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PHYSIOLOGIE - PHYSIOLOGY

Bursting activity and synaptic inhibition in *Aplysia* neuron R15 are mediated by the same ion conductances

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Aplysia neuron R15 is a bursting neuron; it fires bursts of action potentials interspersed with silent periods. This rhythmic activity is generated by a set of ion channels for Na^+, K^+ and Ca^{++}. During the silent period, or interburst, K^+ conductance is high and Na^+ and Ca^{++} conductances are low; during the burst the situation is reversed. Stimulation of the branchial nerve activates an inhibitory synapse that increases K^+ conductance and decreases Na^+ and/or Ca^{++} conductance, mimicking the behavior of the cell during interburst. Moreover, stimulation of the synapse during interburst, when K^+ conductance is already high and Na^+ and Ca^{++} conductances are already low, produces a smaller response than stimulation during the burst. These results indicate that bursting and synaptic inhibition are mediated by the same ionic channels.

Identification of the Edinger-Westphal nucleus in the primate

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The Edinger-Westphal nucleus is defined as the preganglionic parasympathetic group of neurons innervating the ciliary ganglion. Its exact location in the midbrain is still controversial; it was ascertained in 4 monkeys *(Macaca fascicularis)* by injecting retrograde tracer substances into the ciliary ganglion. Horseradish peroxidase and ^{125}I-labelled wheat germ agglutinine (Schwab et al., Brain Res. *152*, 145, 1978) were used. The labelled cells were identified in serial brain sections and mapped by means of an X-Y plotter. They are of medium size and located in a relatively small and elongated cell column bilaterally near the midline at the rostral level of the oculomotor nuclear complex. The so-called anteromedian cell column was not involved.

The dark current, photocurrent and regeneration of rhodopsin in retinal rods

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When 8% of the rhodopsin in an isolated rat retina was bleached, the rod dark current was shut off and remained so in the dark for 10 min. It was then gradually turned on again, but now the photocurrent had a faster kinetics and a light sensitivity 100-fold less than before the bleach. After a full bleach, the dark current remained shut off for over 3 h. Addition of liposome bound 11-cis retinaldehyde to retinas similarly bleached, restored the rhodopsin, the dark current, as well as the kinetics and light sensitivity of the photocurrent. The addition of all-trans retinaldehyde had no effect. The time that the dark current is shut off by light lengthens as more rhodopsin is bleached. For a given bleach, the time can be greatly shortened by regenerating the rhodopsin with 11-cis retinaldehyde. These results suggest that the light released transmitter which closes the dark current, is controlled in part by the formation and removal of opsin. The effect of opsin on the dark current may be removed by its conversion to rhodopsin or to another inactived form.

Neural mechanisms underlying human physiological tremor

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Physiological tremor could be generated by descending signals impinging on alpha motoneurons. These signals, on arrival at the contracting muscle, produce partially fused twitch contractions, i.e. force oscillations. Force oscillations could also be generated by instabilities in either segmental or supraspinal reflex arcs. In the current experiments the contribution of these mechanisms to tremor was investigated. Motor unit activity and force oscillations of tremor were examined during isometric contractions of the first interosseus muscle. The contribution of twitch contractions to tremor was determined by examining their respective spectral components. The results indicate that units firing near recruitment frequencies contribute with greater significance to high frequency components (above 15 Hz) of tremor than units with firing rates above 12/sec. The contribution of reflex arcs to tremor was determined by examining the cross correlations between pairs of motor units. Broad peaks in the cross correlation replicated the shape of the twitch contraction. The peaks' latency was used to separate segmental and supraspinal effects. The peaks' height indicated that a twitch contraction causes, via reflex arcs, maximally a 1% change in the firing rate of an adjacent motor unit.

Study of the ATP-dependent Na^+ and K^+ transport in vesicles reconstituted with kidney Na^+, K^+-ATPase

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Addition of ATP to liposomes reconstituted with Na^+, K^+-ATPase from kidney outer medulla induces active Na^+, K^+-antiport across the vesicular membrane (Anner, Lane, Schwartz and Pitts, Biochim. biophys. Acta *467*, 340, 1977). The coupling ratio of $Na^+ : K^+$ transport is around 2. For better understanding of the molecular mechanism of the ATP-dependent Na^+, K^+-antiport it is important to know if the $Na^+ : K^+$ coupling ratio can be altered. Exposure of the Na^+, K^+-ATPase tetramer to graded trypsinolysis before reconstitution causes a selective Na^+-transport defect which is related to a specific catalytic defect of the unreconstituted enzyme (Anner and Jørgensen, in preparation). A similar decrease of the Na^+-transport capacity appears when the pump molecule is modified by treatment with Na^+, K^+-ATPase inhibitors. Taken together, the results demonstrate that a fraction of the ATP-dependent Na^+-transport is not obligatorily coupled to K^+-transport.

A vascular link between neurohypophysis and anterior pituitary in the rat: Physiological evidence

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Electrical stimulation of the rat neural lobe promotes corticotropin release in vivo. Therefore, we studied the possible role of the short portal vessels in anterior lobe–posterior lobe communication. When 5 sec long trains of impulses (30 Hz) were applied to the pituitary tract, extracellular potassium concentration (K), recorded in the neural lobe with double-barrel microelectrodes, rose within 50 msec from 2.6 ± 0.4 (SEM) mM to 7.6 ± 0.8 mM. In the anterior adenohypophysis (AA), K rose with a delay of

1-4 sec from 3.0±0.3 mM to 5.0±0.4 mM (n=6). K responses in AA also occurred following i.v. nicotine, but not to CRF or vasopressin. Lesion of long portal vessels did not abolish the K response in AA, and dye injected through a vertebral route still reached AA. Results strongly suggest that the potassium travelled through short portal vessels from neurohypophysis to anterior pituitary, and that neural lobe peptides may follow the same path.

Action of strychnine on a postsynaptic inhibition in tench spinal cord

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In tench, the excitatory and inhibitory effects of Mauthner axon excitation on spinal motoneurones were investigated before and after i.m. injection of strychnine. In order to extend the observation time over a number of hours without interfering with the blood circulation of the spinal cord no laminectomy was performed. Electrotonic potentials were recorded unipolarly from the spinal nerves near the vertebral canal to determine the arrival time of the ipsi- and contralateral Mauthner axon impulses, the onset and time course of the ipsilateral monosynaptic excitation and the onset and time course of the contralateral disynaptic inhibition of the motoneurones. The results show that the crossed inhibition is associated with a postsynaptic conductance increase at the sites where the ipsilateral monosynaptic excitation of the motoneurones occurs, and that strychnine progressively reduces the inhibitory conductance increase in a dose dependent manner without affecting the excitatory and inhibitory synaptic delays.

Relationship between perfusion flow rates and hormonal secretion in perfused isolated rat pancreas

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We perfused an isolated rat pancreas with a throughflow of KRBB. PO_2, PCO_2, pH were monitored continuously. Samples for both glucagon and insulin determination were taken at 3-min intervals. The oxygen consumption was measured at different perfusion flow rates (1.5 ml/min to 6.5 ml/min). The oxygen consumption reached a plateau near 4.5-5 ml/min. Hormonal secretions were measured at: a) low flow ('hypoxic flow'): 2.5 ml/min; b) normal flow ('normoxic flow'): 5.5 ml/min. Both insulin and glucagon secretion rates were flow-dependent; the secretion rate was higher at the highest flow rate. It was interesting to note that the secretion of insulin high enough to maintain rat normal insulin pool was only at 'normoxic flow'. This was calculated with an inactivation constant around about 5 min.

Thyroid regulation in Gunn and Wistar rats at different ages

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Gunn rats are characterized by UDP-glucuronyl transferase insufficiency. In 6- to 30-week-old rats, this anomaly (measured by bilirubin plasma levels) was least intense at 6 weeks and at its highest at 15 weeks ($\bar{X}$±SEM: 10.2±0.5 mg%). Plasma T4 evolved in the same way and as expected, was always significantly higher in Gunn compared to Wistar rats. At 6 weeks, plasma T3 and rT3 were highest, but similar, in both strains; at 15 weeks however, rT3 remained abnormally high in Gunn rats (0.09±0.01 ng/ml) suggesting an increased 5- and a lower 5'-desiodase activity. Despite normal T3 and high total and free T4 levels, plasma TSH was increased in 6-week-old Gunn rats (0.32±0.04 vs 0.11±0.01 µg/ml); at 15 weeks, in spite of maximum values for T4 (9.1±0.5 µg%), a decrease of 50% and not total suppression, as expected, was observed; after which, TSH regularly increased up to the 30th week. These results indicate that the pituitary set point for the feedback mechanism is modified in Gunn rats.

Evaluation of corticotropin releasing factors (CRF) secreted by the rat neurohypophysis in vitro

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The structure of the corticotropin releasing factor (CRF) is unknown, but several studies suggest that CRF *extracted* from posterior hypophysial lobes (PL) is distinct from vasopressin. In order to evaluate the CRF released from posterior lobes in vitro, the CRF activity of posterior lobe incubation media was bioassayed using dispersed adenohypophyseal and adrenal cells. Following electrical stimulation of PL at 30 Hz for 15 min (5 sec on, 5 sec off), CRF release increased 146±26(SEM)% (n=8) with respect to control. The CRF activity was completely abolished by treatment with thioglycolate. The vasopressin release upon PL stimulation was estimated to be 7±1 (SEM) mU (n=8). This amount had the same CRF activity as the CRF released from stimulated PL. Results suggest that CRF *released* from posterior lobes is predominantly vasopressin.

Hypothalamus-brainstem connections: Possible role in the CNS modulation of insulin secretion

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Lateral hypothalamic stimulation will elicit an elevation in blood insulin levels as will the initial ingestion of foodstuffs. The CNS organization underlying neurally-mediated increased insulin secretion is not known. Electrophysiological experiments were performed to investigate the neuronal relations between hypothalamic areas known to influence insulin secretion and brainstem areas known to receive sensory input (gustatory) relevant to 'cephalic phase' insulin secretion. Microelectrode recordings were made from secondary gustatory neurons in caudal brainstem (n. tractus solitarius) of male rats under barbituate anesthesia. The results reveal a population of cells responding to both gustatory (chorda tympani nerve) and lateral hypothalamic (LH) electrical stimulation. Both orthodromic and antidromic responses to LH stimulation were encountered. These results suggest a reciprocal relationship between brainstem areas receiving gustatory input and the lateral hypothalamus, and further suggest that the integration of signals facilitatory to insulin secretion may be organized at the brainstem level.

Thyroid hormones and the energetics of active Na-K-transport in mouse skeletal muscle

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In intact soleus muscles from 3 groups of adult mice (hypothyroid, control and hyperthyroid), the reversible work ($\dot{W}$) effected by the active Na-K-transport process

was estimated from in vitro measurements of ouabain-suppressible ^{42}K-influx, intracellular Na-K concentrations and resting membrane potential. Conditions were: 30 °C, bicarbonate-buffered Krebs-Ringer medium enriched with 10 mM Mg and 5 mM glucose. From $\dot{W}$ and the ouabain-suppressible component of basal metabolic rate ($\Delta\dot{E}$) measured under the same conditions in a perfused microcalorimeter, estimates of the maximum energy expenditure ($\Delta\dot{E}+\dot{W}$) and the minimum overall energetic efficiency ($\dot{W}/[\Delta\dot{E}+\dot{W}]$) of active Na-K transport were obtained. Expressed as percent of basal heat production rate, $\Delta\dot{E}+\dot{W}$ was less than 12% in all 3 thyroid states and contributed to thyroid thermogenesis by less than 15%. $\dot{W}/(\Delta\dot{E}+\dot{W})$ was more than 30%.

Recovery function of slow wave sleep: Studies in the rat

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In man, slow wave sleep (SWS = non-REM sleep stages 3 and 4) predominates in the first part of the sleep period, and is enhanced after sleep deprivation (SD). We investigated whether SWS shows a similar pattern in the rat. During the control days, non-REM sleep with a low-frequency EEG (SWS) was high at the beginning of the light phase and then declined progressively, while non-REM sleep with a high-frequency EEG showed an opposite trend. After a 24-h SD by a slowly rotating cylinder, SWS was markedly enhanced even though the total amount of non-REM sleep changed little. After a 12-h SD in the dark phase, SWS showed only a moderate increase which was insensitive to doubling the rotating rate of the cylinder. REM-sleep was little affected by the 12-h SD, but showed a marked rebound after a 24-h SD. The results support the notion that SWS subserves a specific recovery function.

Respiratory, circulatory and ECG changes at 6000 m with and without propranolol

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The physiological changes during standardized exposure to high altitude have been described in a previous paper and were explained as being due to various factors (J. appl. Physiol. *41*, 159, 1976). In order to analyze the role of the sympatho-adrenal system, stepwise ascent to 6000 m was performed in 20 healthy male volunteers, once with betareceptor-blockade (4 × 40 mg propranolol during 14 h before ascent) and once without. - In both groups the results show identical respiratory gases and parallel circulatory changes, although f_{card} and p_{syst} were lower in the propranolol-group. The ECG changes at high altitude (mainly relative lenghtening of Q-T interval and ST-T segment flattening) were clearly reduced following betareceptor-blockade. - The hypoxia-induced ECG changes are therefore mainly due to the activity of the sympatho-adrenal system.

Hypoxanthine and lactate, indicators of tissue-hypoxia?

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Hypoxanthine, a product of anaerobic ATP-catabolism, is thought to be a new indicator of tissue-hypoxia. In 9 volunteers hypoxanthine and lactate were measured during stepwise ascent to 6000 m (350 mm Hg) in a low-pressure chamber. In order to intensify the tissue-hypoxia, leg-exercise was performed during 4 min at the beginning of ascent and at 6000 m. The stay at 6000 m lasted 30 min. The lowest PA_{O2} was 32.2 ± 7.8 mm Hg. To block the normal degradation of hypoxanthine to urate, 4 of the subjects were treated with allopurinol during 4 days before the experiment. The results show that venous lactate - unchanged during the mild work-load at ground level - always increased at 6000 m after exercise, whereas hypoxanthine-changes occurred irregulary. It is concluded that hypoxanthine appears to be unsuitable as indicator of hypoxia in man.

Rapid channel gating develops after onset of neuromuscular transmission

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In ectopic neuromuscular junctions formed de novo in extrasynaptic membrane the gating of synaptic channels becomes fast only after the synapse is functioning. Therefore, the gating behaviour of endplate channels in neonatal rat skeletal muscle was examined. The mean open time τ of synaptic channels was estimated from the time course of miniature endplate currents and from statistical analysis of endplate current fluctuations induced by acetylcholine (ACh). In soleus muscle, τ was around 4 msec (−70 mV, 20-23 °C) up to the 8th postnatal day - similar to the τ-value found in extrasynaptic ACh receptors. Between days 8 and 17, 2 populations of synaptic channels with τ-values of 4 msec and 1 msec could be distinguished, the latter corresponding to the open times of channels in adult endplates. After day 17, only one population of channels with fast gating was resolved. These results indicate that transmission is functioning before a change to rapid channel gating has occurred.

Discrimination of polarized light by the pigeon

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Polarized light is assumed to play a role in the homing of the pigeon. In an attempt to use behaviorally relevant visual stimuli for the experimental analysis of visual behavior in a skinner box, 5 pigeons were trained to discriminate 2 orthogonal planes of polarized light. First the animals had to reach a stable discrimination performance (75% correct on 3 successive days). This required between 574 and 4787 trials. Then, discrimination behavior of birds which performed > 80% correct in a training session was tested in at least 3 nonrewarded 40-trial sessions where stimulus pairs with orthogonal and parallel polarization axis were presented. On the orthogonal discrimination each individual performed above chance (85, 71, 76, 76, 81%). On the parallel task performance was on chance level (50, 51, 42, 48, 47%). Data show that pigeons using the frontal visual field learned to discriminate 2 axis of polarized light. This questions earlier interpretations of similar experiments where only the lateral and upper visual field was considered to be concerned with the analysis of polarized light.

Efferent connections of the rostral interstitial nucleus of the MLF to the ocolumotor complex of the monkey

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It is well-established that parts of the mesencephalon are essential for the generation of vertical eye movements, but

it is only recently that the rostral interstitial nucleus of the medial longitudinal fasciculus (rostral ni) has been identified as a specific subdivision of this system (J.A. Büttner-Ennever and U. Büttner, Brain Res. *151,* 31, 1978). The nucleus lies rostral to the interstitial nucleus of Cajal. The efferents of rostral ni have now been studied using anterograde tracers. Projections to the oculomotor and trochlear nuclei are mainly ipsilateral. There are also labelled terminals in other areas of the brain stem involved in the control of eye movements, such as the paramedian pontine reticular formation (PPRF) and nucleus praepositus. Most of these descending pathways travel in the MLF. These findings confirm the hypothesis that rostral ni is part of the immediate premotor system controlling vertical eye movements.

Energy cost of ventilation in running dogs

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The energy consumption ($\dot{V}_{O_2}$ tot) and the fraction of it required to sustain pulmonary ventilation ($\dot{V}_{O_2}$ vent) and locomotion ($\dot{V}_{O_2}$ loc) were determined on 11 mongrel dogs (11-20 kg) during aerobic treadmill runs at speeds up to 16 km · h^{-1} and inclines 0-15%. $\dot{V}_{O_2}$ vent was determined as the product of observed $\dot{V}_E$(l · min^{-1}) times the ratio $\Delta\dot{V}_{O_2}/\Delta\dot{V}_E$(ml · l^{-1}). $\Delta\dot{V}_{O_2}/\Delta\dot{V}_E$ ratios were obtained for each animal at given external work loads that were accompanied by $\dot{V}_E$ changes occurring either spontaneously or induced by prolonged low caloric diet or by sino-aortic denervation. In the $\dot{V}_E$ range between 10 and 80 l · min^{-1} $\Delta\dot{V}_{O_2}/\Delta\dot{V}_E$ averaged 15 ml of oxygen per l ventilation. During exercise $\dot{V}_{O_2}$ vent varied between 0.35 and 0.65 of $\dot{V}_{O_2}$ tot for work loads below 25% and above 75% of $\dot{V}_{O_2}$ max, respectively. It is concluded that in the dog the efficiency of the ventilatory pump is very low, particularly at high work loads. As a consequence, the efficiency of locomotion (E_{loc}) increases with increasing speeds.

Superficial fraction of membrane phosphate in nerve

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Rabbit vagus nerves were loaded with radiophosphate and the efflux measured as described elsewhere (Ferrero et al., J. Physiol., Lond. *282,* 507, 1978). When, after incubation in Locke, K-free Locke was applied, there was a large, transient increase in the release of inorganic phosphate. Return to Locke lead to a transient decrease in phosphate efflux; the effect of the K-free solution could then be reproduced. When the same experiment was done in Na-free solutions, removal of K also lead to a transient increase in efflux; addition of K, however, did no longer lower the efflux and the K-free effect could not be repeated. Measurements of the ATP content of the nerves showed no significant change in ATP during incubation in K-free Locke for up to 2 h. The results suggest that removal of K causes a release of inorganic phosphate from a superficial compartment that is filled with phosphate from the inside by a Na-dependent process.

Differential retrograde labeling of neural pathways according to their transmitter specificity

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Radioactive transmitter injected in a brain area might label retrogradely and preferentially the projections using this transmitter by specific terminal uptake followed by intraaxonal migration. This hypothesis was tested in pathways in which a GABA-, glycine (Gly)-, serotonin (5HT)-, resp. dopamine (DA)-mediated transmission is established. 6 h after injection into terminal area perikaryal labeling was observed: a) in the rat caudoputamen (CPU) after [^{3}H]-GABA injection into substantia nigra (SN); b) in the n. Raphé dorsalis (RD) after [^{3}H]-5HT injection into SN; c) in SN compacta (A9), A10 (rostral) and A8 (all heavy), and in RD (light) after [^{3}H]-DA injection into CPU; d) same pattern as in c, but heavy in RD after [^{3}H]-5HT injection into CPU; e) in RD and some in the thalamus after [^{3}H]-5HT injection into the rat cortex; f) in the glycinergic pigeon isthmo-tectal neurons after tectal [^{3}H]-Gly injection. Thus by injection of [^{3}H]-transmitter, connectivity and transmitter specificity could be established.

Interaction of stretch (SR) and irritant receptors (IR) in determination of duration of expiration (t_E) and subsequent inspiration (t_I)

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Certain reduction in t_E is necessary before t_I can be influenced by factors accelerating breathing (A. Davies and E. Kohl, J. Physiol. *284,* 59P, 1978). Karczewski (Inserm, Amiens 1976) presented evidence for a memory which carries the effect of lung receptors activity over into successive breaths. Our results illustrate the interaction of these 2 effects. 100-msec inflations and deflations were applied during expiration to lungs of anaesthetized rabbits. SR were intact or blocked by SO_2. Inflations changed $t_E$without equally large changes in t_I. With SR blocked deflations had the same effect. But with SR intact they shortened the following t_I if t_E was reduced to 50% or more, and prolonged the t_I if the shortening of t_E was smaller. We suggest that the reduction of t_I is due to strong stimulation of IR which facilitates central linking of t_I to t_E. Prolonged t_I results from the 'memory' of reduced SR activity when IR were not stimulated enough to cause a large decrease in t_E.

Latency variability of action potentials in bulbar respiratory modulated (RMN) and nonmodulated (NRMN) neurons after stimulation of vagal nerv (VNS) and spinal cord (SCS)

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VNS and SCS elicited antidromic or orthodromic activation in part of the RMN and NRMN. VNS pulses of 50 or 500 µsec duration applied to discriminate lung afferents revealed no essential differences in neuronal response, 500 µsec pulses being more effective. Differences were found in the proportion of descending spinal axons between RMN (12%) and NRMN (67%) and in the conductance velocities being lower in RMN for VNS and SCS. In RMN threshold for antidromic invasion and latency variability changes, being lowest during and highest immediately after neuronal burst discharge. In NRMN the latency variabilities were comparable to that of the RMN but without cyclic changes.

For VNS and SCS latency variabilities are unevenly distributed along the time axis but positively related to averaged latencies. The resulting cluster formation reveals a further possibility to discriminate antidromic and orthodromic activation.

Induction of enzymatic activities in ganglia infected with a neurotropic virus

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As shown previously pseudorabies virus inoculated in the anterior chamber of one eye in the rat is transported by retrograde axonal flow to the superior sympathetic ganglion on the side of the inoculation. There, the virus gives rise to an abnormal spontaneous electrophysiological activity that has been recorded on the postganglionic as well as on the preganglionic nerves. At the time when this activity had reached its maximum we have measured the total amount of proteins and the activity of 3 enzymes involved in the ganglionic function. The activity of the tyrosinehydroxylase (EC 1.14.16.2) was increased by 16% but the cholinesterase (EC 3.1.1.7) did not show any change in its activity. Both are neuronal enzymes. The cholineacetyltransferase (EC 2.3.1.6), which is a presynaptic marker is increased by 25% and acetylcholine is produced and released in a greater amount. The origin of this enzymatic induction, neuronal or viral, will be discussed.

Direct thermogenic response of a mammalian skeletal muscle to noradrenaline (NA) in various thyroid states

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Several workers reported a 50–100% stimulation by NA of O_2 uptake in perfused skeletal muscle at rest. Experiments at high perfusion flow-rates and O_2 partial pressures suggested that most of this effect could be due to perfusate redistribution within the preparation, as a consequence of the vascular action of NA, rather than to the direct stimulation of metabolism in normally oxygenated muscle fibres. Microcalorimetric measurements on perfused soleus muscles from mice, in oxygenated bicarbonate-buffered Krebs-Ringer medium containing 5 mM glucose, at 30 °C, showed a maximum short-term effect of NA (10^{-6} M) much smaller than that reported for perfusion experiments. Expressed as percent of basal heat production rate, it was less than 5% in intact muscles from euthyroid (control), hypothyroid (perchlorate treated) and hyperthyroid (T_3 treated) animals. An *indirect* response of skeletal muscle to NA in nonshivering thermogenesis in vivo is by no means excluded.

