumin, indicating that these membranes are closed vesicles. Electron microscopy shows a population of vesicles of different sizes. The vesicles contain the same glyco- and phospholipids than the native myelin, while under certain conditions of incubation a limited degradation of the myelin basic protein is observed. These vesicles are capable of maintaining transmembrane ionic gradients. Sealing induces a significant decrease for ^{22}Na efflux. This novel approach to the study of myelin should prove useful to investigate the structural and functional properties of this membrane.

Sequence studies of the terminal regions of AMV RNA: Possible redundancy

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Oncorna virus DNA synthesis in vitro initiates close to the 5′ end of the viral RNA (Taylor and Illmensee; Cashion et al.). To attain transcripts of full length, reverse transcriptase must pass from the 5′ end of the template to the 3′ end of the same or another RNA molecule. Some schemes to explain this event postulate a terminal redundancy of the RNA. To test this hypothesis we sequenced the DNA complementary to the 5′ end of avian myeloblastosis virus RNA and established partial sequences of both ends of the RNA itself. The structure deduced for the 5′ terminal region of the RNA is (5′)7me-GpppG^{2Me}**CCAUUCUACCUCU CACCA**CAUUGGUG-UGCACCUGGGUUGAUGGCCGGACCGUCGAUUCCCU-GACGACUACGAGCACCUGCAUGAAGCAGAAGGCUU-CAU ... and that for the 3′ terminus ... **A[U(U,C,U)A]-[C(C,U,C,U,C)A][CCA]**AA$(A)_{100-200}A_{OH}$. Thus our results are compatible with a terminal redundancy of at least 19 nucleosides (bold-faced). Similar results have been reported for Prague Rous sarcoma virus (J. Coffin and W. Haseltine, personal communication; Shine et al.).

30 S ribosomes bind to a non-initiator region of Qβ RNA in absence of fMet tRNA

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Under initiation conditions E. coli ribosomes bind to intact phage RNA mainly at the coat cistron initiation site, to form a 70 S complex. In the absence of fMet tRNA and IF3 30 S ribosomes also bind to MS2 RNA (Szer and Leffler). We incubated [^{32}P] Qβ RNA and ribosomes with or without fMet tRNA, treated the complex with RNAase A, and centrifuged it through a sucrose gradient. In the presence of fMet tRNA 0.17% of the label was in the 70 S, 0.03% in the 30 S region; without fMet tRNA the the values were 0.07% and 0.05%. The [^{32}P] RNAs from both regions were purified and analyzed: the fragments from the 70 S region were derived from the coat initiation site, while the 2 main fragments from the 30 S region did not come from any initiation site. They had the sequence AG**AGGAGGU**p (P-2a) and GG**AAGGAG**Cp (P-4); both are thus partly complementary to the 3′ end of the 16 S rRNA, ... $AUCACCUCCUUA_{OH}$. P-4 is a site to which B. stearothermophilus 70 S ribosomes bind in the absence of fMet tRNA (Steitz). The 30 S complex might have a role as a precursor rearranging on addition of fMet tRNA to the 70 S initiation complex.

Kinetic parameters of glucose transport in jejunal and ileal brush-border vesicles

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Rabbit intestinal brush-border vesicles were prepared, from either proximal jejunum or distal ileum, by the calcium precipitation of the extraneous cellular debris from a mixture of cell membranes which was obtained by mechanical agitation of the intestine. The rate of glucose flux into the vesicles during the first 5 sec of uptake was determined at several concentrations of glucose in the medium. A comparison of the maximal fluxes (J_{max}) and apparent affinity constants (K_T) of the jejunal and ileal vesicles was made. The K_T for the jejunum and ileum were not significantly different and had values of 171 ± 37 and 142 ± 39 μM, respectively. However, the J_{max} for the jejunum was significantly greater than for the ileum; the values were 1.06 ± 0.14 and 0.44 ± 0.07 pmoles/mg Prot./5 sec, respectively. The higher flux observed in the jejunal vesicles is consistant with results obtained with intact tissue. We have now shown that the difference is due to the J_{max} and not the K_T. We hope to determine whether the J_{max} difference is due to different amounts of carrier or different translocation rates.

NMR studies on the Root Effect of gold fish haemoglobin

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The single haemoglobin component of gold fish exhibits the Root Effect, i.e. a profound decrease in O_2 affinity and cooperativity at acid pH. The Root Effect is considered important in regulating gas pressure in the swim

bladder. In studies of the molecular mechanism of the Root Effect we determined the ^{13}C NMR spectrum of the Hb ^{13}CO of the gold fish. Experimental evidence indicates that in Root Effect Hbs the α and β subunits show seizeable differences in their affinities for ligands. In the ^{13}C NMR spectrum we observed 2 resonances assigned to ^{13}CO bound to the α and β subunits. One line is insensitive to pH and organic phosphates whereas the other is markedly shifted upfield at low pH, as has also been found with trout Hb IV. On the ^1H NMR spectrum of CO Hb we observed a resonance similar to that assigned to the methyl protons of Val E11 in mammalian Hbs. The position of the line is pH dependent indicating a conformational change on the distal side of the heme pocket for at least 1 subunit.

10.5 A diffraction of transmembrane proteins in agglutinated erythrocyte membranes

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Distinct meridional and equatorial X-ray diffraction is recorded from sheep erythrocyte membranes agglutinated with phytohemagglutinin M (PHAM). A broad $(10.5\ A)^{-1}$ diffraction ring with strong equatorial accentuation and a meridional reflection at $(1.5\ A)^{-1}$ are observed, which arise from α-helical transmembrane proteins. The equatorial accentuation of the $(10.5\ A)^{-1}$ ring probably is due to the binding of the agglutinin; some local rearrangement of transmembrane α-helices in the region of the receptor is likely. Gel electrophoresis shows that varying amounts of serum albumin (SSA) are bound to the membranes of individual sheep. PHAM and SSA interfere in the agglutination. The agglutination titer can be increased (type 1) or decreased (type 2) by SSA. At low ionic strength type 1-membranes bind moderate/large amounts of SSA, which are mostly displaced in PHAM-agglutination; type 2-membranes bind moderate amounts of SSA, which are retained after PHAM-agglutination. It is concluded that PHAM-receptor and SSA binding sites on the membrane surface are topographically related.

pH-Dependent changes of breathing modes in a globular protein

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In the basic pancreatic trypsin inhibitor (BPTI) the exchange kinetics of individual amide protons measured by ^1H NMR was interpreted by a model which allows for fluctuations of the protein conformation between closed forms, where no exchange of interior amide proton is expected, and opened forms with characteristic solvent accessibility of interior protons. In the treatment of BPTI this model was used with the assumption that at a given temperature the equilibrium between the different forms of the protein is determined by pH. Since BPTI contains no His residues, the conformational transitions in the pH range 2–9 would thus be related entirely to the deprotonation of the carboxylic acid groups at around pH 3.4 and the N-terminus at pH 8.0. It is suggested that considerations along these lines might be of general interest for descriptions of the molecular dynamics of globular proteins.

Crosslinking of human liver NADPH-dependent aldehyde reductase

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NADPH-dependent aldehyde reductase from human liver, a monomeric pyridine nucleotide-dependent dehydrogenase, was reacted with bifunctional reagents of various chain length. The reaction was followed by activity measurements and by SDS-polyacrylamide gel electrophoresis. No intermolecular crosslinking was observed. Intramolecular crosslinks, however, were obtained with dimethyl suberimidate and with dimethyl adipimidate giving rise to the formation of two new electrophoretic bands. A time dependent decrease of activity of 80–90% was observed concomitantly with the formation of the new bands. Crosslinking and inactivation were partially prevented by the presence of NADPH or $NADP^+$. The substrate p-nitrobenzaldehyde had no effect. Glutardialdehyde and dimethyl malonimidate did not crosslink the enzyme and caused only a decrease of activity of less than 50%. No protection was obtained with NADPH or $NADP^+$. These results suggest the presence of at least three amino groups at distances of ca. 7 Å and in such a spatial arrangement that mutual crosslinking inhibits the binding of coenzyme.

Digestion of a subcomponent of complement (Clq) with pepsin, procollagen-peptidase and collagenase

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In order to prepare well-defined fragments of Clq, limited proteolysis was carried out with 3 enzymes: 1. Pepsin was used at 37 °C and pH 4.5, followed by gel filtration on Biogel P10 and P300. Hydroxyproline, absent in the low-molecular fragments after 24 h, progressively increased in the high-molecular weight fractions. 2. Procollagen peptidase incubation revealed that neither type I- nor III-specific enzyme was capable to cleave Clq at pH 7.4. However, Clq formed a high-molecular weight complex with collagen precursors, but not with collagens lacking the polypeptide extension. This can be observed with type I and III procollagen. Using type I, the complex was formed with pro-α_1, but not with pro-α_2 chains. 3. The effect of microbial collagenase on Clq was assessed by measuring the inhibition of the fixation of native ^{125}I-labelled Clq to immune complexes. Digests obtained below 30 °C displayed full inhibitory activity, whereas a progressive decrease of inhibition was observed on digestion at 37 °C.

PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

Role of specific antibodies in the pathogeny of experimental myasthenia

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Experimental myasthenia was induced in 8 rabbits by injecting Torpedo acetylcholine receptor (AcChR). 7 showed a paralysis about 1 week after the 2nd injection. They were bled before they died. 1 rabbit was not paralysed, even more than 3 weeks after the 2nd injection. The 8 animals had high titers of anti-AcChR antibodies as measured with a radioimmunoassay using a ^{125}I-bungarotoxin-AcChR complex. Antibodies directed at the toxin binding site are not detected with this assay. Their level in the course of the disease is of special interest since they could act as curare-like agents. By measuring the degree of inhibition of toxin fixation to the AcChR, we found high levels of these specific antibodies in 1 of the rabbits with clinical signs of myasthenia, but a 4 times lower titer in the nonparalyzed rabbit. This finding could indicate that anti-AcChR antibodies directed at the toxin binding site may play a key role in the induction of the paralysis.

A comparison between electrical energy dissipation and net phosphagen consumption in the torpedo electric organ

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Fragments of electric organ were stimulated; the amount of electrical work accomplished by the tissue was calculated and compared to the net hydrolysis of ATP and creatine phosphate (CrP). Phosphagens used by presynaptic nerve terminals were differentiated from the total consumption by using curare. After 1.5 min of continual stimulation at 10/sec the decrease in phosphagen level was 20–30% and occurred essentially in nerve terminals. ATP and CrP utilization in electroplaques took place only later, with a marked retardation on the physical expenditure, and continued in the first min after the end of stimulation. During the later recovery period, the electrical parameters of the discharge returned to their initial value in about 1 h, whereas full restoration of ATP and CrP contents took 5 h. It is concluded that the initial potential energy for the discharge must be essentially in the form of ionic gradient, since the energy stored as phosphate bounds in ATP and CrP was not found sufficient in the Torpedo organ to ensure balance of the electrical work accomplished by the tissue.

Etude comparée de l'effet inducteur de deux agents psychomodérateurs – Atrium et complexe 1656 – et du phénobarbital sur les enzymes microsomales hépatiques du rat

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L'Atrium et le complexe 1656 sont constitués de 4 molécules de 3 composants: dérivés mono- et di-substitués du phénobarbital et phénobarbital lui-même. L'effet inducteur de ces produits a été étudié chez des rats Wistar mâles traités par voie orale aux doses de 67, 133 et 530 mg/kg pour l'Atrium et de 133 mg/kg pour le complexe 1656. Les doses de phénobarbital et de ses dérivés – le fébarbamate et le difébarbamate – sont calculées sur la base de la constitution de l'Atrium. Les microsomes hépatiques ont été utilisés pour la mesure du taux de protéines, du cytochrome P-450 et de l'activité de l'aminopyrine-N-déméthylase et de l'aniline-p-hydroxylase. Dans ces conditions expérimentales, l'Atrium et le complexe 1656 ont sur l'ensemble des paramètres étudiés un effet inducteur égal entre eux et égal à celui d'une faible dose de phénobarbital de l'ordre de 20 mg/kg. Le monosubstitué a un faible effet inducteur, tandis que le disubstitué n'en a pas. L'effect inducteur de l'Atrium et de son analogue est donc dû essentiellement à la teneur en phénobarbital des complexes.

Effects of baclofen and muscimol on nigro-striatal dopaminergic functions

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Effects of baclofen and muscimol on various behaviours functionally linked to the nigro-striatal dopaminergic system were investigated. Both compounds only marginally blocked conditioned discriminatory avoidance response in the cat. Their effects were, however, greatly enhanced by α-methyl-tyrosine pretreatment. Baclofen (3 mg/kg i.p.) and muscimol (1 mg/kg i.p.) also potentiated the cataleptic and amphetamine-antagonistic action of haloperidol. Interestingly, muscimol alone markedly increased the stimulation induced by amphetamine (stereotypies) if given shortly prior to it. The effect was partially blocked by bicuculline. In contrast to baclofen, muscimol failed to inhibit the apomorphine-induced contraversive turning in 6-OH-dopamine lesioned rats. Some of the observed effects of baclofen and muscimol are compatible with a 'GABA-like' inhibitory action on dopaminergic transmission. Baclofen, apparently, exerts also other effects on striatal functions which are independent of the assumed gabergic feedback loop.

Acetylcholine release: A radiochemical approach in torpedo electric organ

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The release of acetylcholine (ACh) has been investigated in absence of cholinesterase inhibitor, to avoid any change in kinetics of transmitter metabolism. Fragments of electric organ were incubated for 4–5 h in physiological medium in the presence of ^{14}C-acetate and ^{3}H-choline at the same concentration. The tissue was washed overnight and then submitted to stimulation. ^{14}C- and ^{3}H radioactivities were counted in the superfusing solution. During activity, radiolabelled acetate and choline increased in a ratio of 1:1 in the solution; this confirms that, in this tissue, both precursors are equally incorporated into ACh which is liberated on activity. The method was sensitive enough to detect transmitter released by a single nerve impulse. In a short burst of stimulation (20 impulses in 1 sec) the amount released was a function of the ratio Ca^{2+}/Mg^{2+} in the superfusion solution. Various drugs were tested and, surprisingly, the anticholinesterase physostigmine strongly reduced the release of transmitter.

Effects of cholinergic agents on orthophosphate efflux in rabbit vagus

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In desheathed vagus nerves, loaded with radiophosphate by incubation in ^{32}P labelled Locke, the efflux of radiophosphate into inactive Locke was measured as described (Anner et al., J. Physiol. *260*, 667 (1976)). When the Na of the washing solution was replaced by Li or Tris, the phosphate efflux decreased as the intracellular Na was lowered. When Na was replaced by choline, a transient increase in efflux was found. A similar increase was observed when choline was added to Locke. Further, the efflux increased after addition of acetylcholine (1–5 mM) or methacholine (1 mM) to Locke and addition of acetylcholine to choline-Locke was ineffective. The stimulating action of acetylcholine was not much affected by d-tubocurarine (1.5 mM), hexamethonium (1 mM) or atropine (1 mM). Atropine alone decreased the efflux, an effect shared with the local anesthetic tetracain. Thus, although the transient stimulation of phosphate efflux by choline is induced also by other cholinomimetic agents, the effect is not fully explained by activation of classical nicotinic or muscarinic receptors.

Treatment of poisoning with Amanita phalloides in dogs

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In previous studies, several agents have been demonstrated to antagonize in mice or rats the lethality of poisonous principles from the toadstool Amanita phalloides. Curative effects against alpha-amanitin were provided by cytochrome C in mice while penicillin saved rats poisoned with a lethal dose of Amanita phalloides extract (APL). Moreover, prenisolone and silymarin antagonized the lethality of fractionated doses of APL. The current studies were performed to test the antidotal effects of these agents in dogs poisoned with a sublethal dose of APL by assessing biochemical parameters such as SGOT, SGPT, LDH, alcaline phosphatase, bilirubin and blood clotting factors. Groups of 6–8 dogs received the antidotes 5 and 24 h after the ingestion of APL and their hepatic function was followed for one week and compared to the untreated controls.

Analysis of the tachycardia induced by dihydralazine and minoxidil

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In dogs with carotid loops dihydralazine and minoxidil caused a strong tachycardia. The drug-induced increases in heart rate were analyzed by the use of a multiplicative model (European J. Pharmacol. *39*, 192 (1976): $HR_N = HR_O \cdot V \cdot S \cdot W$; where S represents the sympathetic input to the heart, V the parasympathetic input and and W the interaction between S and V. Minoxidil (3 mg/kg p.o.) increased heart rate by 100% of the pre-drug values. The analysis revealed that the minoxidil-induced tachycardia was related to a reduction of V from − 52% to − 8%. S was not directly influenced by the drug. W, however, which in control experiments was reduced by an inhibition of S though V was absent. The intrinsic heart rate was reduced by 9%. Dihydralazine (10 mg/kg p.o.) increased heart rate by 92% of the pre-drug values. The tachycardia after hydralazine was induced by a reduction of V from − 50% to − 28% and by an increase in S from 28% to 59%. W was also absent after dihydralazine and intrinsic rate reduced by 13%.

In vivo accumulation of urate and PAH in renal tissue of rats and rabbits

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In rabbits concentration of ^{14}C-PAH in fresh tissue divided by concentration in plasma (T/P) was 11.0 ± 1.5 in cortex, 6.1 ± 1.0 in external medulla, and 6.5 ± 1.2 in internal medulla (n = 9). T/P urate was 4.1 ± 1.3, 2.3 ± 0.4, 2.4 ± 0.5 (n = 7), and T/P ^3H-inulin 1.7 ± 0.1, 1.3 ± 0.1, 1.9 ± 0.2 (n = 9). Urate and PAH thus accumulate in renal cells or tubular fluid. GFR was 4.9 ± 0.3 (n = 18), C_{PAH} 22.9 ± 1.4 (n = 10), C_{urate} 7.9 ± 1.6 ml/kg min (n = 11). In rats T/P PAH was 6.7 ±1.4 in cortex, 8.1 ± 3.4 in external medulla, and 7.7 ± 1.1 in internal medulla (n = 7). T/P urate was 1.4 ± 0.3, 1.2 ± 0.3, 2.6 ± 0.5 (n = 7), and T/P inulin 2.5 ± 0.2, 2.2 ± 0.5, 2.7 ± 0.5 (n = 7). Thus, PAH, but not urate, was accumulated in renal cells or tubular fluid. GFR was 8.5 ± 0.5 (n = 7), C_{PAH} 22.3 ± 0.6 (n = 7), C_{urate} 5.2 ± 0.4 ml kg/min (n = 7). In rabbits, in which secretory movement for urate predominates, urate accumulates in renal cells or tubular fluid, as does PAH. In rats, which mainly reabsorb urate, no accumulation of urate occurs.

Differences in acetylcholinesterase of neuromuscular and sympathetic junctions

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Cytochemistry of neuromuscular and nerve-electroplaque junctions show that AChE is concentrated as postsynaptic membranes, a strategic place for terminating the action of transmitter. Indeed, AChE inhibitors strongly prolonge the duration of the excitatory postjunctional potential and lead during repetitive stimulation to rapid blockade of transmission. In rat superior cervical ganglia, most of AChE activity was found in the reticulum of neurons, but very little if any in synaptic membranes. The time course of the excitatory postsynaptic potential was not prolonged by anti-AChE drugs and the ganglion remained able to sustain repetitive activity. It is concluded that, in sympathetic ganglion, the main synaptic action of transmitter is not terminated by AChE, but by diffusion far away of the synapse, where hydrolysis can occur. The physiological role of AChE would be to provide precursors for acetylcholine resynthesis as proposed by Emmelin and McIntosh (J. Physiol. *131*, 477 (1956)). It would be interesting to know if AChE plays a similar role in the central nervous system.

Prostaglandin (PG)-release by macrophages in vitro

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Macrophages are believed to be of major importance in the inflammatory process. They have e.g. been found to produce large quantities of inflammatory prostaglandins (PG). Hence it appears of interest to describe the trigger which activates these cells and causes the release of PG. Phagocytosis alone might be such a trigger, however, it could also be that additional activating factors are required. This question was investigated using peritoneal macrophages cultured overnight in serum free medium. They were then exposed to phagocytosable particles either free of complement or coated with complement factors. We found that phagocytosis alone is not sufficient to cause PG-release. However, phagocytosis of complement coated particles causes PG-release. In accordance with Schorlemmer and Allisons observations (H. U. Schorlemmer and A. C. Allison, Immunology *31*, 781 (1976)) we concluded that complement is of decisive importance for macrophage activation even with respect to the release of inflammatory PG.

Compensatory adaptation after renal denervation

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Removal of 1 kidney induces adaptive changes in water and electrolyte excretion by the remaining kidney (G. Peters, Am. J. Physiol. *205*, 1042 (1963); J.-P. Guignard and J. H. Dirks, Am. J. Physiol. *230*, 1225 (1976)). Participation of the autonomous nervous system to this regulation was studied in anaesthetized rabbits after a) α-blockade with phenoxybenzamine or phentolamine, b) β-blockade with propranolol, and c) surgical denervation of the renal pedicle. Fluid balance, the hematocrit and plasma protein concentration were maintained constant throughout the experiments. In the 3 conditions, contralateral kidney exclusion induced an immediate similar increase in water, Na, K and Cl excretion by the remaining kidney. These changes were already significant 5 min after uninephrectomy. Renal blood flow remained unchanged in the 3 groups. GFR and BP increased slightly in α-blocked animals only. It is concluded that the autonomous nervous system plays no role in the excretory adaptation which occurs when nephron mass is acutely reduced.