Retrograde labelling of the suprachiasmatic nucleus (SCN) from the substantia grisea centralis (SGC)

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Injections of horseradish peroxidase (HRP) in the ventral part of the SGC of the woodmouse produce a retrograde labelling of many hypothalamic areas. Both SCN are namely labelled when the median portion of the SGC has been injected. Unilateral injections lead to ipsilateral SCN labelling except when the HRP diffused across the midsagittal plane. The labelling is especially pronounced in the mediodorsal part of the SCN. In the case of intensely labelled SCN some extremely thin axons are found leaving the nucleus in laterodorsal direction. The electron microscopy shows the HRP reaction product to be located inside the SCN cells. Retrograde SCN labelling has been obtained also using Evans blue. These results confirm our previous findings by the Nauta technique that the SCN has an efferent mesencephalic projection (Favrod and Kucera, Experientia *33*, 779, 1977).

Physiology of tight epithelia: induction of reversible leaky states in frog skin

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Transporting epithelia have been classified as tight or leaky, depending on the resistance of their paracellular shunt pathway. We studied a tight epithelium, the frog skin, by following an automatic plot of the total epithelial conductance (K) versus short circuit current. Theophylline induced striking increases in K, up to 17-fold. This effect was additive to that of 'external' hypertonicity and nonabolished by amiloride or ouabain. In contrast, washing of the skin with normal Ringer solutions brought K down to its initial values. Reversibility could be obtained in a stepwise fashion if the skin had been exposed to both theophylline and 'external' hypertonicity. The induction of reversible leaky states in a tight epithelium indicates that tight junctions are remarkably dynamic structures. Our results suggest in addition that theophylline affects the tight junctions of frog skin.

Kainic acid and glutamate in the pigeon optic tectum

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The effect of kainic acid, a structural analogue of monosodium glutamate, was compared with this excitatory amino acid on neurones in the pigeon optic tectum using microiontophoretic techniques. 2 groups of responses could be observed. 1. Neurones which were not excited by kainic acid itself, but showing strong potentiation when applied simultaneously with glutamate. 2. Neurones which were activated by both substances. On the majority of tested cells, kainic acid was less potent and had lower onset of firing than glutamate. In this group no potentiation could be observed. Using glutamate antagonists the effect of kainic acid was less or not antagonized by nuciferine or GDEE. The present results support the idea of different receptor sites for kainic acid and glutamate on tectal neurones.

Effect of retinal ablation on the pool of some amino acids in different layers of the pigeon tectum

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The content per dry weight of alanine, GABA, aspartate, glutamate, glutamine and glycine was measured by dansylchloride/TLC technique. The tissue samples were microdissected from freeze-dried sections obtained from control and degenerated tecta of unilaterally retina ablated pigeons 1, 2 and 4 weeks after operation. The data were pooled for layers 1 and 2, 3–5, 6 and 7, 8–10 and > 10. The reductions ($p < 0.05$) in percent 2/4 weeks after ablation (layers) were: glutamate 36/75 (1 and 2), 23/51 (3–5), 0/30 (6 and 7), aspartate 40/46 (1 and 2), 24/26 (3–5) and GABA 0/20 (3–5), 0/14 (8–10). In all other cases no significant changes were found. The results may indicate a special function for glutamate and possibly aspartate in the pigeon optic nerve.

Development of exploratory behavior in RHA- and RLA-rats

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Exploratory behavior in a maze changes with age. We investigated the development of this behavior with rats 16, 18, 20, 22, 24 and 26 days old. The 2 lines of rats used in this study (RHA/Verh and RLA/Verh) are selected for high resp. low avoidance scores in the suttle box and also show, among other traits, differences in activity. For each line and age group, 10 animals were put 6 times for 6 min, with an interval of 45 min, into a hexagonal maze with automatic registration. For each successive run, blind alleys were changed to inside or outside, and the whole configuration was rotated. Activity increased 3-fold from 16 to 26 days. From the first to the 6th run the activity decreased, this becoming more distinct in older groups. At the ages of 16, 20 and 24 days RHA-rats were significantly more active (Wilcoxon test) than RLA. With increasing age the activity-decline in the course of the run increased, with a stronger increase in the RHA-rats. These data can be interpreted as a smaller increase of habituation with age in RLA- than in RHA-rats.

Morphological and electrophysiological characteristics of cultured hypothalamic neurons

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Horseradish peroxidase was injected into large neurons of the rat hypothalamic supraoptic nucleus area kept in culture for 3–10 weeks. 2–3 dendrites extending up to 800 µm from the cell body and showing minimal branching were observed. Spinous processes a few microns in length were seen on dendrites and cell bodies. Repeatedly branching axons often emerged from a proximal dendrite and could be followed for several millimeters. A proportion of these neurons spontaneously displayed a phasic discharge pattern. The phasic activity of the majority of cells was synaptic in origin. A minority of neurons fulfilled the criteria for establishing that they were true pacemaker cells. Intracellular depolarizing current injection never induced phasic activity, only evoked single spikes. Bursts of a duration similar to that of spontaneous ones, however, could be elicited by field stimulation in the surrounding tissue.

The delta-sleep inducing peptide (DSIP): hypnogenic effects of i.v. injection in rats

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The synthetic delta-sleep inducing nonapeptide (DSIP) develops a hypnogenic effect in rabbits and cats after intraventricular or i.v. injection[1-4]; there remained to test its effects in rat. In EEG tests combined with electro-oculography (EOG) and electromyography (EMG), the i.v. injection of DSIP (30–40 and 80 nmoles/kg) in free-moving rats induced a significant increase in total sleep duration, with complementary decrease in waking state duration. The effect starts 30 min after injection and increases during the whole experimental time (4.5–5 h). The total sleep consisted predominantly of synchronized delta EEG sleep: this fact, together with a facilitating action on a latent ultradian rhythm suggests that DSIP might act in rabbits and rats as a hypnogenic EEG synchronizing modulator.

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2 G.A. Schoenenberger and M. Monnier, Proc. nat. Acad. Sci., Washington *74*, 1282 (1977).
3 M. Monnier et al., Neurosci. Lett. *6*, 9 (1977).
4 Polc et al., Neurosci. Lett. *9*, 33 (1978).

Effects of isolation rearing upon openfield-behavior in the rat

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Male rats reared for different periods in isolation are compared in their openfield-behavior with group-housed animals. 2 repetitions of the trial at intervals of 135 min are followed by one in a slightly modified openfield (3 different objects in it). Among other behavioral changes, the well-known elevated locomotion could be seen after 3, 5, 7, 9 and 12 weeks of isolation. As there was no significant difference in the number of rearings there seems to be an 'uncoupling' between horizontal and vertical exploration in the isolated rat. Repetitions of the trial revealed the same percentual diminution of activities in both groups. Whereas social rats responded to the slightly modified openfield with a fully restored orientation-reaction, isolated rats showed reduced locomotor- and rearing-activities compared with their first trial, a behavior that resembles the reaction of young group-housed rats on the modified openfield. It is supposed that isolated rats are comparable in their openfield-behavior to young rats.

Microelectrophoresis and microinjection

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Combination of microelectrophoresis and pressure injection from the same barrel of a micropipette into the immediate environment of single neurones proved to be a useful method for application of substances whose release by current is unpredictable and variable. We tested noradrenaline and diazepines on the rat cortex. Nonionized substances may also be readily ejected. The concentration and the pH of the solutions in the micropipettes can be kept at physiological values. A negative retaining pressure is sometimes necessary in these cases. Furthermore, we have compared dose-response curves constructed from responses to equally spaced applications with current and pressure and determined the maximal tissue concentration of ejected drugs reached by microelectrophoresis. These tests were performed with glycine and acetylcholine on montoneurones and Renshaw-cells of the cat lumbar cord. Quantitative data on in vitro release from micropipettes will also be presented.

Mauthner axon impulse: External potential and longitudinal-current measurements

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Single excitable sites (e.g. nodes of Ranvier) can be excluded in the myelinated Mauthner axons (MA) of the Tench spinal cord. Intraaxonally evoked impulses seem to arise from a collective of simultaneously excited sites (axon-collaterals?) distributed over a minimal axonal length of 4.2±1 mm (Greeff, Experientia *33*, 780, 1977). In the present study, MA impulses recorded during penetration of the myelin sheath showed growing amplitude and same polarity as recorded intra-axonally, which demonstrates that the axonal membrane underneath the myelin is

not involved in the excitation. In order to locate the multiple excitable sites, the longitudinal currents of the MA impulse were analyzed. An amplifier with a CMRR of more than 1000 at 30 kHz for a source unbalance of 100 kΩ was developed and combined with signal averaging. Longitudinal currents were measured in 0.5-mm intervals at the dorsal surface of the spinal cord. The present results suggest close spacing of the active unmyelinated membrane parts of the MA, less than 2 mm, i.e. the so far reached spatial resolution.

Allosteric binding sites for kainic acid in the pigeon cerebellum

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The displacement of bound [^{3}H]kainic acid by unlabelled kainic acid from crude membrane preparations indicated a homotropic allosteric effect. The displacement curves measured were analyzed by least square approximation of the Hill-equation assuming an allosteric binding site and an independent binding site for kainic acid. For the independent binding site the values for K_d (nM) and n (Hill-coefficient) were set equal to the values obtained with the pigeon optic lobe were only one binding site is present. At 50 nM [^{3}H]kainic acid concentration the kinetic parameters K_d, B_{max} (pmoles/mg prot.) and n for the allosteric and independent binding sites in this order were: 330/35, 106/2.6, 2.15/1.00 for fresh tissue and 335/35, 81/8.2, 2.05/1.00 for frozen tissue. This indicates that in the pigeon cerebellum different binding sites for kainic acid are present, an allosteric one and one which is probably identical to the one present in the optic lobe.

Projections of precentral, premotor and prefrontal cortex to the pontine nuclei of the monkey

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Corticopontine projections were examined with autoradiographic tracer methods in 18 *Macaca fascicularis*. Labelled amino acids were injected into the main body representation areas of the precentral motor cortex and into circumscribed regions of Brodmann's area 6, 8 and 9 of the frontal lobe. The basilar pontine grey receives a marked and predominantly ipsilateral projection from agranular cortex (areas 4 and 6 including the supplementary motor area) mainly in the caudal fields; relatively weak projections were noted from granular frontal cortex. A detailed map reveals that the terminals of the corticopontine projections are sequentially arranged in folded laminae and bear a relatively high degree of somatotopic resolution. The general layout is comparable to that found in other species (Brodal, 1968; Dhanarajan et al., 1977). N. ret. tegm. pontis receives an ipsilateral somatotopic projection from area 4.

Effect of afferent fibre lesion on kainic acid binding in pigeon optic tectum and rat neostriatum

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L-glutamate specific [^{3}H]kainic acid binding was measured with crude membrane preparations in the tectum after unilateral retinal ablation (1-12 weeks survival time) and the rat striatum after uni- and bilateral cortico-striatal pathway degeneration (1-4 weeks). The binding sites were maximally reduced at the longest survival times only by 11% in the tectum and 23% (unilateral) and 26% (bilateral) in the striatum (compared to the contralateral or unoperated structure). In both structures the toxicity of kainic acid depends on this afferent input (McGeer et al., Brain Res. *139*, 381, 1978; Biziere and Coyle, Neurosci. Lett. *8*, 303, 1978; Streit et al., Experientia *35*, 928, 1979). It is therefore concluded that the neurotoxic kainic acid binding sites are most likely not located on the pigeon optic and the rat cortico-striatal fibres.

Antibody induced suppression of digitalis inotropy in vitro

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The effect of sheep anti-digoxin antibody fab-fragments (kindly provided by Dr W. Riesen, Institut für klinisch-experimentelle Tumorforschung der Universität Bern) on isolated calf right ventricular trabecular muscle was investigated. Preparations were held at 35 °C and stimulated at 1 Hz. Contractile force was monitored continuously. At regular intervals intracellular recordings of transmembrane potential were made using conventional microelectrode technique. Either of 2 procedures were followed: Simultaneous application of drug (digoxin 5×10^{-8} to 1×10^{-7} M/l or strophanthidin 1×10^{-7} to 4×10^{-7} M/l) and antibody (in a 2.5-fold molar excess) prevented inotropic action of both digoxin and strophanthidin. Addition of the antibody at the new glycoside induced steady state height of contraction led to reversal of the contractile response (timecourse being similar to drug washout in Tyrode), even so the glycoside exposure was continued. Action potentials underwent only small changes throughout most of the experiments. These findings are in agreement with observations by Butler et al., Fed. Proc. *36* (9), 2235 (1977).

Uptake of ^{3}H-taurine and ^{3}H-β-alanine in CNS cultures

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Biochemical and electrophysiological studies suggest that taurine and β-alanine may be inhibitory transmitter substances in the mammalian CNS. Both amino acids are taken up by high affinity transport mechanisms in brain slices and in synaptosomes. Since tissue culture techniques provide an excellent tool to investigate the cellular localization of the uptake of neurotransmitters, a study was made of the accumulation of ^{3}H-taurine and ^{3}H-β-alanine in cultures of rat cerebellum, brain stem and spinal cord using autoradiography. In spinal cord and brain stem, both amino acids (10^{-7} and 10^{-6} M) were taken up by a small number of neurones of various size and by almost all glial cells. In cerebellar cultures a small proportion of medium-sized neurones, probably interneurones, as well as all glial cells were labelled with ^{3}H-taurine, whereas ^{3}H-β-alanine was only accumulated by glial elements. The uptake of both amino acids was temperature and sodium dependent suggesting an active transport mechanism.

Effects of glutamate and aspartate on cultured glial cells

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It has been shown that GABA depolarized satellite glial (SG) cells of cultured dorsal root ganglia and astrocytes of CNS cultures without producing changes in membrane resistance (Hösli et al., Exp. Brain Res. *33*, 425, 1978). 4-Aminopyridine (4-AP) which blocks K^+-conductance of

excitable membranes reversibly abolished the depolarization by GABA of SG cells. We have studied the action of glutamate and aspartate on cultured astrocytes of rat CNS. Both amino acids caused a depolarization which was not associated with a change in membrane conductance and which was reversibly blocked by 4-AP. The majority of astrocytes depolarized by the amino acids were lying in the vicinity of neurones, whereas isolated glial cells in the outgrowth zone were usually not affected. From these results it is suggested that the action of glutamate and aspartate on glial cells, like that of GABA, is an indirect effect due to the release of K^+ from adjacent neurones.

Angiotensin II sensitive neurones in septal areas of the rat

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Specific angiotensin binding activity has been confined to the brain stem, diencephalon and in particular to the lateral septal region (Sirett et al., Brain Res. *122*, 299, 1977), areas which have been related to dipsogenic action. Angiotensin II, Sar^1-Ala^8-angiotensin were tested microiontophoretically on physiologically and anatomically identified neurones of the lateral and medial septum of the rat. Activation of cell discharges was obtained from 42 out of 105 neurones recorded in the lateral septum and there was a dose-response relationship: The cells that were specifically responsive to AII not only responded to low current but were blocked in their response by application of the competitive antagonist Sar^1-Ala^8-angiotensin. Depression of firing rate following administration of AII was observed on 13 cells, an effect which was not influenced by the antagonist. In contrast most of the medial septal cells were not responsive to angiotensin II suggesting that this peptidergic system is related to the lateral septal region.

Effect of MS and EAE sera on VRR, DR-VRP and DR-DRP in isolated frog spinal cord

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MS and rat EAE sera seem to have a synaptic blocking action. We tested these sera (25%) in the medium perfusing isolated hemisected frog spinal cord. Ventral root reflex responses (VRR; AC rec.), evoked ventral root potentials (DR-VRP; DC rec.) and dorsal root potentials (DR-DRP; DC rec.) were measured, using standardized human or normal rat serum as controls. All sera inhibited these 3 parameters, as well as spontaneous activity, indicating that they contain an unspecific synaptic blocking factor. When the sera were separated into low ($< 25{,}000$) and high ($> 25{,}000$) mol.wt fractions, the blocking factor was found in the low mol.wt fraction. Heat inactivation did not alter the inhibitory effects. Mg^{++}-ions at a concentration equal to that of 25% serum (0.3 mM) quantitatively inhibit VRP and DRP in the same way. We presume that Mg^{++}-ions, not present in frog Ringer solution, mimic this synaptic blocking action. We could not find any synaptic blocking factor related to the pathogenesis of demyelinating diseases.

Auditory localization in the frontal mid-saggital plane: Right and left ear specialization

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15 subjects were tested for their ability to localize sound sources in the frontal mid-saggital plane, in binaural and monaural conditions. Filtered noise is presented randomly in one of 4 loudspeakers aligned vertically with an angular separation of 11°. The scores are based on the subject's reaction time to the change in position of the sound source. Our results show that the left ear performance is 55% and the right ear 34% of the binaural performance. Simple models with 2 identical noisy monaural detectors would predict that each ear's score should be between 71% and 100% of the binaural performance. To explain the low performances in monaural conditions we must consider either that an auxiliary binaural mechanism is involved, or that the right and left channels do not treat the information in the same way: they are complementary and the binaural performance would then be, as observed, equal to the sum of the monaural scores.

Thermoregulation during positive and negative work with the same energy production

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7 male subjects aged 24-28, performed a positive work (PW) of 75 W and a negative work (NW) of −120 W during 50 min each in a gradient layer direct calorimeter. The intensity of PW and NW was chosen in order to submit the subjects to the same total energy production i.e. $(M - |W|)$ for PW and $(M + |W|)$ for NW. The metabolic energy (M), measured from oxygen consumption, was 415 ± 7 W for PW and 220 ± 8 W for NW. During both PW and NW, the time course of the changes in mean skin temperature ($\bar{T}_s$) and tympanic temperature (T_{ty}) was respectively similar. In addition, the rate of heat losses increased in both types of work by the same amount: the rate of heat storage reached zero within 45 min in both conditions. We concluded that: a) the energy absorbed by the body during negative work is entirely converted into heat; b) the changes in T_{ty} and $\bar{T}_s$ depend on the total heat production, and not on M; c) similarly, the main thermoregulatory response, i.e. the stimulation of sweating, is a function of the rise in body temperature and not of variations in M.

Possibilities and limits of microspectrophotometric determination of the oxygen uptake of cell populations in 'bidimensional' tissue cultures

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The hemoglobin (Hb) oxygen donor/respiration indicator method (Barzu and Borza, 1967) has been modified for detailed studies within the intact young chick embryo cultured in vitro (Raddatz and Kucera, 1977). The measurements are influenced by complex diffusion fluxes taking place within the Hb solution in contact with the respiring cells. The kinetics of this diffusion was tested experimentally and analyzed, using a digital simulation, with respect to the Hb dissociation curve. Thus precise conditions for linear measurements have been defined allowing detections of the order of 10^{-11} to 10^{-10} $lO_2 \cdot h^{-1} \cdot 10^{-2}\ mm^{-2}$ of tissue. The measuring time varies from 8 to 60 sec, at 8 sec the spatial resolution is 100 µm in the case of a homogeneous, uniformly respiring tissue. Rapid scannings of the oxygen uptake of differentiating embryonic cell populations will be presented.

Columnar pattern in the cat visual cortex after optokinetic stimulation

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A columnar organization in areas 17 and 18 of the feline cortex was found after applying different optokinetic stimuli in experiments with the ^{14}C-2D-deoxyglucose technique. 5 cats were exposed to 3 kinds of optokinetic stimuli consisting of a) black and white vertical stripes, b) a random-dot pattern and c) an irregularly embossed white wallpaper. The optokinetic stimulation led to the same columnar labelling in all experiments, but with varying intensity. The data suggest that stimulus direction, contrast and velocity are further factors determining the well-known orientation columns.

REM latency shortened for second half of interrupted night sleep

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The laboratory sleep of 8 healthy volunteers was recorded polygraphically during 3 baseline nights, 3 nights with 5 h sleep, and 3 nights of interrupted 5 h sleep (2.5 h wakefulness between first and second half of sleep). REM latency after onset of sleep stage 2 was significantly shorter for the second half of interrupted sleep than for a) the first half of interrupted sleep, b) baseline nights and c) 5-h nights. The results are discussed in relation to the hypotheses that REM latency is influenced by a circadian rhythm and by preceeding NREM sleep time.

Effects of lysine vasopressin (LVP) and ACTH 4-10 on the giant dopamine neuron of *Planorbis corneus*

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The giant dopamine neuron (GDN) of the water snail *Planorbis corneus* provides a model system for correlative electrophysiological, quantitative histochemical and biochemical studies at the level of the individual nerve cell (Lichtensteiger, Felix and Hefti, Experientia *34*, 901, 1978). We have investigated the action of 2 mammalian peptides on the GDN in an in vitro preparation. The firing of GDN in response to a given depolarization induced by current injection, was consistently increased after administration of LVP to the bath (10^{-6} M). Administration of ACTH 4-10 resulted in a decrease of spontaneous activity and the development of a bursting pattern with silent periods. The ionic mechanisms underlying the reaction of GDN as well as its dopamine metabolism are presently being analyzed. Our results indicate that the GDN represents an appropriate model system for studies of peptide effects on electrophysiological parameters and neurotransmitter metabolism in the same identified nerve cell.

Efflux in inorganic phosphate during activity in rabbit vagus nerve

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In garfish olfactory nerve activity is followed by a period of increased release of phosphate (Ritchie and Straub, J. Physiol., Lond. *274*, 539, 1978). A similar extra-release has now been observed in rabbit vagus nerve, loaded for 2 h with radiophosphate. In solutions with 2 mM phosphate and 1 mM K the resting efflux was 2.7×10^{-3} min^{-1} and the extrafractional loss 3.0×10^{-6} impulse^{-1} at 37 °C. Chromatography of the effluent during rest and activity showed that over 98% of the label is located in the inorganic phosphate fraction. When the extracellular Na was replaced by Li the increased release of phosphate was reversibly abolished without much change in the compound action potential. A decrease in extra-release with normal excitability was also seen when ouabain in concentrations below 0.1 µM was applied. The present results suggest that the extra-release of phosphate is due to an increase in intracellular inorganic phosphate resulting from increased breakdown of ATP by faster working of the Na-K-pump.

Increased exploratory efficiency in a complex maze at estrus

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Exploration of a complex maze that included a lighted open field was investigated in 17 female RHA/Verh. rats on estrus and nonestrus nights. Consistent with previous research, b.wt decreased, locomotor activity increased, and open field entries increased at estrus. In addition, the present experiment evaluated exploratory efficiency independent of locomotor activity. Although a comparable number of equidistant photocells were activated during the initial exploration of each new area, the time required to explore these new areas was significantly less at estrus. These data demonstrate that when a measure of exploratory efficiency based on speed is used, rats explore a complex maze more efficiently at estrus.

Colchicine-induced axonal degeneration and demyelination in the developing rabbit optic nerve

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Colchicine, a drug used to inhibit reversibly axoplasmic transport, was injected into the vitreous body of the eye of 5- to 33-day-old rabbits. Myelination in optic nerves was investigated morphologically and by measuring 2 myelin markers, myelin basic protein and 2′,3′-cyclic nucleotide 3′-phosphohydrolase. After colchicine treatment at age of 5 and 10 days, a complete destruction of ganglionic cells, axons and myelin was observed. The injection of colchicine at the age of 15, 25 or 33 days reduced the amount of myelin present in adults by about 50%. Even 4 months after the injection, no remyelination occurred. Differences observed between 15 and 25 or 33 days of age could indicate the presence of 2 neurone populations, one more resistant than the other to colchicine. This study shows that colchicine induces an irreversible destruction of the optic nerve in developing rabbits and that this technique could be of interest as an atraumatic model to study secondary demyelination.

Neuroendocrine regulation and central dopamine (DA) systems in physical and psychological stress

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Systemic injections of certain hormones that are released upon stress, notably of α-melanotropin, influence central

DA systems. In order to investigate whether such interactions are of physiological significance, the functional state of central DA neuron groups was assessed by microfluorimetry in different stress situations. Male rats were subjected either to physical stress (footshock) alone or to physical stress followed 1 day later by a psychological stress (placement in experimental chamber without footshock). The DA neurons of substantia nigra responded to both types of stress with a more pronounced reaction to psychological stress. No significant changes were seen in DA neurons of the ventral tegmental area (A10). The tubero-infundibular DA neurons exhibited signs of increased activity only after physical stress. The possibility of a relationship between the various types of responses of DA neuron groups and neuroendocrine processes is analyzed by determination of hormone levels and injection of antisera.

Myelination of the rabbit optic system during development

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Myelination was studied in the optic system of the rabbit with the use of electron microscopy and 2 biochemical markers, myelin basic protein (BP) and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP). By the age of 5 days, many compact myelin sheaths were already present in the optic nerve even though the eyes do not open until the age of 8-10 days and myelination has not yet started in the optic disc (Narang, Nature *266*, 855, 1977). The early activation of the myelin-related enzyme, CNP, which preceded the increase of BP, is another indication of the presence of this enzyme in oligodendroglial or early myelin membranes. The discrepancy between CNP activity and BP content could also indicate that CNP is a better glial marker and that BP reflects better the amount of myelin present. Comparison of the morphological and biochemical data obtained from 3 different regions along the same axons indicates that in the rabbit optic system, myelination appears to proceed simultaneously, since no direction of myelination could be demonstrated.

The effects of 4 psychotropic drugs on maze exploration of Roman high- and low avoidance rats

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The rats were tested for 4 days in an enclosed, hexagonal maze. Thereafter barriers were introduced, dividing the system into 2 sections, which were connected by an illuminated open field in the center. Photocells indicated the location of a rat in the maze. The data were automatically recorded by a PDP-11 computer. The behavior of drug treated rats differed from that of the controls depending not only upon drug treatment but also upon the different genetic lines and the maze configuration: Chlorpromazine (1.5 mg/kg) reduced maze center activity (MCA) and exploration in both lines with these effects being more pronounced in the second maze configuration. Pentobarbital (7.5 mg/kg) reduced both MCA and exploration for the 2 lines, with a constant increase for the second maze. The predominant effect of imipramine (7.5 mg/kg) was to depress total activity. Chlordiazepoxide (7.5 mg/kg) which initially depressed MCA, subsequently had a pronounced stimulant effect only in the RLA-rats.

Anaerobic efficiency during exercise transients

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Aerobic (M_{ae}) and anaerobic (M_{an}) energy productions were determined during the immediate transition to different work loads. In 5 subjects exercising at 30, 50 and 70% $\dot{V}_{O2}$max for 8 min, respectively, in a direct calorimeter, M_{an} was obtained by solving the heat balance equation: $M_{ae} + M_{an} = H + W + S$ where M_{ae} is measured by indirect calorimetry, H (total heat losses) by direct calorimetry, W (external work) by ergometry and S (heat storage) by thermometry using esophageal, skin and muscular temperatures. The fraction of W due to M_{ae} was: $W_{ae} = M_{ae}$ work $\times \eta_{ae}$, where η_{ae} = net efficiency of aerobic work; η_{ae} was $26.6 \pm 0.5\%$. The fraction of W due to M_{an} was: $W_{an} = W - W_{ae}$. The efficiency of anaerobic work (η_{an}) was: $\eta_{an} = W_{an}/M_{an}$. Our values for η_{an} were: $35 \pm 1\%$, $36 \pm 1\%$ and $36 \pm 1\%$ for each work load, respectively. η_{an} may be considered to approach the value of the contraction-coupling efficiency of muscles, whereas η_{ae} results from the product: $\eta_{ae} = \eta_{an} \times \eta_p$, where η_p is the phosphorylative-coupling efficiency.

Conductance effects of isobutyl-methyl-xanthine (IBMX) on frog skin

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Theophylline (T) is a classical tool for examining the postulates of Sutherland on cAMP, but some of its effects remain poorly understood. We report here results obtained in frog skin with IBMX, a potent analogue of T. The following parameters were monitored: potential difference (PD), short circuit current (SCC) and total electrical conductance (K). Overall, the effects of IBMX and T were comparable, IBMX being more potent than T by 1-2 orders of magnitude. In Cl (but not in SO_4) Ringers, IBMX decreased PD by 30-50% before any detectable change in SCC, sometimes at concentrations as low as 10^{-6} M. The increase in K was several fold, reversible and dose-dependent, but its profile varied somewhat. Thus IBMX, like T, but at micromolar concentrations, had a Cl-dependent effect on conductance which may be exerted, at least in part, on tight junctions. Further work is needed to determine the structure-activity relationship underlying this effect and its relation, if any, to phosphodiesterase inhibition.

Respiratory responses to vagal and hypothalamic electrical stimulation during sleep in cats

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During synchronized sleep electrical stimulation of the vagus nerve elicited responses similar to those of pulmonary inflation or deflation. In contrast, during desynchronized sleep the responses to the same stimulation were variable or even failed to appear. Electrical stimulation of the anterior hypothalamus eliciting phase switching or chronotropic respiratory effects during synchronized sleep was ineffective during desynchronized sleep. These results support the hypothesis of a release of brain stem respiratory mechanisms from hypothalamic regulatory influences during desynchronized sleep (P.L. Parmeggiani, Brain Res. 7, 350, 1968).

Mechanosensory modification of ciliary activity: Involvement of Ca^{++} and Mg^{++} in membrane currents

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Mechanical stimulation of the anterior end of the ciliate Stylonychia produces a predominantely Ca^{++}-dependent depolarization consisting of 3 components (de Peyer and Machemer, J. comp. Physiol. *127*, 255, 1978). High frequency cine analysis of the ciliary response shows that, upon this depolarization, the cirri reverse the orientation of the power stroke together with increased frequency of beating. Depolarizing step pulses under voltage clamp condition induce an early Ca^{++} inward current and a reversal response of the cirri, whereas inward currents evoked by mechanostimulation in absence of voltage displacement fail to activate the cilia (de Peyer and Machemer, Nature *276*, 285, 1978). If Ca^{++} is replaced by Mg^{++}, mechanical stimulation produces a depolarizing receptor potential, which does not elicit an action potential nor trigger a reversal of the cirri. In voltage clamp the receptor inward current is little affected, while depolarizing voltage steps fail to induce an inward current.

The effects of LH-RH and angiotensin II on neurones in the organum vasculosum lamina terminalis (OVLT)

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Luteinizing hormone-releasing hormone (LH-RH) has been localized in the lamina terminalis region of the brain but the physiological action of the peptide in this region is unknown. By surgically opening the ventral brain to visualize the optic chiasm, a 5-barrelled pipette for microiontophoresis was lowered into the lamina terminalis. LH-RH and angiotensin II were applied to recorded units. LH-RH either inhibited these neurons or was without effect, whereas angiotensin was mostly excitatory. No excitatory effects of LH-RH were recorded. The results may reflect a negative feedback effect of LH-RH and show that both peptides have a neural action on cells in lamina terminalis. The opposite effects seen with angiotensin and LH-RH may be related to their different roles in sex behavior and water balance.

Site differences in the fatty acid composition of s.c. adipose tissue of obese women

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Adipose tissue was obtained by needle biopsy from 3 s.c. sites (thigh, abdomen, upper arm) in 22 obese women. Fatty acid composition was determined using GLC; results relate to 11 fatty acids. The FA composition of adipose tissue from arm and abdomen was remarkably similar, with the exception of levels of lauric acid. The majority of saturated FA were present in smaller proportions whilst the majority of unsaturated FA were present in larger proportions in the thigh than in the 2 other sites. Highly significant intersite differences were found for 6 of the major FA and for both total amounts of saturated and unsaturated FA and their ratios. 6 of the subjects were given an energy-restricted diet (3400 kJ/day) for 1 month. FA composition of the 3 sites was determined before and after the dieting period. This regimen did not alter the intersite differences described above. It did however induce a shift in the proportion of saturated to unsaturated FA in favour of the latter, within a given site.

Internal power in cycling

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Internal power (W_i) in cycling was calculated from graphical analysis of leg motion assuming that: a) the angular speed is constant, and b) the only moving body parts are the lower limbs. For a subject of average size W_i (w) increases linearly with the cube of the pedal frequency (f, s^{-1}): $W_i = c\ f^3$, where $c = 5.14$. The external power in level cycling (W_e) increases with the speed (v, m/s): $W_e = 3.2\ v + 0.19\ v^3$ (calm air, sea level, 'dropped' posture, racing bike, smooth surface) (P. E. di Prampero et al., 1978). Hence, the total mechanical power ($W_t = W_e + W_i$) in the above conditions is described by: $W_t = 3.2\ v + 0.19\ v^3 + c\ f^3$. As v and f are known for aerobic (~ 6 min) and anaerobic (~ 10 sec) record performances, c can be calculated by an iterative procedure from the above equation. It amounts to 7.0 $J \cdot s^{-4}$, a value close to that obtained from graphical analysis. It can then be concluded that W_i accounts for about 6–8% of the total power in maximal effort.

Utilization rate of a 100 g glucose load during prolonged exercise in obese subjects

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Using naturally labelled ^{13}C-glucose, ingested glucose (IG) oxidation was investigated in 6 exercising obese subjects (149±2% ideal weight). 1 h after a 100-g glucose load, they pedaled for 2 h at 20% $V_{O_2}max$ (53±3 W). Total carbohydrate (CHO) oxidation was calculated from the nonprotein RQ and IG oxidation from expired $^{13}CO_2$ using mass spectrometry. Expired CO_2 became enriched with ^{13}C 45 min after the glucose load, and $^{13}CO_2$ equilibrated with the CO_2-bicarbonate pool after 1 h exercise. During the 2nd h of exercise, the relative contribution of CHO, lipids and proteins represented 54, 41 and 4%, respectively, of total energy expenditure; 29% of this energy was provided by IG oxidation. During the 2-h exercise period, 41±5 g of IG were oxidized. When compared to results obtained on resting obese and controls, it can be concluded that oxidation of IG is impaired in the obese at rest, but during exercise IG is utilized to the same extent as by the controls.

Role of lipids in photosynthetic membrane function as revealed by lipolytic treatment

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Thylakoids (CP) or subchloroplast particles (SubCPII, SubCPI) enriched in photosystem II (PSII) and photosystem I (PSI) were treated with a potato lipolytic acyl hydrolase (LAH). In CP, the whole electron transport chain (PSII + PSI) underwent considerable loss in activity whilst electron flow through PSII or PSI was still preserved. Photophosphorylations were the first functions to be totally inactivated. LAH catalyzed a limited lipid hydrolysis, mainly hydrolyzing phosphatidylcholine. SubCPII and SubCPI electron flows were more resistant to LAH than when they were integrated into CP. However, lipid hydrolysis was weak in SubCPII but almost total in SubCPI. It is suggested that lipids (except a few tightly bound molecules)

are not an absolute requirement for the separated PSII and PSI photochemical activities and that phosphatidylcholine could be closely associated with the functional link between PSII and PSI probably at the plastocyanin level.

Influence of internal pH on the slow inward current and the contraction of Purkinje fibres from mammalian heart

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Purkinje fibres were used to study the influence of internal pH on action potential configuration, slow inward current (i_{si}) and contraction. The internal pH was altered by addition and removal of NH_4Cl or by changes in pCO_2 in the bathing solution. The external pH (7.40) and the Ca^{++}-activity ($a_{Ca++} = 1.07$ mM) were kept constant throughout. Acidification of internal pH by increasing the pCO_2 from 40 to 100 mm Hg produced a decrease of the amplitude of the plateau and the size of the i_{si} concomitant with a reduction of the contraction. Internal alkalinization upon addition of NH_4^+ markedly increased the 3 parameters considered. These results suggest that a reduced Ca-sensitivity of the myofilaments (A. Fabiato and F. Fabiato, J. Physiol. *276*, 233, 1978) might not be the only explanation for the impaired contractility during acidosis.

Release of newly synthesized ^{3}H-glutamate from rat corticostriatal pathway, in vitro

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Degeneration of the corticostriatal pathway leads in striatum to a decrease in glutamate pool and uptake, suggesting there a transmitter role for glutamate. However, a calcium-dependent release of glutamate from corticostriatal nerve terminals has not been demonstrated. Using a technique described (Reubi et al., Neurosci. Lett. *10*, 171, 1978) in vitro release of ^{3}H-glutamate, newly synthesized from ^{3}H-glutamine, was measured in rat striatal slices. 47 mM KCl induced a 20-fold increase of spontaneous release, which was calcium-dependent. In the same experiments, a calcium-dependent release of ^{3}H-GABA was simultaneously observed. Thus glutamate and GABA are likely transmitters in the striatum. 2 weeks after ablation of frontal and dorsolateral cortex, when the corticostriatal neurons are degenerated, a large decrease in K^+-evoked glutamate release was observed in comparison to intact striatum, while the GABA release was not decreased. This supports the hypothesis that glutamate is a neurotransmitter in the corticostriatal pathway.

Binaural representation in the medial geniculate body

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Single unit activity was recorded in the medial geniculate body (MGB) in response to clicks, noise and tone bursts delivered simultaneously to both ears or only to the ipsi- or contralateral ear. Over 600 units were studied in nitrous oxide anaesthetized cats and 13 different types of binaural interactions have been distinguished according to monaural and binaural responses. The overall distribution of these codes is the same for tone, noise and clicks. For a given unit the probability of this binaural code to be identical for noise, tone and clicks is only 0.1, and it is 0.5 for belonging to the same ear dominant class. In the pars lateralis the great majority of units (90%) have binaural inputs, with predominancy of the contralateral influence (60%). Only 10% of the units are monaural (3% ipsi, 7% contra). Units having an ipsilateral dominance (25%) are mainly clustered in a limited 400 µm wide frontal section close to the anterior part of the medial third of the MGB. In the other subnuclei the binaural interactions and their distributions are quite similar.

Origin of secretion after acute ischaemia of dog ileum

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Immediately after a total ischaemia of 1 h, there is a profuse secretion of water, Na^+ and Cl^- into the lumen of the ischaemic loop. The villous mucosa is damaged and no longer transports amino acids and sugars in vitro; crypt structure is preserved. When dogs are loaded with phenol red by i.v. infusion, no dye appears in the lumen of control or ischaemic loops, suggesting that secretion is not due to passive filtration across denuded villi. In dogs made uraemic by bilateral nephrectomy prior to the ischaemia, the passage of urea into the lumen is the same in control and ischaemic loops, indicating that water flow from blood to lumen is unaltered by the ischaemia. Unidirectional Na^+ flux measurements reveal that the lumen-blood flux is reduced in ischaemic intestine, but the reverse flux is hardly changed. To interpret these findings, it is proposed that in the control intestine, crypt cells secrete water and electrolytes, whereas villous cells absorb them; when this balance is upset by ischaemia, net secretion results.

Putative morphological correlates of dendritic release sites for dopamine in rat substantia nigra

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The rat nigral dendrites were investigated systematically by means of thin section and freeze fracture electron microscopy. Typical axodendritic synapses were frequently encountered; they may be of Gray 1 or 2 type. No direct contacts between dendrites were observed, rather the dendrites were consistently ensheathed by intervening glial lamellae, which in turn were frequently interconnected by gap junctions. The most conspicuous form of interneural contacts were the large intermediate junctions (attachment plaques) between axon terminals and dendrites which appeared frequently in close connection with typical synapses. These contacts were characterized by subjunctional SER cisterns on the dendritic side but failed to display synaptic vesicles on either side of the junction. Since dopamine is known to be localized in the SER fraction the problem arises as to whether these dendro-axonic attachment plaques may represent the release site of dopamine.

Mental maze learning in humans

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An experimental procedure was developed for automatic recording (PDP-11) of mental maze learning processes in humans: A key board of 49 keys arranged as a 7×7 square is placed in front of a subject (S). Some keys activate the presentation of a picture in front of the S, while the remaining keys do not produce a picture (blacks). The arrangement of the picture-rewarded keys can be selected by the experimenter, but is not known to the S. The S has to locate all keys producing pictures in a minimum of time and with a minimum of picture repetitions and blacks. Over 7 successive trials considerable improvement of performance was observed if the arrangement of picture-

rewarded keys was complex but remained the same over the 7 trials. If the arrangement was varied from trial to trial, learning occurred only if the variation of the arrangement allowed the S to develop strategies (generalization from one arrangement to another). The method described permits the concomitant recording of psychophysiological parameters.

Prenatal development of monoamines in the rat

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The pattern of the development of catecholamines (CA) was traced in neurons and nonneuronal cells of rat fetus with histochemical and biochemical techniques. CA were detected first in the early midgestational period around ED 10 in the epithelial cells of the visceral yolk sac, an important nutritive organ. Subsequently, CA are seen in sympathetic ganglion cells and then in the CNS. In the CNS, CA neurons appear in a rostrocaudal sequence: hypothalamus, midbrain and lower brainstem. Within a few hours after their first appearance, the CA and serotonin cells develop into most conspicuous cell clusters. The ascending projections to di- and telencephalon develop during the second half of gestation. The neocortex is innervated by CA fibres in a characteristic bifurcated pattern sparing the cortical plate until shortly before birth.

The influence of medial hypothalamic lesions on conditioned emotional response in pigeons

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To determine whether the medial hypothalamus (MH) plays a role in the control of emotional behavior in pigeons, animals were tested in a CER paradigm before and after MH lesions. In this operant test situation, the animals received 4 unavoidable shocks in 1 h, each preceded by the 2-min presentation of a red light (CS). Throughout the session pecking was reinforced on a VI-30 schedule. Pigeons typically exhibit a reduction in response rate during CS presentation in such training sessions. To evaluate the degree of suppression, pecking during the 2-min CS was compared to that during a control interval. Bilateral MH lesions substantially reduced this conditioned suppression. Although control pigeons normally show an enhanced pecking rate over training sessions and following the introduction of the shock procedure, the rates observed in lesioned pigeons were even greater. To evaluate the possible contribution of altered feeding motivation following MH lesions, food deprived pigeons were also tested in the same operant paradigm.

Influences of dopamine on substantia nigra pars reticulata neurones

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The dopamine neurones of substantia nigra pars compacta have dendrites which reach into pars reticulata and liberate dopamine (DA) with nerve impulses. The targets of this dendritically released DA may be neighbouring dopamine neurones since they are known to be sensitive to DA. In the present experiments on rats we found that also nondopaminergic pars reticulata neurones were influenced by iontophoretically applied DA. Quantitatively these neurones were less sensitive to the depressant action of DA, and in a minority of neurones excitation to DA was seen. Some of these neurones projected to ventromedial thalamus. The data suggest a functional connection between the DA system and the motor system at the level of substantia nigra, possibly of a modulatory nature.

Effects of thyroid status on catecholamine-induced increase in reducing equivalents in rat brown adipose tissue

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Steady-state supply of reducing equivalents to the respiratory chain was evaluated in brown adipose tissue (BAT) by monitoring the pyridine nucleotide redox states via the surface fluorescence. BAT samples were stimulated with isoprenaline (β-agonist), phenylephrine (α-agonist) and norepinephrine. Hypothyroidism was induced by adding 2-mercapto-1-methyl-imidazol to the drinking water. In control rats of the same age, all 3 analogues produced a steady state increase in the redox ratio. Their potency was found to be isoprenaline $\gg$ norepinephrine > phenylephrine. In hypothyroid rats the isoprenaline response was greatly reduced (by 93%), the phenylephrine response was reduced less (by 36%) and the norepinephrine response was not affected. For each analogue the dose response curve was shifted to the right. The order of potency to produce maximum response was norepinephrine > isoprenaline = phenylephrine. We conclude that thyroid status affects predominantly the β-receptors.

Dynamic JULESZ random-dot stereograms and correlograms evoke sustained brain potentials in humans

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On-line computer-generated (100 frames/sec) JULESZ random-dot correlograms alternated in 720-msec epochs between (1a) binocularly correlated (flat) and (1b) binocularly uncorrelated ('wooly') conditions, and stereograms between (2a) binocularly correlated (as 1a) and (2b) binocularly partially disparate (depth) conditions. Onset of the correlated conditions (1a and 2a) evoked in all 11 ss a late (starting around 200 msec) sustained negativity over anterior scalp areas, whereas onset of the uncorrelated (1b) or partially disparate (2b) conditions evoked a late sustained positivity over anterior areas, independent of attention to conditions a or b. The results are reminiscent of CNV/P 300 observations, and suggest a 'hard-wired' general brain response to binocularly uncorrelated or disparate visual input.

Facilitation and inhibition of learning by intracranial injection of substance P

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Using various passive avoidance learning procedures the effects of posttrial injection of substance P (SP) into different brain regions shown to contain relatively high endogenous SP levels, were investigated. In the substantia nigra of rats 500 ng SP led to severe retention impairment. Similarly, 50 ng SP into the medial amygdaloid nucleus caused amnesia for a step-down avoidance task. In contrast to these findings, SP injection into the ventromedial hypothalamus influenced retention neither in a step-down

avoidance nor in an alcove avoidance task, whereas in the lateral hypothalamus 50 ng SP strongly facilitated learning of the step-down avoidance as well as the alcove avoidance task. These results suggest a mediating role of SP in memory processing. Additionally, it should be mentioned that these effects of posttrial SP injection parallel the effects of posttrial electrical stimulation of the corresponding brain regions on passive avoidance learning.

Optic nerve dependent kainic acid toxicity in optic tectum

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Kainic acid has been shown to induce characteristic lesions in neurons receiving a presumable glutamate input, a toxicity which does not appear when this input is missing (Biziere and Coyle, Neurosci. Lett. *8*, 303, 1978). Following optic nerve degeneration in pigeon, glutamate level and uptake decrease in the tectum (Henke et al., J. Neurochem. *26*, 131, 1976; Fonnum and Henke, Experientia *35*, 931, 1979). After tectal injection of kainic acid (0.5-2.0 µg in 0.5 µl), the following light microscopic changes were observed: a) within 1-48 h important neurophil vacuolization restricted to deep half of layer 5, rich in retinal terminals, b) within 1 h to 21 days, progressive neuronal cell loss throughout the tectum. These toxic effects were not observed 2-12 weeks after contralateral retinal ablation but could be partly restored by combined glutamate (0.2 mg) and kainate injection. Thus in the pigeon tectum, kainic acid neurotoxicity is dependent upon an intact retinal input, a finding consistent with a special role of glutamate in layer 5 terminals.

Infusion of natural or artificial cerebrospinal fluid into the lateral ventricle of rat: Depression of motor activity and paradoxical sleep

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Motor activity and sleep in the rat were recorded during and after infusion of natural (nCSF) or artificial cerebrospinal fluid (aCSF) into the lateral ventricle. In the dark phase of the 12 h light-12 h dark cycle, motor activity was depressed for several hours after a 15-min infusion of nCSF or aCSF at a rate of 3 µl/min, or after a 45-min infusion of aCSF at 1 µl/min. Infusions of nCSF or aCSF (20 min, 3 µl/min) during the light phase did not affect the sleep states during the infusion, but caused in the post-infusion period a long-lasting selective depression of paradoxical sleep. The possibility that endogenous activity and sleep modifying factors are released or transmitted by the infusates, will be further investigated by infusing nCSF obtained from sleep-deprived animals.

Hepatic 'osmoreceptors': Role in neurohypophyseal hormone release?

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Antidromic compound action potentials (CAP), recorded in the rat pituitary tract, were previously shown to decrease whenever the endogenous electrical activity increased, and CAP decreases correlated with neurohypophyseal hormone release. CAP decreased 11.2±4.7(SEM)% (n=7) when 4% NaCl (0.2 ml) was infused into the hepatic portal vein (PV) and 16.4±4.0% (n=7) when the saline solution was infused into the jugular vein. Responses after injection of 0.9% NaCl were not significant. CAP decreased 15.7±3.7% (n=4) following superfusion of PV with 0.1 ml of 4% NaCl, and 24.4±6.0% (n=24) and 18.4±3.0% (n=3) following electrical stimulation of PV tissue and sciatic nerve, respectively. Xylocaine, applied to PV tissue and spinal cord (T13-L3 level), but not vagotomy, abolished the CAP decrease in response to electrical stimulation of PV and to PV superfusions. Data suggest that nerve fibres, or pain- and osmoreceptors situated in PV tissue, but not intrahepatic osmoreceptors, are implicated in neurohypophyseal control.