An unexpected effect of strychnine on vascular smooth muscle

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In strips of rabbit main pulmonary artery (RMPA), strychnine (S) (10^{-3} M) increased the membrane potential of the vascular smooth muscle cells from –60 mV to –78 mV, as measured with intracellular glass microelectrodes. This hyperpolarizing effect was concentration-dependent in the range between 10^{-4} M and 10^{-3} M. Since the rate of the hyperpolarization was enhanced with increasing concentrations of S and since quaternary S was ineffective, S is likely to act from the inside of the cell membrane. For a 10fold change of K_0, the slope of the relationship between membrane potential and log M concentrations of KCl was 43 mV in controls and 54 mV in the presence of strychnine (10^{-3} M). It seems, therefore, that an increase in potassium permeability of the cell membrane is responsible for the S-induced hyperpolarization. Both, ^{45}Ca uptake into the vascular smooth muscle cells and contractions of the strips of RMPA in in response to high KCl were inhibited by S in a concentration-dependent manner suggesting an impairment of Ca influx into the vascular smooth muscle cells.

Adrenergic receptors in glioblast cultures from newborn rat cortex

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Primary glioblast cultures from newborn rat cerebral cortex carry α- and β-receptors coupled to adenyl cyclase. Adenyl cyclase was measured in cells cultured for 11–13 days by prelabeling with ^{14}C-adenine (H. Shimizu et al., J. Neurochem. *16*, 1609 (1969)) in the presence of 3-isobutyl-1-methylxanthine. Isoproterenol (ISO) (0.01 μM) stimulates adenyl cyclase activity up to 50-fold over the control value, in agreement with Gilman and Schreier (Molec. Pharmacol. *8*, 410 (1972)). When noradrenaline (NA) alone is used as the agonist, maximal adenyl cyclase activity is only 70% that obtained with ISO, but NA in combination with the α-antagonists phenoxybenzamine or phentolamine stimulates adenyl cyclase to the same extent as does ISO. Clonidine, an α-agonist, inhibits ISO stimulated adenyl cyclase to the level obtained with NA alone. In summary, α-agonists partially inhibit the stimulation of adenyl cyclase induced by β-agonists in primary glioblast cultures.

Metabolites of vaso-active antihypertensives as mediators of the circulatory effects

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The relaxant effects of various vaso-active antihypertensives were determined in strips of rabbit main pulmonary artery contracted by noradrenaline. While sodium nitroprusside inhibited the noradrenaline-induced contractions at low concentrations (10^{-7}–10^{-6} M), more than 1000fold higher concentrations of hydralazine, dihydralazine and minoxidil were required for similar relaxation. These in vitro findings contrast with the powerful hypotensive action of the latter drugs. Pretreatment of spontaneously hypertensive rats with proadifen (SKF 525A) (100 mg/kg p.o.) – an inhibitor of mixed function oxidases of microsomes – prevented the fall in blood pressure produced under control conditions by hydralazine, dihydralazine, minoxidil and guancydine. In contrast, proadifen did not influence the hypotension observed after sodium nitroprusside, diazoxide or guanidine. Proadifen had no effect on blood pressure. It is concluded that metabolites of hydralazine, dihydralazine, minoxidil and guancydine are largely perhaps even totally responsible for the hypotensive action of these drugs.

Subcellular distribution of dopamine (DA) in substantia nigra

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In homogenates from substantia nigra of individual male rats the subcellular distribution of DA was studied by differential and continuous as well as discontinuous density gradient centrifugation on a microscale. The various fractions were assessed by known markers and electron microscopy. Crude mitochondrial fraction (P_2) contained 40–60% of DA and noradrenaline (NA). On continuous sucrose density gradients DA-containing particles peaked at the density where synaptosomes are found. Na showed an almost identical distribution. Following an injection of 6-hydroxydopamine into the fourth ventricle, Na in substantia nigra decreased by 56% and DA only by 17%. The subcellular distribution of DA remained unchanged. Electron microscopy of P_2 and of the part of the continuous density gradient containing the DA peak showed characteristic synaptosomes and circular bodies of equal size devoid of synaptic vesicles. The results indicate that at least half of nigral DA is contained in particles. These are not identical with NA synaptosomes, and probably are derived from dendrites (dendrosomes).

Role of cardiac and vascular beta-receptors in the action of phentolamine in anaesthetized cats

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Phentolamine (Phe) has been used for vasodilator therapy of heart failure. As a mechanism of vasodilatation an indirect stimulation of vascular beta-receptors has been discussed in addition to alpha-blockade and direct effects (Gould and Reddy, Am. Heart J. *92*, 397 (1976)). Phe was infused to anaesthetized cats at rates of 20 and 50 $\mu g/kg \times min$ after pretreatment with either NaCl ($N = 6$), Propranolol 10 (Prop. $N = 8$) or Metoprolol 5 $\mu g/kg \times min$ (Meto, $N = 8$) for 30 min. In this dose Prob markedly blocked vascular beta-receptors in the cat hind limb, whereas the cardioselective betablocker Meto up to 25 $\mu g/kg \times min$ had no such effect. Phe caused max. increases in heart rate (13 ± 2 beats/min) and cardiac output (88 ± 33 ml/min; both $p < 0.025$, paired t-test) which were abolished by both Prop and Meto. The max. fall in blood pressure (BP) induced by Phe was -13 ± 5 mm Hg after NaCl, -11 ± 3 after Prop and -13 ± 5 after Meto (all $p < 0.05$). As the fall in BP was not altered by blockade of vascular beta-receptors, an involvement of these sites in the vasodilating action of Phe seems unlikely.

Regulatory drinking in the pigeon

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Water-satiated pigeons drank after haemorrhage or injections of hypertonic NaCl (i.v.), polyethylene glycol (PEG) (i.p. or i.v.), dextran (i.v.), avian kidney extract (i.v.), porcine renin (i.v.), angiotensinamide (i.v. infusion) or isoprenaline (s.c.). Drinking responses to hypertonic NaCl (0.625–5 ml 2 M i.v./kg) plus PEG (0.625–5 ml 50% w/w i.p./kg) were additive. Water intake following 0.625 ml 2 M NaCl solution/kg, but not following 2.5 ml 2 M NaCl/kg was additively increased by bleeding (5 ml/kg). Drinking after dehydration or PEG (i.p.) was diminished by i.v. isotonic NaCl solution, before offering water. – The drinking response to i.p. plus i.v. PEG was greater than to either stimulus alone, suggesting that i.p. PEG may induce drinking without causing hypovolaemia. The dipsogenic effects of i.v. PEG or dextran may be due to 'contraction' of interstitial fluid volume or to an increase of plasma oncotic pressure per se.

Pharmacological control of salivary secretion in a blood-sucking arthropod

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In ixodid ticks fluid secretion by isolated salivary glands can be triggered by substituted β-phenylethylamines (of which dopamine (DA) is the most potent), by apomorphine and by ergot alkaloids. Phenoxybenzamine, flupenthioxol, phentolamine, propranolol and dichloroisoprenaline all block DA-induced secretion, but only at extremely high antagonist: agonist concentration ratios. Spiperone and pimozide increase the maximum response of the gland twofold. Such observations suggest a receptor type distinct from α-, β-adrenergic and DA-receptors. Pilocarpine (PC), inactive in vitro, is an agonist in vivo. Atropine, reserpine and guanethidine selectively inhibit PC-induced secretion, indicating the latter's indirect mode of action. Isosmotic saline injected into the haemolymph also stimulates salivation, an effect which is reserpine-sensitive but atropine-insensitive; the latter observations suggest a nervous pathway distinct from that stimulated by injected PC.

Lisuride and D-LSD: Effects on the monoaminergic system in the rat brain

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The ergot derivative lisuride hydrogen maleate (LIS) injected i.p. to rats in doses as low as 0.05 mg/kg induced, similarly to D-LSD, a decrease of homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA) in the whole brain with minor changes of the dopamine (DA) and 5-hydroxytryptamine (5-HT) levels. In the striatum and the limbic forebrain, LIS reduced HVA to about the same degree. LIS and to a lesser extent D-LSD induced a dose-dependent increase of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate in the whole brain. In the n. caudatus and in the n. accumbens septi, LIS and D-LSD counteracted the accumulation of L-DOPA induced by reserpine plus NSD 1015 (a decarboxylase inhibitor). In striatal homogenates LIS and D-LSD blocked the DA-induced activation of the adenylate cyclase (AC), whilst D-LSD, but not LIS, stimulated per se AC. In limbic slices, both drugs antagonized the activation of AC induced by noradrenaline (NA). These data indicate that both LIS and D-LSD stimulate pre- and postsynaptic DA and 5-HT receptors and block NA receptors at higher doses.

Clonidine-induced diuresis and increase of water intake in rats

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The relationship between the diuretic and the dipsogenic effects of clonidine (C) and their time courses was investigated by measuring urine flow (V), urinary sodium excretion ($U_{Na}V$) and water intake between 1 and 24 h after C (9.5–950 μg/kg s.c.) in normal rats. C (9.5 to 300 μg/kg) acutely and dose-dependently increased V and UN_aV with a maximal effect within the first h. This acute effect persisted with treatment up to 3 weeks. Pentobarbital anesthesia (40 mg/kg i.p.) abolished the initial pressor response to C and augmented the depressor one, thus suppressing the diuresis. Water intake was acutely diminished after high, sedative doses of C, whereas a delayed increase in water intake was observed 6 to 24 h after C. This water intake was suppressed by nephrectomy or bilateral ureteral ligation before C. – Pentobarbital interferes with the diuretic effect of C by changing its action on arterial blood pressure. C appears to stimulate water intake in rats by inducing a renal water loss.

Some effects of muscimol in mice, rats and cats

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Muscimol (M) in doses below 10 mg · kg^{-1} i.p. did not protect mice from convulsions induced by either pentetrazol, strychnine, bicuculline, picrotoxin, thiosemicarbazide or electroshock, but was effective against 3-mercaptopropionic acid (ED 50 = 0.46 mg · kg^{-1} i.p.). Convulsions and death occurred after 10 mg · kg^{-1} M. In rats, M (1–3 mg · kg^{-1} i.p.) induced catalepsy; a synergistic effect was found with haloperidol and diazepam. Unilateral injections of M (10 μg in 2 μl) into the caudate or the substantia nigra (SN) induced vigorous turning away from the injected side. M markedly reduced the depressant effect of picrotoxin on potentials evoked in the cat SN by caudate stimulation. M in doses of 0.3 and 1.0 mg · kg^{-1} i.p. reduced REM sleep (by 20% and 40% respectively) and increased the number of non-REM PGO waves (slightly and by 30% respectively) in cats; at 3 mg · kg^{-1}, M induced ataxia, hypotonia, clonic seizures with cortical spikes and waves. The effects of the GABA mimetic M on the whole animal differ considerably from those of agents believed to enhance GABA ergic transmission, e.g. benzodiazepines.

Mechanisms for the exagerated sedative effect of pentobarbital in rats with experimental hepatic failure

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The exagerated response of patients with advanced liver disease to sedative drugs may be related to reduced hepatic drug metabolism but could also be explained by an increased sensitivity of the brain. Pentobarbital sleeping times were therefore investigated in 6 male rats with chronic hepatic failure due to end to side portocaval anastomosis (PCA) and in 6 sham operated controls (SOC). In both groups the sleeping time correlated linearly with the logarithm of the dose ($r = 0.96$). In rats with PCA the slope was 5 times steeper than in SOC, suggesting a corresponding reduction in hepatic metabolism. The extrapolated sleep threshold dose was 15 mg/kg after PCA and 18 mg/kg in SOC. Upon awakening plasma and brain concentrations, measured by GLC, were lower in rats with PCA (9.6 ± 2.7 μg/ml SD and 17.6 ± 3.6 μg/ml) than in SOC (13.5 ± 1.2 μg/g and 24.3 ± 1.4 μg/g respectively, $p < 0.005$). It is concluded that in the rat with PCA the brain is significantly more sensitive to pentobarbital than in SOC.

Induction of hypothermia in men and hyperthermia in women after 200 mg i.v. 1-5HTP

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To what extent are central serotoninergic mechanisms involved in temperature regulation in man? A new tool to investigate this question is the soluble 1-5HTP-ethylester (Ro 3-5940, 200 mg) infused after peripheral decarboxylase inhibition (Ro 4-4602). An initial rise was followed by a long-lasting hypothermia in men ($N = 7$) and hyperthermia in women ($N = 6$). This sex-specific divergent pattern of temperature response to 1–5HTP was significant from 2 to 4 h after end of infusion. Although initially peak plasma levels of 1-5HTP were higher in men than women, no later differences were found. Thus pharmacokinetic differences alone are insufficient to account for the opposite temperature response, rather, a gender-determined biochemical organization of the CNS may be responsible. Greater prolactin and cortisol stimulation in women than men after 1-5HTP support such an interpretation. These results indicate a role for central 5HT mechanisms in thermoregulation in man, but underline the importance of considering sex specific factors in pharmacological studies.

Cytochrome P 450: Subcellular site of apoprotein synthesis and heme incorporation

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Induction of cytochrome P450 (P450), the terminal oxidase in drug metabolism requires synthesis of apoprotein in rough (R) endoplasmic reticulum (ER) and heme in mitochondria (MITO). Recent studies suggest that structural contact of RER and MITO may be essential for final assembly of the holocytochrome (Z. physiol. Chem, *357*, 1041 (1976)). We investigated the subcellular distribution of precursor incorporation into heme and apoprotein moieties of P450. Rats were injected with labeled δ-aminolevulinic acid or leucine and sacrificed after 4 h. Heme and apoprotein of P450 were isolated from rough microsomes (RM), smooth microsomes (SM) and RER separated from MITO-RER complexes (RERm). Radioactivity of P450-heme and drug-induced apoprotein synthesis was highest in RERm as compared to RM and SM. The data suggest heterogeneity of ER-membranes in regard to P450 synthesis and support the concept that MITO-RER complexes operate as functional units in this process.

GABA-receptor identification by ^{3}H(+)bicuculline-methiodide binding in rat CNS

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The inhibitory action of GABA in the CNS is selectively antagonized by (+)bicuculline or (+)bicuculline-methiodide (BM). ^{3}H-BM binds to cerebellar synaptic membranes in a stereospecific saturable fashion with K_D of 350 nM and a maximum binding of 4.5 pmoles/mg protein. The inhibition of ^{3}H-BM-binding by a variety of compounds closely parallels their ability to inhibit or mimic the synaptic inhibitory action of GABA: ^{3}H-BM is displacable by (+)bicuculline (ED_{50} = 75 nM), muscimol (75 nM), GABA (350 nM) and 3-aminopropanesulfonic acid (400 nM), while (−)bicuculline, diaminobutyrate, nipecotic acid, glutamate, aspartate and glycine are inactive. There is a 8fold difference in the extent of ^{3}H-BM-binding in different CNS regions: it is highest in cortex and cerebellum and lowest in spinal cord. In subcellular fractionation ^{3}H-BM-binding is most enriched in crude synaptic membranes. These findings indicate that BM-binding represents an interaction with the post-synaptic GABA-receptor.

Effects of morphine on lateral hypothalamic self-stimulation

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The effects of systemic morphine injections on lateral hypothalamic self-stimulation were examined in 3 studies. Experiment 1: In naive rats injections of 8 mg/kg morphine, but not of 2 mg/kg resulted in a significant increase in the threshold for lateral hypothalamic self-stimulation. Experiment 2: Animals were addicted during a 10-day-period of 2 morphine injections per day at doses increasing daily in 10 mg/kg steps up to 100 mg/kg, injected for 3 days. Than the animals were injected 200 mg/kg once daily. Self-stimulation thresholds were found to be significantly lower 2 h compared to 22 h after the 200 mg/kg injection of morphine. Experiment 3: 16 mg/kg of morphine caused a suppression of self-stimulation. However, when the animals were given continuously priming stimulation and were hand shaped towards the lever whenever they left it, the suppression could be reversed. However, the animals which remained unstimulated showed a significantly better recovery.

Central dopamine-receptor stimulation by LSD and lisuride

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Lisuride, a methylisoergolenyl derivative, provoked signs of central stimulation in rats similar to those observed after LSD. We therefore compared lisuride with LSD in Ungerstedt's rotation model in rats with unilateral 6-OH-DA-lesions in the nigro-striatal pathway and as antagonists of the neuroleptic-induced catalepsy. Lisuride and LSD induced dose-dependent turning towards the unlesioned side. Lisuride was 10 to 20 times more potent and considerably longer acting than LSD. Haloperidol blocked the lisuride-induced rotation completely, whereas the LSD-induced turning was only reduced partially. Lisuride and LSD, injected 3 h after the neuroleptic prochlorperazine, antagonized the neuroleptic-induced catalepsy in a dose-dependent manner. The effect of lisuride lasted for at least 3 h, that of LSD about 1 h. Since qualitatively the same effects are observed with the dopamine-mimetic apomorphine, it is proposed that LSD and lisuride stimulate central dopamine-receptors.

Properties of the insulin-degrading system of rats poisoned with N-monomethylacetamide (NMMAA)

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Crystalline insulin added in vitro to plasma from rats with NMMAA-induced diabetes has been shown to be destroyed at 37 °C, but not at 0 °C (Experientia *32*, 781 (1976)). – Ultrafiltrates of the insulin-destroying plasma samples did not inactive insulin: the degradation thus appears to depend on macromolecular components of the plasma. Dialysis of the samples did not decrease the insulin-destroying power. – The degradation process was blocked by thiol reagents such as N-ethylmaleimide (10 mM), iodoacetamide (10 mM) or ethacrynic acid (5 mM). It was not influenced by exogenous reduced glutathione (GSH 300 μM). The thiol reagents thus do not appear to block the inactivation of insulin by binding GSH. The inhibiting effect is thought do be due to the blocking of one or several SH-dependent enzymes. The activity of these enzymes was slightly depressed by sodium edetate (5 mM). – The inactivation of insulin by plasma of rats with NMMAA diabetes followed first-order kinetics. The destroying activity appeared to be correlated with the severity of diabetes.

Pharmacokinetics of $^{14}CO_2$-breath tests

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Measurement of specific activity (SA) of $^{14}CO_2$ in breath has recently been introduced as a noninvasive method to assess hepatic demethylation of appropriately labelled drugs in man. The kinetics of this test, however, are inadequately understood. Therefore, 1.5 mMol (2 μCi) of ^{14}C-glycodiazine, which is eliminated by demethylation only, were administrered to 5 subjects. Plasma concentrations of glycodiazine and SA of $^{14}CO_2$ were determined by HPLC and liquid scintillation counting, respectively. Using a 2 compartment open model and nonlinear least square regression analysis, the first order rate constants of demethylation (k_{el}) were 0.14, 0.23, 0.45, 0.95, 0.99 h^{-1}, and the terminal plasma disappearance rate constants (β) were 0.10, 0.14, 0.17, 0.19, 0.18 h^{-1}. Independently calculated terminal disappearance rate constants of SA (k_b) were 0.08, 0.13, 0.17, 0.21, 0.20 h^{-1}. Since k_b is linearly correlated with β (r^2 = 0.97) and not with k_{el}, it does not directly reflect the demethylation rate but rather the rate of loss of drug from the body during the phase of pseudoequilibrium.

Effects of systemic muscimol and GABA in the spinal cord and superior cervical ganglion of the cat

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The effects of muscimol and GABA on spinal cord were studied in spinal cats, their actions on autonomic ganglion cells in the superior cervical ganglion (SGC) of anaesthetized cats. Muscimol (0.3 to 3 mg · kg^{-1} i.v.) produced a long-lasting depolarization of primary afferent (PA) endings as measured by changes in the steady potential of dorsal rootlets and the PA excitability. Segmental dorsal root potentials, monosynaptic and polysynaptic reflexes, and spontaneous γ-motoneurone activity were depressed. Motoneurones were slightly hyperpolarized as indicated by recording from ventral rootlets. GABA produced similar effects but was about $^1/_{100}$ as potent as muscimol. Close-arterial injections of muscimol and GABA to the SCG depolarized sympathetic ganglion cells and depressed ganglionic transmission, muscimol being approximately 10 times more potent than GABA. Bicuculline reduced the effects of muscimol and GABA on spinal cord and SCG. The effects of the two GABA receptor stimulants differ from those of agents enhancing GABA-ergic transmission, e.g. benzodiazepines.

Micropuncture study of urea excretion by the rabbit kidney

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Pentobarbital-anesthetized rabbits were injected with 32 mU/kg ADH i.v. and infused with saline (0.1 ml/min) and ADH (0.6 mU/kg · min). Urine flow was 0.02 ± 0.002 ml/kg · min · 1 kidney, GFR 3.5 ± 0.4 ml/kg · min · 1 kidney, U/P_{urea} 28 ± 3 and fractional excretion of urea 0.20 ± 0.05 (n = 7). TF/P_{inulin} was 1.35 ± 0.02 in early and 1.74 ± 0.03 in late proximal tubules; TF/P_{urea}: TF/P_{inulin} was 0.87 ± 0.03 (n = 10) in early and 0.70 ± 0.03 in late proximal tubules. No addition of urea occurred between late proximal and early distal puncture sites, since TF/P_{urea}:TF/P_{inulin} was 0.71 ± 0.05 in early distal, whereas TF/P_{inulin} was 2.35 ± 0.06 (n ± 8), and TF/P_{osm} 0.75 ± 0.02. In late distal tubules TF/P_{urea}: TF/P_{inulin} was 0.52 ± 0.11, TF/P_{inulin} 5.1 ± 0.9 and TF/P_{osm} 0.95 ± 0.04 (n = 6). – In 5 rabbits dehydrated for 48 h and receiving ADH, urea concentration was 470 ± 20 mM in urine and 337 ± 38 mM in papillary tissue water. Non-diuretic rabbits, thus, differ from rats by the absence of recycling of urea to Henle's loop, and by non-equilibration of urine and papillary water urea concentrations.

Aldosterone and spironolactone dependent Na^+ transport in toad bladder epithelium

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The in vitro physiological response to aldosterone in the urinary bladder of the toad is characterized by an increased rate of short circuit current (= SCC i.e. Na^+ transport) which is apparent only after a latent period of 45 to 90 min. The effect of the competitive antagonist spironolactone (SC9420) was compared in 2 conditions: a) anti-induction by addition of SC9420 (10^{-5} M) 30 min before aldosterone ($2 \cdot 10^{-8}$ M), b) deinduction by addition of SC9420 6 h after a full induction by aldosterone. In the anti-induction condition, the response to aldosterone was almost completely inhibited by SC9420. In the deinduction condition, SCC first decreased rapidly (as early as 15 min after SC9420 addition) and later at a much slower rate. The effects of SC9420 in both conditions were fully reversible upon addition of a high dose of aldosterone ($2.5 \cdot 10^{-5}$ M). These results suggest that 1 of the components induced by aldosterone has a fast turn-over and can be rapidly inhibited by the interaction of SC9420 with the agonist-receptor complex.

Granular and extragranular action of psychotropic drugs on monoaminergic neurons

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Reserpine-like and neuroleptic drugs enhance the dopamine (DA) turnover in the striatum by different primary mechanisms, i.e. interference with presynaptic granular DA stores and blockade of pre- and/or postsynaptic DA receptors respectively. In order to differentiate between these 2 mechanisms, the presynaptic DA storage in rats was abolished by reserpine (5 mg/kg i.p.) and the subsequently enhanced striatal DA turnover restituted to virtually normal values by injection of apomorphine (0.3–1 mg/kg i.p., 4 h after reserpine), a DA receptor agonist. In these animals administration of various types of drugs with reserpine-like action no longer caused an enhancement of the striatal DA turnover measured by the accumulation of endogenous dopa after inhibition of cerebral dopa decarboxylase with 3-hydroxy-benzylhydrazine. In contrast, neuroleptics like haloperidol, chlorpromazine and clozapine still enhanced the turnover of the amine. Both types of compounds were found in the series of benzo[a]quinolizine derivatives.

Effects of alpha methyl-p-tyrosine on exploratory behavior in two rat strains

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The effects of alpha methyl-p-tyrosine (AMT, 30 and 60 mg/kg i.p., 60 min injection-test interval) on exploratory locomotion patterns were investigated in 72 5.5-month-old female rats of the Roman High- and Low-Avoidance strains (RHA, RLA). Each rat was tested once a day during 6 consecutive days in a complex maze (Bättig et al., Pharmacol. Biochem. and Behavior *4*, 435 (1976)). On the last testing day, AMT was combined with nicotine (0.2 mg/kg s.c., 30 min injection-test interval). The results showed that 30 mg/kg AMT did not alter locomotor activity and exploratory locomotor patterns in the RHA strain, while 60 mg/kg AMT decreased locomotor activity without altering exploratory locomotor patterns. In contrast, both 30 and 60 mg/kg AMT increased locomotor activity and stimulated maze exploration in the RLA strain. AMT in combination with nicotine resulted in an inhibition of locomotor activity and maze exploration in both strains. The results showed the importance of genetic components in this specific drug action, and that AMT can alter the effects of nicotine.

Selective induction of epoxide hydratase in rat liver

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Epoxide hydratase catalyses the inactivation of mutagenic epoxides from polycyclic hydrocarbons to the corresponding dihydrodiols, but may thereby provide precursors for the mutagenically even more active dihydrodiol epoxides. To elucidate the role of epoxide hydratase in the mechanism of tumor formation caused by polycyclic hydrocarbons, a selective epoxide hydratase inducer has been developed. Trans-stilbene oxide, a potent epoxide hydratase inducer (max. induction: about 300% of control) showed after different doses and mode of application no significant effect on the monooxygenase system. The induction of epoxide hydratase was dose dependent up to sublethal amounts of trans-stilbene oxide and in the whole range the cytochrome P-450 content, the benzo(a)pyrene monooxygenase and aminopyrine N-demethylase activity were never significantly increased. SDS-gel electrophoresis performed with liver microsomes after pretreatment with trans-stilbene oxide indicated the presence of higher amounts of epoxide hydratase rather than an activation of the enzyme.

Resealed red cell ghosts – A model system to study catecholamine effects on cation permeability

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We studied the properties of resealed ghosts from chicken erythrocytes (CEG) and rat reticulocytes (RRG) which do contain adrenergic β-receptors as a system possibly suited to test the effects of β-receptor stimulants on cation permeability. In the absence of Ca CEG did not reseal to K and Na after reversal of hemolysis. In the presence of Ca (10^{-4} M) or Mg (10^{-2} M) the permeability to Na and K was lowered to the normal value. The need for Ca or Mg to reseal CEG made this system ill suited to study the cyclase system. By contrast, about 30% of the RRG did reseal to K and Na in the absence of Ca. Below pH 7 Ca increased the yield of RRG sealed to Na and K. A maximum was observed at 2×10^{-6} M Ca. Above pH 7.2 Ca was without effect on the resealing. In sealed RRG an increase in intracellular Ca^{++} from 5×10^{-8} to 3×10^{-7} M (pH 7) caused a large and specific increase in K permeability. Isoprenaline increased cAMP in resealed Ca-free RRG almost 50fold and tended to increase Ca influx which could be monitored by following the Ca-induced K efflux.

Antispermatogenic activity of an indenopyridine derivative

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The indenopyridine derivative (4aRS, 5SR, 9bRS)-2-ethyl-1,3,4,4a,5,9b-hexahydro-7-methyl-5-p-tolyl-2H-indeno[1,2-c]pyridine hydrochloride was found to be an antispermatogenic agent in rats and dogs. Following a single oral dose of 30 mg/kg the testes of rats were drastically reduced in weight for a period of several weeks. Within 24 h after drug application degenerative changes in the seminiferous tubules were observed. Spermatids as well as spermatocytes became pycnotic, occasionally forming characteristic multinucleated associations. Even after 30 mg/kg and more numbers of spermatogonia and Sertoli cells remained in the control range. Serum-LH and -FSH of chronically treated rats were slightly increased, indicating that the compound does not act by suppressing gonadotropin secretion. Leydig cell function was not affected since there was no reduction in seminal vesicle weight. These findings suggest that the compound is acting in the testis at the vascular or tubular level.

Differential temperature response to i.v. 1-5HTP in male and prooestrus female rats

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The observation that i.v. 1-5HTP induced hypothermia in men and hyperthermia in women led to a parallel investigation in rats. All animals were given a peripheral decarboxylase inhibitor (Ro 4-4602, 12.5 mg/kg i.p.) and 90 min later 1-5HTP-ethyl-ester (Ro 3-5940, i.v.) or saline. L-5HTP induced a marked, long-lasting hyperthermia in male rats. This was significantly different from the parallel control group at 50 mg/kg 1-5HTP, 20 mg/kg, but no longer at 8 mg/kg ($N = 10$ for each dosage). In contrast, 20 mg/kg 1-5HTP in prooestrus female rats did not modify temperature ($N = 12$). These results of a dose dependent temperature response to 1-5HTP add to the evidence involving central 5HT mechanisms in temperature regulation. However the difference between male and prooestrus females in both the pretreatment basal temperature and the response to the same dose of 1-5HTP, probably reflects a gender-determined biochemical state of CNS function.

Benzodiazepines, barbiturates and hippocampal inhibition

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Benzodiazepines (BD) and barbiturates (BA) have been shown to enhance GABA-mediated pre- and postsynaptic inhibition at different sites in the CNS (Refs in: Polc and Haefely, Archs Pharmacol. *294*, 121; Nicoll et al., Nature *258*, 625) but GABA-antagonistic effects of BD have also been reported (Steiner and Felix, Nature *260*, 346). We investigated the action of BD and BA on the GABA-mediated recurrent inhibition of hippocampal CA1 pyramidal cells. The inhibitory period, following single shocks to fimbria or alveus, was prolonged by systemic BA. Microiontophoretic or systemic BD did not specifically influence the inhibitory period, as an apparent prolongation could not be dissociated from a general decrease in the probability of firing. However, with population spikes, elicited by a double-shock technique, it was possible to demonstrate recurrent inhibition which was potentiated by systemic BD or BA.

Central tryptaminergic effects of 26-059. A novel serotonin derivative

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26-059 (N,N'-bis[2-(5-hydroxy-3-indolyl)ethyl]hexamethylenediaminedihydrochloride) was compared with the physiological precursor of serotonin (5-HT), d, l-5-hydroxytryptophan (5-HTP). In the cat, pretreated with 0.5 mg · kg^{-1} i.p. reserpine, 26-059 reduces the number of PGO-waves (ED_{50} 0.8 mg · kg^{-1} i.v.) similar to 5-HTP (ED_{50} 4.0 mg · kg^{-1} i.v.). 5-HT was inactive in doses up to 30 mg · kg^{-1} i.v. In mice and rats, 26-059 enhanced locomotor activity and excitability. In rats equipped for chronic recording of the electrocorticogram, 26-059 altered the sleep wakefulness cycle. After a dose of 0.1 mg · kg^{-1} i.p. the analysis of the different stages over 6 h showed that wakefulness was prolonged by 57%, rapid eye movement and slow wave sleep were shortened by 35% and 15%, respectively. Neurochemical data on rat brain 5-HT content and turnover support the hypothesis that 26-059 passes the blood brain barrier and acts as central tryptaminergic stimulant.

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

Studies on the conservation of untranslated regions in mRNA of immunoglobulin L chains

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The mRNA of immunoglobulin L chains contains an important region (between the polyA and the region coding for the Constant part) which is not translated (= 3'UR). Its function is unknown and an important problem is the degree of sequence conservation of that unexpressed region among the mRNA of different immunoglobulin chains. Short ^{3}H-cDNA (3'UR) was prepared from different mRNAs and cross-hybridization studies showed that kappa and lambda chain 3'UR were not related, whereas complete cross hybridization was observed among different kappa chains. The hybrids formed between plasmid-purified short cDNA and mRNA of different kappa chains showed identical melting curves and digestion of the hybrids with nuclease S 1 left the size of the ^{3}H-cDNA intact under denaturing conditions. Fragments were prepared corresponding to the 3'portion of plasmid-purified ^{125}I L chain mRNA of different kappa chains and compared by fingerprint analysis. These data were also indicative of complete sequence conservation in the 3'UR of different kappa chain mRNAs.

Ribosomal DNA in spores in Physarum polycephalum

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Using RNA-DNA hybridization techniques spores of the true slime mold Physarum polycephalum were found to contain 320 genes, each coding for 19 S and 26 S rRNA. Hydridization of rRNA to spore DNA fractionated on CsCl density gradients shows that the sequences coding for 19 S and 26 S RNA are located at a satellite position (1.714 g/cm^{3}) of greater density than the main band DNA (1.702 g/cm^{3}). The data demonstrate that in spores ribosomal DNA is not degraded and that no amplification of these genes takes place in hatching amoebae. The DNA content of spores (0.6 pg/spore) and the number of extrachromosomal rRNA genes present suggest that spores are in G2 phase.

The effect of heat shock on Drosophila melanogaster

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When Drosophila melanogaster, normally grown at 25 °C, is exposed to 37 °C for short times, it appears that a few specific genes are turned on, while most of the other genes, active in the cells before the heat shock, are turned off. The products of the heat shock genes have been characterized: at least 6 distinct polypeptides are synthesized in response to heat shock, and we have identified 6 new species of mRNA each of which codes in vitro for one of the heat shock proteins. The synthesis of most of the proteins made at 25 °C in the cells, is suppressed after the heat shock although their messenger RNAs apparently not degraded. This control, probably at the translational level, will be discussed.

A collection of hybrid plasmid clones corresponding to the entire Drosophila melanogaster genome

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Drosophila offers a number of advantages for studying gene organization and expression in higher eukaryotes. 1. There is an extensive background of classical genetic studies; 2. it has a small genome (1.6×10^{8} bp), and 3. specific DNA sequences can be readily located on the giant chromosomes by in situ hybridization. Using the newly developed techniques for the cloning of specific DNA fragments by insertion into bacterial vectors in vitro, we have collected 25,000 hybrid plasmid clones using the ampicillin resistant plasmid pSF 2124 as a cloning vehicle. The average size of the cloned DNA fragments is about 12,000 bp, so that our clone collection corresponds statistically to the entire Drosophila genome. We are now screening these clones for DNA sequences that are complementary to Drosophila t-RNAs and to m-RNAs isolated from different stages during early embryogenesis.

Restriction endonuclease cleavage maps of bacteriophage P1 DNA

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Several restriction endonuclease cleavage maps of bacteriophage P1c1^{ts} DNA were established by reciprocal double digestions. The following restriction endonucleases were used (the number of cleavage sites per P1 genome is given in parenthesis): PstI (1), HindIII (3), BglII (11), BamHI (14), EcoRI (26). All HindIII and BglII fragments, 12 out of 14 BamHI fragments, half of the fragments produced by EcoRI and the cleavage site of PstI could thus be ordered on the P1 map. The preferential starting point for packaging the phage DNA was also established; because of the terminal redundancy of phage DNA, an additional band present in less than molar amounts is formed in phage DNA digests but not in P1 plasmid digests. The fragments containing the DNA flanked by inverted repeat sequences that hybridizes with the G loop of phage Mu were localized on the P1 map.

Reversible induction of growth control in transformed cells

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Succinylated Concanavalin A (SCA) is a non-agglutinating protein that binds to cell surfaces. It inhibits the growth not only of untransformed fibroblasts (3T3), as has been shown before, but also of cells that have been transformed with the oncogenic virus SV40. 3T3 cells stop growing at a certain density in G_1 of the cell cycle. The density reached by transformed cells is much higher and they do not accumulate in G_1. SCA inhibits the growth of transformed cells at lower densities in a non-toxic and reversible fashion. It was shown by flow microfluorometry, autoradiography, as well as quantitative ^{3}H Thymidin pulses that even these transformed cells accumulate in G_1, as do untransformed cells. Experiments will be presented, which rule out a direct effect of SCA on serum components and support an effect on the cell surface.

Positional stability of nucleosomes on DNA

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The chromatin of eukaryotic cells and of the papovaviruses polyoma and SV40 is composed of subunits (nucleosomes) each containing about 140 to 200 basepairs of DNA and the four histones H2A, H2B, H3 and H4. It seems reasonable that under certain conditions, for example during transcription and replication of DNA, nucleosomes may move from one region of DNA to another. Such movement might occur either by dissociation of the histone component from DNA, or by energetically more favourable slipping or rolling. I have constructed hybrid molecules of polyoma DNA with a radioactively labelled segment joined via the $EcoR_1$ nuclease site to an unlabelled segment containing nucleosomes. Nucleosome movement can then be detected as protection of the labelled DNA against digestion by staphylococcal nuclease. Results so far confirm that dissociation of the histones and DNA in nucleosomes occurs only in greater than 0.8 M NaCl. Experiments are underway to find whether nucleosomes migrate along DNA in physiological conditions and whether migration is influenced by transcription of the DNA.

A temperature-sensitive T4 rIIB mutation affecting synthesis of the mutant gene product

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HD263, a temperature-sensitive mutation in the rIIB gene of bacteriophage T4, affects the synthesis of the rIIB protein in a temperature-dependent way. A variety of experiments suggest that the mutation acts at the level of translation. The synthesis of rIIB protein primed by RNA extracted from mutant infected cells is temperature sensitive in an in vitro system, especially when the mRNA to ribosome ratio is increased. Comparison of the methionine-containing tryptic peptides (including the N-terminal peptides) of rIIB protein synthesized in vivo and in vitro failed to reveal any difference between mutant and wild-type proteins.

The subcellular distribution of particle-bound negative charges in rat brain

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Ferritin cationized with Girard's reagent T [$(CH_3)_3$-$NCH_2CONHNH_2$] was used as a marker for negative electric charges on subcellular components of rat brain. Pre- and postsynaptic membranes did not bind cationic ferritin (CF), either on their outer or their inner surface. Vesicular membranes, however, were heavily labelled with CF. Post- and presynaptic densities appeared to carry a large number of negative charges. CF was also bound within the synaptic cleft. The density varied, however, from synapse to synapse; in general, binding within the cleft was significantly reduced when the synaptosomes were pretreated with 1 M NaCl. CF was observed in high density on the outer surface of myelin sheaths and even between the lamellae at the intraperiod lines. Neurotubules and neurofilaments bound CF in significant amounts. The results are discussed in relation to the mechanism of neurotransmission and to the binding and transport of drugs.

Studies on the product synthesized in vitro by the influenza virion-associated polymerase

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The RNA-dependent RNA polymerase associated with influenza virion was activated in vitro. In addition to the 4 ribonucleoside triphosphates, CH_3COOK, and Mg^{2+} (2 mM), ribosomes (10–20 µg) from HeLa cells had to be present. Under these conditions, ApG or GpG stimulated the polymerase activity about 3fold. The ribosomes, but not the dinucleotides, could be replaced by tRNA from rat liver (200 µg). Transcription with Mg^{2+} and ribosomes gave an RNA product of the same size as transcription with Mg^{2+}, ribosomes and the dinucleotides, namely

between 5 S and 20 S (analyzed by centrifugation on a sucrose gradient). However, transcription with Mg^{2+} and ApG without ribosomes resulted in an RNA of a smaller size. Annealing for 4 h at 65 °C rendered the RNA 100% ribonuclease resistant. The transcription product had a base composition complementary to the influenza genome (negative strand) and was therefore plus-stranded. Poly(A) was not detected.

Extra DNA in forebrain cortical neurons

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Cytophotometric and biochemical techniques show that neurons from rat, mouse, rabbit and pigeon forebrain contain more than a diploid amount of DNA. In adult rats the mean DNA content per cortical neuron comes to 3.5 c. The synthesis of this extra DNA starts a few h before birth and continues postnatally up to the age of 30 days. Postnatal labelling with [^{3}H]thymidine results in the incorporation of radioactivity into the neuronal DNA as shown by autoradiography and density gradient centrifugation (neutral and alkaline CsCl, neutral Cs_2SO_4). The recovered radioactive material is rendered acid soluble by digestion with pancreatic DNase. Double labelling of neuronal DNA with [^{14}C]thymidine given prenatally to pregnant rats and [^{3}H]BUdR injected postnatally into the lateral ventricles of the baby animals yields a density profile (in neutral and alkaline CsCl) that is consistent with a semiconservative mode of replication of the extra DNA.

Isolation and some properties of mRNA for immunoglobulin heavy chains

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The mRNA coding for mouse immunoglobulin light (L) chain can be isolated from plasmocytoma tumors and much has been learned recently about its structure and about its translation into Pre-L chain. Almost nothing is known, however, about the mRNA for immunoglobulin heavy (H) chains. RNA was prepared from ER-bound polysomes, fractionated by oligo-dT-cellulose chromatography and poly A-RNA was sedimented through a sucrose gradient. The translation of RNA from each fraction was studied in vitro and the product was analyzed by SDS acrylamide gel electrophoresis and radioautography. H chain mRNA activity was detected in RNA fractions about 17 S (γ chains). RNA from these fractions was pooled and refractionated on sucrose gradient with the same analysis for H chain mRNA activity. The nature of the cell-free translation product, the size of the H chain mRNA, estimated on acrylamide gels in formamide, and the extent of the purification of H chain mRNA achieved will be discussed.

Ecdysone, the ovarian hormone and intestinal proteases in mosquitos

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Female mosquitos generally require blood meals for oogenesis, which is under neuro-endocrine control. The blood meal is digested by proteolytic enzymes among which the most prominent is a trypsin-like enzyme, referred to as mosquito trypsin. In Aedes aegypti its activity is confined to the posterior midgut and to the time of blood digestion. Based on our earlier results, we have interpreted the secretagogue stimulation as induction of trypsin by the substrate, which can be any soluble protein. Recently we have found the endocrine system to be involved in the process of trypsin induction. In ovariectomized females trypsin activity is consistently reduced by 50%, and full activity can be restored by reimplantation of an active ovary. Injections of 8-ecdysone into ovariectomized females bring about the same result: restoration to the maximal enzyme activity. We conclude that for ecdysone, the ovarian hormone, a new function has been found. Beside stimulation of vitellogenesis in the fat body of blood-fed females, it is promoting the process of induction of mosquito trypsin in the midgut cells.

Oligodeoxyribonucleotides primed in vitro reverse transcription of various RNAs

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We have found that all RNA species so far examined can be efficiently transcribed in vitro into DNA using the Avian myeloblastosis virus (AMV) DNA polymerase and small oligodeoxyribonucleotides. The range of RNA templates tested include: heat denatured Rous Sarcoma Virus (RSV) RNA, chicken ribosomal RNAs, rabbit globin messenger RNA, bacteriophage R17 RNA and E. coli t-RNA^{PHE}. The relative efficiency of each RNA as a template for in vitro reverse transcription as well as the complementarity of DNA product and RNA template have been determined. In addition it has been shown that the oligodeoxyribonucleotides directly prime the synthesis of DNA and remain covalently attached to the product DNA after the reaction. In the case of heat denatured RSV RNA and chicken 28S r-RNA the transcription process has been followed in situ, using our recently developed procedure of analysis (J.-L. Darlix, P. A. Bromley and P.-F. Spahr, submitted to J. Virol.).

Guinea-pig seminal vesicle epithelium: Effects of castration

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Seminal vesicle epithelium was isolated from intact animals and 4- and 8-day castrates. Its ultrastructure was investigated. The height of cells and number of secretory granules decreased after castration, whereas number of lipid inclusions and of lysosomes increased. Castration induced ribosomes to dissociate from endo-

plasmic reticulum and caused the latter to dilate. Nuclei were isolated and their apparent RNA-polymerase I and II activities were measured. Both activities were reduced to about $^1/_3$ of values found in nuclei of intact animals 4 days after castration. No further decrease occurred between days 4 and 8 of castration. Additional experiments revealed that this reduction of apparent nuclear RNA-polymerase activities was not due to an increased nuclear RNase activity but was due to a diminished access of the RNA-polymerases to the nuclear template and a decreased activity of the polymerases which was presumably due to a decrease in the number of enzyme molecules. J.C.L. and C.M.V. are at the Mayo Clinic, Rochester, Minnesota, USA, where this work has been performed.