Enzyme and binding assays in 4 regions of pigeon brain

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Choline acetyltransferase, glutamate and aromatic amino acid decarboxylase (ChAT, GAD, AAD) activities (nmoles/h/mg prot.) were measured in whole homogenates of paleostriatum (P) and 3 regions of the pigeon visual system, wulst (W), ectostriatum (E) and tectum opticum (T). Binding (fmoles/mg prot.) of quinuclidinyl benzilate (QNB) at 1.9 nM final conc., muscimol 2.8 nM, dihydroalprenolol (DHA) 15 nM, serotonin 7 nM, kainic acid 60 nM, strychnine 2 nM and etorphine 1.3 nM was measured with crude membrane preparations. The results are: GAD: W 82, E 84, T 159, P 178; muscimol binding: W 170, E 180, T 105, P 86. AAD: W 3, E+T <2, P 28; DHA/serotonin binding: W 166/254, T 238/131, P 210/119. ChAT: W 46, E 14, T 98, P 117 (paralleled acetylcholinesterase staining); QNB: W 304, E 232, T 214, P 450. Kainic acid/strychnine/etorphine binding: W 185/14/127, E 113/n.d./103, T 197/71/144, P 332/32/125.

Glycerol as a freeze-protecting agent in frog skin: A functional and morphological survey

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When a frog skin mounted in an experimental chamber is exposed to 20% glycerol the following bioelectric changes are seen: a) Glycerol outside only: Rapid and irreversible drop of PD and R. b) Glycerol from both sides: Same effect as a but recovery after 60-90 min. c) Glycerol from inside only: Slight fall of PD, however rapid increase of R to 4 times the initial value. d) Starting like c but adding glycerol outside after stabilization of R: Slight fall of R but rapid increase of PD, giving a protected skin with apparently normal transport functions. PFEF (protected freeze exchange fixation): Protected skins are rapidly frozen in liquid propane, then exchange-fixed at −60 °C as follows: Methanol +3% buffered glutaraldehyde+slowly decreasing concentrations of glycerol (from 15 to 0%). After 48 h the skins are embedded in the usual way in Epon and processed for EM work. By virtue of the glycerol protection the zone of EM detectable ice cristal damage is moved away from the tissue surface from a depth of 4 µm to a depth of 20 µm.

Distribution and classification of pigeon retinal ganglion cells projecting to the thalamus

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Topography and morphology of retinal ganglion cells projecting to the dorsolateral thalamus (DLL) were studied in the pigeon by HRP retrograde tracing. Distribution and size of labelled perikarya were plotted from tangential

sections of flat mounted retinal pieces. Labelled cells were predominantly distributed along the horizontal meridian. Rostral injections resulted in labelled neurons mainly (75%) in the temporal retina. Central injections yielded a naso-temporal distribution. After caudal injections most (74%) labelled cells were found in the nasal retina. The fovea never contained any labelled neurons. 2 classes of retinal ganglion cells could be distinguished: small, medium and large. The majority of the labelled cells belonged to the class of large cells. Thus, DLL receives a retinotopically organized input originating in a distinct medium sized class of ganglion cells distributed along the horizontal meridian including part of the binocular segment of the retina.

Influence of internal pH on r_i of Purkinje fibres from mammalian heart

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Conventional cable analysis was performed to detect changes in the internal longitudinal resistance (r_i) and the membrane resistance (r_m). The internal pH was altered by addition and by removal of NH_4Cl or by changes in pCO_2 in the bathing solution. The external pH (7.40) and Ca^{++}-activity ($a_{Ca^{++}} = 1.07$ mM) were kept constant. Addition of 15 mM NH_4Cl or reduction in pCO_2 from 40 to 0 mm Hg to cause an intracellular alkalosis revealed a transient or constant decrease in r_i ranging up to 50%. Removal of NH_4^+ or increase in pCO_2 from 40 to 100 mm Hg to cause an intracellular acidosis resulted in a transient increase in r_i of up to 50%. It appeared as if the effect of NH_4^+-removal could be potentiated by simultaneously increasing the pCO_2. A decrease in r_i was usually associated with a decrease in r_m and a depolarization, whereas an increase in r_i gave the opposite effects on r_m and membrane potential. These results are consistent with the hypothesis that the cell-to-cell coupling might be influenced by the intracellular pH (Turin and Warner, Nature *270*, 56, 1977).

Structural and functional definition of the motor cortex (arm area) in monkeys

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The posterior and anterior borders of the motor cortex were delimited by microstimulation in awake monkeys and by relating foci of low-threshold effects to cytoarchitectonic fields. Corticospinal cells were mapped electrophysiologically and by HRP labelling. Rostrally, the motor cortex includes only the most caudal portion of area 6 (presence of corticospinal neurones and positive stimulation effects). Caudally, corticospinal cells were found also in the postcentral cortex (areas 3, 1, 2, 5), but microstimulation was not effective beyond area 4. This indicates that the postcentral cortex should not be considered as a motor area (the postcentral corticospinal neurones modulating somatosensory transmission only). Alternatively, the postcentral corticospinal neurones may be too sparse for detection of motor effects by microstimulation.

Corticopontine projections from sensory and motor areas in the rat

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The pontine nuclei (PN) constitute the most important relay for signals transmitted from the cerebral cortex to the cerebellum. The linkage of various cortical fields with the PN was studied with degeneration and autoradiographic techniques as well as with retrograde labelling of cortical neurones following HRP injections into the PN. The projection was powerful from the sensorimotor cortex (especially from the face area), moderate from the visual cortex and weak, from the auditory cortex. The visual and the auditory cortices were found to project mainly to the lateral and rostral PN. The outflow from a small cortical area often projected to 2 or more discrete territories in the PN. These foci could overlap with foci receiving fibres from other cortical areas. These observations will be discussed in relation with previous findings in monkeys.

Mass-fragmentographic identification of endogenous glycine release upon stimulation of neural pathway

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Glycine might be a transmitter in neurons of nucleus isthmi pars parvo cellularis Ipc projecting to the pigeon tectum since, after [^{14}C] or [^{3}H]-glycine injection into tectum, radioactive glycine collected by a push-pull cannula placed near injection site increased significantly during electrical stimulation of Ipc (Reubi and Cuénod, Brain Res. *112*, 347, 1976). In this report the release of *endogenous* glycine was measured, using the same technique. Glycine was detected in the push-pull cannula perfusate by highly specific mass-fragmentography (selected ion monitoring) of its N-pentafluoropropionylhexafluoroisopropylester. Ipc stimulation (20–40 Hz) induced a 5-fold increase in glycine concentration. Endogenous glutamic acid remained at constant level. Endogenous glycine release upon pathway stimulation strongly support the hypothesis that glycine is a transmitter in Ipc-tectal neurons.

Activity of adenosine 5′-phosphosulfate (APS) sulfotransferase in greening primary leaves of *Phaseolus vulgaris* L.

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In spinach, APS sulfotransferase (APSSTase) is localized predominantly in the chloroplasts. Chloroplast development experiments with *Euglena* indicate, however, that in this organism the enzyme isn't a typical chloroplast enzyme. These conflicting results prompted us to measure the extractable APSSTase activity in greening bean leaves. We included O-acetyl-L-serine sulfhydrylase (OASSase) in our measurements, an enzyme which does not appear to be predominantly localized is the chloroplasts of either *Euglena* or spinach. Before illumination the organisms were imbibed for 24 h and cultivated for 3 days in the dark-grown plants. In the light, the specific activity of OASSase remained constant at 1.5 µmoles cysteine formed per min per mg protein for 4 days. The extractable APSSTase activity, however, increased from 25 to 150 nmoles per h per mg protein. These results indicate that in the primary leaves of bean APSSTase and OASSase have a similar intracellular distribution as in spinach.

BIOCHEMIE - BIOCHIMIE - BIOCHEMISTRY

M_{412} decay inhibition in the photocycle of bacteriorhodopsin by phenylisothiocyanate

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Chemical modification of bacteriorhodopsin by a hydrophobic probe has been investigated for delineation of related structural and functional characteristics of the membrane-integrated protein. Upon treatment of purple membranes of *Halobacterium halobium* with phenylisothiocyanate covalent, concentration dependent modification of bacteriorhodopsin was obtained. The incorporation rate saturated at approximately one mole phenylisothiocyanate per 1 mole of bacteriorhodopsin. The inhibition studies performed with phenylisothiocyanate resulted in reduction of the relative M_{412} decay rate constant in the photocycle of bacteriorhodopsin. A comparable phenylisothiocyanate concentration dependence was obtained for inhibition as described for the chemical modification. The M_{412} decay rate constant tended to saturate at 60% of the control value. These findings indicate a significant relation between chemical modification of the protein and retardation of the photocycle.

Damage of mitochondria induced by microsomal lipidperoxidation

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Carbontetrachloride is metabolized in the microsomal membrane to the radicals $CCL_3^{\cdot}$ and $Cl^{\cdot}$. The radicals lead to peroxidation of microsomal membrane lipids. Since it is known that lipidperoxides can serve as chain initiators in the peroxidation of unsaturated fatty acids we investigated whether lipidperoxides formed in microsomal membranes cause damage also in mitochondrial membranes. If such a long range damage to mitochondria were due to lipidperoxides it should be lower or absent in the presence of glutathione peroxidase which reduces organic peroxides ($ROOH + 2\ GSH \rightarrow ROH + GSSG$). We found that in vitro incubation of microsomal and mitochondrial membranes in the presence of NADPH and CCL_4 leads to peroxidation of both types of membrane. Glutathione peroxidase showed a strong protection only on the peroxidation of mitochondrial membranes suggesting that lipidperoxides formed in microsomes or metabolites derived from cause a long range damage in mitochondria.

Effect of iron chelators and specific *E. coli* bovine milk antibody on the growth of *E. coli*

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Enteric bacteria requiring iron for growth produce their own chelators (enterochelins), which transport iron to the cell. Bullen et al. (Br. med. J. *1*, 69, 1972) have shown that growth of *E. coli* is prevented in the presence of lactoferrin and specific human milk antibody. We observed a similar effect with native lactoferrin in bovine colostral whey proteins and media containing ovotransferrin. Between pH 7.5 and 8.0 the growth of *E. coli* was inhibited for at least 24 h by 200 µg/ml ovotransferrin, once internal iron reserves were exhausted. Bovine colostral whey preparations (100 µg/ml protein) with specific *E. coli* antibody activity inhibited growth for 5–7 h. A slight synergetic effect was found on simultaneous addition of specific antibody together with ovotransferrin. The iron chelators EDTA and desferrioxamine also inhibit the growth of *E. coli* at concentrations of 0.07 mM and 0.01 mM, respectively.

Effect of portal infusion of somatostatin (SRIF) on the exocrine function of rat pancreas (amylase, lipase and trypsin)

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When compared to control rats treated with saline, SRIF (100, 500 and 1000 µg/kg/h) infused into the portal vein of rats stimulated the secretion of pancreatic enzymes without affecting the output of pancreatico-biliary juice. Maximal stimulation of amylase, lipase and trypsin was observed immediately after the beginning of SRIF perfusion. Although the amount of enzymes secreted was dose-dependent, the stimulation lasted for about 30 min whatever the dose perfused was. Somatostatin induced a nonparallel secretion of pancreatic enzymes: all doses of SRIF induced a preferential stimulation of lipase, when compared to tryptic and amylase activities. This immediate effect cannot be due to preferential synthesis of a particular enzyme; it could be supposed that somatostatin acts differentially upon the intracellular transport and emeiocytosis of the considered enzymes.

Macrophages are activated by endocytosis of α2-macroglobulin-protease complexes to produce neutral proteases

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α2-macroglobulin (α2M) inactivates proteases released during inflammatory processes. After cleavage of one peptide bond the α2M molecule entraps the enzyme, sterically hindering access to the active site by protein substrates. α2M-protease complexes are readily taken up by macrophages. Uninduced peritoneal macrophages from 3-month-old Swiss mice were purified and cultured on ^{125}I-labelled fibrin surfaces. Proteolysis was followed by radioactivity released in the culture supernatants. Uninduced macrophages had little effect. Exposure to α2M-trypsin complexes dramatically increased proteolytic activity after 3 days. No proteolysis was detected after 1 day, very little after 2 days. Cycloheximide and prednisone inhibited the release of proteases by macrophages. Thus, endocytosis of α2M-trypsin complexe activates macrophages to produce and release proteases. α2M-protease complexes might play a role in the mechanism of self-perpetuation of inflammatory reactions.

A rabbit antiserum precipitating human galactosyltransferase

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Human galactosyltransferase (GT) was purified from pooled milk and malignant ascites from an individual using affinity chromatography on N-acetylglucosamine-agarose and alpha-lactalbumin-agarose. Both enzymes were homogeneous on SDS-polyacrylamide electrophoresis. Human milk galactosyltransferase was injected into rabbits in its native state (GT-n) and as a polymerisate obtained by a

cross-linking agent (GT-c). A low-dose injection scheme was applied. The rabbits produced precipitating antibodies against GT-n as revealed by the Ouchterlony technique. The anti-GT-n-antiserum reacted with native GT, polymerized GT and human milk; but no reaction was observed against sheep and cow's milk. The anti-GT-c-antiserum reacted against native and polymerized GT by forming 2 precipitation lines one of which was identical with the line obtained by using anti-GT-n-antiserum. In addition, anti-GT-c-antiserum cross-reacted with sheep and cow's milk. Pure ascites galactosyltransferase appeared to be immunologically identical with milk galactosyltransferase using the anti-GT-c-antiserum.

The effect of glycosidases on ligand-binding properties of purified acetylcholine receptor (AcChR)

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Although purified AcChR from a wide variety of tissues has been extensively characterized, relatively little attention has been paid in its carbohydrate content. Thus an attempt was made to modify the carbohydrate content of AcChR using sugar hydrolases and to determine the consequent effects on various AcChR properties. Incubation of receptor with a mixture of exoglycosidases or purified α-mannosidase reduced its concanavalin A-binding capacity by 13.9% and 63%, respectively. The binding capacity for the AcChR-specific neurotoxin, α-bungarotoxin, was also decreased, by 3.6% and 18%, respectively. A decrease of toxin-binding activity (16%) was also observed under the influence of an endoglycosidase (mannose-N-acetylglucosamine specific). Total AcChR carbohydrate content was measured in the experiment with endoglycosidase and a 32% drop was measured. The immunochemistry of modified AcChR is under investigation.

Saccharine-induced cephalic phase insulin secretion in normal rats and rats with denervated islets

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The effect of oral sweet stimuli on changes of peripheral blood levels of insulin and glucose was measured in unanesthetized, freely moving normal and islet-transplanted rats bearing chronic cardiac catheters for continuous blood sampling and oral fistulas. In normal rats, saccharine (1 ml, 0.15% in 1 min) produced a significant insulin response peaking at the second or third poststimulus minute, without any changes in glycemia. Tap water produced a significant, smaller insulin response. In rats with chemically destroyed B-cells but with functionally adequate islet transplants in the liver (adequacy assessed by normal glucose tolerance), the saccharine-induced insulin response was absent. These findings support the concept of a neurally mediated preabsorptive insulin reflex secretion upon oral sweet stimulation.

Hydration of a steroid epoxide by epoxide hydratase

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A radiometric assay for the determination of epoxide hydratase activity with a steroid epoxide (16α,17α-epoxy-1,3,5(10)-estratrien-3-ol) has been developed. The radioactivity measured after extraction of the product into ethylacetate resulted from the estratrien 3,16β,17α-triol. The apparent K_m-values with liver and testes microsomes were 10 μM; and 6.3 μM, respectively. The specific activity of epoxide hydratase with 16α,17α-epoxy-estratrienol as substrate was about twice as high as that with benzo(a)pyrene 4,5-oxide in all investigated organs (1.6-2.2-fold). Immunoprecipitation of solubilized liver microsomes with antibodies raised against homogeneous epoxide hydratase was measured with 16,17-epoxy-estratrienol, benzo(a)pyrene 4,5-oxide and styrene oxide. The extent of precipitation of activity towards all 3 substrates was the same which suggests that all 3 reactions are catalyzed by the same microsomal enzyme.

Does peroxidation of microsomal membrane lipids change the interaction of membrane proteins?

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The interaction of the microsomal NADPH-cytochrome P450-reductase and cytochrome P450 is an unresolved problem. Several authors postulate an interaction of the components by collision and movement in the transverse plane of the membrane. During incubation of microsomes in the presence of NADPH, Fe(2+) and EDTA, the activity of NADPH-cytochrome P450-reductase remained constant, whereas a loss of cytochrome P450 concomitant with lipid peroxidation was observed. The relative loss of cytochrome P450 in phenobarbital-pretreated rat liver microsomes was larger than in the control microsomes. It has previously been demonstrated, that lipid peroxidation leads to a cross-linking of membrane components. In an attempt to investigate the interaction of NADPH-cytochrome P450-reductase with cytochrome P450, the kinetics and extent of cytochrome P450 reduction were studied with peroxidized membranes and correlated with the degree of lipid peroxidation.

A hybrid consisting of 2 polyoma DNA units linked to a bacterial plasmid causes lytic infection in mouse fibroblasts

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Polyoma (PY) DNA was ligated to pBR322 through the BamHI site and cloned in *E. coli* HB101. 26 of 28 recombinants consisted of one unit each of PY and pBR322 DNA. 2 plasmids consisted of 1 pBR322 and 2 PY DNA units linked head-to-tail; one had a ~800 b.p. deletion in the PY moiety. None of the hybrids with only 1 polyoma DNA unit caused lytic infection of permissive cells, but cleavage with BamHI gave rise to a specific infectivity similar to that of BamHI-cleaved PY DNA. In contrast, supercoiled PY-pBR322 DNA hybrids containing 2 or 1.9 units of PY DNA caused lytic infection; the specific infectivities (based on full-length PY units) were 10% and 1%, respectively, of form I PY DNA. This shows that infectivity of PY DNA-containing hybrids most likely depends on excision of full-length PY DNA by legitimate recombination.

A localized difference in the solution conformation between ferri- and ferrocytochrome c

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The binding sites for ubiquinone-cytochrome c reductase (complex III) and for cytochrome c oxidase (complex IV)

on cytochrome c are virtually identical implying that cytochrome c oscillates between complexes III and IV during electron transfer (Rieder and Bosshard, FEBS Lett. *92*, 223, 1978). The necessary release of cytochrome c from complexes III and IV following the electron transfer reaction could be triggered by the localized conformational difference between ferri- and ferrocytochrome c which we observe at an area adjacent to the common binding site for complexes III and IV. The conformational difference was deduced from the slower rates of reaction with acetic anhydride of lysine residues 39, 53 and 55, but not of any of the remaining 16 lysine residues of ferrocytochrome c. Reaction rates were compared by the method of differential chemical modification (Rieder and Bosshard, J. biol. Chem. *253*, 6045, 1978). The crystal structures of ferri- and ferrocytochrome c are indistinguishable.

Studies on the regulation of malate dehydrogenase isoenzymes of *Schizosaccharomyces pombe* in continuous culture

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In batch cultures, with glucose as the substrate, *S. pombe* exhibits a secondary monoauxic growth, indicating the absence of the enzymes of the glyoxylic acid cycle. Previous experiments (Flury et al., Biochim. biophys. Acta *341*, 465, 1974) demonstrated the existence of 2 electrophoretically distinct isoenzymes of malate dehydrogenase (MDH), one being repressed by glucose. The X-D-diagram from a carbon-limited chemostat was characteristic for a glucose-sensitive organism. In derepressed cells 2 isoenzymes were present and were localized in the mitochondria and the cytoplasm, respectively. At dilution rates D above D_R the cytoplasmic isoenzyme disappeared. The kinetics of the regulation after pulsing derepressed growing cells with glucose indicated that besides the regulation of the MDH isoenzymes on the level of protein synthesis, another mechanism was involved, controlling the already existing isoenzymes.

Purification and characterization of murine T-cell specific surface antigens

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The Thy-1 molecule, the major membrane glycoprotein of mouse thymocyte, was isolated from culture medium of thymus fragments. Antigenic activity of the fractions obtained during the successive purification steps was assessed by inhibition of complement-mediated cytotoxicity of xenogenic anti-Thy-1 antiserum. A pure protein of apparent mol.wt of 25,000 daltons was obtained by several steps including gel-filtration, ion exchange chromatography and preparative isoelectric focusing. Whereas the water soluble protein showed 100% reactivity with xenogenic anti-Thy-1 antiserum when tested by indirect immunoprecipitation, it did not express the Thy-1.2 allogenic specificities. This might be explained by the lower degree of glycosylation observed between the purified protein and the native membrane antigens. However, peptide maps and 2-dimensional electrophoretic analysis revealed no major differences in the protein moiety of the water-soluble Thy-1 molecule and the membrane components obtained by immuno-precipitation from cell extracts with allo- and xenogenic antisera.

The primary structure of porcine plasminogen

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The polypeptide chain of porcine plasminogen (mol.wt approx. 90,000) was cleaved with CNBr. Using gel-filtration the NH_2-terminal fragment (58 amino acids) and an undecapeptide were separated from the bulk of disulfide-bridged fragments. This latter fraction, after reduction and carboxamidomethylation yielded an additional 6 fragments in the mol.wt range 2000 to 30,000 daltons. The fragmentation pattern differs from that of human plasminogen probably due to different constellations at Met-x bonds. The purified fragments were characterized by their amino acid composition and by partial sequence determination by the automated Edman method. These data compared with the known primary structure of human plasminogen allowed the assignment and localization of the CNBr-fragments within the porcine plasminogen polypeptide chain. Besides homologous regions the partial sequence of porcine plasminogen also shows distinct sequences differing considerably from the primary structure of human plasminogen.

Formation of cysteine from adenosine 5′-phosphosulfate (APS) in spinach chloroplast extract

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Reduced glutathione (GSH) has been proposed to act as carrier in SO_4''-reduction from APS in chlorella. In spinach chloroplasts a concentration of 3 mM GSH has been reported. Therefore we set up a reconstituted chloroplast system containing 3 mM GSH, in which SO_4'', given as ^{35}S-APS, is reduced to the thiol level and incorporated into cysteine. The reaction depends on GSH, APS, O-acetyl-L-serine and reduced ferredoxin, produced in the light with thylakoid membranes and ascorbate/dichlorophenolindophenol as e^- donor. The rate of cysteine formation found is about 1 nmole/mg prot./h. With oxidized glutathione replacing GSH no appreciable cysteine formation is observed. When GSH is replaced by dithiothreitol similar rates of SO_4''-reduction are found. The addition of different spinach thioredoxin fractions does not enhance the reaction.

Regulatory effects of a 17,000 mol.wt component in phosphorylase kinase

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Rabbit muscle phosphorylase kinase preparations (PhK) contain a heat-stable 17,000 mol.wt component (17k) (Cohen et al., FEBS Lett. *92*, 287, 1978) closely related to the Ca-dependent regulatory protein (CDR) of phosphodiesterase (PDE) and adenylate cyclase (AC). Both heat-denatured PhK and isolated 17k stimulated PDE and AC, 17k being as efficient in PDE activation as CDR. Gel-filtration of PhK fractions incubated with EGTA (PhK^-) or with Ca^{2+} + CDR (PhK^+) gave ratios of 17k/monomer of 0.2 for PhK^- and 0.7 for PhK^+, as determined by both gel-electrophoresis and PDE assay. Complete removal of 17k from PhK could not be achieved by use of either DEAE 52 or phosphocellulose, nor by immobilized Troponin-I. Both PhK and PhK^-, but not PhK^+, showed reversible Ca-dependent binding to immobilized CDR, indicating that

PhK^+ is probably saturated with CDR. Isolated 17k as well as CDR stimulated PhK activity at pH 6.8, but not at pH 8.2; this activation was inhibited by trifluoperazine.