Activation of macrophages by supernatants of Con A-stimulated lymphocytes

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Mouse peritoneal exudate macrophages (PEM) were infected in vitro by the protozoan parasite Leishmania enriettii, then exposed to supernatants (SN) of spleen lymphocytes cultured for 72 h in presence of 5 μg/ml Concanavalin A. This resulted in macrophage activation, culminating in destruction of the intracellular microorganisms, which was complete within 20 h of the addition of active SN. A 6 h exposure of PEM to active SN (6 h pulse), followed by incubation in normal medium, was sufficient to induce full activation. When parasitized PEM were treated with 3 mg/ml trypsin for 30 min prior to a 6 h pulse of active SN, considerable inhibition of activation was observed, suggesting that enzyme treatment removed from the cell surface a receptor for the factor present in active SN. Parasite destruction in activated PEM correlated with an increase in lysosome content of the latter cells. Leishmania killing could be prevented by addition of hydrocortisone during exposure to active SN, perhaps reflecting an inhibitory effect of the drug on phagosome-lysosome fusion.

Transcriptase of the parainfluenza virus SV5

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An RNA-dependent RNA polymerase ('transcriptase') is described in SV5 virions and infected cells. Its activity was higher in the presence of Mn^{++} ions than Mg^{++}, and was stimulated by polyamines. The RNA synthesized in vitro by detergent-disrupted SV5 was fully complementary to virion (minus-strand) RNA and had a sedimentation coefficient of 16–18 S both in regular and in denaturing sucrose gradients. Its complexity was determined by ribonuclease T1 fingerprinting and by saturation hybridization with radioactive genome RNA, and was consistent with the size estimate obtained from sedimentation analyses: only about one-fifth of the SV5 genome, possibly one mRNA species, was transcribed in vitro. Viral transcription complexes were isolated from disrupted virions and infected cells, and their polypeptide composition was studied by polyacrylamide gel electrophoresis. Both complexes contain nucleocapsid protein NP, viral protein 5 (MW ~ 50,000 daltons) and a small amount of the high-molecular weight protein L.

Transcription of the histone gene – 'operon' of Drosophila melanogaster

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We have isolated and identified labelled histone messengers of Drosophila tissue culture cells. After UV-irradiation of the cells followed by pulse labelling of the RNA, the relative proportion of these messenger RNAs changes. The same difference is observed analyzing either polysomal or total cellular RNA. These results are consistent with the existence in Drosophila of a high molecular weight RNA containing the 5 histone RNAs, which is then processed into the final messenger molecules.

Bindungsstudien mit statischer Fluoreszenzpolarisation

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Es wurde eine Apparatur zur Messung der statischen Fluoreszenzpolarisation für Bindungsstudien von fluoreszierenden Liganden mit Biopolymeren (z.B. Hormon-Rezeptor-, Hapten-Antikörper, Substrat-Enzym-Bindung) entwickelt. Ein automatisches, kontinuierliches Misch- und Verdünnungssystem, das mit minimalen Mengen der kostbaren Substanzen auskommt, garantiert eine hohe Messgenauigkeit und Reproduzierbarkeit des Titrationsvorganges. Das von einem Laser angeregte Fluoreszenzlicht der Probe wird von einem Photon-Counting-System detektiert, wobei die Messdaten digital verarbeitet werden. Die Bestimmung der Bindungsparameter aus den experimentellen Werten erfolgt durch ein auf dem Computer durchgeführtes nichtlineares Anpassungsverfahren. Die Messgenauigkeit der Apparatur ermöglicht die Bestimmung der Bindungsparameter von unmarkierten Liganden durch Verdrängungsexperimente mit guter Relevanz. Eigens für diesen Zweck durchgeführte Computersimulationen lassen genaue Abschätzungen der experimentellen Fehler zu.

Synthesis of muscle specific proteins in myogenic cell cultures

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During differentiation of chicken myogenic cells to myotubes a transition of the B-creatine kinase (B–CK) to the muscle specific M–CK with its increased concomitant accumulation occurs. Cell cultures were pulse-labelled with ^{3}H-leucine in standard medium and its incorporation into soluble, acid precipitable protein was measured. A linear increase of incorporation was observed 15–30 min after the start of labelling up to at least 4 h. Cultures at different stages of differentiation were pulse labelled for 2 h, extracted for soluble protein and CK was isolated by immunological methods. The specificity of the method was verified by analysis of the purified CK on SDS-gels. The major part of the radioactivity was found in a band comigrating with carrier-CK. The ratio of incorporation into M–CK to incorporation into total soluble protein increased and seemed to stabilize at a higher level, but the values for this ratio for B=CK decreased, during myogenic differentiation. The change in rates of synthesis of CK-subunits seems to be implied in the regulation of the CK-transition.

Simian virus 40 salt-stable protein-DNA complex

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High concentrations of salt (NaCl or CsCl) do not remove all protein from isolated preparations of ^{35}S-methionine-labelled SV40 chromatin. By a variety of techniques I have been analyzing the nature and specificity of this putative protein-DNA complex. When unfixed SV40 chromatin is banded to equilibrium in CsCl density gradients, the major protein species found in the DNA fractions is the virion structural protein, VP1. However, VP1 is the major species found in every fraction of the gradient in the general region of RNA and DNA. When SV40 chromatin is sedimented in sucrose velocity gradients containing 1 M NaCl, the major protein found in every fraction above 10–15 S is again VP1. Prior treatment of the extract with DNase I does not qualitatively change this result. I am currently investigating whether or not there really is a protein bound tightly to SV40 DNA in high salt, or whether a fraction of the VP1 is indeed associated with the viral DNA.

Morphological study of glucagon release by the A-cell of the islet of Langerhans

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The morphological events underlying glucagon release by the A-cell of the islet of Langerhans were studied quantitatively in thin sections and freeze-fracture replicas. Criteria for release were: a) margination of secretory granules in thin sections and b) exocytotic figures in freeze-fracture replicas. Stimuli of glucagon secretion were 40 mM arginine in the absence of glucose or a deprivation of calcium in the presence of 16.5 mM glucose. Biochemical measurements of glucagon secretion indicated that the 2 stimuli induced isolated islets to release glucagon over a 30 min incubation period. 40 mM arginine was found to increase the margination of secretory granules, while deprivation of calcium had no effect on this parameter. Similarly, arginine increased significantly the number of exocytotic figures on A-cell membranes, while the absence of calcium had no effect. These results indicate that exocytosis represents a significant mechanism of glucagon release following arginine stimulation but that glucagon secretion in response to calcium deprivation may occur via other mechanism(s).

Excision of the r-determinant from R100.1

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The formation of the r-determinant element of R100.1, pLCl, has been shown to be mediated by the 2 insertion sequences (IS1) which flank the resistance genes. This has been demonstrated by an analysis of the homology between the various EcoRI generated fragments of R100.1, pLC1 and RTF derivative, pAR132, using both DNA: DNA hybridization on membrane filters and heteroduplex analysis under the electron microscope.

Contractile proteins in human cancer cells

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To study contractile proteins in human cancer cells, we have used: a) immunofluorescent staining of actin and myosin; b) identification by means of electron microscopy of cytoplasmic filaments. We examined: basal cell carcinoma (4), squamous cell carcinoma (3), infiltrating mammary carcinoma with (5) or without (5) fibrosis. Controls were normal human skin (3) and mammary glands (3). In controls, immunofluorescent staining of epithelial cells was very weak. Cancer cells were strongly positive for actin and myosin. At electron microscopic examination, no filaments were present in normal epidermal cells with the exception of tonofilaments; in mammary epithelium, few microfilaments were present at the peripheral part of the cells facing the lumen or in microvilli. Tumor cells from the skin and mammary gland contained many microfilaments and larger filaments. These changes in the content and/or organization of cytoplasmic contractile proteins may be of potential importance in evaluating malignant growth.

Antiserum against human cold-insoluble globulin reacts with a cell attachment factor from horse serum

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Cold-insoluble globulin (CIG; mol. wt 220,000, estimated by polyacrylamide gel electrophoresis in SDS) was purified from thrombin-treated Cohn Fraction I (CF-I) of human plasma by chromatography on DEAE-Sephadex and Sephadex G-200. Rabbit antisera against CIG (injected antigen was ca. 95% pure) reacted in double immunodiffusion tests with 3 components of CF-I, the most prominent of which appeared serologically identical to a single crossreacting activity in purified CIG or horse serum (HS). Rabbit anti-human-fibrinogen reacted with CF-I, but not with CIG. Fractionation of HS by the method of Klebe (Nature *250*, 248 (1974)) yielded material promoting adhesion to a gelatinized substratum of chick myoblasts maintained in serum-free medium. Throughout its further purification by chromatography on DEAE-Sephadex and crosslinked Sepharose 4B, cell attachment activity was correlated with the presence of a protein of mol. wt 220,000 (SDS gel) and with ability to react with anti-human-CIG. CIG, which itself mediates attachment of chick myoblasts, must be similar to the HS attachment factor.

Evidence for a repressed cytoplasmic globin ribonucleoprotein complex

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The globin-specific mRNA of duck reticulocytes is associated in vivo with proteins, which differ in their composition if the mRNA is actively translated in polyribosomes or if it is free in the cytoplasm. These 2 classes of ribonucleoprotein complexes have been tested for their ability to direct protein synthesis in the wheat germ cell-free system. The polyribosomal mRNP stimu-

lates the globin synthesis with the same efficiency as does corresponding amount of purified 9S mRNA. The free cytoplasmic mRNP, in contrast, is not translated in vitro, whereas the deproteinized mRNA directs globin synthesis as efficiently as the polyribosomal mRNA. Moreover, the free cytoplasmic mRNP inhibits the translation of other mRNA or mRNP. The level of inhibition is dependent on the concentration of the free mRNP and equally effective against the purified 9S mRNA and the polyribosomal mRNP. The inhibitory effect is due to a factor which is not released by high-salt treatment of the free particle. These data represent the first direct biochemical demonstration of 'Informosomes' as 'masked' forms of mRNA (A. S. Spirin, Eur. J. Biochem. *10*, 20 (1969)).

Effect of osmolarity of glutaraldehyde fixatives on rat lung

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The validity of the notion that in glutaraldehyde fixation the fixative vehicle rather than the total solution should be made isotonic was tested on lung tissue by transmission and scanning electron microscopy and morphometry. Hypo-, iso- and hypertonic fixatives (osmolarity ranges for total solution 190–660 mosm, buffer 40–400 mosm, glutaraldehyde 150–250 mosm {1.5–2.5%}) were instilled through the trachea. Expecting the delicate lung membranes to act as an osmometer we used shape changes of erythrocytes (EC) in small vessels to judge the osmotic effect on tissue. We found that the hypotonic fixatives lead to spheric deformation of EC, whereas crenated forms occurred with all hypertonic solutions including those with isotonic buffer. Isotonic fixatives (300–350 mosm) preserved the biconcave discoid form of EC. Hypertonic fixatives extracted plasma water from the alveolar capillaries; as a result plasma gelified and the air blood tissue barrier became squashed down onto EC. We conclude that the osmotic effect of glutaraldehyde cannot be disregarded.

The location of the $tRNA_1^{met}$ gene(s) within Xenopus laevis $tDNA_1^{met}$

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3.1 kb repeat units of $tDNA_1^{met}$ from the frog Xenopus laevis have been cloned in a λ vector. One of these repeats, which appears to be typical of the majority, has been digested with several restriction enzymes and the products tested for their ability to hybridize with $tRNA_1^{met}$. These studies suggest that the $tRNA_1^{met}$ gene(s) are confined to a 1.16 kb region within the 3.1 kb repeat units. To define the locations more precisely, a search has been made for the predicted alignments of cleavage sites of those enzymes that should cut the gene sequence. There are 4 such enzymes. The effects of 2 of these, Hha I and Hae III, indicate that each repeat unit may contain two $tRNA_1^{met}$ genes. Both potential coding sequences are on the same DNA strand, which thus suggests the polarity of transcription, and they are some 0.67 kb apart. Attempts are being made to test these assignments by hybridization, by mapping the cleavage sites of the other two enzymes that should cut the gene sequence, and by DNA sequence analysis.

Tryptic fingerprint analysis of Herpes simplex virus nucleocapsid proteins

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Nucleocapsids of HSV-1 and HSV-2 contain 6 major polypeptides of similar molecular size. However, studies have shown differences in the electrophoretic mobilities of the major capsid protein, VP155. This difference probably reflects differences in structure. Our goal was to study the nature of the difference by tryptic fingerprint analysis. Herpes-infected cells were labeled with (^{35}S)-methionine for HSV-1 and ^{3}H-methionine for HSV-2. VP155 was cut out from 15% gels, eluted, oxidized and digested with trypsin. Ion exchange chromatography showed that the major methionine peptides of VP155 for HSV-1 and HSV-2 were clearly different. The nature of this difference is under investigation.

In vitro transcription of the total sequences of Rous Sarcoma Virus RNA

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Conditions have been found which promote the efficient reverse transcription of all the Rous Sarcoma Virus (RSV) RNA sequences by avian myeloblastosis virus (AMV) DNA polymerase. The complete transcription has been demonstrated in 2 ways. First, using our recently developed procedure (J.-L. Darlix, P. A. Bromley and P. F. Spahr, submitted to J. Virol.) to follow in situ the reverse transcription process we have found that after 1 h all the RSV RNA sequences have been transcribed with an efficiency varying from 30% to 90%. Second, the DNA made under these conditions was shown to protect from nuclease digestion all the RSV RNA sequences after hybridization at a DNA to RNA weight ratio of 0.25. The complementary DNA made is 99% single stranded and after 1 h of transcription has a chain length varying from 1500 to 5200 nucleotides residues. A detailed analysis of the reverse transcription process using both procedures strongly suggests that initiation of RSV DNA synthesis in vitro by AMV DNA polymerase takes place at 3 to 4 sites on the RSV RNA.

Cycle lumineux et continu en phagosomes de l'épithélium pigmenté rétinien

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L'influence du cycle lumineux sur le contenu en phagosomes (segments externes des photorécepteurs phagocytés), ainsi que la distribution intracellulaire d'enzymes hydrolytiques (Cytidine-monophosphatase, Arylsulfatase) sont étudiés dans l'épithélium pigmenté rétinien de Rana esculenta. Les animaux ont été soumis à des cycles de 12 h d'obscurité/12 h de lumière. Les yeux ont été prélevés et fixés pour la microscopie électronique et la cytochimie ultrastructurale à différents temps avant et après l'enclenchement de la lumière. Les observations sur coupes semi-fines montrent une quasi absence de phagosomes à l'obscurité. Ces observations sont confirmées et quantifiées en microscopie électronique. Le nombre de phagosomes est fonction du cycle lumineux: il augmente dès l'enclenchement de la lumière, pour atteindre un maximum 1–2 h après. Leur nombre diminue pendant l'obscu-

rité, pour atteindre un minimum maintenu jusqu'à l'enclenchement de la lumière. La distribution intracellulaire des enzymes ne semble être ni en relation avec le contenu en phagosomes, ni avec les conditions d'illumination.

Cytological changes related to induction of fluid secretion in a salivary gland

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Ixodid ticks eliminate excess ions and water ingested with the blood meal via the salivary glands. Previous studies showed that the rate of secretion by isolated glands increases 60fold over the first 4 days of feeding. Concommittently, remarkable cytological changes occur in the type III acinus. In unfed ticks, inconspicious water cells are squeezed between the vacuolar and cap cells. In feeding ticks there is little change in the small cap cell, but the apical surface of the protein-secreting vacuolar cell is very rich in microvilli. The water cell becomes very prominent, rich in mitochondria and its plasma membrane surface increases dramatically, forming a tortuous labyrinth of extracellular space. Although this cell undoubtedly plays a fundamental rôle in fluid secretion, the precise rôle played by the layer of vacuolar and cap cells (which is interposed between the acinar lumen and the water cell layer) remains uncertain.

Swiss STEM project: Progress report

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The development of a high resolution scanning transmission electron microscope adapted for biological investigations is approaching completion (A. Engel, J. Dubochet and E. Kellenberger, J. ultrastruct. Res. *57*, 322 (1976)). The HB-5 microscope from Vacuum Generators has been interfaced to a Varian V-73 minicomputer which controls storage, treatment and display of image signals. The system has been designed to allow a precise control of the surface and environment of the specimen and to handle images recorded at minimum electron dose. Images comparing favorably with micrographs taken by conventional microscopes have easily been obtained for every thin specimen we have investigated. Type I pili and sex pili of E. coli were studied in more detail and in both cases their fine structure could be resolved for the first time by electron microscopy. The possibility of finding for every specimen the best compromise between 2 contradictory requirements – little beam damage (low dose) and high signal to noise ratio (high dose) – is the biggest advantage of our STEM.

Changes of aortic endothelium during different types of hypertension

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In rats, 1 week after ligature of the aorta between the 2 renal arteries, hypertension develops with high levels of plasma renin activity (PRA). The aortic endothelial cells of hypertensive animals contain important amounts of cytoplasmic microfilaments and 'en face' preparations of endothelial cells fix anti-actin antibodies. Permeability to horse radish peroxydase (HRP) through the endothelial layer of hypertensive animals is increased when compared to normal animals. 40 days after ligature of the aorta, the animals are still hypertensive but the level of PRA is normal; the aortic endothelial cells do not contain microfilaments; the immunofluorescent staining of 'en face' preparations of aortic endothelial cells is similar to that of normal animals and there is no increased permeability to HRP. Results similar to those seen in 40 days hypertensive animals are seen during hypertension induced by unilateral nephrectomy and feeding with a 6% NaCl rich diet. Probably aortic endothelial cells react differently to different types of hypertension.

In vitro-phosphorylation of histones in intact nuclei and extracted chromatin of skeletal muscle

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In order to gain information about the structural changes occurring in chromatin of postmitotic cells during differentiation and aging histones are phosphorylated in intact nuclei and in extracted chromatin of dog skeletal muscles by means of an exogenous histone kinase and ATP-γ^{32}P. Experimental conditions for maximal histone phosphorylation are described. The activity of an endogenous histone phosphatase present within the nuclei is overcome by an excess of added histone kinase, which is demonstrated to enter the nuclei. The phosphorylation pattern is different between histones phosphorylated in extracted chromatin and in intact nuclei, indicating that chromatin does not retain its structure during extraction from the nucleus.

Synthese und biologische Eigenschaften von Derivaten des Adrenocorticotropin-(1–24)-tetrakosipeptids ($ACTH_{1-24}$) mit verkürztem N-Terminus

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Das Weglassen einer zunehmenden Anzahl von Aminosäuren im N-terminalen Befehlsbereich von $ACTH_{1-24}$ führt zu abnehmender Wirksamkeit (potency) und reduziertem maximalem Effekt (intrinsic activity) der Hormonderivate. Dies wurde durch chemische Synthese und biologische Prüfung der hochgereinigten Polypeptide $ACTH_{5-24}$, $ACTH_{6-24}$, $ACTH_{7-24}$ und $ACTH_{8-24}$ bewiesen. Steroidogenese und Akkumulation von cyclo-Adenosin-3′,5′-monophosphat (cAMP) in Nebennierenrindenzellen erfordern mindestens die Sequenz His-Phe-Arg-Trp--, d.h. $ACTH_{6-24}$, Lipolyse in Fettzellen mindestens Glu-His-Phe-Arg-Trp--, d.h. $ACTH_{5-24}$. Dennoch wird die membrangebundene Adenylatcyclase von Nebenrinden- und Fettzellen schon durch die Sequenz Phe-Arg-Trp--, d.h. durch $ACTH_{7-24}$, stimuliert ($ACTH_{8-24}$ ist inaktiv). Die Stimulierung membrangebundener Adenylatcyclase durch ACTH scheint keine genügende Bedingung für die Erzeugung von Steroidogenese und Lipolyse zu sein.

The effect of colchicine and vinblastine on the ultrastructure of rat leukemia cells

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The influence of colchicine and vinblastine on the ultrastructure of cells of the undifferentiated rat leukemia L 5222 is demonstrated by transmission and scanning electron microscopy. Untreated cells adhering to glass are round with regularly arranged microvilli or polarized with a prominent protrusion at one pole and conspicuous ruffles at the other pole. In addition, the surface of polarized cells is made up of microvilli and a few ridges and blebs. L 5222 cells possess an appreciable amount of microtubules. Treatment with colchicine (10^{-4} M) and vinblastine (5×10^{-5} M) results in complete loss of microtubules. In addition, vinblastine induces paracristal formation. The surface structure of L 5222 cells is drastically altered by treatment with both alkaloids: the regular pattern of surface extensions is replaced by irregularly arranged ridges, folds and blebs alternating with smooth areas. These findings suggest that for L 5222 cells the microtubules, among other functions, are responsible for the surface architecture.

Growth stimulation of normal human diploid fibroblasts (Wi-38) by hydrocortisone

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Wi-38 cells provide an excellent model to study aging at the cellular level. They have a limited life span of approximately 40 in vitro cell doubling generations. Hydrocortisone (HC) has a growth promoting effect on these cells. 0.5 µg HC/ml, added to the medium with 10% fetal calf serum, increased the cell density by 30%. This effect was only observed when the drug was added to suspended cells after trypsinization. In long term experiments the cells were subcultivated at a constant time interval of 4 days and a split ratio of 1:2, beginning between the 20th and 24th generation. Without HC the cell density began to decrease steadily around the 34th, and the cells died at the 46th generation. Addition of 0.5 µg HC/ml at each cell split extended the cell doubling potential for 15 generations and cell death occurred abruptly at the 50th generation. A similar effect was also observed at lower serum concentrations. In addition to growth promotion, HC favored the adherence of the cells to the surface. No correlation could be established between both effects.