The effects of inhibitors of oxidation on proton translocation by cytochrome oxidase

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According to the chemi-osmotic hypothesis of Mitchell the role of cytochrome oxidase in proton translocation by mitochondria is to carry out vectorial electron transport. Recent work in this laboratory indicates that, concomitantly with electron transport, cytochrome oxidase in addition carries out proton translocation. Ferrocytochrome c induces proton extrusion from reconstituted cytochrome oxidase vesicles and this is abolished by protonophores. The stoichiometry of protons translocated per electron equivalent is approximately one. Azide and cyanide are well-established inhibitors of oxidative activities in cytochrome oxidase. We demonstrate here that these compounds also inhibit ferrocytochrome c induced proton extrusion from reconstituted cytochrome oxidase vesicles comparably with their effects on ferrocytochrome c oxidation. These parallel inhibitions provide further evidence in support of proton translocation being a general property of cytochrome oxidase.

Structural and functional properties of a protein binding to C4b

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We have recently reported the purification of a plasma macroglobulin binding to sepharose-IgG. This β1-glycoprotein has a mol.wt of about 500 kdaltons, a sedimentation coefficient of 12 S, and is composed of 5 to 6 disulfide-linked subunits of 95 kdaltons. Its serum concentration is about 0.3 mg/ml. These properties resemble those of a recently described C4b-binding protein. The protein bound to sepharose-IgG has been shown to form stable complexes with C4b, but not with native C4. In the presence of purified C3b-inactivator (C3b-INA) and of this protein, the α'-chain of C4b is cleaved into 3 fragments. One of these is released (C4d), while the 2 other remain disulfide-linked to the rest of the molecule (C4c). Native C4 is not affected. Neither the C4b-binding protein nor C3b-INA alone cleave C4b. This report confirms recent results showing that a unique serum protein, distinct from β1H, serves as a cofactor for C4b cleavage by C3b-INA. Its possible role as a cofactor for cleavage of C3b is currently under investigation.

Lactase-phlorizin hydrolase from the small intestine of the suckling rat: purification and kinetic analysis of proximal and distal forms of the enzyme

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The initial report of the existence of 2 different forms of lactasephlorizin hydrolase in the small intestine of suckling rats (J. R. Green and H. P. Hauri, FEBS Lett. *84*, 233, 1977) has been confirmed and investigated further. The 2 forms of the enzyme were purified in high yield from the proximal and distal regions, respectively, of the suckling rat small intestine by a modification of the method of Schlegel-Haueter et al. (Biochim. biophys. Acta *258*, 506, 1972). During chromatography on DEAE cellulose at pH 6.0 the proximal form of the enzyme eluted at a lower ionic strength than the distal one. However, kinetic analysis indicated that K_m, pH activity curves, phlorizin hydrolase stability at 45 °C and lactase lability at 45 °C were all similar for the 2 purified enzymes.

Interaction of human red cell membrane acetylcholinesterase with lipids

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Pure human erythrocyte membrane acetylcholinesterase is an enzymatically active dimer in presence of detergent micelles. In absence of detergents the dimers losses its activity when diluted to a concentration which prevents self-aggregation of the enzyme. The effect of phospholipids on the activity of the dimeric form of the enzyme was investigated by diluting the dimer into sonicated lipid suspensions. Total human erythrocyte membrane lipids are able to fully preserve activity at 2 mg lipids/ml after 30 min of incubation. Individual lipids (PS > PI > PC = PE > SM) preserved activity, though to a lesser extent than total erythrocyte lipids. Except for PI and PE enzyme activity remained stable for at least 5 h. It was further shown, that only the dimer is incorporated to any extent into preformed liposomes, whereas the aggregated enzyme is inable to do so.

Plasminolysis fragments of fibronectins that retain antigenic and attachment-mediating activity

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Mild plasmin digestion cleaves the region containing the interchain disulfides from horse serum and human plasma fibronectins (FN), leading to the appearance of prominent bands (SDS gels, reducing and nonreducing conditions) at ca. 200 and 210 K, 20–30 K smaller than the reduced subunits of the intact FNs. Like the FNs, these fragments bound to gelatine-sepharose; they were isolated by elution with 4 M urea. Purified fragments reacted with antibodies against human FN in immunodiffusion tests and were 80–90% as active as the corresponding FNs in promoting myoblast attachment as assayed at 20 h (method: E.C. Puri and D.C. Turner, Exp. Cell Res. *115*, 159, 1978), but were less than 10% as active at 2 h. Disulfide-linked dimers are thus not essential for mediating cell attachment. Horse and human FNs reduced and alkylated under denaturing conditions did not form precipitin lines with antibody, bind to gelatin-sepharose, or promote myoblast attachment at 2 or 20 h.

Structure and dynamics of the active site of mitochondrial aspartate aminotransferase

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The knowledge of the spatial structure combined with sequence information has provided a static picture of the active site of the holoenzyme in the pyridoxal form. The molecular activity of non-cross-linked microcrystals without diffusional rate-limitation (thickness < 1 μm) is 3% of that of the enzyme in solution. Microspectrophotometric measurements with polarized light were performed on

single crystals. The extinction was determined as a function of crystal orientation. While the results for the pyridoxal form were as expected from the X-ray structure, those in the presence of substrates or substrate analogs indicated rotatory movement of the coenzyme during the catalytic process. High resolution crystallographic studies of different functional states of the enzyme are underway.

Characterization of cAMP-dependent protein kinases in GH_3-cells

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The cAMP-dependent protein kinase activity of GH_3-cells is predominantly found in the cytosol fraction. 2 forms of isoenzymes could be detected: PK-I (Corbin type I) and PK-II (Corbin type II). These 2 forms have been characterized by ion-exchange chromatography, gel-filtration and polyacrylamide gel-electrophoresis under nondenaturating conditions. The holoenzyme (R_2C_2) of PK-I has a mol.wt of 240,000 with cAMP-binding proteins (R_2) of 124,000 mol.wt, whereas PK-II displays a mol.wt of 185,000 (R_2C_2), with a cAMP-binding protein dimer (R_2) of 102,000 mol.wt. In addition, a cAMP-binding protein with mol.wt of 36,000 could be detected, which is presumably derived from the 102,000 R_2-dimer. The catalytic subunits (C_2) exhibited a mol.wt of 40,000 for both isoenzymes. There is evidence that these protein kinases are activated during the release of prolactin after TRH-stimulation.

Induction of alkaline phosphatase activity by diphosphonates in cultured bone cells

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The diphosphonates are compounds similar to PPi but containing a P-C-P instead of a P-O-P bond. 2 diphosphonates, dichloromethane diphosphonate (Cl_2MDP) and ethane-1-hydroxy-1,1-diphosphonate (EHDP), were investigated for their effect on alkaline phosphatase. Cl_2MDP increased the activity by a factor of 30, EHDP being less effective, whereas neither diphosphonate had any effect on acid phosphatase. The effect was dependent on protein synthesis, but independent on cell growth, therefore excluding cell selection. After subculturing treated cells in a diphosphonate-free medium, the enzyme activity fell almost to control levels. The physicochemical characteristics of the control and stimulated enzyme were indistinguishable. Since alkaline phosphatase can be stimulated by substrates in cultured cells, it is possible that the diphosphonates, which are analogues of PPi (a substrate of alkaline phosphatase) but which are not hydrolyzed by the enzyme, act by a similar mechanism.

Glycoprotein changes in a mouse melanoma variant with reduced metastasis

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Glycoproteins were compared in a metastasizing cell line (F_1) of B16 melanoma, and a WGA-resistant clone (Wa-4), which has reduced metastatic capacity. Cell surface proteins were labelled with lactoperoxidase-iodination and ^3H-reduction after periodate or galactose oxidase treatment and analyzed by SDS gel-electrophoresis. Differences were revealed in the relative mobilities of several major bands. Binding of ^{125}I-WGA to cellular proteins in gels following electrophoresis showed that some major components detected in F_1 cells were not seen in Wa-4 cells. Analysis of glycopeptides prepared by pronase digestion revealed differences in the complex N-glycosidic carbohydrate chains, whereas the mannose-rich N-glycosidic chains and the O-glycosidic chains were similar. Structural analysis revealed that the change in Wa-4 cells involved reduction of the sialic acid residues bound to C-3 galactose to half and a concomitant increase in the fucose bound to C-3 of 4-substituted N-acetylglucosamine.

The structure of mitochondrial aspartate aminotransferase at 3.2 Å resolution

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Mitochondrial aspartate aminotransferase, a pyridoxal phosphate dependent enzyme, is an α_2 dimer of 2×401 amino acids. In an electron density map at 3.2 Å resolution, the course of the polypeptide chain could clearly be followed in both subunits. Side chain densities could be identified with the aid of the amino acid sequence. The subunits are related by a molecular dyad. The spatial structure, a novel one, contains per subunit 15 helices and a mainly parallel, 7-stranded twisted β-sheet behind the coenzyme. In total, the secondary structure is estimated as 50% α-helix, 12% β-sheet and 12% β-turns. The interactions between the subunits are mainly hydrophobic. One wall of the active site pocket is provided by the neighboring subunit. The aldimine linkage between Lys-258 and the coenzyme can be seen. Cys-166, which is susceptible to syncatalytic changes in reactivity is 25 Å away from the coenzyme.

Inhibition of vitamin B_{12}-binding to transcobalamin II

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Two fractions (45 and 66) isolated from media fermented by *Coryne-bacterium* sp. effectively inhibited the binding of vitamin B_{12} to transcobalamin II (TC II). TC II is a B12-binding protein in human serum, essential for transporting B12 to various cells. The inhibition of B_{12}-binding to TC II was determined by our immunochemical method based on the precipitation of labelled TC II by insolubilized anti-TC II antiserum (radioimmunosorbent technique). Partially purified 45 exhibited a single spot in TLC and no UV-absorption. 45 completely inhibited the binding of B_{12} to unsaturated TC II at R.T. in a dose-dependent, concentration-independent fashion. A 16-fold excess of 45 displaced 70% of labelled B_{12} from TC II-B_{12}. Accordingly, excess B_{12} could, under certain conditions, displace 45 from the TC II-inhibitor complex. The second inhibitor, 66, was purified and identified as a protein of mol.wt 47,000 (UV max. at 278 nm) with cytotoxic activity towards Eagle's KB-cells. The inhibiting properties of 66 were similar to those described for 45, although 66 appeared to be even more effective in displacing B_{12} from its binding site on TC II.

Properties of antibodies to peripheral and intrinsic red cell membrane proteins

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Antibodies have been raised in rabbits against the major peripheral and intrinsic red cell membrane proteins purified by preparative SDS-PAGE. The specificity of these reagents has been tested by complement mediated cytotoxicity on ^{51}Cr-labelled red cells, direct immunoreaction with

membrane proteins resolved on SDS-PAGE or indirect immunoprecipitation from membrane extracts of labelled red cells. Whereas antibodies to band 3 revealed only antigenic determinants located on the cytoplasmic portion of this transmembrane protein, antibodies to the major sialoglycopeptides were directed to both the cell surface and cytoplasmic segments of the protein. In addition, differences in antigenicity could be detected between glycophorin A and B, 2 of the 3 species of red cell sialoglycopeptides previously described. Finally, a close structural association between band 3 and spectrin was suggested by the co-precipitability of these 2 membrane components from NP.40 cell extracts when specific antibodies to either protein were used.

Studies on the specificity of a fluorogenic peptide substrate for enteropeptidase

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The N-terminal sequence of trypsinogen, -$(Asp)_4$-Lys-, constitutes the major substrate recognition and specificity determinant for enteropeptidase. Hesford et al. (FEBS Lett. *71*, 279, 1976) synthesized a naphthylamine derivative of this sequence and demonstrated its hydrolysis by enteropeptidase. The specificity of the peptide Gly-(L-Asp)$_4$-L-Lys-2-naphthylamide for the direct estimation of enteropeptidase has now been investigated in more detail. There is evidence that more than one intestinal enzyme is capable of attacking the peptide. Duodenal juice from patients with congenital enteropeptidase deficiency still hydrolyzes the substrate, but at a much slower rate than normal juice. The fractionation of extracts of rat intestinal mucosa by gelfiltration partially resolves several components which can attack the substrate. These additional reactions can be modulated by alterations in the assay conditions and by the judicious use of various inhibitors.

Glycerol transport in blood stream form of *Trypanosoma brucei*

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Glycerol penetrates in the long slender blood stream form of *T. brucei* through a passive mediated carrier. The analogs 1.2- or 1.3-propanediol are not translocated by the glycerol carrier, they penetrate by free diffusion. In opposition to other trypanosomes, D-glucose does not interact with the glycerol transport system. Under aerobiosis, the observed uptake accounts for the coupled reactions of penetration and phosphorylation, free glycerol cannot be detected inside the cells. In contrast, under anaerobiosis, glycerol which is then a glycolytic end-product, is produced through the reversal of glycerol kinase. In this case intracellular glycerol accumulation is prevented by the carrier facilitated efflux.

Internalization of lactogenic hormones in mammary cells

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Internalization of lactogenic hormones (oPRL) was followed in mammary cells in order to characterize the cellular events which allow these hormones to exert their effect on gene expression. Cells (5×10^6) were incubated at 4 °C for 6 days with 25 pM ^{125}I-oPRL, washed, brought to 37 °C and processed for light and EM autoradiography. At 4 °C, all the grains were at the cell surface. At 37 °C, 30%, 50% and 65% of the grains at 5, 15 and 30 min, respectively, were associated with intracellular membranes, and 8%, 18% and 25% with nuclei. Upon elution from cells or from purified nuclei, ^{125}I-material comigrated in SDS-PAGE with native oPRL, except in cells incubated 15 and 30 min at 37 °C, in which some lower mol.wt hormone was found, indicating intracellular degradation. The correlation of rapid uptake of oPRL and nuclear localization with fast increase in rate of casein transcription suggest that oPRL, alone or associated with its receptor, acts intracellularly on nuclear structures.

Variant and conserved amino acid residues in the spatial structure of aspartate aminotransferases

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Both immunological and amino acid sequence studies have shown that the mitochondrial (m) and cytosolic (c) isoenzymes of aspartate aminotransferase (AAT) are homologous proteins. A comparison of the sequence of mAAT from chicken (determined to 85%) with those of mAAT and cAAT from pig shows that the degree of interspecies sequence identity between the 2 mitochondrial isoenzymes (about 85%) markedly exceeds that between cAAT and mAAT from pig (48%). Furthermore, the 2 mitochondrial isoenzymes crystallize roughly isomorphously. All significant differences in electron density correlate with individual amino acid substitutions. As expected, highly conserved segments of the AAT sequence are found in the interior of the molecule, at the active site, and at the subunit interface.

Ca^{2+} translocation in Ehrlich ascites tumor cells

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Ca^{2+} uptake into Ehrlich ascites tumor cells was studied at 0 °C in the presence of mitochondrial inhibitors, conditions that minimized complications caused by excretion or sequestration of Ca^{2+} by organelles. Under these conditions ruthenium red inhibited Ca^{2+} uptake, but other previously implicated ions, such as P_i or Mg^{2+} had no effect. Valinomycin either inhibited or slightly stimulated Ca^{2} uptake depending on the size and orientation of the K^+ gradient across the membrane. Nigericin inhibited Ca^{2} transport. These data suggest an electrogenic uptake of Ca^{2+}, perhaps (associated with an excretion of protons) by an H^+/Ca^{2+} antiport mechanism. Glucose inhibited Ca^{2+} uptake suggesting the possibility of an energy-driven Ca^{2+} expulsion mechanism. Consistent with this possibility it was found that plasmamembranes of ascites tumor cells contain a Ca^{2+}-dependent ATPase. These preparations when incorporated into liposomes in an inside-out orientation, catalyzed an ATP-dependent uptake of Ca^{2+}.

Study on insulin secretion by genetically obese rats

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The in vivo secretion of insulin and the possible involvement of the vagus nerve were studied in pair-fed (i.e.

nonhyperphagic) anesthetized genetically obese *(fa/fa)* rats. All experimental groups consisted of 6 animals. 4-week-old obese rats had already increased basal insulin levels (4 ng/ml versus 1.5 ng/ml for controls), an abnormality that became more marked at 9 weeks of age (10 ng/ml). Following an i.v. glucose bolus to 4-week-old rats, insulin secretion was greater (14.3±1.3 ng/ml) in obese than in controls (6.6±0.5 ng/ml). The over-insulin secretion, tested under identical conditions, became more marked in 9-week-old obese rats, i.e. 25.6±1.6 ng/ml versus 8.6±0.8 ng/ml) for controls. In these experiments, while glucose disappearance was more rapid in the 4-week-old obese animals than in controls, it was identical to controls at 9 weeks of age. This indicated progressive appearance of insulin resistance. Using an i.v. arginine bolus, analogous results were observed with regard to insulin secretion in obese animals, although the abnormalities were even more marked than with glucose. In all the experiments mentioned above acute bilateral abdominal vagotomy failed to reverse the hyperinsulinemia of obese animals toward normal, in contrast to what has been reported in rats made hyperinsulinemic by hypothalamic lesions. In conclusion: obese *fa/fa* rats have basal and stimulated insulin secretion that is greater than normal, an abnormality that worsens with age, and is apparently not vagally-mediated.

A new method to determine the interaction of lectins with polysaccharides and glycoproteins: An application of ellipsometry

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Ellipsometry is the measurement of the effect of reflection on the state of polarization of light. Such measurements may be used to estimate the thickness of a film adsorbed on a reflecting material. This rapid method has now been used to measure the thickness of layers adsorbed specifically from lectin solutions on a film of polysaccharide or glycoprotein deposited on the surface of nickel-coated glass slide. The interaction of tetravalent concanavalin A (ConA) and divalent succinyl-ConA with yeast mannan was studied in function of the lectin concentration. By extrapolating to infinite concentration, the thickness of bound succ-ConA and ConA was found to be 42 and 77 nm, respectively. By Scatchard analysis, the binding constant of both molecules was found in the same order of magnitude ($0.2\text{-}1\times10^7$ M^{-1}). Ceruloplasmin, fetuin, the carcinoembryonic antigen (CEA) and a β-galactosidase from *Aspergillus niger* reacted with wheat germ agglutinin, soya bean agglutin, the *Ricinus communis* lectin RCA_I and ConA in agreement with the qualitative sugar composition of the glycoproteins.

Effects of extracellular Ca^{2+} and Mg^{2+} on hepatic α-adrenergic Ca^{2+} release and glycogenolytic response

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α-adrenergic activation of glycogenolysis in perfused rat liver is paralleled by a net release of Ca^{2+}. By attempting to modify the Ca^{2+} efflux we tried to relate the amount of Ca^{2+} released with the metabolic response. Livers were perfused in a nonrecirculating system. Ca^{2+} and Mg^{2+} were added in varying concentrations. Chlortetracycline (CTC) fluorescence was used to probe calcium movements in response to phenylephrine (0.5 μM). The release of glucose and lactate was measured as an index of glycogenolysis. Extracellular Ca^{2+}/Mg^{2+} ratios were positively correlated with CTC-fluorescence changes ($p < 0.05$) and with metabolic responses ($p < 0.01$). CTC-fluorescence changes and metabolic responses were correlated as well ($p < 0.01$). Extracellular Ca^{2+} and Mg^{2+} may compete for uptake into the phenylephrine sensitive pool. Release of Ca^{2+} may be the trigger for the metabolic response.

Interaction between artificial membranes and sheep red cell membranes

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Ruminants have a) a low content of lecithin and b) a phospholipase A_2 in their red cell membranes. In order to understand the function of this enzyme we have studied the interactions between liposomes and sheep red cell ghosts. When sheep red cell membranes are incubated in presence of sonicated dispersions of lecithins, a net uptake of the phospholipid by the ghosts occurs. In presence of ethylene diamine tetraacetate (phospholipase inhibited) the uptaken lecithin remains unaffected whereas in presence of calcium it is rapidly hydrolyzed, whereby the liberated fatty acid is transferred to the liposome. External lecithin from liposomes and sheep serum and phosphatidylethanolamine tend to chase the uptaken lecithin whereas sphingomyelin does not. The uptake of lecithin is independent of the cholesterol content of the liposomes but is temperature-dependent with a maximum near 30 °C. It increases with ionic strength of the medium and shows a pH-dependence with a minimum at pH 7.

A comparison of eosinophil and neutrophil peroxydases from horse leukocytes

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Neutrophil peroxydases (NP) are well-characterized, whereas the eosinophil peroxydases (EP) are much less known. Our method (Experientia *34*, 1654, 1978) for the isolation of pure horse eosinophils permitted a comparative study of EP and NP. By discelectrophoresis (pH 4.8) NP can be separated into 2 bands, one being probably due to the catalase. The EP can be separated into at least 5-6 isoenzymes, 4 bands of which migrate much faster than NP. After homogenization in water, 6% of NP and 50% of EP are dissolved. A_{430}/A_{280} of our NP-preparation was 0.6-0.68 and A_{415}/A_{280} of the EP 0.8. 50% inhibition of the NP was obtained by 4×10^{-7} M CN^- and 2.6×10^{-5} M N_3^-, that of the EP by 9.1×10^{-8} M CN^- and 7.8×10^{-5} M N_3^-. Both peroxydases are less inhibited by 3-amino-1,2,4-triazol (NP 50% at 0.13 M, EP 50% 6.8×10^{-4} M). EP as well NP show NADH and NADPH-oxydase activity (450-550 mU/mg protein). These activities can be activated over 100-fold by 2,4-dichlorphenol (DCP) (for a 100× NADH-ox EP activation: 3×10^{-5} M DCP; NP: 3×10^{-3} M DCP) and inhibited by CN^-.

Abnormal water balance in experimental and genetic obesity

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Experimental obesity produced by stereotaxic lesions of the ventromedial hypothalamus (VHM) resulted in hyperphagia, polydipsia, decreased water retention and urine osmo-

lality, enhanced excretion of total solute and urea. A 24 h water deprivation test unmasked the unability of VMH-lesioned rats to raise antidiuretic hormone (ADH) in urine. It is suggested that VMH destruction induced an impairment of ADH production resulting in a state of diabetes insipidus which in turn elicited compensatory drinking. Genetic obese *(fa/fa)* rats exhibited hyperphagia, polydipsia, decreased water retention, enhanced excretion of total solute and urea. Under water deprivation, obese rats produced as much ADH in urine as lean rats and despite of that excreted larger volumes of diluted urine. Prostaglandins PGE_2 levels in urine of *fa/fa* rats exceeded control values by a factor 3. As PGE_2 are known to antagonize the action of ADH, it is proposed that the overproduction of PGE_2 by *fa/fa* rats might account for their impaired water retention.

Covalent modification of human erythrocyte band 3 and phosphate transport inhibition by hydrophobic arylisothiocyanates

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The covalent chemical modification of human erythrocyte band 3 by phenyl-(^{14}C)-isothiocyanate has been confirmed by purification of the labelled protein. After labelling of human erythrocyte ghosts with phenyl-(^{14}C)-isothiocyanate band 3 was isolated. Under defined conditions a ratio of 0.5 mole phenylisothiocyanate bound per mole polypeptide was determined in the purified, electrophoretically homogeneous protein. The chemical modification of red cells with phenylisothiocyanate resulted in 60% inhibition of phosphate influx compared to 79% for the similarly hydrophobic 2-naphthylisothiocyanate and 74% for the characterized phosphate transport inhibitor p-sulphophenyl isothiocyanate. Further insights into the chemical architecture of the anion transport protein are expected to be gained by studying the structural properties of the chemically modified peptide(s).

A comparative study of horse and human eosinophilic leukocytes

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The aim of this study was the comparison of the properties of horse eosinophils (HoE) with those of the human eosinophils (HE), more difficult to isolate in pure state. The following results were obtained with cells isolated according to Experientia *34*, 1654 (1978). In the Boyden chamber, the eosinophils of both species show chemotaxis against swine serum. During phagocytosis of zymosan, opsonized with isologic serum, the O_2-consumption by HE (7.1 nmoles O_{at}/min/10^6c.) was greater than that of HoE (1.08 nmoles) and the O_2^--production twice higher in HoE than in HE. The HoE-peroxydase can be separated by discelectrophoresis into 5-6 and the HE-peroxydase into 3-4 isoenzymes. 2 ATPases and 3 ac. phosphatases can be separated in the HoE- and only 1 ATPase and 2 ac. phosphatases in the HE-extract. The number and the migration of the SOD-, catalase- and LDH-isoenzymes are identical in both cell species. The specific activities of β-glucuronidase, cathepsin D and ac. phosphatase are similar in both cell types. The activity of LDH, peroxydase and alk. phosphatase in the HoE is twice as high as that of HE, that of the arylsulfatase in about 3 times and that of the catalase 10 times greater in HE than in HoE.