Generation of small plasmids following integration of F-like R factors into the E. coli chromosome

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The F-like transmissible R plasmids R1, R100-1 and R6.5 are able to suppress the effects of a dnaA mutation by integrating into the chromosome, a phenomenon which has been called integrative suppression. We have found that this integration is accompanied by the appearance in the cell of smaller covalently closed circular molecules (ccc) in the majority of the Hfr's produced. One of these elements, called pLC1, was studied by analysis of its partial denaturation pattern in the electron microscope and of the electrophoretic mobility of EcoR1 digestion products on agarose gels. The results indicate that pLC1 is the r-determinant of R100.1 (i,e. the part of 100.1 carrying all but one of the drug resistance markers). Transformation experiments and preliminary results from cloning experiments between pLC1 and the temperature sensitive plasmid pSC201 have not demonstrated an autonomous replication capability for the r-determinant.

Temperature and hydration dependent confirmational changes of the polar headgroup of L-α-dipalmitoyl lecithin

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Oriented layers of L-α-dipalmitoyl lecithin have been prepared on a sample plate for attenuated total reflection infrared measurements. Spectra have been recorded at temperatures between 22 °C and 87 °C and at humidities of 200 ppm and 30,000 ppm (~ 90% rel. hum. at 25 °C), respectively. Drastic conformational changes in the polar headgroup occur continuously at temperatures above ~ 60 °C. The results may be summarized as follows: Conformational changes are much more prominent at high humidity – The existence of an equilibrium between gauche and trans isomers of the choline part (U. P. Fringeli, Z. Naturforsch., c, in press; P. Rihak et al., Chem. Phys. Lip. submitted) is confirmed by the temperature dependence of the relative populations – The intermolecular interaction between $-N(CH_3)_3^+$ and the fatty acid ester groups is reduced synchronously by the conformational change of the choline part – At high temperatures the conformation of the phosphate group is significantly altered as reflected by the PO_2^- and POC-stretching vibrations. The number of different conformations is probably reduced – The conformations of the fatty acids ester groups have not been affected significantly neither by temperature nor by humidity.

Alteration of sciatic nerve myelin in streptozotocin-diabetes

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Nerves of rats with streptozotocin-diabetes show impaired conduction velocity which can be prevented by insulin or by feeding 1% myoinositol. Myelin sheath of sciatic nerves in diabetic rats was studied by freeze-fracture and the number and size of protein-containing particles of the membranes assessed quantitatively. Streptozotocin-diabetes reduced the number of particles per nm^2 from 140.4 ± 4.7, $n = 5$ (control) to 123.3 ± 2.4, $n = 5$, $p < 0.02$ on the P-face of the membrane and from 38.4 ± 2.3, $n = 5$ (control) to 32.8 ± 1.2, $n = 5$, $p < 0.05$ on the E-face. Nerves from streptozotocin-diabetic animals treated with insulin or fed with 1% myoinositol showed no change in the concentration of particles: 138.4 ± 5.5, $n = 6$ (P-face), and 39.3 ± 1.8, $n = 6$ (E-face) for insulin-treated animals; 141.1 ± 4.7, $n = 6$ (P-face) and 38.6 ± 1.7, $n = 6$ (E-face) for rats fed

with 1% myoinositol. The size of particles remained constant in all experimental conditions. These results indicate a reversible change in the organization of the sciatic nerve myelin sheath membranes induced by diabetes.

Purification of globin and immunoglobulin cDNA and mRNA on columns of plasmid DNA

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One of the possible usefulness of the recently constructed bacterial plasmids containing specific mammalian gene sequence (Rougeon, Kourilsky and Mach, Nucl. Ac. Res. *2*, 2365 (1975)) is preparative hybridization for the purpose of purification of specific complementary sequences, either cDNA or mRNA. Plasmid DNA carrying gene sequences from mouse globin or immunoglobulin light chain was sonnicated and covalently bound to cellulose as described by Noyes and Stark (Cell, 1976). The binding of the DNA is done under denaturing conditions (80% DMSO), after diazotization of aminocellulose. The efficiency of coupling of ^{3}H-DNA was found to be about 60%. DNA-cellulose was prepared with DNA from specific recombinant plasmids and was used to hybridize cDNA or mRNA, in 50% formamide and with gentle shaking. Washing and elution of the purified cDNA or mRNA were performed either in batch form or in a column. Different properties of these plasmid DNA-cellulose columns and their use for the purification of unique cDNA or mRNA from crude preparations will be discussed.

Analysis of E. gracilis chloroplast rDNA by cleavage with endo R. EcoR1 and endo R. BamH1.

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Circular DNA of E. gracilis chloroplasts has a total of about 139,400 kb. Endo R. BamH1 splits the DNA into 6 fragments (P. W. Gray and R. B. Hallick, in: Proc. Genetics and Biogenesis of chloroplasts and mitochondria, Munich 1976) and we showed that endo R. EcoR1 generates 22 fragments. The EcoR1 fragments E, K, N (7.9, 3.2, 2.4 kb) and the BamH1 fragments D and E and F (7.3, 5.8, 5.8 kb) are shown to hybridize to 165 and 235 chloroplast rRNA. The BamH1 fragments D and E and F carry EcoR1 sites. The EcoR1 fragments E and K carry BamH1 sites. The EcoR1 fragment N seems to be located within the BamH1 fragments D and E and F. A physical map of the rDNA region is presented.

A light- and electron-microscopic study of hairy cell leukemia

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In a patient with splenomegaly and pancytopenia the diagnosis of hairy cell leukemia (HCL) suggested by previous bone marrow biopsy was verified by cytological studies of a spleen cell suspension obtained at splenectomy. Histologically there was a diffuse lymphomatous infiltration of the spleen by tartrate-resistent acid phosphatase positive cells. Incubation of unfixed cells in suspension with anti-human gammaglobulin (HGG)-FITC revealed a spotty fluorescence of the cell surface. Scanning EM (SEM) of cells attached to cover slips show numerous slender finger-like surface projections morphologically different from short microvilli of normal lymphocytes and from ruffled projections of monocytes. Transmission EM (TEM) confirmed the presence of hairy projections with microfilaments and in addition intracytoplasmic ribosome-lamellar complexes. It thus appears that in many cases HCL may be diagnosed by a single bone marrow biopsy, however, histochemistry, TEM and SEM of cells from spleen and leukemic blood may be of additional diagnostic value.

Isolation of messenger RNA coding for egg shell protein of Ascaris lumbricoides

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Poly(A)-containing RNA from polyploid uterine epithelial cells of A. lumbricoides has been isolated by chromatography on poly(U)-sepharose. The bulk of poly(A)-containing RNA migrates as 18S RNA in formamide-polyacrylamide gels. Translation of this RNA in a cell-free wheat germ system produces a polypeptide with identical migration behavior in SDS-urea-polyacrylamide gels as the polypeptide isolated from the proteinaceous egg shell. Moreover, the 2 proteins reveal almost identical peptide patterns in fingerprint analysis. The native egg shell protein has been identified as a glycoprotein with a mol. wt of about 10,000 D, as determined by SDS-polyacrylamide gel electrophoresis.

Protein patterns of oocytes from wild-type and 'grandchildless' females in Drosophila subobscura

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^{35}S-methionine was injected into the abdomen of adult females and incorporation was allowed for 10 h. Oocytes were dissected and cut into defined fragments. Proteins were extracted in 6 M urea and 2% NP-40 and analyzed by two-dimensional gel electrophoresis which separates according to size and charge. When the anterior fragment of stage 10 egg chambers containing the nurse cells is compared to the posterior fragment which consists mostly of the growing oocyte, one protein (spot) was found to be specific for the anterior fragment, while 10 proteins were specific for the posterior fragment. 2 of these proteins could also be detected in mature oocytes in the anterior and posterior fragments respectively. When fragments of mature oocytes from 'grandchildless' mutant flies (which give rise to embryos lacking pole cells) were compared with the corresponding fragments of wild-type oocytes several differences were observed in both posterior and anterior proteins.

The organization of genes for transfer RNA and ribosomal RNA in amoebae and plasmodia of Physarum polycephalum

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Using RNA-DNA hybridization techniques nuclei from both amoebae and plasmodia of the true slime mould Physarum polycephalum were found to contain 275 genes each coding for 5.8S, 19S and 26S rRNA, 685 genes for 5S rRNA and 1050 genes for tRNA. Hybridization of these RNA species to both amoebal and plasmodial DNA fractionated on isopycnic CsCl gradients reveal that the 5.8S, 19S and 26S rRNA genes are located at a satellite position ($\varrho = 1.714$ g/cm^3) with respect to the main band of DNA, whereas 4S and 5S RNA genes are located exclusively in the main band peak of DNA ($\varrho = 1.702$ g/cm^3). This result was confirmed by demonstrating that only the 5.8S, 19S and 26S rRNA species hybridize to purified plasmodial ribosomal DNA. The 19S and 26S rRNA genes are localized on extrachromosomal DNA molecules of a discrete size (38 million daltons) in amoebae as well as in plasmodia.

Biosynthesis of brush border glycoproteins by human small intestinal mucosa in organ culture

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Glycoprotein synthesis was measured by the incorporation of ^{14}C-glucosamine. After 24 h of culture, the radioactivity peaks on SDS/polyacrylamide gels of isolated brush border membranes were found exclusively in the high molecular weight region and corresponded to protein bands identified as maltase-glucoamylase, lactase, sucrase-isomaltase, enterokinase and alkaline phosphatase. Enzymatic activity could not be assigned to the three remaining labelled bands. Most of these glycoproteins were already labelled after 5 h. Newly glycosylated brush border enzymes remained predominantly associated with the brush border of intact cells with little release into the medium up to 24 h. Turnover was measured by the pulse-chase technique. It was found that newly glycosylated brush border enzymes turn over very slowly probably due to the absence of bile acids or pancreatic enzymes. These results may prove to be useful for the study of the regulation of normal brush border glycoprotein synthesis and in disease in man.

Surface structure of the Tetrathyridium of Mesocestoides corti by TEM and SEM

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Filamentous and blade-like microtriches occur on the body surface of these cestode larvae as shown by TEM and SEM by Hess and Guggenheim (Z. Parasitenk. (1977)). The filamentous or cylindrical form, 3–7 μm long, has a tubular, 0.9 μm thick base and a slightly thinner, filamentous shaft. The spatulate or blade-like type is a large 2,5 μm high transverse blade with a posteriorly directed support which tapers distally. The filamentous microtriches have flexible shafts and are certainly involved in food uptake. The blade-like forms are rigid and considered to be of importance in tissue penetration function by preventing a retreat. On the anterior extremity of the Tetrathyridium and on the suckers (zone A), both microthrix forms are seen, more posteriorly (zone B), only spatulate microtriches occur and on the posterior part of the larvae (zone C) only filamentous forms are found. The border between zone A and B is distinct while zone B merges imperceptibly into zone C. The scolex bears club-shaped and filamentous sensory processes.

In vitro lambda DNA packaging and its applications

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DNA on its way to become filled into the phage λ head passes through a series of complexes which can be isolated and characterized by the pattern of in vitro complementation they exhibit. In vitro packaging is polar and size independent: if a digest of λ DNA with restriction endonuclease(s) is offered, the fragment containing the the left molecular end of the DNA is selectively packaged. In this in vitro packaging system normal λ DNA can be replaced by recombinant λ DNA molecules that have been made by cleaving λ and E. coli DNA with restriction endonucleases and ligating the fragments together. By this means E. coli genes (and others, if required) can be made infectious. This process is efficient, nonselective (since the DNA is only a passive packaging substrate) and can be performed safely. As one application of these methods the gene(s) for the bacterial function groE, which is (are) responsible for proper prehead assembly in λ by means of somehow controlling a protein cleavage and fusion activity, was (were) isolated as λ transducing phages.

Analysis of the RNA synthesized in vitro by the intracellular influenza RNA polymerase

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A ribonucleoprotein complex containing the influenza RNA-dependent RNA polymerase was isolated from the cytoplasm of cells infected with influenza virus. RNA synthesized in vitro by this ribonucleoprotein-polymerase complex shows a similar sedimentation pattern on sucrose gradient as the segmented viral RNA. This newly synthesized RNA was separated into 2 fractions after poly(U)-Sepharose chromatography. The unbound RNA has a base composition complementary to the genome (negative strand) of influenza virus and is therefore plus-stranded. The bound RNA is also complementary to the influenza genome but has in addition a poly(A) sequence linked to it. Both RNA fractions show the same sedimentation pattern on sucrose gradient. Preliminary experiments suggests the presence of poly(U) sequences in both fractions of RNA. No evidence for an in vitro methylation of the newly synthesized RNA has yet been found. ApG which stimulates the polymerase activity did not modify the characteristics of the in vitro product.

Geometry of a foraminiferal shell structure reflecting ectoplasmatic functions

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Foraminifera grow successively adding new chambers to their shell. The protoplasmic body coheres through passages between neighboring chambers. As a response to mechanical or chemical irritation, the protoplasm withdraws from late chambers. Species where the reticulopods extrude from the last chamber's openings are unable to move when irritated and live therefore on plants in freely circulating seawater. Others live on the sediment-water interface depending on active movement out of local pollution by accumulation of decaying organic matter. During irritation in hostile environments their motility is kept up by extruding the reticulopods from canal systems connecting all chambers of the growth series to outer space. Different taxonomic groups construct canal systems by covering incised sutures with different shell elements. This is reflected by different geometric patterns in canal systems but their basic geometry meets always the functional requirements connecting early chambers directly to the ambient environment.

Retinoic acid binding protein (RABP) in normal, preneoplastic and neoplastic human mammary tissue

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Tumorgrowth is recognized to occur in 3 distinct phases: initiation, preneoplasia and transformation. Recently Vitamin A and its oxidation product retinoic acid has been implicated to be involved in the initiation of tumors; i.e. retinoic acid could inhibit and reverse tumorgrowth. However, the molecular mechanisms by which retinoids act remain to be established. The detection of a specific retinoic acid binding protein (RABP) in human breast and lung tumor, but not in the surrounding tissue may indicate that retinoids exert their physiological effect by such binding proteins. Though the molecular mechanism is still unknown we have strong evidence that the appearance of RABP parallels the developing of tumors. In normal tissue of 8 patients no RABP could be detected, but 55% of proliferative dysplasias (20 patients) and 53% of primary carcinomas (21 patients) contained RABP. The binding protein was determined with agar gel electrophoresis and sucrose density gradients.

DNA polymerase-α, -β and -γ activities in different tissues during pre- and postnatal development

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The activities of the 3 known DNA polymerases-α, -β, and -γ were determined in rat brain neurons, cardiac muscle, spleen and liver and were correlated with the rate of cell proliferation during perinatal development. In neurons and cardiac muscle, which stop dividing before birth, DNA polymerase-α activity drops precipitously from a high level with the approach of term and disappears at approximately 2 weeks postnatal age. In contrast, spleen α-polymerase activity is almost absent in late gestation, when there is little cell division, and increases abruptly after birth with the sudden onset of cell proliferation. In liver, the slowly decreasing rate of cell multiplication is paralleled by a gradual decline of α-polymerase activity. These data give further evidence for an involvement of DNA polymerase-α in DNA replication. DNA polymerase activities -β and -γ show no consistent correlation with the extent of cell division. This indicates that these enzyme activities might be responsible for repair processes rather than for DNA replication.

Restriction cleavage analysis of specialized transducing phage P1Cm

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A large number of P1Cm, i.e. plaque-forming specialized transducing phages carrying the chloramphenicol resistance gene derived from the R plasmid NR1, has been isolated. Restriction cleavage analysis of their DNA reveals that the Cm segment can be integrated at least at 5 different sites into the P1 genome. Among the 5 sites, 2 are located in the gene region responsible for P1-specific restriction and modification and another in the region identical to the G-loop of phage Mu. In addition a non-essential region of the P1 genome was identified, since several P1Cm carry a deletion adjacent to the site of Cm integration without being affected in their plaque-forming ability. These P1Cm are defective in a new type of DNA modification function. On the basis of the results obtained, the physical map is aligned with the genetic map of P1.

Dissociation of phage λ and phage λ polyheads with citraconic anhydride

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Citraconic anhydride is a reversible acylating agent for the free amino groups of proteins. Due to the conversion of a positive to a negative charge, protein-protein interactions are weakened and protein complexes such as bacteriophage capsids dissociate. The acylation of phage λ ghosts and the dissociation that it causes can be followed by centrifugation, gel electrophoresis and electron microscopy. Phage proteins are sequentially dissociated by increasing the concentration of the agent. The acylated proteins can be well separated by gel filtration. After de-acylation at low pH the protein pD regains its biological activity and will complement D^--lysates in vitro and bind to pD-free enlarged preheads. Phage λ polyheads citraconylated to a degree that does not cause dissociation, show a much better resolved surface structure, as seen in negatively stained preparations in the electron microscope. The resolution allows to estimate the position of both the pD and pE protein monomers within the capsomers.

A cold sensitive DNA mutant of E. coli: dnaAcos

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We have isolated cold sensitive suppressor mutations of dnaAt46; they seem intragenic and could not be separated from dnAt46 by Pl-transduction. Like dnaAt46 they affect chromosome replication but in the opposite way: at

42 °C dnaAcos grows normally, but below 37 °C it overproduces DNA with respect to proteins. Cells can still divide several times but the DNA/mass ratio increases drastically. Eventually cells become deformed and lyse. dnaAcos mutants carrying the plasmid λdv grow normally at low temperatures. The gene P product of λdv seems alone responsible for the suppression of dnaAcos: several λdv plasmid mutants in gene P do not suppress dnaAcos, although they can be propagated by the strain at the permissive temperature (42 °C). Gene P product of phage λ is known to interact with dnaB, but not with dnaA product. Competition of the plasmid for dnaB product could lower the initiation potential of the cell, correcting dnaAcos induced overinitiation, but this hypothesis remains to be tested.

In vitro study of phagocytosis by retinal pigment epithelium (RPE)

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In order to study some aspects of recognition, endocytosis and post-engulfment phenomenon of phagocytosis in RPE, we have used an in vitro model. Histiotypical cultures of adult pig's RPE were stimulated with polystyrene micropheres (1.0; 0.3; 0.1 μm) during 1–48 h with and without colchicine added. The cells were observed by scanning and transmission electron microscopy. EM cytochemistry (AcPase, Arylsulfatase) and biochemical assays (AcPase, β-glucoronidase) were used to follow variations in lysosomal enzyme localization and intra- and extracellular concentration. After a latency period of 4–8 h, microspheres were surrounded by newly formed microvilli. During the same period, lysosomes increased in number when compared to unstimulated cells. Sphere diameter appeared not to be a determinating factor for recognition. Cells incubated with colchicine still phagocytized microspheres; however fusion of primary lysosomes with phagosomes was inhibited. Presently we are investigating the release of hydrolytic enzymes by activated cells into the medium.

Peptide analyses of envelope glycoproteins of Rous Sarcoma Virus

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The 2 envelope glycoproteins of Rous Sarcoma Virus (Pr-C), gp 85 and gp 37, can be purified from ^{35}S methionine labeled virus on an agarose column in 6 M Guanidine Hydrochloride. Tryptic peptides of gp 85 were prepared by removal of sialic acid residues followed by oxidation and trypsin digestion. The peptides were bound to a cation exchange column (Chromobead Type P) and eluted with an exponential pH gradient. 18 ^{35}S methionine labeled peptides could be separated in this way. Gp 37 aggregates in 6 M Guanidine HCl and is eluted from the agarose column with the flow through. It has been purified further by SDS PAGE. The analyses of the tryptic peptides of this protein should enable us to tell whether gp 37 is related to gp 85. A protein which moves slightly slower than gp 85 on a SDS Polyacrylamidgel can be immunoprecipitated from pulse-labeled chicken embryo fibroblasts infected with Pr-C using an anti gp 85 serum. The tryptic peptide map of this protein will be analyzed and compared to the peptide maps of gp 85 and gp 37.

Regulation of the expression of bacteriophage T 4 Genes 32 and 43

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We have examined the mechanism of the self-regulation of bacteriophage T4D genes 32 and 43. It has been demonstrated that the rate of P32 synthesis can increase substantially in situations in which new RNA synthesis is blocked either by the drug rifampicin or by inactivation of a temperature sensitive host RNA polymerase. In addition, RNA's have been extracted from various infections which have large differences in the in vivo rate of P32 synthesis. Determination of the in vitro capacity of these RNA's to direct the translation of P32 indicates only small differences in the relative amounts of gene 32 mRNA in such infections. These results are interpreted as suggesting gene 32 self-regulation operates by regulation of the translation of gene 32 mRNA. In contrast, experiments with gene 43 mutants show that overproduction of P43 is blocked when further RNA synthesis is prevented. Thus, the self-regulation of gene 43 appears to operate by control of transcription.

Intramembranous particle distribution at the node of Ranvier in mammalian spinal cord axons

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The plasma membrane of myelinated axons has been examined by freeze etching electron microscopy in cat and rat spinal cord. A relatively high concentration of small (below 10 nm) and large particles (10–20 nm) was found at the nodal as compared with the internodal axolemma. This difference was particularly striking at the EF face. Nodal particle distribution was not significantly different between EF and PF faces. The concentration of large intramembranous particles at the nodal was many times greater than at the internodal axolemma. It is suggested that these large particles may be specifically related to the mechanism of excitability.

α-Melanotropin-macromolecule complexes

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Polypeptide hormones covalently bound to macromolecules (proteins or viruses) are important for the generation of highly specific antibodies, for their isolation and characterization, and for the localization of hormone receptors. N^{α}-bromoacetyl-α-melanotropin [^{3}H] (α-MSH′) was attached to albumin and tobacco mosaic virus (TMV) which both had first been substituted by thio-succinic acid [^{14}C.] Purification of the albumin-α-MSH′ complex by extensive dialysis, electro dialysis and Sephadex gel chromatography; characterization by polyacrylamide gel electrophoresis. Substitution rate: 5 · · · 12 α-MSH′ molecules per albumin depending on the reaction conditions. This complex exhibited a relatively high biological activity of $\sim 10^{7}$ U/g in the in vitro frog skin assay. The

TMV-α-MSH' complex had similar activity and was capable of aggregating α-MSH antibodies as was shown by electron microscopy. These results clearly demonstrate a receptor stimulation by polypeptide hormone macromolecule complexes.

Cell cycle dependent effects of amethopterin on cellular thymidine triphosphate pools

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Amethopterin inhibits de novo synthesis of thymidine nucleotides; the expected decrease of intracellular thymidine triphosphate (dTTP) content after addition of the inhibitor to cell cultures was, however, reported to be absent or small or to occur rather slowly (e.g. C. N. Baumunk et al., Cancer Res. *31*, 1930 (1971)). In our studies, cellular dTTP was determined at various times after addition of amethopterin to asynchronous and synchronous CHO cultures. If the medium contained undialyzed fetal calf serum, dTTP content decreased slowly. Similarly, in asynchronous cultures incubated in medium with dialyzed serum, dTTP content was as high as 10 pmoles/10^6 cells at 30 min after addition of amethopterin. If, however, synchronous S-phase cell populations were incubated in medium with dialyzed serum, dTTP content within 10 min decreased to 1.5–3 pmoles/10^6 cells. Failure of amethopterin to cause rapid depletion of intracellular dTTP may, therefore, reflect maintenance of thymidine nucleotide pools by cells that are not in S phase.

Mapping of the resistance genes of R100.1 and pLCl

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We have established a correlation between the antibiotic resistance genes and the EcoRI generated fragments of both R100.1 and pLCl by cloning these fragments on the plasmid pCR1. Our results provide genetic support for a model in which the r-determinant excision is mediated by the IS1 sequences flanking the resistance genes.

Cytoplasmic differentiations reflected by shell morphology in foraminifera

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The mineralized shell of canaliculate foraminifera (marine protozoans) is differentiated into a series of interconnected chamber cavities and a complex canal system located within the chamber walls. Connections between chamber and canal system are restricted to a few, small openings in each chamber. The chamber system, which is not in direct contact with the ambient environment, contains 'endoplasm' with ultrastructural features of metabolically active cells: nuclei, mitochondria, microbodies, dictyosomes, endoplasmic reticulum, numerous vacuolar types and symbionts if present. The canal system which connects the chambers directly with the exterior of the shell, contains 'ectoplasm' reflecting transport and motility functions. Ectoplasm is characterized by microtubuli, small mitochondria and a few particular vacuolar types, exactly as free reticulopodia of other foraminifera. Thus in nummulitids and elphidiids, the division of labour between endoplasm and ectoplasm, the two distinct parts of the large protoplast, is clearly expressed by shell morphology.

The influence of serum lipids on the morphology of neuroblastoma cells

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Culturing of neuroblastoma cells in lipid-free serum allows spontaneous neurite extension and the formation of an extensive network similar to that induced by glial conditioned medium. Neurites are rapidly retracted upon the addition of fresh serum, lipoproteins or serum lipids bound to bovine serum albumin. Both the retraction of neurites and the inhibition of their formation can be brought about by the free-fatty acid fraction of serum lipids. Coupled with studies on the effect of phospholipids, tryglycerides, cholesterol, etc. on neuroblastoma morphology it is clear that the composition and level of exogenous lipid has a strong influence on morphological differentiation. Glial conditioned medium may owe its activity to both a reduced fatty acid content (cf serum) and the presence of specific protein-bound lipids which trigger morphological differentiation.

Purification of mouse globin and immunoglobulin genes with mercurated plasmid DNA

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Direct structural studies of immunoglobulin genes ultimately require the purification and/or cloning of these genes. Since a bacterial recombinant plasmid carrying a gene sequence derived from a light chain mRNA had been constructed, the DNA from this plasmid was used to attempt the purification of complementary sequences (cDNA, mRNA or DNA strands from cellular DNA fragments). Mercurated plasmid DNA was hybridized in liquid with denatured DNA from mouse embryos or plasmocytomas, previously digested with endonucleases EcoR1 or BAM I. The hybrids were selectively trapped on a column of SH-Sepharose and after washing, strand separation of the hybrids was achieved on the column and the complementary strands recovered by elution. The DNA was further fractionated on alkaline sucrose gradient with elimination of DNA shorter than 1000 bases long. Titration of DNA purified from 50 mg of cellular DNA indicated more than a 1000fold enrichment in globin or L chain specific sequences with about 40% recovery. A second round of purification could be performed with little further loss in specific gene sequences.

Fluorescence microscopy of 5HT organelles in normal and storage pool deficient blood platelets (P)

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Mepacrine accumulates selectively in the serotonin (5HT) storage organelles of blood P enabling the microscopic observation of these organelles as fluorescent dots; on irradiation the P emit flashes (Experientia *31*, 742 (1975)).

The number of dots per isolated P exposed to 5×10^{-5} M mepacrine averaged 6.3 ± 0.1 in man, 17.0 ± 0.6 in rabbit, 10.8 ± 0.3 in cat, 8.8 ± 0.2 in guinea pig, 8.5 ± 0.3 in mouse and 5.6 ± 0.3 in rat (± SE, n ≥ 51). The slightly lower number of microfluorimetrically measured flashes did not differ significantly from the dot number in these healthy P. P with storage pool deficiencies of fawn-hooded rats (storage pool disease), C57Bl/6J-bg/bg mice (Chediak-Higashi syndrome) and a patient (Hermansky-Pudlak syndrome) had a deficient mepacrine accumulation. The following values refer respectively to fluorescent dots, flashes, fluorescence intensity, ATP and 5HT of isolated P (controls = 100%): fawn-hooded rats 86 ± 4, 27 ± 5, 81 ± 3, 33 ± 4 and 20 ± 4%; bg mice 65 ± 3, 5 ± 1, 47 ± 5, 47 ± 5 and < 1%; patient 11 ± 2, not determined, 68 ± 5, 48 ± 3 and 3 ± 1% (± SE, all p versus controls < 0.01).

Histone content in human lymphocytes from young and old donors

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The distribution of 0.25 N HCl extractable histone fractions from human lymphocytes is not uniform in young and old donors. At older ages histone H1 is increased. In order to locate the origin of this H1 increase we prepared lymphocyte nuclei by an aqueous nonidet method and compared the amount of histone extracted from these nuclei with that extracted from whole lymphocytes. There was no significant quantitative or qualitative difference between young and old donors. Per nucleus we found H1 = 1.26 ± 0.1, H2A + H2B + H3 = 4.19 ± 0.289, H4 = 1.23 ± 0.072 pg. In the cytoplasmic fraction, however, we found that H1 increases from 1.27 pg per cell from young donors to 2.52 pg of H1 per cell from old donors. The question as to whether parts of H1 are lost from the nucleus during preparation is currently being examined by a non-aqueous isolation procedure of nuclei.

cAMP-Rezeptoren und die periodische Aktivierung von Adenylzyklase bei Dictyostelium

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Aggregationsreife Zellen reagieren chemotaktisch auf cAMP (Konijn Adv. Cyclic Nucleotide Res. *1*, 17 (1972)) und geben es periodisch ab. Ein Verstärkermechanismus für cAMP-Pulse ermöglicht die Weiterleitung der chemotaktischen Signale von Zelle zu Zelle. cAMP-Rezeptoren an der Zelloberfläche messen Änderungen der extrazellulären cAMP-Konzentration (Gerisch und Malchow, Adv. Cyclic Nucleoside Res. *7*, 49 (1976)). Extrazelluläres cAMP führt zu einer pulsweisen Aktivierung der Adenylzyklase. Das Enzym wird auch spontan periodisch aktiviert. Spontane Oszillationen sind mit einer periodischen Erhöhung der extrazellulären Protonenkonzentration verbunden. Zugabe von cAMP löst ebenfalls eine pH-Erniedrigung aus, wobei die Wirksamkeit von Analogen ihrer Affinität zum cAMP-Rezeptor entspricht (Nanjundiah und Malchow, Hoppe Seyler's Z. Physiol. Chem. *357*, 273 (1976)). Extrazelluläres cAMP verschiebt auch die Phase der Oszillationen, d.h. das oszillierende System ist an die cAMP-Rezeptoren gekoppelt.

Preparation of Qβ DNA-containing plasmids and their use for sequence analysis

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With the advent of new techniques for DNA sequencing (Sanger and Coulson; Maxam and Gilbert) it seemed that RNA sequencing might be accelerated by the use of DNA transcripts. Since we are analyzing Qβ RNA, we prepared Qβ DNA-containing plasmids: a poly(A) tail was added to Qβ RNA fragments (500–2000 nucleotides) with terminal riboadenylate transferase. A double-stranded DNA copy was synthesized using reverse transcriptase and oligo(dT) (Maniatis et al.), and inserted into plasmid PCRI using the poly(dA)-poly(dT) tail technique. After transfection, more than 200 Qβ DNA-containing clones were identified by in situ hybridization with [^{32}P] Qβ RNA. The Qβ DNA inserts were identified by hybridization with characteristic oligonucleotides of Qβ plus and minus strands, with known map positions. Most or all of the Qβ genome was represented in the plasmid collection. The Qβ specific segment of one plasmid was excised by S_1 nuclease in the presence of formamide (Hofstetter et al.). The 1800 nucleotide long fragment including most of the replicase cistron and the 3′ terminal region is being sequenced.

Characterization of human histone genes by restriction analysis

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We have previously shown that the histone genes of human placenta DNA are repeated 30–40fold (Wilson et al., BBRC *61*, 404 (1974); Wilson and Melli, J. Mol. Biol., 1977, in press). The analysis of the histone genes in CsCl gradients, using the hybridization technique, has shown that histone DNA has a higher GC content as compared with the main band DNA. This observation allowed the isolation of a DNA enriched in histone gene sequences which could be used to study human histone DNA by digestion with restriction enzymes. After digestion the DNA was electrophoresed in agarose gels and transferred to a Millipore filter with the Southern technique. Separate and combined digestion with EcoRI and HindIII suggests the existence of a gene unit of approximately 14.3 kb.

Synthesis in vitro of Semliki Forest virus (SFV) messenger RNAs containing poly(A) tracts

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In SFV or sindbis virus infected cells 2 major virus-specific single-stranded RNAs are regularly present: the 42S genomic viral RNA (mol. wt 4×10^6) and the 26S RNA (mol. wt 1.6×10^6). The 26S RNA is the major messenger RNA and represents one third of the 42S RNA and is derived from the 3′ end of the genomic RNA. Both species contain at their 3′ end a poly(A) tract. Using an unfractionated extract from SFV infected BHK-21 cells which catalyzes the synthesis of all virus-specific RNAs, it is found that the in vitro synthesized 42S and 26S

RNAs are complexed with protein as messenger ribonucleoproteins (mRNP). These mRNPs exist at least in part of ribosomal initiation and elongation complexes. Between 70 and 80% of the phenol extracted in vitro synthesized 42S and 26S RNA are bound to oligo(dT)-cellulose and can be eluted either with 50% formamide or at low ionic strength at elevated temperature. The length of the poly(A) on the viral RNAs synthesized in vitro during a 30 min reaction and isolated by adsorption on oligo(dT)-cellulose exceeds that of the poly(A) tracts on viral mRNAs synthesized in vivo.

Serum-induced reversal of morphological differentiation of neuroblastoma cells

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Change of medium causes reversal of morphological differentiation in neuroblastoma cells previously grown in glia conditioned medium. The degree of reversal correlates with the serum concentration in the new medium. At a certain serum concentration the reversal activity is quantitatively antagonized by increasing amount of serum-free glia conditioned medium. Excess of lipids or fatty acids can inactivate the serum reversal activity. These results are consistent with a scavenger role for a serum apolipoprotein(s) on the neuroblastoma cell membrane.

Antagonistic effect between serum-free glial conditioned medium and fatty acids on the morphological differentiation of neuroblastoma cells

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Glial cells in tissue culture release a macromolecular factor which can induce morphological differentiation of neuroblastoma cells. We now report that neuroplastoma cells are able to extend neurites when grown in a medium supplemented with delipidated serum. The slight variance in the neuroblastoma cells growth rate in lipid-free medium cannot explain the high degree of spontaneous morphological differentiation. Fatty acids, especially oleic acid, added to the delipidated serum, prevent this spontaneous morphological differentiation. The effect of oleic acid is quantitatively antagonized by the addition of increasing amount of serum-free glial conditioned medium. These results suggest that the previously described glial factor may exert its effect through modification of the properties of the neuroblastoma cell membrane.

Polyoma virus-specific RNA in cytoplasm and detergent-washed nuclei of productively-infected mouse cells

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We previously reported that only 5–10% of late polyoma virus-specific RNA synthesized in the nucleus of productively-infected mouse cells is transported to the cytoplasm (Acheson, Experientia *32*, 784 (1976)). In those experiments, perinuclear ribosomes remained associated with the nuclear fraction. To test whether virus-specific RNA associated with the perinuclear cytoplasm might differ from the bulk of virus-specific cytoplasmic RNA, we applied the Penman (JMB *17*, 117 (1966)) method of nuclear isolation. Cells are lysed in NP-40 at pH 8.5 in a low salt buffer. The nuclear pellet is washed in an NP-40-deoxycholate detergent mixture. High mol. wt nuclear RNA is extracted with 2% triisopropylnaphthalenesulfonate/phenol/chloroform after a brief treatment with DNAse. RNA is extracted from each of the two cytoplasmic fractions with 1% SDS/phenol/chloroform. The nuclear fraction contains only 8% of the total 18S RNA, a measure of cytoplasmic contamination. Nuclear contamination of the cytoplasmic fractions is about 2%. Polyoma-specific RNA present in these 3 fractions will be analyzed.

Specific and non-specific stimulation of human cells producing monoclonal IgM

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In a patient with IgM paraproteinemia the monoclonal IgM (14 mg/ml) agglutinated murine erythrocytes with a titer of 1:140,000 at 4 °C and 1:4000 at 37 °C. Lymphoid cells were isolated from the patient's peripheral blood and stimulated with mitogens (PHA, ConA, PWM) and with murine erythrocytes. ^{3}H-Thymidine incorporation, surface and cytoplasmic immunoglobulins (IgM and idiotype) were assayed before and after 5 and 7 days of culture. Cells carrying idiotypic determinants could not be stimulated by the erythrocytes. A normal blastogenic response could be induced by mitogens, but no idiotypic determinants could be demonstrated on the surface or in the cytoplasm of the lymphoblasts.

Similarities and differences in the structure of some Xenopus laevis tDNAs

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The reiterated genes coding for $tRNA_1^{met}$ of Xenopus laevis are found within tandemly-linked 3.1 kb repeat units. DNA fragments of this length, highly purified for $tRNA_1^{met}$ genes, been cloned in a λ vector via their cohesive HindIII ends. Of the 10 clones examined that hybridize with unfractionated 4S RNA, 8 also hybridize with $tRNA_1^{met}$ and possess the distribution of EcoRI sites typical of $tDNA_1^{met}$. 2, however, have a completely different EcoRI restriction pattern. One of these hybridizes with $tRNA_1^{met}$ but the other does not and may therefore code for other tRNA species. Comparison of the restriction maps of these 2 cloned fragments and of a typical $tDNA_1^{met}$ repeat unit shows that these fragments share some common restriction sites, but that a significant amount of sequence divergence exists. The locations and possible significance of these sequence similarities and differences will be discussed.

Localization of protein P24 in T4 capsids and aberrant preheads

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When T4 capsids are extracted with 7 M urea at pH 11.0 the P24 is quantitatively removed together with a small amount of P23. The residual capsids appear normal in negatively stained preparations, except that they have clearly visible holes at the vertices (including that at the apex), suggesting that P24 is located principally if not exclusively in these positions. Immuno-electron microscopy supports this view. When either wild type phage or aberrant preheads are treated with antibody prepared against soluble purified P24, the particles agglutinate vertex-to-vertex. The surface of the particles is free of antibody, except at the vertices. There, clumps of antibodies are seen on free particles and bridges can be seen linking the vertices of agglutinated ones. Clumps and bridges have been shown to be antibody by their reaction with ferritin-tagged anti-rabbit γ globulin. The reaction of the anti-P24 is stronger with the preheads than with phage. We have not determined whether this reflects a change in the antigenicity of the P24 during capsid maturation or a change in accessibility of the protein on the surface of the particle.

DNA folding in the 140 base pair nucleosome

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Nuclease digestions of chromatin represent a powerful tool for the elucidation of chromatin structure. 2 classes of nucleases acting at different levels of structural organization of chromatin are known, a) nucleases that preferentially attack the link of adjacent nucleosomes and b) those that cleave the DNA within the structurally conserved 140 base pair 11 S monosome. DNase I (pancreatic DNase) which belongs to the second class nicks each strand at sites spaced by multiples of 10 bases. Pancreatic DNase digestion of the 140 base pair nucleosome labeled at the 5'-ends followed by a quantitative analysis of the labeled single-stranded fragments shows that both DNA strands are exposed every 10 bases. The probability distribution of nicking might indicate a twofold symmetry of the 140 base pair nucleosome. In particular it is shown that the predominant fragment of 80 bases is derived from several regions within the 140 base pairs. Its possible significance with respect to chromatin structure is discussed.

Comparison between the effect of nerve growth factor (NGF) on sympathetic ganglia and adrenal medulla

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In previous experiments it has been shown that NGF has no general growth promoting effect on adrenal medulla. This, together with the fact that administration of NGF-antiserum to newborn rats does not affect the adrenal medulla, while leading to an extensive destruction of the peripheral sympathetic nervous system, was taken as evidence that the adrenal medulla does not respond to NGF. However, recent experiments have shown that NGF elicits a selective induction of tyrosine hydroxylase (TH) and dopamine B-hydroxylase (DBH) both in sympathetic ganglia and adrenal medullae of newborn and adult rats. This selective enzyme induction does not depend on intact preganglionic cholinergic fibres. Moreover, light and electron microscopic autoradiograms showed that intravenously injected ^{125}I-NGF is not only accumulated in postganglionic adrenergic neurons but also with high selectivity in adrenal chromaffin cells. It is concluded that the effect of NGF of the adrenal medulla is confined to the selective induction of TH and DBH whereas the general growth promoting effect observed in adrenergic neurons is absent.

Oligo(U) as primer for reverse transcriptase

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AMV reverse transcriptase transcribes RNA into cDNA when appropriately primed. Commonly, oligo(dT) is used for templates with 3' terminal poly(A). The resulting products have 5' terminal dT-tracts of variable length which may interfere with subsequent reactions (i.e.: Maxam and Gilbert sequencing procedures). To avoid such problems we have used oligo(rU) primers, which can subsequently by hydrolyzed off the product by alkali. Poly(U) was fragmented by partial alkaline hydrolysis, treated with acid to open cyclic phosphates and digested with alkaline phosphatase. Fractionation was by chromatography on Tener columns, which yielded oligomers of discrete sizes, or on Sephadex G-100, which gave a heterogeneous preparation (15 to 50 residues). In a globin mRNA-directed reaction, the most active preparations (chain length 12–20) gave up to 60–80% the incorporation found with oligo(dT). At oligo(U)/template ratios of about 1 mole/mole, the length of the cDNA product corresponded to that of the mRNA. At higher oligo(U)/template ratios the size of the product decreased, presumably because of internal starts.

Comparison of three computerized methods for particle size distribution analysis

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From the variety of mathematical methods for derivation of the particle size distribution from their plane sections, 3 were selected and programmed for computer application. These methods were: transformation according to Wicksell (Biometr. Z. *17*, 84 (1925)), transformation of Giger and Riedwyl (Biometr. Z. *12*, 156 (1970)) and the transformation described by Schwartz (J. Microsc. *96*, 25 (1972)). With each of these programs one and the same data set was treated. This set was formed by 5000 measurements of the diameter of renal corpuscles done on paraffin section of 5 normal kidneys of healthy adult (250 g) rats. On the basis of results obtained the different methods will be compared and some principles governing their choice and application will be analyzed. The impact of the derived distributions of glomerular sizes on the interpretation of glomerular number will be discussed.

Ultrastructural changes in rat liver during bile acid induced choleresis

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It has been suggested that the canalicular plasma membrane (CPM) and the Golgi apparatus play an important role in bile formation. These structures were, therefore, measured by morphometry in bile fistula rats after induction of choleresis with either taurocholate (TC, 800 nmoles/min 100 g b.wt), the major bile acid of the rat, or dehydrocholate (DHC, 330 nmoles/min 100 g b.wt), a synthetic bile acid, and in controls (saline infusion). Although similar increases in bile flow (65% and 69%) and in the volume density of bile canaliculi (22% and 20%) occurred after TC and DHC, the surface density of CPM and Golgi membranes decreased (47% and 37%, respectively) after DHC and did not change significantly after TC. The diminution of CPM was accompanied by a qualitatively observed loss of microvilli but could not be related to biliary excretion of phospholipids, which was 17.5, 9.6 and 10.3 nmoles/ming liver after TC, DHC and saline infusion, respectively. The data indicate that the choleresis induced by the synthetic bile acid DHC and by the natural bile acid TC are associated with different ultrastructural changes.

mRNA for creatine kinase in RNA from chicken embryos and myogenic cell cultures

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Our studies on macromolecular differentiation during myogenesis are extended to investigations of the presence of mRNA coding for the muscle specific protein M-creatine kinase (M-CK) in myogenic cell cultures. Total cytoplasmic and polysomal RNA was obtained by phenol extraction or $CsCl_2$ centrifugation, its analysis on SDS-sucrose density gradients demonstrated it to be of large size with no apparent signs of degradation. ^{3}H-uridine labelled RNA from cultures was enriched for poly-A sequences by chromatography on oligo dT cellulose and analyzed on density gradients. It sedimented over a wide range with a peak in the 18S rRNA region. The translational activity was tested in a cell free protein synthesizing system and followed the profile of radioactivity in the gradients. The sizes of the products primed by polysomal RNA from 48 h cultures extended up to a mol. wt of 100,000. The peptides were subjected to immunoadsorption on anti M-CK Sepharose, bound labelled material was eluted and analyzed on SDS-gels. A substantial part of radioactivity migrated with carrier CK.