In vivo distribution of fluorescently labelled cytoskeletal components microinjected into human fibroblasts

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Graessmann's microinjection technique was chosen to introduce fluorescently labelled cytoskeletal proteins into fibroblasts. The injected cells were examined during culture by fluorescence microscopy. After 1-2 h, rhodamine-labelled actin exhibited a distinct network of fluorescent filaments, resembling the stress fibres observed by classical immunofluorescence microscopy. Thus, the injected protein seems both to enter the intrinsic actin pool of the cell and to participate in an assembly and disassembly of filamentous structures. Examination of the fluorescent fibres by reflection contrast microscopy indicated their close locatin to the substratum. Injected myosin SF-1 formed similar structures, whereas BSA was homogenously distributed at the beginning and then segregated into cytoplasmic granules. The method described will be used to investigate other proteins in their effect on dynamic aspects of cytoskeleton organization.

Role of a membrane-bound glycoprotein in the translocation of polymeric immunoglobulins through epithelia

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Rabbit secretory component (SC), a glycoprotein synthetized and secreted into milk by mammary gland epithelial cells, binds with high affinity to mammary membranes and cells ($K_a = 4.6 \times 10^8$ M^{-1}, $k_{1+} \cong 3 \times 10^6$ M^{-1} min^{-1}, $k_{1-} \cong 6 \times 10^{-3}$ min^{-1}). Number of binding sites varies according to the degree of occupancy by endogenous SC (3150±1250 sites per cell, n = 6). Liver membranes also express SC at their surface, but do not bind further SC, whereas heart membranes do not express nor bind SC. Rabbit dimeric $(IgA)_2$, isolated from sIgA by dissociation in 3 M KSCN interacts with membrane bound SC both on liver and mammary membranes, as well as on mammary cells ($K_a \cong 1 \times 10^9$ M^{-1}, $k_{1+} \cong 6 \times 10^6$ M^{-1} min^{-1}, $k_{1-} = 6 \times 10^{-3}$ min^{-1}). No binding to heart membranes occurs. $(IgA)_2$ binding is inhibited by free SC and Fab anti-SC. Thus binding of SC to epithelial cells constitutes the first step in the translocation of polymeric immunoglobulins across epithelia.

Plasma membrane ATPase activity in different yeasts

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Differences have been found in pH-optimum and specific ATPase activity in plasma membranes of yeasts. In order to examine whether these differences are due to variations in yeast strains or species we checked ATPase activity of plasma membranes in 3 strains of *Saccharomyces cerevisiae* and in *Candida tropicalis* grown on glucose or hexadecane. In all the yeasts tested the ATPase optimum was near pH 7 except for that in *C. tropicalis* grown on hexadecane, where the pH-optimum was comparatively broad and shifted towards pH 8. This fact points to the importance of the growth conditions. A sharper pH-optimum and a higher specific activity of the ATPase could be obtained by using sucrose density gradients for purification of the plasma membranes. Finally we compared membrane proteins

which could be labelled by incubation of the membranes of both yeast species with [γ-^{32}P]ATP and Mg^{++}. In both cases phosphoproteins with mol.wts of 110,000 and 160,000 could be detected. In *C. tropicalis* also phosphoproteins with lower mol.wts were visible on gel-electrophoresis.

Studies on isolated lysine-binding regions of human plasminogen

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Native human plasminogen was partially fragmented with elastase. By affinity chromatography on Lysine-Biogel, followed by gel-filtration on sephadex G-75 2 fragments were isolated which still had intact binding sites for ω-aminocarboxylic acids, such as lysine or 6-aminohexanoic acid. The smaller fragment (approx. 10,000 daltons) corresponds according to amino acid composition and sequence to the 4th kringle of the Magnusson plasminogen model (H. Claeys et al., FEBS Lett. *61*, 20, 1976). The larger fragment (approx. 40,000 daltons) which is situated in the NH_2-terminal part of the original polypeptide chain, yielded after chymotryptic digestion a subfragment comprising the region of the first kringle which also had lysine binding capacity. In the 2 isolated kringles the nature of the lysine binding site and its behavior towards 6-aminohexanoic acid was studied by specific derivatization of certain amino acid residues and also by equilibrium dialysis in the presence of 6-aminohexanoic acid.

Transmembrane protein structure in the erythrocyte ghost

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The conformation and arrangement of membrane proteins can be studied by X-ray diffraction from oriented membrane specimens in the region of 10.5 A. The orientation of the membranes is determined from the meridional reflections from the lamellar stacking. Oriented erythrocyte ghost pellets produce equatorial 10.5 A diffraction and a meridional 1.5 A reflection; the ratio ε of the equatorial and the meridional intensities of the 10.5 A band is $\varepsilon \simeq 1.6$-4.0 in human and sheep ghosts. Thus a significant part of the membrane polypeptide chains oriented normal to the membrane are arranged as side-to-side packed α-helices; from biochemical data it is tentatively concluded that they belong to the major transmembrane proteins glycophorin and band 3. Changes in ε are observed if the glycophorin-specific phytohemagglutinin is bound to membrane receptors; ε also depends on membrane disorientation, but ligand-induced rearrangements of transmembrane proteins cannot be excluded. Analogous studies with human lymphocyte membranes were initiated.

The mechanism of spectrin phosphorylation in human erythrocyte ghosts

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Phosphorylation and dephosphorylation by Mg-ATP of spectrin component 2 has been shown to be related to the shape of the cell. We tested the hypothesis whether this reaction is catalyzed by both extrinsic kinase and phosphatase or occurs within the spectrin molecule itself. With 0.5 M NaCl we thus removed the kinases (i.e. casein kinase), which have been suggested to catalyze the above reaction (Avruch and Fairbanks, J. Cell Physiol. *89*, 815, 1976). This treatment did not abolish spectrin phosphorylation. It reduced, however, the phosphorylation level at 2 mM ATP from 0.6 to 0.36 moles phosphorous per mole of component 2. Accordingly, the critical ATP concentration to produce disc cells was increased in these salt-treated ghosts. Dephosphorylation was not affected by salt extraction. These results are consistent with the second hypothesis, i.e., spectrin being a self-phosphorylating and -dephosphorylating system.

Specific binding of ^{3}H-spiroperidol in the absence of dopamine-sensitive adenylate cyclase in the A10 cell region of the rat brain

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The 2 major dopamine (DA) neurone systems in the rat brain are the nigrostriatal A9- and the mesolimbic A10-systems. The A9-cell body-dendritic region (substantia nigra) contains a DA-stimulated adenylate cyclase and specific spiroperidol binding receptors. These parameters were investigated in the A10-cell body region (area ventralis tegmenti) punched out from frozen brain sections. In contrast to the A9-cell region, DA failed to stimulate adenylate cyclase in the A10-cell region, although the enzyme was stimulated by GMPPNP (a GTP analogue) and NaF. However, specific binding of spiroperidol could be measured in the A10- as well as in the A9-cell region. This binding was stereospecific, saturable, heat sensitive and showed a linear dependence on protein. This suggests a) that DA receptors are present in the A10-cell region, and b) that these DA-receptors are unrelated to adenylate cyclase, unlike one type of DA-receptor found in the A9-cell region.

Regulatory role of glutathione peroxidase in rat liver mitochondria

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In selenium deficiency the mitochondrial glutathione peroxidase activity is decreased to 0-5% of control. The Arrhenius plot of the Ca^{++}-transport shows 2 breaks in selenium supplemented animals (at 24 °C and 12 °C), whereas in selenium-deficient animals the break at 12 °C is lacking. After loading mitochondria with Ca^{++} a rapid Ca^{++}-release and a simultaneous oxidation of endogenous NAD(P)H (due to the action of glutathione reductase) can be induced by an organic hydroperoxide or H_2O_2 only when glutathione peroxidase is present in the matrix (selenium supplemented). No Ca^{++}-release and no oxidation of NAD(P)H is observed in selenium deficiency. Peroxide-induced oxidation of the mitochondrial NAD(P)H is reversible in the presence of succinate when no Ca^{++} has been taken up. In the presence of accumulated Ca^{++}, however, NAD(P) is not re-reduced, and a simultaneous Ca^{++}-efflux into the medium takes place. The possibility that the transport of Ca^{++} and pyridine nucleotides is coupled will be discussed.

Paracatalytic modification of aldolase: A side reaction of the catalytic cycle resulting in irreversible blocking of 2 active site residues

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For enzymes producing carbanion intermediates, the combination of the normal substrate with a suitable electron-

acceptor has been proposed as a highly specific binary system for their active site-directed modification. Such paracatalytic enzyme modifications are due to transiently reactive intermediates which are generated during the oxidation of the enzyme-activated substrate and which, without being released from the enzyme, modify groups at the active site (Meth. Enzym. *46*, 48, 1977). For example, fructose-1,6-P_2 aldolase from rabbit muscle is completely inactivated in the presence of substrate in saturating concentration and 0.5 mM hexacyanoferrate(III) (pH 7.6, 25 °C). Concomitantly, one triose phosphate derivative per subunit is covalently incorporated. Peptide analysis showed that the triose phosphate derivative forms an intrachain cross-link between the active site residues Lys-146 and Lys-227.

Acetylcholine receptor from *Torpedo marmorata* or acetylcholinesterase from human red cells: Incorporation from Triton X-100 containing solutions into liposomes

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The purification of 2 amphiphilic membrane proteins, the acetylcholine receptor from *Torpedo marmorata* and acetylcholinesterase from human red cells involves solubilization in presence of micellar concentrations of nonionic detergents such as Triton X-100. We have investigated the possibility of introducing these 2 proteins directly from Triton X-100 containing solutions into lipid bilayers without using sonication nor dialyzing the detergent. The method uses phosphatidylcholine as lipid and Amberlite XAD-2 as detergent removing agent. The recovery of incorporated protein in the liposomes exceeded 80%. The vesicle size averaged 220 Å. The final Triton X-100 concentration in the liposomes was inferior to 1% (w/w) receptor or acetylcholinesterase.

Removal of senescent human red cells from circulation: A receptor-protein for autologous IgG

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Senescent human red cells are removed from circulation by macrophage-phagocytosis. Macrophages recognize old cells because they contain bound, autologous IgG (M. Kay, PNAS *72*, 3521, 1975). Here we report the first attempt to isolate the red cell membrane bound receptor for autologous IgG. Autologous or allogenic IgG binds to glycophorin-enriched vesicles (GEV). IgG binding above control (with absorbed IgG) is 3-4-fold higher with GEV from old than young cells (10–15% of population). Trypsin treatment abolishes binding. Cleavage of 20% of sialic acid does not reduce binding. When IgG is absorbed on GEV from old cells, the supernate has lost its capability to induce erythrocyte-phagocytosis.

Purification and chemical modification of melittin

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Melittin, a basic and hydrophobic polypeptide is the main component of bee venom. It displays marked detergent-like properties. Usually, it is purified by gel-filtration and cation-exchange chromatography. The self-aggregation properties of melittin interfere with both chromatographic steps; association to a tetramer takes place at high peptide and high salt concentrations, giving rise to double and asymmetric peaks, which can overlap with other venom constituents. Conditions have been developed which allow melittin to elute in monomeric form. The purified material was shown to be homogeneous by electrophoretic analysis and was devoid of phospholipase activity. Chemical modifications of the peptide have been performed to introduce specific radioactive labels.

Characterization of an endogenous inhibitor of ^{3}H-muscimol binding isolated from rat brain

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A heat stable, endogenous inhibitor of high affinity GABA binding to rat brain synaptic membranes has been purified from whole rat brain by Costa et al. (PNAS *75*, 4024, 1978). We also have purified this so-called endogenous GABA inhibitor according to their methods by fractionating whole rat brain extracts. Partially purified fractions were characterized in binding assays and SDS gel-electrophoresis. Our data strongly suggest that the inhibitor is GABA. The compound which inhibited ^{3}H-muscimol binding a) could be removed by dialysis, b) runs at the same place as ^{3}H-GABA on G-100 sephadex, and c) is characterized as GABA by amino acid analysis. Fractions containing inhibiting activity before dialysis when analyzed by SDS gel-electrophoresis were devoid of any coomassie blue stainable material. The identity of the endogenous inhibitor of GABA binding with GABA would not be surprising since it has been shown (Olsen et al., Life Sci. *22*, 1653, 1978) that GABA is released into the supernatant when synaptosomal membranes during extraction are exposed to osmotic shock or frozen.

Amniotic testosterone in the prenatal sex determination

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Fetal testicle produces important amounts of testosterone (T) mainly at the time of sexual differentiation. Plasma T in male fetuses rises at the 12th week of pregnancy and amniotic T is increased between the 16th and 20th week. During this period nonconjugated amniotic T was measured in 30 male and 30 female fetuses, after control of sex by caryotype. Mean levels (±SEM) in males (312 pg/ml±25) were significantly different ($p<0.001$) from those in females fetuses (118 pg/ml±8.9). The ranges were respectively 155-730 pg/ml and 36-240 pg/ml. These results indicate that in approximately 85% of the cases the sex of the fetus in utero can be determined by this procedure. When associated with the determination of sexual chromatin in amniotic fluid, whose results are also not absolute, it should allow to diagnose rapidly and with a high degree of security the sex of the fetus in cases of recessive diseases associated with the sex.

The subunits of Cl are held together both by Ca^{++}-dependent as well as Ca^{++}-independent forces

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We have examined the role of calcium in maintaining Cl structural integrity. By equilibrium dialysis of Cl against ^{45}-

Ca^{++}-containing buffer, we have found 1 atom of Ca^{++} to be exchangeable into the Cl molecule. No Ca^{++} was taken up by either Clq alone or by a mixture of Clr and Cls, suggesting that the Ca^{++} serves as a bridge between the Clq and the Clr, s subunits. The Ca^{++}-free form of Cl is still active when the assay is performed at room temperature. However, when the Ca^{++}-free form is exposed to 37 °C, it dissociates into Clq and Clr, s subunits. When Ca^{++} is added back, Cl does not dissociate at 37 °C. Dissociation of native Cl into its subunits by EDTA (2 mM) is not mediated by chelation of Ca^{++}, as previously thought, since the EDTA effect was not abrogated by adding a 15-fold molar excess of Ca^{++}. The interaction of EDTA with Cl is being studied using equilibrium dialysis of Cl against ^{14}C-labelled EDTA.

The purification in 2 steps of the first component of complement (Cl) in its nonactivated form

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Present methods for Cl purification in the nonactivated form are both time-consuming, and give low yields, as they require the separate purification of the 3 subunits Clq, r, s, followed by their reconstitution. As a result work on the early steps of complement activation has been hampered. We have developed a rapid isolation procedure for Cl which gives both good yields and maintains Cl in its nonactivated form. Human serum at 2 °C is passed over a sepharose IgG column in the presence of 0.1 mM p-nitrophenyl p-guanidinobenzoate (NPGB). After extensive washing with veronal-buffered saline-NPGB, Cl is eluted at fast flow-rate in the same buffer adjusted to 0.4 M NaCl. In more than 20 preparations Cl was obtained in at least 70% yield and represents 30–40% of the total protein eluted. The contaminants include IgG, C4 and C4b-binding protein and are removed by gel-filtration on an ACA 22 column. SDS-PAGE analysis of the Cl peak reveals only the Clq, Clr and Cls subunits. The reduced gels show no Clr or Cls cleavage when NPGB is included in the procedure. When NPGB is omitted, a significant amount of cleaved Clr and Cls is observed. The kinetics of this native Cl are compared with those of Cl prepared by previously published procedures.

Creatine kinase (CPK): NMR and fluorescence evidence for ADP interaction with aromatic residue

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Complexes of CPK with ADP were studied by ^1H-NMR at 360 MHz, using the intermolecular nuclear Overhauser effect observed in NOE-difference spectra. The data show that besides 2 aliphatic signals observed previously (James, Biochemistry *15*, 4724, 1976), additional NOEs occur in the aromatic region between 6,5 and 8 ppm. These NOEs are compatible with the location of one or several aromatic side chains near the adenine ring in the CPK-ADP complex. Independent evidence for an interaction between the purine and aromatic amino acids comes also from quenching studies of protein fluorescence. Binding of ADP, IDP and GDP reduces Trp-emission of CPK by a resonance transfer mechanism. The critical distance from ADP to Trp as calculated from the Förster overlap integral is maximally 5 Å, suggesting that a Trp is located at or near the purine binding site of CPK.

Thermodynamics of Ca- and Mg-binding to parvalbumin

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Parvalbumins are known to bind 2 g-atoms Ca^{2+} per mole, but considerable controversy remains on the relative affinities of the 2 sites. Ca-binding and Mg/Ca-exchange of carp parvalbumin were studied by equilibrium dialysis. Scatchard analysis gave for both sites equilibrium constants of $K_{Ca}=3\times10^9\ M^{-1}$ and $K_{Mg/Ca}=3\times10^4\ M^{-1}$, with an apparent Ca-affinity in 1 mM Mg^{2+} of $K'_{Ca}=3\times10^7\ M^{-1}$. Binding of Ca^{2+} and Mg^{2+} was fully competitive, with $K_{Mg}=1\times10^5\ M^{-1}$. Microcalorimetry was used to determine enthalpy and entropy changes associated with Ca-binding and Mg/Ca-exchange. Values of $\Delta H^0_{Ca}=-9.0\ kcal\times mol^{-1}$, $\Delta S^0_{Ca}=+13.2$ e.u., and $\Delta H^0_{Mg/Ca}=-6.0\ kcal\times mol^{-1}$, $\Delta S^0_{Mg/Ca}=+0.4$ e.u. per metal-binding site were obtained, leading to $\Delta H^0_{Mg}=-3.0\ kcal\times mol^{-1}$ and $\Delta S^0_{Mg}=+12.8$ e.u. for Mg-binding. This demonstrates that metal-complex formation of parvalbumin is driven by both enthalpy and entropy contributions, whereas Mg/Ca-exchange displays only negligible entropy change.

Involvement of disulfide-linked protein aggregates in sugar cataracts

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In a recent study (PNAS *75*, 2918, 1978) on diabetic cataract formation, we found that the nonenzymatic glycosylation of lens proteins in vitro increases the susceptibility to sulfhydryl oxidation and formation of high mol.wt (HMW) aggregates which scatter light. The addition of reducing agents could prevent or reverse the aggregates. We have extended these studies to the cataracts of diabetic and galactosemic rats. Following the disruption of the lens, a cloudy solution was obtained only from the cataractous lenses. The absorbance at 550 nm of the solution of both cataracts could be decreased by more than 50% upon reduction with dithioerythritol. The presence of HMW aggregates was ascertained with sucrose density gradient centrifugation. The mol.wt of the aggregates was greater than 150×10^6 daltons (Adenovirus) and could be decreased upon reduction with DTE. These data suggest that experimental sugar cataracts have, in common with human cataracts, the presence of HMW aggregates partly linked by disulfide bonds.

Formation of glucose-6-phosphate from glyceraldehyde-3-phosphate by rat liver cytosol and its regulation by the ratio of Mg^{2+}/Ca^{2+}

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Under optimal conditions, glucose-6-phosphate (G6P) was formed from glyceraldehyde-3-phosphate (GAP) by liver cytosol from either fed or fasted rats at a rate of 4.9 ± 0.5 $\mu moles\cdot min^{-1}\cdot g$ liver wet $weight^{-1}$. G6P-formation from GAP was inhibited by Ca^{2+} and stimulated by Mg^{2+}. The regulatory step was found to be at the fructose-1,6-bisphosphatase reaction. The conversion rate correlated linearly with the ratio of free Mg^{2+}/free Ca^{2+}, provided that the ratio was lower than 16. Inhibition of G6P-formation at 1 mM Ca^{2+} ($Mg^{2+}/Ca^{2+}=10$) gradually increased from 3% at 56 µM GAP to 46% at 670 µM GAP. These variations

are related to differences in fructose-1,6-bisphosphate concentrations resulting from increasing GAP concentrations. The findings imply a role of Ca^{2+} in the regulation of gluconeogenesis. It is suggested that stimulation of gluconeogenesis by some hormones may be induced by a lowering of the cytosolic Ca^{2+}-concentration.

Effects of lithium on glycogen deposition in isolated rat hepatocytes

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Lithium at concentrations as low as 1 mM stimulated glycogen production from glucose in isolated hepatocytes from 24 h fasted rats. The activity of glycogen synthase *a* was already increased 5 min after the addition of lithium, whereas the stimulation of net glycogen deposition required a lag phase of about 20 min. Preincubation of liver cells with lithium in the absence of glucose led to an increased stimulation of glycogen production during the subsequent incubation with glucose. The stimulatory effect of lithium was additive to those of potassium and insulin. Lithium antagonized the inhibitory action of suboptimal doses of glucagon on glycogen production, but had no effect on the total cAMP contents of the incubated liver cells. From a comparison of our results with those of other authors working with isolated diaphragm, it can be concluded that lithium, in contrast to potassium, has a similar effect on glycogen deposition in both liver and muscle tissue.

Release of acetylcholinesterase (AChE) from human erythrocytes by membrane vesiculation

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Up to 50% of total erythrocyte membrane AChE could be released without hemolysis when intact erythrocytes were incubated with sonicated dimyristoylphosphatidylcholine (DMPC). DMPC addition induced a release of vesicles from the erythrocyte membranes. The vesicles were purified by 2 centrifugation steps which separated them from intact erythrocytes and added DMPC. The vesicle-lipid composition was similar to that observed in native erythrocytes. AChE, glycophorin, band 3, band 6 and hemoglobin were the major protein components. No spectrin was detectable. Freeze-fracture electron microscopy showed large (1000 Å diameter) structures with protein particles imbedded in the lipid bilayer. The vesicles described provide a promising system for further study of lipid-protein and possible protein-protein interactions of erythrocyte membrane proteins.

Separation of factor VIII multimers under physiological conditions

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The structure and function of factor VIII are still a matter of ignorance and controversy. Under denaturing conditions, i.e. in the presence of SDS, purified factor VIII-related protein was fractionated on polyacrylamid-agarose electrophoretic gels into a series of multimers with mol.wts ranging from 1.5 to $\sim 20 \times 10^6$. Since the specific activities of factor VIII seem to depend on the molecular size of the corresponding protein moiety efforts were undertaken to isolate and characterize native factor VIII aggregates of various sizes. We have, therefore, designed an electrophoretic system which enabled us to fractionate factor VIII multimers without the use of SDS. The gels consisted of 2% polyacrylamide and 0.25% agarose. Electrophoresis was carried out in a 0.04 M Tris-0.06 M Tes buffer of pH 7.5. Our results show that migration of factor VIII multimers is rather a function of size than charge. Preliminary experiments revealed that the gels are also applicable for preparative electrophoresis; factor VIII activities were recovered in the eluted fractions.

Histidine as metal ligands at the active site of *Neurospora* tyrosinase

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The involvement of histidyl residues as ligands to the active-site copper of *Neurospora* tyrosinase was investigated by differential dye-sensitized photoinactivation studies. The enzymatic activity of the holoenzyme is essentially unaffected by photooxidation in the presence of methylene blue; in contrast the aopenzyme progressively looses its ability to be reactivated with Cu^{2+}. Under these conditions, 3 out of totally 9 histidyl residues are preferentially destroyed in the apoenzyme, whereas the number of histidyl residues in the native enzyme remains constant. From a comparison of the relative yields of the histidine-containing peptides in conjunction with the sequence information of this monooxygenase, it is suggested that histidyl residue 188, 193 and 289 participate in the binding of the active-site copper of *Neurospora* tyrosinase.

Effects of steroids, drugs and vitamins on the liberation of granule enzymes from intact horse eosinophilic and neutrophilic leukocytes

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There is some evidence that eosinophilic leukocytes, in contrast to neutrophilic leukocytes, have a more secretory character and probably have some importance in modulation of inflammation (inactivation of SRS-A, PAF and histamine) by liberation of their granule enzymes. We tested comparatively the effect of different substances on the liberation of eosinophilic and neutrophilic granule enzymes. On both cell types, histamine, diphenylhydramine, epinephrine and hydrocortisone showed almost no action on granule enzyme release such as peroxydase, β-glucuronidase, acid-phosphatase and cathepsin D. But vitamin A, E, D_2 and progesterone stimulated especially the eosinophilic leukocytes in releasing the granule enzymes, with maximum enzyme liberation after a 20-min incubation. Under the same conditions the neutrophilic granule enzyme release only reached its maximum after $2\frac{1}{2}$ h and the liberated activities were 10-30 times smaller than that of the eosinophilic granule enzymes. Colchicine seems to have only a minor inhibition effect on the enzyme release.

Identical biological effects of pancreatic glucagon and a purified moiety of canine gastric immunoreactive glucagon

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Since in the dog, the gastric fundus contains the largest amount of glucagon immunoreactivity (IRG), a fraction of

the IRG of mucosal scrapes of 105 canine stomachs was extracted and purified until immunological homogeneity was obtained When immunoequivalent amounts (300-2500 pg/ml) of either types of glucagon were used, the same biological responses with respect to glycogenolysis as well as to urea, lactate, and pyruvate production were observed. Liver cyclic AMP was also raised to the same extent by either one of these hormones. We conclude that this moiety of gastric IRG is apparently identical to pancreatic glucagon, since a) their mol.wts, elution properties in ion exchange chromatography and their electrophoretic mobility are indistinguishable, and b) both hormones elicited identical biological effects in isolated rat hepatocytes.

Specific labelling of membrane protein(s) involved in the cyclitol transport in *Klebsiella aerogenes*

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The carrier of the cyclitol transport system in *K. aerogenes* has a mol.wt of 33,000-35,000 daltons as found by specific labelling, based upon a) comparison of membranes of induced and noninduced cells (amino acids incorporation and p-diazobenzene-[^{35}S]-sulfonic acid), b) reactivity in substrate protected and nonprotected cells (N-ethylmaleimide). Only with the N-ethylmaleimide technique, 2 other proteins were also labelled, but their relation to the transport system is not yet clarified.

Nitrate reduction of a thermophilic strain of *Bacillus stearothermophilus*

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The energetics and efficiency of growth of a prototrophic, facultative anaerobic strain of *B. stearothermophilus* have been investigated under conditions with and without nitrate at 65 °C in batch culture. These studies give evidence for the existence of a nitrate- and also a nitrite-reducing system in the organism. Support for this sort of an anaerobic respiration comes from microcalorimetric growth experiments, where 7-8 times the energy production is obtained as compared with glycolytic fermentation (in form of heat evolution and synthesis of cell material). The greater amount of the energy liberated by the catabolic steps of glucose degradation is released as heat. The stored amount of energy which corresponds to the produced cell substance has been determined by combustion calorimetry. Under aerobic conditions aerobic and anaerobic respiration are used simultaneously at late logarithmic growth phase, so that oxygen does not seem to repress the synthesis of the nitrate-reducing system.

NMR studies of the reversible thermal denaturation of the basic pancreatic trypsin inhibitor

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The reversible denaturation of the basic pancreatic trypsin inhibitor (BPTI) and derivatives obtained by selective reduction of disulfide bonds was studied with ^{1}H NMR at 360 MHz. The thermal stability of the modified species is markedly reduced, even though their solution conformation is nearly identical with the native protein. Observation of several individually assigned resolved resonance lines allowed to monitor different regions of the protein during the unfolding and refolding transitions. While no deviations from a cooperative 2-state transition could so far be detected for BPTI, different transition temperatures for different molecular regions were observed in the modified inhibitors, where high stability was evidenced for regions containing hydrophobic amino acid side chains. Saturation transfer experiments showed that the unfolding rate is of the order of 1 sec^{-1}. The present observations are consistent with a previously proposed dynamic domain model of globular protein conformations.

Attachment of acetylcholinesterase (AChE) to human erythrocyte membranes

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Erythrocyte AChE activity is found exclusively on the outside of red blood cells. To investigate its attachment to the membrane erythrocyte ghosts were incubated with either phospholipase C, sphingomyelinase C, phospholipase A_2 or various combinations of these 3 enzymes and subsequent AChE release was studied. Breakdown products of phospholipase A_2-action were removed with fatty acid poor bovine serum albumin. Not more than 8% of the AChE were released from the membranes. The total AChE activity was not decreased by phospholipase-action. 2 possible explanations for this behavior are: 1. AChE is bound to a membrane spanning protein which causes its tight attachment to the membrane and stability against phospholipid breakdown. 2. AChE itself is an integral membrane protein with only the active site located on the outer side of the membrane.

Abnormalities of the endocrine pancreas following hypothalamic lesions in the rat

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The arginine-induced secretion of insulin, glucagon and somatostatin was studied in perfused pancreases from control and nonhyperphagic ventromedial hypothalamic (VMH)-lesioned rats. Compared to controls, pancreases from VMH-lesioned rats secreted more insulin and glucagon and less somatostatin. These abnormalities were restored toward normal upon perfusion with atropine. These data support the concept that, in VMH-lesioned rats, increased parasympathetic activity may prevail and be responsible for the observed abnormal endocrine secretions. In any situation tested during this study, low somatostatin release always corresponded to high secretion of insulin and glucagon. Mechanistically, these findings suggest that the D-cell responsiveness could modulate the release of insulin and glucagon. However, in VMH-lesioned rats, it is also possible that the putative increased cholinergic activity may influence the A-, B- and D-cells individually, without necessarily implicating intercellular relationships.

Optical properties of Co(II)-substituted *Neurospora* tyrosinase

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Substitution of Co(II) for the native Cu(II) ions of *Neurospora* tyrosinase gives rise to a violet colored cobalt derivative with a stoichiometry of 2.0 ± 0.2 g atoms of Co bound

per mole protein. The energies and intensities ($\varepsilon_{608}=470$, $\varepsilon_{564}=490$, $\varepsilon_{524}=390$ $M^{-1}cm^{-1}$) of the electronic transitions in the visible region together with the low CD-intensities ($\theta_{564}=1800$ deg cm^2 $dmole^{-1}$) indicate a distorted tetrahedral coordination geometry of the cobalt center. These findings differ remarkably from the well-known low mol.wt Co(II)-complexes which bind O_2 reversibly and which are all coordinated octahedrally.

Phosphate influx into synaptosomes isolated from rabbit brain cortex

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Synaptosomes were prepared from rabbit brain cortex by a modification of the method of Gray and Whittaker (J. Anat. *96*, 79, 1962). After isolation on a sucrose gradient the synaptosomes were suspended in phosphate-free Locke. ^{32}P-phosphate labelled Locke was then added to the suspension and after 15 sec incubation an aliquot was removed and filtered on a Sartorius filter with a pore size of 0.45 µm. After washing with nonradioactive Locke, the filter and the filtrate were counted. There was an increase in uptake of phosphate with a tendency towards saturation at high phosphate concentrations; the apparent K_m was 0.49 mM and the V_{max} 10.4 nmoles/*min* per mg protein. With longer incubations the uptake reached a steady level after 2–3 min. Replacing the Na by HEPES, choline or sucrose caused a large decrease in the uptake of phosphate, suggesting that the phosphate influx is mediated by a Na-dependent transport system.

Comparative studies on lactate dehydrogenases (LDHs) from thermophilic *Bacillus stearothermophilus* and mesophilic *Bacillus megaterium*

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Thermostable LDH from *B. stearothermophilus* and thermolabile LDH from *B. megaterium* have been isolated by affinity chromatography and crystallized by vapor diffusion technique. Apo- and holoenzym form different crystal types. The significant difference in thermostability between these 2 LDH's is shown by activity and thermal transition ($\Delta pH/\Delta T$) measurements. At small pyruvate concentrations both enzymes are activated by fructose-1,6-di-phosphate, yielding smaller K_m-(pyruvate) and higher V_{max}-values. Their isoelectric points, amino acid compositions and N-terminal sequences have been determined and compared.

Identification of polymorphic proteins in pigeon brain extracts by 2-dimensional gel-electrophoresis

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The proteins of the brain extracts of 85 individual pigeons *(Columba livia)* were separated by 2-dimensional gel-electrophoresis. The method is a modification of O'Farrell's technique and separates proteins first by charge and then by mol.wt. In the population of pigeons examined were 3 proteins, A, B and D which occurred each in 2 forms. The position on the gel of these 6 proteins corresponded to about the following isoelectric points (pH) and mol.wts (kdaltons): Protein A_1: 6.4/43; A_2: 6.6/43; B_1: 5.7/41; B_2: 5.8/40; D_1: 6.2/22; D_2: 6.2/21. The variant forms are genetically determined, since protein A, B and D occurred each in 3 phenotypes (A_1, A_1A_2 and A_2; B_1, B_1B_2 and B_2; D_1, D_1D_2 and D_2) corresponding to the 3 possible genotypes. From the observed frequencies of the phenotypes the following allele frequencies were calculated: Allele A_1: 0.72; A_2: 0.28; B_1: 0.15; B_2: 0.85; D_1: 0.73; D_2: 0.27. A weakly stained protein C occurred in 4 different forms and 6 phenotypes.

The purification of ferredoxin-thioredoxin-reductase from spinach

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In the spinach chloroplast a number of enzymes is activated by light. This activation, probably due to a reduction on the level of the enzyme molecule, is mediated by ferredoxin (Fd), reduced in the light through the e^- transport chain, ferredoxin-thioredoxin-reductase, a novel enzyme, and enzyme specific thioredoxins (Th). A simple and rapid purification procedure has been developed that allows the concomitant isolation and complete separation of Fd-Th-reductase and Th. Using acid, acetone and (NH_4) SO_4 precipitation, gel-filtration, ion exchange and affinity chromatography the procedure yields pure proteins. From 1 kg of spinach leaves 1–1.5 mg of Fd-Th-reductase are obtained. The enzyme has a mol.wt of 38,000 daltons as determined by gel-filtration and SDS gel-electrophoresis. With saturating amounts of chloroplastic fructose 1,6-bisphosphatase and Th the Fd-Th-reductase shows a specific activity of 60 µmoles of reductase dependent fructose 1,6-bisphosphate hydrolysis per min and per mg protein.

The primary structure of allophycocyanin and comparative studies with C-phycocyanin and C-phycoerythrin

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Allophycocyanin (APC) serves as receptor of light energy from C-phycocyanin and as transmitter to chlorophyll a. APC is one of several biliproteins which are aggregated to phycobilisomes, a macromolecular light-collecting protein-pigment complex in cyanobacteria. APC consists of 2 subunits (17,200 daltons each). The α- and β-subunits were cleaved into 4 and 5 cyanogenbromide fragments, respectively. The automated Edman degradation of these fragments, using polybrene as carrier, provided more than 90% of the total amino acid sequence. Overlapping fragments were obtained by additional cleavage methods. The comparision of the homologies of APC with CPC shows an unexpected poor homology of the α-CPC subunit to the other subunits of APC and CPC. Sequence studies of biliproteins will give an insight into the phylogenetic development of the phycobilisomes and into the structure-function relationship.

Isolation and identification of the membrane containing ceramide-galactosyltransferase and cerebroside-sulfotransferase in mouse brain

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In order to identify the membranes containing the enzymes ceramide-galactosyltransferase (EC 2.1.2.48) and cerebroside-sulfotransferase (EC 2.8.2.11), we worked out a system, which allows the localization of these enzymes in mouse brain microsomes. Homogenates of 16-day-old mouse brains were centrifuged for 20 min at 17,000 × g and the supernatant was further fractionated on a continuous sucrose gradient ranging from 0.8 to 1.3 M sucrose. The gradient was assayed for marker enzymes of myelin, plasma membranes, cytosol, Golgi membranes, ER membranes and lysosomes. The fractions were characterized further by electron microscopy. The results suggest that ceramide-galactosyltransferase and cerebroside-sulfotransferase are both located in the Golgi membranes of mouse brain, as shown before for rat kidney with other methods.

Interaction of phenylisothiocyanate and nitroxide isothiocyanate with sarcoplasmic Ca^{2+}-ATPase

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Sarcoplasmic reticulum membranes were modified by phenylisothiocyanate and the equally hydrophobic spin-label 2,2,6,6-tetramethyl-4-isothiocyanate piperidine nitroxide. Covalently-bound phenyl-(^{14}C)-isothiocyanate was found to be predominantly associated with the Ca^{2+}-ATPase. Preincubation of sarcoplasmic reticulum membranes with nitroxide isothiocyanate followed by phenyl-(^{14}C)-isothiocyanate resulted in reduction of radioactivity incorporation. Nitroxide isothiocyanate binding did not affect the ATPase activity, whereas phenylisothiocyanate induced significant inhibition of the enzymatic activity even upon preincubation with nitroxide isothiocyanate. In comparison with the nitroxide isothiocyanate the specific interaction site of phenylisothiocyanate with the Ca^{2+}-ATPase was concluded to be of topologically different nature.

Spin label modification of the chloroplast proteolipid

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A spin label analog of DCCD (dicyclohexylcarbodiimide), NCCD (N[2,2,6,6-tetramethylpiperidyl-1-oxyl]-N′ [cyclohexyl]carbodiimide) has been used to label the DCCD-binding proteolipid of the chloroplast membrane ATPase complex. The EPR spectrum of the NCCD-labelled proteolipid in chloroplast membranes is highly immobilized, $\tau c = 3$-4 μsec. Removal of ATPase CF_1 did not decrease immobilization nor allow ascorbate reduction of the label. Reconstitution of the isolated proteolipid with lipids yielded a less contrained spectrum ($\tau c = 3$ nsec) than in situ. With modified labelling conditions, spin interaction could be demonstrated between NCCD-labelled proteolipids. It is concluded that the NCCD-binding site is inaccessible to the bulk phase. Immobilization noted does not appear to be due to interaction with either CF_1 or lipid. Spin interaction data indicate the proteolipid exists in a polymeric form.

Drug metabolizing enzymes in specialized regions of the endoplasmic reticulum

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Enzymes of the endoplasmic reticulum were partially separated by centrifugation in continuous sucrose gradients containing low concentration of detergent into 4 fractions in which the following enzymes were enriched: a) glucose-6-phosphatase; b) cytochrome P-450, NADPH cytochrome c reductase, 7-ethoxycoumarin-0-deethylase; c) NADH cytochrome c reductase, cytochrome b_5, epoxide hydratase, UDP glucuronyl transferase; d) glutathione-S-transferases. The distribution of epoxide hydratase was identical whether determined with styrene oxide or benzo(a)pyrene-4,5-oxide as substrate giving further support to the conclusion that both substrates are hydrated by the same enzyme. The fact that epoxide hydratase, UDP glucuronyl transferase and microsomal glutathione-S-transferase activities were not enriched in the same fractions as the cytochrome P-450 monooxygenase system argues against a firm spatial association of any of the former enzymes with the monooxygenase system.

Brush-border membrane enzymes and proteins of the human foetal small intestine

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Saccharidase, peptidase and alkaline phosphatase enzymes were assayed in homogenates and purified brush-border membrane fractions prepared from the small intestine of human foetuses aged 10–29 weeks. The protein, glycoprotein and enzyme components of the brush-border membranes were analyzed by polyacrylamide gel-electrophoresis using the split-gel technique. The observed increase in the activity of several brush-border enzymes during prenatal development was concomitant with an increase in the intensity of specific protein bands on stained gels. As foetus age increased there was a general shift in the location of the maximum enzyme activities from the distal to the proximal region of the gut. There was no difference in the mobility on gels, and therefore no change in apparent mol. wt, of the enzyme bands from foetal brush-borders in comparison with those from the small intestines of infants and adults.

Immunochemical detection of M-protein

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The 165K M-protein of chicken skeletal muscle was purified by an improved method and injected into rabbits. The antibodies obtained were specific for M-protein as shown by immunodiffusion tests and by the formation of immunoprecipitates only at 165K when SDS gels of muscle extracts were overlaid with antibody-containing agarose gels. The sensitive overlay method distinguishes M-protein from 165K glycogen debranching enzyme; it was used to show: 1. Extraction of M-protein from skeletal myofibrils is rapid at high salt, but is incomplete even after 72 h at low salt. 2. M-protein is present in chicken heart myofibrils (which lack an M-line), but is less readily extracted at high or low salt. Immunofluorescence studies confirmed that M-protein is located in the H-zone of adult skeletal and heart myofibrils. Cultured smooth muscle cells and elongated myoblasts and small myotubes in cultures derived

from embryonic skeletal muscle, showed a stippled, stress fibre-like, fluorescence pattern. Mature myotubes had typical H-zone striations; fibroblasts did not stain.

Proton nuclear magnetic resonance (NMR) studies of metallothioneins

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The structure of metallothioneins and their apoproteins (thioneins) from equine liver and kidney have been investigated in ^1H_2O and ^2H_2O by ^{1}H-NMR spectroscopy at 270 MHz. The major differences occur at the low field side of the water resonance and are associated exclusively with amide protons resonances. The main conclusions are: a) great similarity between metallothioneins from different species; b) existence of a well-defined tertiary structure; c) existence of minor metal-dependent differences in tertiary structures; d) confirmation of metal-binding to the sulfur ligands of cysteinyl side chains; e) existence of residual ordered structure in thionein consistent with performed metal binding sites. It is suggested that the primary metal-binding -Cys-X-Cys-sequences are stabilized by hydrogen bonds from serine side chains to the peptide backbone.

Purification and characterization of a dihydrodioldehydrogenase

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A dihydrodioldehydrogenase has been purified to apparent homogeneity from the cytosol fraction of rat liver by ammonium sulfate precipitation, chromatography on DEAE-cellulose, interfacial salting in and gel-filtration on sephadex G-100 superfine. Electrophoretic analysis at the final stage shows one single protein band and during analytical ultracentrifugation one single symmetrical peak was observed. The dehydrogenase consists of one peptide chain of mol.wt 35,000 and an isoelectric point of 6.2. The substrate specificity indicates that selectively 3α-hydroxysteroids were oxidized using NAD^+ as well as $NADP^+$ as cofactor. Furthermore the enzyme is able to catalyze the reduction of 3-ketosteroids. As cofactor NADH or NADPH can be used.

Is the heavy form of acetylcholinesterase a marker for synaptogenesis?

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In rat nerve-muscle cultures, the heavy form of acetylcholinesterase (AcChE) (16S) has been proposed as a biochemical marker for synapse formation (Koenig and Vigny, Nature *271*, 75, 1978). We have tested this hypothesis on 11-day embryonic chick muscle and 8-day embryonic chick ciliary ganglion cultures. 3 major molecular forms of AcChE were found (19S, 11S, 6.5S) using sedimentation analysis (5-20% sucrose; 175,000×g; 16 h). No neural induction of the 19S form could be demonstrated as a) it was already present in muscle or ganglia cultured individually and b) it did not increase in co-cultures although muscle contractions occurred due to synapse formation. We conclude that the heavy form of AcChE cannot be a marker for synaptogenesis in the 8-day chick ciliary ganglion and 11-day muscle cultures.

Human brain aldehyde reductases

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Human brain contains multiple molecular forms of aldehyde reductase (AR) (Eur. J. Biochem. *37*, 69, 1973). The 2 main forms (AR 1, AR 3), are NADPH-dependent and show a partially overlapping substrate specificity, reducing aromatic aldehydes (4-NO_2^-, d-CO_2-benzaldehyde), 1,2-dicarbonyl compounds (CH_3^-, C_6H_5-glyoxal) as well as certain quinones (camphorquinone). In addition to the common substrates AR 1 reduces menadione (Vit. K_3) and AR 3 reduces uronic acids (glucuronate) and open chain sugar aldehydes (glyceraldehyde). For both enzymes maximal rates of reduction are obtained at pH 6-6.5. AR 1 is inhibited by the flavonoids quercetine, quercitrine and rutine (I_{50} ca. 1 µM). Ouchterlony immunodiffusion gave one precipitate band between AR 3 and antibodies against AR from human liver (J. biol. Chem. *252*, 3821, 1977), whereas AR 1 did not cross-react with the liver enzyme. Our findings suggest identity of AR 3 with aldehyde reductase (EC 1.1.1.2) and L-hexonate dehydrogenase (EC 1.1.1.19) occurring ubiquitously in mammals. AR 1 cannot be assigned to any classified enzyme.

Arabinitol, a metabolite of *Candida albicans*

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In sugar analyses by GC of the yeast *Candida albicans* a hitherto unknown component was found and caracterized as a 5 C atoms polyol. By MS this pentitol was identified as arabinitol. This and other sugar alcohols have been found in the urine of healthy subjects and were significantly either elevated or decreased in diseases like diabetes mellitus or chronic renal failure. *C. albicans*, if cultured either in serum, yeast nitrogen broth or in a glucose solution, produces arabinitol with a concomitant consumption of glucose. In an in vivo experimental model, rabbits were infected with *C. albicans* to get a systemic candidiasis. Their urines contained considerable amounts of arabinitol and their blood glucose was diminished compared to normal. A relatively low concentration of arabinitol in serum can be explained by a rapid clearance of this compound from the blood.

Simultaneous radioenzymatic assay of free and conjugated adrenaline (A), noradrenaline (NA), dopamine (DA) and epinine (E)

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Modifications of the COMT single isotope radioenzymatic method (Da Prada and Zürcher, Life Sci. *19*, 1161, 1976) resulted in increased speed, specificity and sensitivity of the assay. This improved method allows the simultaneous measurement of pg amounts of free and, following acid hydrolysis, conjugated A, NA, DA and E in tissues, as well as in plasma, cerebrospinal fluid (CSF) and urine. For the first time by this method minute amounts of endogenous E have been measured in hypothalamus and the adrenal gland. In normal volunteers the plasma NA concentrations was significantly higher in standing than in recumbent position (459±50 vs 272±31 pg/ml, respectively). The concentration of NA, A and DA found in the human CSF was 358+71; 22±3 and 55±25 pg/ml, respectively. In human plasma and urine A and NA (~ 70%) as well as DA (> 98%) were present mainly as sulfate and/or glucuronide conjugates.

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Drug-mediated induction of cytochrome P450 in hepatocyte culture: Modulation by dexamethasone

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Differential induction of several forms of cytochrome P450 (P450) has previously been demonstrated in serum-free cultures of chick embryo hepatocytes (CEL-C; Althaus et al., J. biol. Chem., in press). This induction is modified in vivo by steroids such as dexamethasone (DEX). We tested if DEX modulates induction of P450 by its known effects on the proliferative state of hepatocytes. Results. DEX (10^{-14} to 10^{-6} M) added in combination with phenobarbital (1.57 mM) or β-naphthoflavone (0.02 mM) caused a dose-dependent increase in the drug-mediated induction of P450. This effect of DEX was mimicked by addition of hydroxyurea (HU, 10^{-5} to 10^{-2} M) or cytosine-1-β-D-arabinofuranoside (ARA-C, 10^{-8} to 10^{-5} M) to drug exposed CEL-C. A significant inhibition of ^{3}H-thymidine incorporation into DNA was observed in CEL-C after DEX, HU and ARA-C. These data suggest that the effect of DEX on drug-mediated induction of P450 may be related to its effects on the cell cycle.