ATP production and Ca^{2+} uptake by mitochondria in chick embryo fibroblasts during replication of Semliki Forest Virus

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Chick embryo fibroblasts were infected with Semliki Forest Virus at a multiplicity of 20 PFU/cell. Using different substrates, ADP/O quotients and acceptor control ratios (ACR) of isolated mitochondria were measured in the course of infection. Ca^{2+}/O quotients and corresponding ACR were determined of mitochondria in situ. $^{45}Ca^{2+}$ uptake was studied in the same system. 5 h after infection (hpi) ADP/O and Ca^{2+}/O quotients, corresponding ACR and $^{45}Ca^{2+}$ uptake were elevated as compared to controls whereas 10 hpi these parameters were reduced. The latter observations are indicative of impaired mitochondrial ATP production and Ca^{2+} regulation. This is also reflected by high permeability of the inner mitochondrial membrane revealed by cytochemical methods. Stimulation of $^{45}Ca^{2+}$ uptake was observed already at 0 and 2.5 hpi. It is one of the earliest biochemical changes in virus infected cells and could have implications on the regulation of different enzyme systems.

Digital analysis of the heart development on the organ level

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Quantitative expression of the heart morphogenesis in digital form is needed for correlation with the underlying cellular and subcellular events. The point count morphometry was applied on semithin serial section of chick embryonic heart in the period between the 3rd and 5th ed in intervals of 8 h. The lumen occupied 27–38 relative volume percents (rvp) of the bulbus, the wall represented 63–72 rvp. 20–24 rvp of the wall consisted of myocardium, 14–22 rvp of intercushion mesenchyme. The bulbar cushions occupied 34–66 rvp of the bulbus. Most important were the distal ventral bulbar cushion (2–14 rvp) and the proximal left bulbar cushion (6–16 rvp). Statistical evaluation demonstrated a significant decrease in the importance of the myocardium between 3rd ed 8 h and 4th ed 8 h. The growth maximum in the distal ventral bulbar cushion occurred between 3rd ed 16 h and 4th ed. Its size significantly decreased 8 h later. Similar reduction took place on the proximal left bulbar cushion between 3rd ed 16 h and 4th ed.

Replication of the prophage P1 during the cell cycle of Escherichia coli

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We have followed, by DNA-DNA hybridization, the variation in the number of copies of prophage P1 relative to 2 chromosomal markers when the doubling time of the host cells is modified by a change in carbon source. The ratio of P1/chromosome terminus undergoes a two-fold decrease when the cell doubling time increases from 24 to 215 min, whereas the ratio of P1/chromosome origin increases 1.4fold; both ratios tend towards unity at slow growth rates. This suggests that the replication of prophage P1 is not simultaneous with chromosome initiation or chromosome termination. The chromosome replication time is unaffected by the presence of P1, and remains constant over the range of doubling times studied, with a value of about 40 min. Following amino acid starvation, the P1/chromosome origin ratio increases from 0.7 to 0.9, suggesting that P1 retains the ability to replicate after chromosome initiation has stopped and in the absence of essential amino acids. The results are discussed with reference to similar studies done on F and R1.

A method for separating myogenic from fibrogenic cells in primary cultures of embryonic chick muscle

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Trypsinized cells washed thoroughly with serum-free medium are cultured (37 °C, 5% CO_2 in air, gelatinized tissue culture plates) in a medium consisting of 83.3% L-15, 16.7% 150 mM $NaHCO_3$, 2% high mol. wt (excluded from Sephadex G-25) fraction of 12-day chick embryo extract, supplemented with insulin (200 ng/ml, added daily) and antibiotics. After 2 days nearly all the myogenic cells are in suspension (mostly as small aggregates), while almost all the fibroblasts adhere to the dish. If cultured further, many of the suspended cells fuse to form multinucleate 'myoballs' that remain viable for at least 1 week. If the suspended cells are instead decanted after 2 days into new dishes and cultured in the same medium supplemented with horse serum, the cells attach within 6–8 h and go on to produce highly fused cultures nearly devoid of fibroblasts. Fibroblast contamination is greater if nonattached cells from older cultures are subcultured. Some attachment is seen with 0.05% serum; maximal attachment requires 0.5%.

Nature des fragments d'ADN apparaissant lors de la réplication

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Les fragments d'Okazaki, formés lors de la réplication de l'ADN, contiennent une sous-classe capable d'être séparée de l'ADN total en milieu neutre. Ces fragments, purifiés par chromatographie sur hydroxyapatite, sont plus petits que les fragments d'Okazaki et s'hybrident aux deux brins de l'ADN parental, alors que les fragments d'Okazaki sont partiellement polarisés et s'hybrident préférentiellement au seul brin allant dans la direction 5′–3′.

Intercellular junctions in rat parathyroid gland: A freeze-fracture study

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Freeze-fracture replicas of the rat parathyroid glands revealed that the chief cells carry specific membrane specializations which represent 2 types of intercellular junctions, namely tight and gap junctions. The presence of such intercellular contacts characterized by distinct functional implications might be relevant to the functions of this endocrine gland.

Uranaffin reaction: A new cytochemical technique for the localization of adenine nucleotides in organelles storing biogenic amines

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Amine storage organelles of aldehyde-fixed prior to dehydration rabbit platelets have a strong affinity for uranyl ions and appear highly electron dense when examined by electron microscopy; both the matrix and membrane of these organelles are intensely stained. This affinity, which is also shown by platelets of other species, including healthy human donors, is independent of the presence of amine. Indeed, megakaryocytes and reserpinized platelets, which contain no cytochemically demonstrable amine, show a positive uranaffin reaction. However, platelets and megakaryocytes of species with storage pool deficiency (low ATP), including patients with Hermansky-Pudlak syndrome, are uranaffin negative. The cytochemical reaction, probably the result of an interaction between UO_2^{++} ions and phosphate groups of 5′-phosphonucleotides, is also observed in adrenal medulla, sympathetic nerve terminals and ganglion cells, suggesting that the technique will be of considerable value in identification of aminergic neurons and in further elucidation of amine storage mechanisms.

Temperature and hydration dependent conformational changes of choline and glycerophosphorylcholine

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Infrared spectra of choline chloride and glycerophosphorylcholine · $CdCl_2$ were measured by ATR (attenuated total reflection) technique. The absorption bands were assigned to the normal vibrations with help of the normal coordinates analysis. It has been found that the bands at 890 cm^{-1} and 920 cm^{-1} correspond to the same stretching mode of the choline skelet in gauche and trans conformation respectively. Changes of the relative intensities of these 2 bands with temperature and water content in the spectra of choline chloride were interpreted in terms of conformational equilibrium between the 2 conformers. At the temperature of the phase transition in choline chloride ($\sim$ 80 °C) the unique presence of gauche conformation is destroyed and internal disorder about the C–C axis takes place. Relative large amounts of choline molecules occur in trans conformation. The temperature and/or range of the phase transition is influenced by increasing water content which also favours the trans conformation. Changes were also found in the spectra of glycerophosphorylcholine at different temperatures and water contents. They are probably associated with both the choline and phosphate group.

Organization and function of the chloroplast DNA of Chlamydomonas

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The chloroplast DNA of Chlamydomonas reinhardi has been digested with the restriction enzymes EcoRI, BamI, BglII, HindIII and SalI. The sum of the mol. wts of the chloroplast DNA fragments produced by the first 3 enzymes equals 120×10^6 daltons. Most of the EcoRI fragments and several Bam fragments have been cloned in E. coli. The chloroplast-plasmid hybrids have been examined by analytical ultracentrifugation and by electron microscope heteroduplex analysis. The chloroplast ribosomal RNA genes are located on four EcoRI fragments, of which 2 are present in 2 molar amounts and on 2 Bam fragments also present in 2 molar amounts. These 6 fragments have been cloned and a partial restric-

tion map of this chloroplast DNA region has been established. In addition, some tRNA genes have been located. Several chloroplast DNA-plasmid hybrids have been used in an in vitro coupled E. coli transcription-translation system, in which several polypeptides coded for by the chloroplast DNA can be recognized.

Vitellogenin mRNA in Xenopus during primary and secondary stimulation by estrogen

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Estrogen induces in the liver of male Xenopus laevis synthesis of the yolk precursor protein vitellogenin. Using cDNA hybridization vitellogenin mRNA was first detected at a level of 0.06% of the cytoplasmic poly(A)-containing RNA 12 h after primary stimulation with estrogen. On the 7th day of hormone treatment it had accumulated to an average of 34%, corresponding to about 35,000 mRNA molecules per cell. As judged from the incorporation of $^{32}PO_4$ into blood plasma proteins, onset and extent of vitellogenin synthesis is correlated with the abundance of vitellogenin mRNA in the cytoplasm. 41 days after estrogen injection, when vitellogenin synthesis had ceased, the abundance of vitellogenin mRNA was reduced to $< 0.03\%$. Secondary stimulation by estrogen resulted in a 30fold faster accumulation of vitellogenin mRNA in the cytoplasm within 12 h. Therefore the enhanced appearance of vitellogenin during secondary stimulation, known as 'memory' effect, results from a more rapid accumulation of vitellogenin mRNA rather than unmasking of pre-existing mRNA.

Changes of thymidine nucleotide metabolism in the cell cycle

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CHO and P-815 cells were incubated at 0 °C or 37 °C with tracer amounts of ^{3}H-thymidine, then washed at 0 °C or 37 °C and reincubated at 37 °C in nonradioactive medium. Within 10 min, 21–26% of labeled intracellular thymidine phosphates were released, in the form of thymidine, into the culture medium. During incubation of cells at 0 °C, ^{3}H-thymidine was incorporated into the intracellular nucleotide pool, but not into DNA. After 10 min reincubation of cells in nonradioactive medium at 37 °C, pool radioactivity had decreased to 30% of the initial value, while 45% were incorporated into DNA and 25% released into the medium. In contrast, in synchronous cultures consisting predominantly of S phase cells, under the same conditions pool radioactivity had decreased to 10%, whereas only 15% appeared in the medium as compared to 75% in DNA. Catabolic degradation of thymidine nucleotides thus seems to be more important in cells not engaged in DNA synthesis and may represent a regulatory mechanism preventing the accumulation of large amounts of thymidine nucleotides within the cell.

Histone DNA sequence organization

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The cloned histone gene cluster of the sea urchin Psammechinus miliaris (about 6000 base pairs long) was subjected to sequence analysis using both the chemical procedure of Maxam and Gilbert and the enzymatic method developed by Sanger and Coulson. In both methods a sequence of 50–100 nucleotides starting from a defined position in the DNA can be directly read off a polyacrylamide slab gel. Using numerous restriction enzyme cleavage sites, about 25% of the 6 kb histone DNA repeat unit have been sequenced, with the aim of determining the primary structure of coding regions as well as control sequences for transcription, translation and precursor RNA maturation. The sequences obtained up to now include regions of the H2B, H3, H2A and H1 genes as well as segments of the AT-rich spacer DNA. Particular emphasis was put on sequences immediately preceding or following a coding region since they might be involved in the control of histone mRNA translation.

Excision of insert from hybrid plasmids containing poly(dA)-poly(dT) links

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Hybrid plasmids are frequently constructed by elongating a fragment of double-stranded DNA at both 3′ termini with poly(dA) and hybridizing it to linearized plasmid DNA carrying poly(dT) tails. Using the hybrid plasmid Pβ G (Maniatis et al.), which consists of plasmid PMB9 with an insert of rabbit globin DNA 600 BP long, flanked by 40 and 115 AT-pairs respectively, we found conditions to specifically and efficiently excise the insert. Under conditions of partial denaturation AT-rich regions become preferentially susceptible to the single-strand specific nuclease S_1. Partial denaturation and cleavage can be attained either by digesting in 45–55% formamide at 50 °C in the presence of excess nuclease S_1, or by first partially denaturing the DNA in the presence of formaldehyde, diluting and then carrying out the digestion under standard conditions. The method was applied to many other hybrid plasmids, both analytically, to determine the size of the insert, and for preparative purposes. When DNA from phage T7, λ or ∅ 29 was subjected to a similar procedure discrete, specific band patterns were found.

In vitro activation of macrophages by phagocytosis

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Resting and activated peritoneal macrophages (MPH), i.e. obtained from untreated and thioglycollate-treated mice, respectively, were compared in vitro. Activated MPH contain about twice as much protein and LDH per cell than resting MPH. They release into the medium large quantities of lysosomal glycosidases and of plasminogen activator at nearly constant rate for up to 2 weeks, starting from the beginning of culturing. Resting MPH release similar amounts of lysosomal glycosidases per mg of cell

protein, but very little plasminogen activator. Release of the glycosidases, however, starts only after a lag period of 2 days. When during this lag resting MPH are allowed to phagocytose zymosan, they rapidly become activated and soon behave like thioglycollate-induced MPH. Release of lysosomal glycosidases is a true secretory process since it is independent of on-going phagocytosis. Considerable de novo synthesis of these enzymes is indicated by their accumulation in the medium in amounts largely exceeding the intracellular levels and by blockade in the presence of cycloheximide.

Inhibition of oncornavirus reverse transcriptase by β-lapachone

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β-Lapachone, a naphtoquinone derivative isolated from various tropical trees has an inhibitory effect on reverse transcriptase of avian myeloblastosis virus (AMV). At a drug concentration of about 10 μM 50% inhibition is obtained. β-Lapachone was chosen as one of the most potent of a large number of related derivatives. It inhibits both crude reverse transcriptase from lysed virus or highly purified enzyme, the template/primer being either endogenous RNA or a variety of synthetic polynucleotides. Inhibition is also observed with reverse transcriptase from Rauscher murine leukaemia virus. Further studies of the specificity of the reaction are in progress. The mechanism of action appears to involve the protein itself, since competition experiments using template/primer or substrate did not show any significant change in the pattern of inhibition, whereas large amounts of protein reduced the inhibitory effect. The detailed mode of action of β-Lapachone is currently being further evaluated.

Selectivity of trans-synaptic migration of macromolecules following retrograde axonal transport in the rat superior cervical ganglion (SCG)

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2 macromolecules, nerve growth factor (NGF) and tetanus toxin (TT), have been shown to be transported retrogradely in adrenergic ganglion cells. EM autoradiography of the SCG 14 h after injection into the anterior eye chamber and the salivary gland revealed labelled adrenergic neurons. In addition, a pronounced labelling of presynaptic cholinergic nerve terminals innervating the labelled ganglion cells was observed after injection of TT but not after injection of NGF. In order to decide whether the absence of trans-synaptic migration of NGF is due to a lack of receptors on the terminals of preganglionic fibres, wheat germ agglutinin was injected. This lectin is known to be taken up by all nerve terminals studied. A highly efficient retrograde transport in the adrenergic neurons was observed, which, however, was not followed by trans-synaptic migration of the label. Similar negative results were obtained with phytohaemagglutinin and ricin II. These results suggest that trans-synaptic migration of macromolecules following retrograde transport may not depend primarily on the presence of receptors on the presynaptic terminals of the preganglionic neuron. As a working hypothesis a selective release by the adrenergic ganglion cell can be postulated.

Properties and developmental transition of hemoglobins in Xenopus

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Electrophoretic separation of hemoglobins revealed 5–9 fractions for premetamorphic larvae and 3–4 fractions for adult toads. Larval Hb (Hb_L) comprise 2–3 predominating components, whereas adult Hb (Hb_A) contain 1–2 major components. Immunologically no common antigenic properties could be detected between total Hb_L and total Hb_A suggesting differential composition of these proteins. Estimations of the relative amount of Hb_A from electropherograms showed a marked change in the proportion of Hb_A from 30 to 80% to occur within 10 weeks after the completion of metamorphosis. 'In vivo' labelling with ^{3}H-leucine allowed detection of Hb_A synthesis already during metamorphosis suggesting involvement of the thyroid hormones. However, chemical thyroidectomy of larval stages, which suppressed the morphological transformation, only resulted in a delayed appearance of the Hb_A. It is concluded that initiation of synthesis of Hb_A, if it is at all controlled by the thyroid hormones, requires a much lower hormone concentration than the morphological transformation.

Preclinical experiments with the biomedical pion beam of the S.I.N.: Mutation induction in male germ cells of Drosophila

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Dose responses of dominant and sex-linked recessive lethals, sex chromosome loss, partial loss of the Y-chromosome and autosomal translocations were determined following negative pion and 140 kV X-ray irradiation. RBE values ranging from 0.5 to 3.5 were obtained depending on the dose level, type of mutation and developmental stage of the germ cells tested.

Hybrid plasmids containing T4 DNA

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When bacteriophage T4 DNA is digested with restriction endonucleases Eco RI or Hind III a large number of fragments are obtained. The genes present on the restriction fragments can be determined by a DNA transformation assay. By ligation of fragments to plasmid DNA digested with the same endonucleases, we have obtained hybrid plasmids which contain various segments of the phage genome. The portion of the genome present can be determined by a marker rescue assay. Analysis of hybrid plasmids thus far obtained suggests that certain restriction fragments cannot be cloned, at least with the 2 plasmid vectors employed.

Origin and direction of replication of R100.1 and an RTF derivative, pAR132, in synchronized culture

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R100.1 and its RTF derivative, pAR132, were isolated from E. coli K12 cultures after synchronization of initiation of replication by sequential amino acid and thymine starvation. Plasmids isolated as covalently closed circular DNA after a short pulse of ^{3}H-thymidine at the time of initiation followed by a 30 min chase in unlabelled thymidine and thymine, were analyzed by electron microscope-autoradiography of partially denatured molecules. This analysis showed that in both the R100.1 and pAR132 populations, replication is predominantly unidirectional in a single sense from an origin in the region of Kb5–Kb10 on the R100 map. A more precise mapping of the replication origin, at Kb8.8 on the R100 map, was obtained by partial denaturation analysis of fork positions in replicative intermediates of the plasmids isolated in total cell lysates from synchronized cultures. This study also showed that approximately 90% of the replicative intermediates analyzed, in both R100.1 and pAR132 populations, replicated unidirectionally in a single sense from the Kb8.8 origin.

Tumor-stroma relationships in human colon carcinomas grafted to the nude mouse

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Human solid tumors growing in nude mice induce a murine stroma which consists of connective trabeculae, vascular bed and various types of infiltrating cells. The nature of the human tumor cells as well as that of the mouse stroma may be demonstrated on cryostat sections by anti-species antibody reactivity in immunofluorescence or by karyotypic analysis. Light microscopy quantitative analysis of the xenograft constituents, such as tumor cells, necrotic areas and connective-vascular bed, indicates that their relative proportions appear to be similar when serial transfers of the graft are compared for a same time interval following the subcutaneous inoculation to the nude mouse. 2 colo-rectal tumors, a poorly and a well-differentiated carcinoma, have been studied. The present results indicate that a) the amount of autologous stroma within the patient's tumor is generally greater than in the xenograft, b) the ability to induce a given amount of heterologous stroma from the mouse may be closely related to the successful take of the particular xenograft.

Modified SDS-polyacrylamide gels crosslinked with diallyl-tartardiamide (DATD)

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We were interested in solubilizing gels for scintillation counting. According to Anker (FEBS-Letters 7, 293 (1970)) gels were crosslinked with DATD instead of methylenebisacrylamide (MBA). We modified the 'mole for mole DATD in place of MBA' prescription to a ratio of acrylamide (ACAM) to DATD of 73:27 (ACAM = 7.5%). We could surmount swelling to 30% over the initial length and got gels similar with MBA-gels (ACAM = 7.5%, ACAM:MBA = 95:5). Our gels have following characteristics: 1. gels are transparent; 2. crystal violet is, bromphenol blue is not migrating with the front; 3. gels build up spontaneously spacer-gels; 4. gel slices are soluble in 2% periodic acid; 5. gels show as sharp protein bands as MBA-gels; 6. compared to MBA-gels, low mol. wt proteins migrate similarly, but with increasing mol. wts protein migration increases too; 7. proteins too big to enter MBA-gels do enter DATD-gels; 8. log mol. wts in correlation with the relative mobility of marker proteins fit a line; the coefficient of the determination of linear regression (r^2) is in case of DATD slightly higher than in MBA-gels.

Subunit structure of chromatin from Physarum polycephalum

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Limited digestion of isolated nuclei from Physarum with micrococcal nuclease reveals DNA fragments which are multimers of a repeating subunit. The size of the subunit was calculated from acrylamide-agarose gels calibrated with rat liver DNA fragments. The subunit structure differs from that of higher eukaryotes: each subunit contains only 170–175 base pairs as compared to 190–200 base pairs in higher eukaryotes. However, a quasi limit digest of 140 base pairs is obtained with Physarum chromatin as with higher eukaryotes. The basic repetitive structure of chromatin from diploid plasmodia and haploid amoebae is the same. The extrachromosomal ribosomal DNA located in the nucleoli, is arranged in a similar chromatin structure. Ribosomal chromatin is somewhat more slowly digested by micrococcal nuclease than bulk chromatin as determined by acid soluble products after different digestion times. Isolated DNA fragments after chromatin digestion hybridize equally well with 19 + 26S rRNA as does unfragmented DNA.

Qualities and projections of sensory neurons in a homeotic appendage of Drosophila

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Hungry Drosophila flies extend their proboscis when their tarsi come into contact with a sugar solution. Pro-, meso- and metathoracic legs differ from one another in their responsiveness. In the homeotic mutant Antennapedia ($Antp^{73b}$) of D. melanogaster stimulation of an antennal leg (which corresponds morphologically to a mesothoracic leg) elicits a proboscis extension response (PER) comparable to that of a mesothoracic leg. Since the PER cannot be evoked by gustatory stimulation of wild-type antennae these data prove the legness of the chemosensory neurons in the transformed appendage. Wallerian degeneration reveals that the sensory axons from the homeotic leg do not terminate in normal leg projection areas in the thoracic ganglion, but predominantly within normal antennal centers of the brain. How the PER can be evoked despite the missing homeotic projection to normal leg centers may be answered by a small group of sensory axons which pass from the antennal leg directly into the mushroom bodies, centers assumed to mediate information processing of gustatory input.

Role of cellular locomotion in leukemic infiltration

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2 transplantable rat leukemias with different locomotive behavior in vitro, as recorded by time lapse cinemicrography, were studied in view of their capacity to infiltrate the chick embryo mesonephros in organ culture. L 5222 leukemia has a high percentage of locomoting cells which assume a conspicuous polarized shape, whereas BNML is practically non-locomotive. After inoculation on mesonephros explants, the 2 leukemias displayed a completely different behavior. According to histological evaluation, L 5222 had started to infiltrate at 2 h and extensively colonized the mesonephros fragments at 24 h. Many of the infiltrated cells were fixed in the characteristic locomotive configuration. BNML, on the other hand, showed only a very slight infiltration recognizable at 24 h. At 25 °C and 18 °C, both leukemias were not capable to infiltrate, which corresponds with their inability to locomote at subnormal temperatures. Thus, there is good agreement between the locomotive and the infiltrative capacity of the 2 leukemias.

Experimental anatomical identification of electromotoneurons in the spinal cord of Sternarchus albifrons

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Horseradish peroxidase was injected into the electric organ of 6 animals. The spinal cord was examined light- and electronmicroscopically after 3–4 days survival time. Tracer was found almost exclusively in large unipolar neurons situated medially and slightly dorsally to the central canal. These neurons are identical with those found to be AchE-negative by Sandri et al. (Brain Research *111*, 157 (1976)). After control injections into the skeletal muscles of the trunk the tracer was carried to the multipolar motoneurons located ventrally and laterally in the spinal cord but not to the former pool of neurons. Thus, 2 types of motoneurons exist; they are differentially localized and provide a separate innervation to 2 distinctly different effector organs.

Possible role of catecholamines and cAMP in the regenerating forelimb of the Newt

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The cAMP concentrations were measured in regenerates of the Newt forelimb at progressive stages of development. 2fold increase in cAMP was detected at the wound healing-, medium early bud-, and late bud-stages, the range of unchanged levels of cAMP in other stages being 4.9 to 6.8 pmoles per mg protein. Previous work (Experientia *33*, 48 (1977)) showed that the response of the cAMP generating system to noradrenaline (NA) was also stage-dependent. The increased sensitivity to NA is shown now to overlap the peaks of cAMP in normal conditions of regeneration. Pharmacological characterization of the catecholamine response, using β-adrenergic agonist (isoproterenol) or antagonist (propranolol), indicates that the receptors are of a β-type. Furthermore, various pieces of tissue removed from normal limb (i.e. skin) contained much less cAMP than regenerates and were insensitive to NA. These results would suggest a role for cAMP in the process of regeneration, preceded by a possible involvement of β-adrenergic receptors at various stages of development.

Particulate protein kinases and cAMP-binding proteins in calf ovaries

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Protein kinase (PK) and cAMP-binding activities were identified in the subcellular fractions isolated from calf ovaries. Sephadex G-200 gel filtration of the Triton X-100 solubilized enzymes revealed cAMP-dependent protein kinases of 230,000 mol. wt in the nuclear, microsomal and cytosol fractions, and of 80,000 mol. wt in the nuclear and mitochondrial fractions. Both cAMP-dependent enzymes appeared to have similar properties in the subcellular fractions. The nuclear fraction prepared in the presence and absence of the protease inhibitor PMSF exhibited cAMP-dependent PK of 230,000 mol. wt and 80,000 respectively. The conversion to the smaller enzyme was accompanied with little loss in activity. All particulate fractions contained a cAMP-independent PK of 40,000 mol. wt which was cAMP-independent due to the lack of inhibition with either the rabbi muscle inhibitor or the regulatory subunit of the holoenzyme. A cAMP-binding protein of 40,000 mol. wt was the major binding component in the nuclear fraction.

A nonanucleotide complementary to the 16S rRNA end inhibits ribosome binding

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Shine and Dalgarno proposed that the 3′ terminus of ribosomal 16S RNA is essential in protein initiation by hydrogen bonding to a purine-rich region preceding the initiator sequence. To test this, we searched for a blocking oligonucleotide binding specifically to the 3′ terminus of 16S rRNA. [^{32}P] Qβ RNA was totally digested by RNAase A and the resulting oligonucleotides were incubated with ribosomes. Upon sucrose gradient centrifugation, radioactivity was found in the position of 30S ribosomes. It contained an oligonucleotide, **AGAGGAGGU**p (P-2a), which can form 8 base pairs with the 3′ terminal region, ... AUC**ACCUCCUU**A$_{OH}$, of 16S rRNA. The interaction between the 2 regions was demonstrated by a technique similar to that of Jakes and Steitz. Purified P-2a, which is not derived from an initiator sequence, was a potent inhibitor of initiation complex formation. Thus, the incorporation of [^{32}P] Qβ RNA (2 pmoles) into a 70S complex was 90% inhibited when the ribosomes (5 pmoles) were preincubated with 20 pmoles of P-2a. Similar oligonucleotides isolated at random gave no significant inhibition.

Membrane retrieval in neurosecretory axons

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Endocytosis, following stimulated exocytotic hormone release in axons of rat and hamster neural lobes, was studied by a) tracing the uptake of horseradish peroxidase (HRP) and b) freeze-fracture. After an i.v. injection of HRP, reaction product appeared mainly in vacuoles as large as or larger than the secretory granules. The relative volume occupied by these structures increased 3fold after haemorrhage or electrical stimulation of the pituitary stalk. Microvesicles also contained reaction product but the microvesicular uptake was not secretion related and gave rise to vesicles different from those found in clusters. En-face-views of the split plasmalemma of freeze-fractured axons revealed invaginations whose number doubled in stimulated glands. Large vacuoles, presumably derived from these invaginations were apparent in the cytoplasm of cross-fractured axons. It is proposed that the membrane retrieval associated with hormone release occurs by a form of macropinocytosis or 'reverse exocytosis'.

Direct evidence for the condensation of nucleosomes by histone H1

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In chromatin containing all histone proteins the distances between the nucleosomes are small (less than 20 Å) and very regular. However, if histone H1 is selectively removed at NaCl concentrations between 100 and 450 mM, the chromatin fibres appear with their nucleosomes well-separated from each other by distances of 250 Å or more. The removal of histone H1 does not occur in the presence of AG 50 W-X2 without prior NaCl treatment. It is slow at 50 mM NaCl and is fast and complete at 100–450 mM NaCl. However, salt treatment (up to 450 mM) without resin, with subsequent dialysis, leads to an appearance in the electron microscope which is almost indistinguishable from native chromatin, implying reconstitution of the chromatin fibres during dialysis. Treatments of the chromatin with further increasing NaCl concentrations leads to progressively fewer nucleosomes and to DNA filaments of variable thickness.

Electron microscopic and immunologic studies of the surface structure of bacteriophage λ head

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The bacteriophage λ head contains 2 major proteins arranged in a composite icosahedral surface lattice: gpE and gpD. The latter is not present in DNA-free head precursor particles (preheads), and can be added in vitro after DNA packing. In previous studies with tubular forms (polyheads) we had described 2 morphologically distinct types of capsomer. However, the assignment of each type to gpE or gpD was based on indirect evidence. We therefore examined in more detail the following questions: a) What is the orientation and shape of the single subunit in each type of capsomer? b) Which type of capsomer, the hexameric one or the trimeric one corresponds to gpE and which to gpD? To answer question b we labelled polyheads with anti gpE or anti gpD monovalent antibodies (Fab fragments). Our results indicate that gpD is most likely clustered in trimers and forms the capsomers protruding from the polyhead surface.

Growth pattern of human progenitor cells in agar

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Growth pattern of individual in-vitro-colony-forming cells (CFU-C) in agar culture has been determined by microscopic examination and cell counting of precisely localized colonies at 24 h intervals, following plating of human bone marrow: 1. Colonies grow from single progenitor cells rather than from initially aggregated cells. 2. Quantitation of CFU-C by scoring colonies on day 14 clearly underestimates the total number of colony-forming cells. Some colonies in these asynchronous cultures reach maximal size between day 6 and 13 and disappear thereafter. Some colonies fuse, some others become apparent at various times of cultivation. Estimates of CFU-C in the original population do thus only reflect colony-forming ability on a given day and are arbitrary: CFU concept is limited to an operational sense. It neither corresponds to a precise physiological state of differentiation of hemopoietic cells (heterogeneity of colony sizes and colony cell types) nor does it account for all in-vitro-colony-forming cells.

Dissociation of lytic and mitogenic action of SV 40 in permissive (monkey) cells

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Confluent monkey kidney cell cultures (arrested in G_1) were infected with temperature-sensitive mutants mapping in the early gene (ts A58 and ts A28, provided by P. Tegtmeyer). At 33 °C they induced a normal lytic infection: expression of the early viral gene (i.e. synthesis of early 19S mRNA and of T-antigen) is followed by an abortive mitosis (burst of overall cellular RNA synthesis, chromatin duplication but no prophase); this is paralleled by replication of viral DNA and expression of late viral functions leading to assembly of progeny virus and cell death. At 41 °C these mutants are unable to replicate their DNA and to express late viral functions. However, early 19S mRNA and T-antigen are synthesized, followed by cellular chromatin duplication (S-phase), prophase and complete or incomplete mitosis. This sequence is closely similar to that observed during abortive infection of nonpermissive cells with wild-type SV 40. Our results suggest that SV 40 T-antigen (or a derivative) acts as mitogen and that the mitogenic function is distinct from the function required for initiation of viral DNA replication.

Intramembranous particles at the presynaptic active zone in rat spinal cord

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The distribution of large (8.7–13.7 nm) and small (5.0–8.7 nm) particles in the presynaptic membrane was investigated in the spinal cord of unanesthetized and anesthetized rats by freeze etching electron microscopy. Both EF and PF faces were examined. The presynaptic region was subdivided into an active and a surrounding zone. The density of large particles was found to be significantly higher in the active as compared with the surrounding zone in both unanesthetized and anesthetized rats. Thus, the presence of large particles represents an important feature of the active zone. Considerably more large particles were found in the waking than in the barbiturized state. This difference is paralleled by a vast increase of vesicle attachment sites in the presynaptic membrane of unanesthetized animals. It is suggested that the large particles may represent calcium channels and thus provide the morphological substrate for the mechanism of excitation-secretion coupling.

In vitro cleavage of RSV polypeptide precursor

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The polypeptide precursor pr76 to the internal viral group specific (gs) antigen proteins of Rous sarcoma virus (RSV), synthesized in a cell-free system of ascites cells, has been processed in vitro into the internal viral proteins by the addition of one of the purified internal viral proteins, p15, to the cell-free system. The entire disrupted Rous sarcoma virus likewise stimulates the in vitro cleavage process, however the Rauscher murine leukemia virus did not. Autocatalytic cleavage of the RSV polypeptide precursor pr76, which contains the peptide sequence of p15, is not observed.

In vitro RNA-synthesis by nuclei isolated from Con A-stimulated lymphocytes

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Our interest has been focused on the change of RNA-metabolism during cell-differentiation because of its likely involvement in the control of cell growth. Detergent isolated nuclei are a suitable and clean system for in vitro studies. Lymphocytes from mouse spleen were stimulated with the T-cell mitogen Con A. Cell-nuclei were isolated with 0.25% NP-40 and the incorporation of ^{3}H-UTP into TCA-precipitable material has been determined. Since this material is rapidly degraded by RNase we conclude that it is RNA. The rate of synthesis is maximal 50 h after Con A-stimulation. In agreement with results from intact cells, the rate of RNA-synthesis is doubled in nuclei isolated from Con A-stimulated cells compared with nuclei from control cells. RNA-synthesis stops 10 min after incubation with ^{3}H-UTP at 25 °C. No degradation occurs for at least 3 h. It is concluded that in this system only elongation of RNA-chains is observed. However in the presence of ammonium-sulfate the RNA-synthesis continues for more than 2 h, indicating that initiation is taking place.

Age dependent differences of the stainable DNA content in somatic nuclei of honeybee determined by quantitative cytofluorometry

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Neuronal cell nuclei in G_0 phase from the corpora pedunculata of the honeybee (Apis mellifica) were stained with the fluorochrome BAO (bis-(4-amino-phenyl)-1, 3, 4-oxadiazol). The amount of BAO stainable DNA measured in 2 size classes of diploid neuronal nuclei as a function of the age of the adult workers was found not to be constant during the first 6 days of life. For the smaller nuclei the measurable DNA decreased between day 1 and 2, increased up to day 5 and decreased again between day 5 and 6. In the larger nuclei the DNA amount remained more or less constant. In males no difference in BAO DNA amount between the 2 size classes of nuclei was found. Both showed a strong decrease at day 1. These changes are correlated to changes of the amount of RNA isolated from whole brains (O. Kuhn, E. Kubli and E. Hauschteck-Jungen, Experientia *28*, 982 (1972)) and coincide with different age dependent activities of the bees.

Electron microscopic localization of cDNA hybridized to its RNA template

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cDNA synthesized on purified vitellogenin mRNA from Xenopus liver was hybridized to the template using a modified reaction mixture of 73% formamide, 3.6 M urea, 0.5 M NaCl, 30 mM Tris and 1 mM EDTA pH 8.5 at 22 °C to avoid degradation of the RNA. The hybrids formed under these conditions had the same T_m as those obtained in standard hybridization buffer. For electron microscopy the hybrids were spread in 80% formamide, 4 M urea, 30 mM Tris and 1 mM EDTA pH 8.5. Under these low salt conditions the majority of the hybrids were still resistant to S1 nuclease. Contour length measurements proved that most of the RNA molecules were still intact showing the expected mol. wt of 2.3×10^6. The length of the hybridized cDNA corresponded to an average mol. wt of 0.26×10^6. In about 75% of the hybrid molecules the cDNA was located at one end of the RNA. Since cDNA synthesis is dependent upon oligo(dT) as primer, we assume that the doublestranded segment corresponds to the 3′ end of the mRNA.

SEM observations on adult, fetal and cultured bovine esophagus epithelia

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The surface of the stratified epithelium of esophagus of adult cattle, of bovine fetuses, and of fetal bovine esophagus in organ culture has been examined by scanning electron microscopy. The plasmalemma of the esophagus cells of adults shows the characteristic winding folds already known as microplicae from squamous epithelia of other species. The cells of 6 fetuses (CRL: 20–40 cm) examined did not exhibit microplicae but short irregularly distributed microvilli. Esophagus from the same fetuses was maintained for several weeks in small petridishes

under conditions known to be suitable for trachea organ culture. Slight changes in surface structure become noticeable after 3 days and increase with time. After some days of culture the microvilli are grouped in small clusters on most cells. Later single microvilli grow to very long protrusions. In some cells the microvilli sit on large microplicae, on others short microvilli are arranged in lines forming microplicae-like structures.

Binding of ATP analogues to intact myosin and isolated myosin heads

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The contractile protein myosin consisting in part of a double chain α-helical rod bears at one end 2 globular head portions each with an active site. Reports of studies with pyrophosphate and ADP indicate that between 1 and 2 moles of ligand bind per myosin. We measured the binding of both ligands as well as the ATP analogue, adenylylimidodiphosphate, which cannot be split by the enzyme, under various conditions by equilibrium dialysis, high speed centrifugation and thiol group reactivity changes. Myosin was consistently found to bind 2 moles ligand per molecule. However, the binding occurred invariably with 2 apparent binding constants which differ by a factor ranging from 50 to about 5000. On the other hand, one mole of ADP was found to bind to isolated myosin heads with only one apparent binding constant (K = about 8×10^6 M^{-1} at 25 °C) corresponding to the higher K value found with myosin. The absence of a second binding constant in isolated heads suggests that the two-step binding process found with myosin arises from negative cooperativity between the 2 heads of the intact molecule.

DNA-binding proteins in the unfertilized egg of Drosophila melanogaster

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In prokaryotic systems, proteins regulating gene activity bind to specific DNA sequences. We have attempted to isolate such proteins from unfertilized Drosophila eggs. Several proteins which seem to bind specifically to Drosophila DNA have been purified using DNA-cellulose-chromatography, Sephadex-gel filtration, SDS-polyacrylamidegel-electrophoresis and isoelectric focussing. These proteins have mol. wts between 10,000 and 70,000 d and isoelectric points between pI 4.8 and 7.4. In a filter binding assay these proteins bind less than 1% of total ^{3}H-labeled Drosophila-DNA but the binding is not competed by an excess of Salmon-DNA. When Drosophila DNA is digested with the restriction enzyme RI and fractionated on Agarose gels, these proteins bind to 1 or 2 successive fractions of restriction fragments. This suggests that these proteins recognize specific DNA-sequences but final proof of sequence specificity can only come from pure DNA-sequences. Thus we have inserted the above restriction fragments into the plasmids pSC101 and pSF2124. The filter binding assay is used for the selection of hybrid plasmid containing the specific sequences.

Motility of oocyte nuclei and follicles in a Dipteran insect

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Time-lapse films of in vitro cultured ovaries of the paedogenetic gall midge Heteropeza pygmaea (Dipt.) reveal 2 kinetic phenomena: 1. pulsation of oocyte nuclei and 2. rotation of follicles (D. F. Went, Dev. Biol. *55* (1977)). The oocyte nuclei are continuously deformed by a pulsating movement which lasts until follicles have been formed and are released by the ovary. 1 pulsation to and fro takes 5–7 min. A sheath of microfilaments associated with microtubules covers large areas of the nuclear envelope. Following their formation, the follicles start to rotate within the ovary. 1 revolution takes between 3 h and 7 h. The direction of rotation inside of the ovary is at random. However, after release from the ovary into the culture medium, the follicles continue to rotate around the longitudinal egg axis. The only conspicuous surface differentiations of the follicle cells revealed by electron microscopy are microvilli.

Restriction site mapping of a poxvirus DNA

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Rabbitpox virus (strain Utrecht) DNA was isolated from purified virions and cleaved with selected restriction endonucleases. HindIII produced 15 fragments and SstI at least 10 fragments which could be resolved by agarose gel electrophoresis. By summation of the mol. wts of the fragments the size of the rabbitpox virus genome was found to be approx. 117×10^6 daltons. Restriction sites were mapped using 3 independent approaches. a) Individual fragments from HindIII and SstI digests were recovered from the gels, digested with the other enzyme and compared to a combined digestion of the entire DNA with both enzymes. b) Partial digestion products were isolated, completely cleaved and the resulting fragments identified. c) Isolated restriction fragments were labelled in vitro with ^{32}P-dATP and hybridized to filter-bound fragments produced by the other enzyme. By combining the data obtained from these experiments the tentative arrangement of the fragments produced by HindIII and SstI was determined.

Determination of K^+ and Na^+ activities in salivary gland nuclei of Chironomus tentans by ion selective microelectrodes

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Different ionic milieus influence differentially the condensation of explanted salivary gland chromosomes. In addition it has been shown that the total ionic content of salivary gland nuclei changes during larval development. Since we expect the free ion content to be less than the total content, but only the former to be responsible for the observed effect on chromosome condensation, we decided to monitor the activities of K^+ and Na^+ during larval development. Double barreled microelectrodes (outer ⌀ 1 µm) for K or Na based on neutral carriers are used. Electrode slope and selectivity factors are determined by a standard addition method ($K^{Pot}_{KNa} = 0.001$;

$K^{Pot}_{NaK} = 0.1$). Salivary glands of Chironomus tentans are explanted in an insect tissue culture medium. Electrodes are implanted by micromanipulation under optical control. Signals of ion activity and cell membrane potential are measured simultaneously by a specially designed high impedance differential amplifier and monitored on a chart recorder. Initial findings for the 4th larval stage were: $a_K = 60 \pm 6$ mM and $a_{Na} = 20$ mM.

Guanosine and deoxyguanosine toxicity for a Drosophila cell line

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A new Drosophila tissue culture medium, ZH1%, has been developed in which purine auxotrophic cells are unable to proliferate. A purine prototrophic clonal Drosophila cell line, KcAlo, has been tested in medium ZH1% for its growth response to varying concentrations of different purines. The concentrations producing a growth inhibition of 50% (or more) were: 10^{-3} M adenine (A); 10^{-3} M adenosine (AR); 10^{-4} M deoxyadenosine; higher than solubility guanine; 10^{-5} M guanosine (GR); 10^{-5} M deoxyguanosine (GdR); higher than 10^{-5} M hypoxanthine; higher than 10^{-3} M inosine (HR); 10^{-3} M deoxyinosine; 10^{-3} M purine; higher than 10^{-3} M xanthine. The toxicity of GR could be counteracted by either A, AR or HR; none of the other purines nor any of the natural pyrimidines (bases, ribosides or deoxyribosides) could reduce the effect of GR. The toxicity of GdR, on the other hand, was unchanged by any of the purines listed above, but completely reversed by deoxycytidine. Of all other pyrimidines only deoxyuridine and thymidine partially counteracted GdR.

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