Metabolism of [1-Me-^{14}C]paraxanthine in the rat: Identification of a new metabolite

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The physiological properties and the metabolism of paraxanthine (1,7-dimethylxanthine), a metabolite of caffeine detected in human urine in 1883, are unknown. Labelled paraxanthine was synthesized and administered to 6 rats by tail vein injection. 24 h later, the radioactivity collected in $^{14}CO_2$, feces and urine amounted respectively to 2.3±0.2% (mean±SEM), 7±1% and 85±3%. The 1-methyl group was stable and the formation of 7-methylxanthine and 7-methyluric acid is a minor metabolic pathway. The organs studied contained a maximum of 0.2% while 4% was found in the intestinal tract. No accumulation of radioactivity was detected by whole animal body autoradiography. 2-dimensional thin-layer chromatography of whole urine showed that unchanged paraxanthine was quantitatively the most important compound (52±3% of urine radioactivity). 1-methylxanthine (11±1%) and 1-methyluric acid (21±3%) identified, demonstrated the metabolic importance of demethylation in the 7-position. 1,7-dimethyluric acid and the newly identified 4-amino-5[N-methyl-formylamino]1-methyluracil corresponded both to 15±2% of urine radioactivity. 3-hydroxy derivatives of 1-methyl and 1,7-dimethylxanthine were not detected in the urine of the rat.

Toxicity and mutagenicity of moulds of the *Aspergillus glaucus* group: Identification of physcion and 3 related anthraquinones as main toxic constituents from *A. chevalieri*

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Organic extracts of cultures of several strains of the *Aspergillus glaucus* group were screened for their production of known mycotoxins (by TLC) and for acute toxicity in the mouse. No aflatoxin, sterigmatocystin, ochratoxin A, rubratoxin and patulin was found. One strain of *A. chevalieri*, ZT 8268, was selected for further investigations because of its toxicity in the mouse. 4 anthraquinone derivatives, physcion, physcion-anthranols A and B and erythroglaucin were isolated and identified by UV, IR, NMR and mass spectrometry. Toxicity of these compounds was tested in mice, chicken embryos, 1-day-old cockerels and in the 'Salmonella/microsome' test. They were found to be toxic after i.p. injection in the mouse and in the chicken embryo test and they were positive in the 'Salmonella/microsome' test. No toxic effects could be demonstrated after oral administration in mice and in 1-day-old cockerels.

Monoaminergic mechanisms mediating asymmetry in the unilateral labyrinthectomized frog

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Most animals and man show tilting of the head after loss of one labyrinth or destruction of one vestibular nuclear complex. Compensation of body asymmetry is established within few days. Excitement, motor activity and many drugs disturb balance again, isoproterenol is the strongest influencing monoaminergic pathways. Passive straightening of the head reinstalls symmetry as long as no active movement occurs. Section of contralateral cervical dorsal roots removes asymmetry too. Inhibition of movement, e.g. by blocking dopaminergic pathways by spiroperidol, prevents the labyrinthectomized frog from circling. Propranolol has the same effect without the sedative action of spiroperidol and antagonizes isoproterenol. The unbalancing action of apomorphine and L-DOPA is antagonized by spiroperidol and by propranolol, purposeful moving is preserved with the latter only.

No interference of 1-methionine with the aminopyrine breath test

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Collection of $^{14}CO_2$ in breath after application of ^{14}C-aminopyrine (AP) has become accepted as a quantitative liver function test. In vitro, Waydhas et al. (Eur. J. Biochem. *89*, 143, 1978) observed an increase in $^{14}CO_2$ derived from ^{14}C-AP in the presence of 0.1 moles 1-methionine (M). This was due to increased formate oxidation rather than AP demethylation. Since M deprivation may be important in patients, $^{14}CO_2$ exhalation was investigated after i.v. administration of ^{14}C-AP (40 µmoles/kg, 2 µCi) to rats. Peak $^{14}CO_2$ output was 0.81±SD 0.18% dose/min, and $^{14}CO_2$ disappearance 1.49±0.19%/min (n=6). Compared to controls, M (670 µmoles/kg i.v.) produced no change in $^{14}CO_2$ output. No effect of M was seen in M-deprivation or after phenobarbital pretreatment and 10 times higher doses of AP. Analogous experiments in normal dogs also showed no effect of M. It is concluded that single doses of M do not interfere with the AP breath test.

Analysis of catecholamines (CA) as pentafluorobenzoyl (PFB) derivatives in the pg range by gas-liquid chromatography with electron capture detection (ECD)

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For their sensitive and quantitative gaschromatographic estimation the CA dopamine and noradrenaline were trans-

formed into the stable and high ECD-specific PFB-derivatives. With PFB-chloride as acylating agent and presence of pyridine ng of the CA could be reproducible acylated. Down to 20-50 pg of the CA could be detected after gaschromatographic separation on a silylated glass column (1 m/2,5 mm) packed with 5% OV-17 on Chromosorb W, aw, DMCS treated, 80/100 mesh at 265 °C. The high adsorption-activity of the PFB-CA could be eliminated by optimizing the reaction (e.g. minimizing the reagent concentrations) and gaschromatographic conditions (e.g. use of a different injection technique with a priming substance). Linearity of the method is shown for 50-500 ng of the CA detecting them as pg. The application of the method to biological material is demonstrated by measurement of dopamine in the corpus striatum of the rat.

Gamma-glutamyl transpeptidase (GGT) - an early marker for hepatocarcinogens in rats

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GGT is a positive marker for preneoplastic and neoplastic rat hepatocytes. In an attempt to demonstrate a raised liver enzyme activity in rats, serial percutaneous fine-needle aspiration biopsies were taken from animals treated with the hepatocarcinogen, N-nitrosomorpholine (10 mg/100 ml drinking water). The biopsy fragments (2-5 mg liver tissue) were smeared on glass slides and stained cytochemically for GGT, or homogenized and assayed biochemically. Few hepatocytes with a cytochemical staining reaction could be detected already in some rats after 1 week, and after 4 weeks all smears contained positive cells. Liver GGT activity was significantly raised already after 2 days and showed a continuous increase with time. Marked individual variations in tissue GGT levels were observed. This is due to the focal occurrence of preneoplastic, GGT-positive lesions. The method thus allows early detection of the effect of a hepatocarcinogen in the living animal.

Effects of colchicine on the microvascular endothelium in acute inflammation

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Changes in microcirculation are essential to the development of inflammation, especially inflammatory oedema. This symptom results mainly from increased extravasation of components of blood plasma. This process is regulated by the vascular endothelium. How this regulation is achieved in inflamed connective tissue remains unclear. We tried to gain insight into the role of the vascular endothelium in inflammation by correlating the formation of inflammatory oedema with ultramorphological changes of microvascular endothelial cells in inflamed tissue. Inflammation was elicited in the cremaster of rats by injecting the irritating compound carrageenan. In addition, in one group of animals colchicine which depending on the site of inflammation, reduces or enhances oedema formation, and ^{131}I-iodinated serum albumin was administered i.v. 1 h later the cremaster was removed and the oedema measured by defining the content of ^{131}I. In ultrathin sections of the same muscle the endothelium of small vessels was also analyzed. We found that the volume density of vacuoles in the endothelial cells correlated with the degree of oedema formation. This result shows how drugs may modulate oedema formation by influencing the vascular thoroughfare in inflamed tissue.

Effects of an isolated myocardial ischemia in dogs on plasma catecholamines, local metabolism and hemodynamic function

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Clinical and some experimental findings indicate an increase in circulating catecholamines in ischemic heart disease following acute coronary occlusion. The object of this study was to determine the effect of an acute decrease in flow in the left anterior descending coronary artery to 25% and 0% of normal flow on hemodynamic function, on local metabolism (lactate, pH, pCO_2, O_2 sat) and on levels of plasma catecholamines. Both a 75% reduction in flow and total occlusion had marked effects on contractility and biochemical parameters. Surprisingly, despite these clear signs of ischemia sustained coronary occlusion caused no distinct increase in plasma catecholamines either in the coronary venous blood from the ischemic and nonischemic parts of the myocardium or in the systemic blood. Left ventricular performance remained almost unchanged. There is an indication of the nonischemic myocardium to compensate the hemodynamic function of the damaged ventricular wall.

Species- and enzyme induction-dependent activation of a polycyclic aromatic hydrocarbon metabolite to mutagenic derivatives

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5,6-Dihydroxy-5,6-dihydrodibenz(a,h)-anthracene (5,6 Diol), a K-region metabolite of dibenz(a,h)anthracene (DBA), itself not mutagenic, could be activated to a strong mutagen for *Salmonella typhimurium* by mouse liver microsomes much more efficiently than DBA. However this strong activation was observed only after enzyme induction by phenobarbital or Aroclor 1254. Microsomes from control or β-naphthoflavone-treated mice, or from rats treated with various inducers did not activate 5,6 Diol or had only weak effects. Mutagenicity of the metabolically activated 5,6 Diol could be reduced by GSH and by an unknown cytoplasmic enzyme not requiring GSH. Rats, besides having much lower activation by microsomes, were more efficient in this cytoplasmic inactivation. The ability of tissue preparations to activate DBA was not related to their ability to activate 5,6 Diol. The results show that different patterns in activating and inactivating enzymes in different animal species are critical contributors to corresponding differences in toxic effects.

Incorporation and metabolism of radioactive acetate in electric organ of *Torpedo*

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The electric organ of *Torpedo* is able to use external acetate as efficiency as choline for the synthesis of ACh (Israël and Tuček, J. Neurochem. *22*, 487, 1974). Fragments of electric organ were incubated in physiological medium with ^{3}H or ^{14}C-labelled acetate and TCA-extracts were analyzed by high-voltage electrophoresis and thin-layer chromatography. Approximately 40% of the total incorporated radioactivity was in the form of ACh. Moreover, 3 other labelled metabolites of acetate were found, representing 5, 15 and 40% respectively. The subcellular distribution of these substances was determined by comparing the incorporation into pure

synaptosomes with that into other fractions of tissue. The 3 unknown compounds were studied: according to chromatographic and other properties, the first is presumed to be glutamate, the second aspartate and the third is under investigation.

Central effects of a novel GABA mimetic

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The drug, 3-methyl-3-azabicyclo[3.1.0]hexane-1-carboxylic acid (Sandoz 26-585), meets the essential requirements of a GABA mimetic because it displays a GABA-like inhibitory action which can be blocked by known GABA-antagonists in model systems which measure drug effects specifically on central GABA receptors. Application of 26-585 to the perfusate at concentrations between 10 µM and 1 mM reversibly inhibited spontaneous activity of cultured rat Purkinje cells, and this effect could be antagonized partially by 10 µM picrotoxin. Similarly, microiontophoresis of 26-585 or GABA onto rat Purkinje neurons recorded extracellularly in vivo reduced their firing rate. In binding studies, the same concentration of 26-585 ($IC_{50} = 1.2 \times 10^{-5}$ M) was required to displace either ^{3}H-GABA or ^{3}H-muscimol bound to dialyzed cerebellar membranes. Drug concentrations up to 10 mM did not affect ^{3}H-GABA uptake into rat cortical synaptosomes.

Inhibition of shock-induced fighting by nicotine

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After 2 training sessions (of 50 foot-shocks each, with a shock intensity of 3 mA, shock duration of 1 sec and an intershock interval of 10 sec) for shock-induced fighting in a closed chamber, 32 pairs of 7-month-old male RHA/Verh rats were given 4 further sessions, also 4–5 days apart, preceded by injections of physiological saline, or nicotine in doses of 0.1, 0.2 or 0.4 mg/kg. The injections were given 30 min before testing, in a latin square sequence. Defining the responses seen as 'no response' (1, 2*), 'defensive posture' (3*) and 'fight/attack' (4, 5*) (* see Experientia *34*, 897, 1978), it was shown that nicotine inhibited shock-induced fighting at all dosage levels. With the 0.4 mg/kg dose, the inhibition was partly due to general depression, as 'no response' was increased from control levels with this dose. The inhibition caused by the 0.1 and 0.2 mg/kg dosages, however, was characterized by replacement of the 'fight/attack' response with the 'defensive posture' response, thus demonstrating, for the first time, an inhibition of 'aggression' in rats by nicotine.

Serotonin receptors in *Aplysia:* Analysis by [^{3}H]-LSD binding, adenylate cyclase activation and electrical response in neuron R15

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The binding of the serotonin (5HT) antagonist/partial agonist [^{3}H]-LSD to membranes is a simple assay for putative 5HT receptors. To be sure that these binding sites represent physiological 5HT receptors, one must show a) that distribution of binding in several tissues parallels tissue sensitivity to 5HT, and b) that pharmacology of the binding and of 5HT-induced physiological responses are the same. Studies on *Aplysia* ganglia and muscle tissues indicate that criterion a is fulfilled. Criterion b is under study in 2 bioassay systems, the 5HT-sensitive adenylate cyclase in *Aplysia* ganglia and muscles, and 5HT-induced hyperpolarization in neuron R15. Results thus far indicate that the binding assay is indeed a valid measure of physiological 5HT receptors.

Kinetics of acetylcholine release: A study using 4-aminopyridine

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Acetylcholine (ACh) release has been investigated in the electric organ of *Torpedo* marmorata by measuring, a) the amount of ACh released in response to one or a few nerve impulses, b) the electrical response, c) the corresponding changes in the content of tissue ACh. Modified saline solutions and the drug 4-aminopyridine were used to either decrease or increase the efficiency of the release mechanism. When 2 paired stimuli were given separated by various time intervals, less ACh was released in the second impulse than in the first. The degree of this depression was a function of the amount liberated in the first impulse. Recovery from the depression was achieved in a few seconds. These data provided kinetics for a functional model in which a membrane 'operator' can bind a finite amount of internal ACh and discharge it into the cleft when activated by calcium entry.

Localization of thiamine in the electric organ of *Torpedo*

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When compared to other tissues, the electric organ of *Torpedo* contains a surprisingly high amount of total thiamine (120 nmoles/g); 38% of this in the form of thiamine triphosphate (TTP). An histofluorescence analysis of the tissue shows that thiamine is mainly located in nerves and axon terminals. This is substantiated by the finding that thiamine (mainly in the form of TTP) is most abundant in purely cholinergic nerve endings isolated from the tissue. The intrasynaptosomal volume is 3.14 µl/g of original tissue (Morel et al., J. Neurochem. *30*, 1553, 1977); therefore we can calculate a total thiamine concentration of 16 mM. In synaptosomes, the specific activities of ACh and TTP are nearly the same (80 nmoles/mg prot.). We have already demonstrated that thiamine is implicated in synaptic transmission (Eder et al., Nature *264*, 186, 1976). TTP might be the form of thiamine which is involved in ACh metabolism and release.

Locomotor behavior of rats in a residential maze

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A method for monitoring spontaneous locomotor behavior of rats is described using a residential maze connected to a computer (PDP11). The maze consists of 6 concentric alleys connected near the peripheral ends by a hexagonal alley. The subjects' locomotion is registered by 18 optical IR gates. Status changes of each gate are stored on a disk file and can be retrieved for complete session reconstruction and data analysis. The maze is contained in a wooden box for sound isolation. Food and water are provided separately at 2 alley ends. An experimental session lasts for 1 day with a computer-controlled light-dark cycle. The following parameters are generated: local activity (defined as successive crossings of the same gate) and locomotor activity

(crossings of different gates) in function of time and maze location, preferred paths, stereotypies, water and food consumption compared to home-cage data and amount and patterns of defecation. First experiments with rats of different ages show a relatively high variability between rats, but a very consistent behavior in successive sessions of the same subject spaced 2-3 weeks apart.

Correlation between in vivo anticonvulsant activity and in vitro inhibition of carbonic anhydrase activity of sulfonamides

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The best documented effect of anticonvulsant sulfonamides, such as acetazolamide, methazolamide and sultiame, is the inhibition of the enzyme carbonic anhydrase (CA: E.C. 4.2.1.1); consequently the antiepileptic effect is thought to be mediated through inhibition of this enzyme, mainly in the brain. Support for this was found with some new synthesized sulfonamides, where a correlation ($r=0.70$) between antielectroshock activity (5 mA; 50 Hz; 0.35 s; ear electrodes) in mice and inhibition of beef erythrocyte CA in vitro was observed. It could be shown that the sulfonamides can be divided in 2 classes with different biological activities: 1. Sulfonamides that inhibit CA without having any anticonvulsant activity. 2. Sulfonamides that inhibit CA and have an anticonvulsant activity. Within the first class of sulfonamides there were many diuretics, whereas in the second group most anticonvulsants have only a weak diuretic activity. It is proposed that the new anticonvulsant sulfonamides act more specifically on CA in the brain and not on the renal enzyme.

Galactose breath test (GBT): Differences in rate limitation between diabetics and nondiabetics

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Recently, breath collection of $^{14}CO_2$ (GBT) following application of uniformly labelled ^{14}C-galactose (CG) has been suggested as a liver function test. This procedure might prove to be clinically useful provided its rate limiting metabolic step is not affected by disturbances in carbohydrate metabolism. Consequently, we administered CG i.v. (0.5 g kg^{-1}; 2 μCi) to 10 diabetics and measured both galactose elimination capacity (GEC) and GBT. In the diabetics, GBT ($3.3 \pm$SD 1.9% $\cdot$ kg $\cdot$ μmoles^{-1} $\cdot$ min^{-1}) did not parallel GEC (5.6 ± 1.3 mg min^{-1} kg^{-1}; $r=0.33$). In contrast, 10 nondiabetic controls matched for similar GEC exhibited a good correlation between GBT (7.7 ± 3.4) and GEC ($r=0.72$). Since the plasma disappearance of bromsulphthalein was similar in both groups, it is concluded that in diabetics galactokinase is no longer the primary determinant for the fate of CG thus rendering GBT unsuitable for testing liver function.

Dopamine-induced hyperpolarization of neurons in the cat caudate nucleus

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We have previously reported (P.L. Herrling et al., Neurosci. Lett., suppl. 1, S258, 1978) that iontophoretic application of dopamine (DA) always resulted in a depolarization of the membrane of caudate neurons that is accompanied by a reduction of the firing rate. This last effect was not a Na^+-inactivation. We now report that with a slightly modified method, where the distance between the tips of the recording and iontophoretic electrodes was reduced from previously 100 μm to 40-50 μm, it was possible to measure hyperpolarization and reduced firing rates following DA-application in 30% of the neurons recorded so far. The remaining neurons depolarized as before. During the hyperpolarizations a significant increase of the amplitude of the cortically evoked EPSP occurred. These findings might possibly be explained by the existence of depolarizing DA-receptors on the dendrites and hyperpolarizing DA-receptors on the soma and axon hillock of some caudate neurons.

Toxic effects of heavy metals on rat alveolar macrophages in culture

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Alveolar macrophages are potential targets for air pollutants. Toxic effects of solutions of Cd, Ni, Cr^{VI} and Hg salts - trace metals associated with energy production from fossil fuel - were studied in vitro using cultures of rat alveolar macrophages obtained by pulmonary lavage. Exposure was for 20 h. Trypan blue (TB) uptake and loss of β-glucuronidase were used as measures of cell death. Phagocytosis was measured as uptake of yeast. Results. TB uptake was dose-dependent with all 4 metals tested. Approximate LD_{50}'s were 1.3×10^{-5} M for Cd; 4.5×10^{-3} M for Ni; 2×10^{-4} M for Cr, and 5×10^{-4} M for Hg. Dying cells leaked β-glucuronidase; loss of this lysosomal enzyme was dose-dependent with Cd, but extent of enzyme loss was lower than expected from TB uptake. Phagocytosis was depressed after Cd and Ni, with ED_{50}'s 10 and 5 times lower than LD_{50}'s, respectively. These data indicate greater sensitivity of the phagocytosis test as compared to the TB test. This experimental system may be used in screening other groups of pollutants.

Metabolic patterns in hepatocyte monolayers (HM) derived from atrophic (AL) and regenerating livers (RL)

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The effects of liver disease on hepatic drug metabolism in man are ill-defined. Since AL after porta-caval shunt and RL after partial hepatectomy may be regarded as disease models, we have studied microsomal, cytosolic and peroxisomal enzyme kinetics in rat HM from AL and RL, using ^{14}C-aminopyrine (AP), ^{14}C-ornithine (OR), and ^{14}C-uric acid (UA) as substrates. $^{14}CO_2$ was captured on a filter soaked with 0.5 n NaOH and suspended over the HM. $^{14}CO_2$-yield per mg protein (CY) was linear over 5 h for all substrates. CY from OR was 52 dpm min^{-1} in RL-HM, as compared to 30 in AL-HM. In agreement with results in vivo, V_{max} for AP-demethylation in RL-HM was 6.4 pmoles min^{-1} and K_m was 0.16 mmoles. Whereas in AL-HM CY from AP was decreased paralleling P-450 content, CY from UA was doubled paralleling uricase content. Thus, using a simple yet quantitative approach, changes due to AL or RL may be demonstrated in HM, opening the way to studies of biopsy-derived hepatocytes from liver patients.

In vivo covalent binding to rat liver DNA of macromolecule-bound aflatoxin B_1: A relais toxicity study

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Groundnut cakes used for animal nutrition generally contain aflatoxin B_1 (AFB_1). A considerable fraction of a dose of AFB_1 is bound covalently to liver macromolecules and is taken up in this form by humans eating such liver. AFB_1 is strongly hepatocarcinogenic. The carcinogenic potency of the covalently bound aflatoxins is not known, but was estimated on the basis of DNA binding, a valuable endpoint in carcinogenicity testing: Rats were orally administered ^{14}C- or ^{3}H-AFB_1. After 6 h, their liver macromolecules were isolated (containing about $1/30$ of the dose of AFB_1 covalently bound) and administered p.o. to another rat. After 9–12 h, liver DNA was isolated from this second rat. No radioactivity was detected on this DNA. On the basis of our limit of detection of 1.2 cpm it was calculated that macromolecule-bound AFB_1 is at least 140 times (^{14}C-experiment) or 4000 times (^{3}H-experiment) less potent with respect to DNA binding than AFB_1 itself.

Effects of hydergine and bromocriptine on maze acquisition in rats

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In a Lashley maze rats were trained in 4 consecutive trials to obtain a liquid reward. Starting time (ST), running time (RT) and number of errors (NE) were measured. Hydergine (H, 3 or 10 mg/kg s.c.) given 4 h before each trial, reduced ST, RT and NE as compared to solvent controls. The effect of H became more pronounced as the number of trials and treatments increased. When 3 mg/kg s.c. of H were given 2 h before each trial ST and RT were prolonged while NE was reduced. Daily treatment with bromocriptine (3 or 10 mg/kg s.c.), 4 h before trial, also resulted in a decrease in NE, independent of alterations in ST and RT. The results indicate that during treatment with the 2 ergot derivatives cognitive performance of the rat may be increased (reduction of NE) independent of changes in motor performance (changes in ST and RT).

Comparison of the beta-sympathomimetic stimulated cAMP accumulation in vagus nerve and in the superior cervical ganglion

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In rabbit vagus, an accumulation of cAMP occurs upon incubation of the tissue with beta-sympathomimetics. It was attempted to characterize this effect, in part by comparison to the cAMP accumulation occurring in the superior cervical ganglion (SCG) under similar conditions. It was observed that isoproterenol is in the vagus about 10 times more potent than in the SCG. Moreover, in the vagus salbutamol and isoetharine were found to be equipotent to isoproterenol, suggesting that the receptor involved is of the beta-2 type. Accordingly, only high concentrations of the beta-1 blocker practolol reduced the cAMP accumulation in the vagus, and the substance was without agonistic effect on the cAMP content. Upon sequential stimulation with isoproterenol, the amplitude of the effect decreased in the SCG, but not in the vagus. Finally, when the tissues were pretreated by incubation with protease, the effect of salbutamol on the cAMP level was enhanced in vagus nerve, but not in SCG.

Determination of the anthelmintic drug mebendazole in plasma

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In the treatment of patients with echinococcosis mebendazole (M) has been only partially successful. Although large doses have been advocated, little is known about therapeutic plasma concentrations. To determine M in plasma a HPLC procedure was developed. M together with the internal standard ciclobendazole was extracted with chloroform at pH 11. The extract was analyzed isocratically on a LiChrosorb SI 60 column with a mobile phase consisting of acetonitrile:water-saturated chloroform:ammonia (75:92.5:0.1) at 307 nm. Reproducibility was 3–10% CV (20–200 ng/ml). Rodents (natural intermediate hosts for *Echinococcus multilocularis*) receiving effective oral doses of M showed plasma concentrations between 150 and 600 ng/ml, but patients on chronic therapy (40 mg/kg/day) only between 6 and 117 ng/ml. It is concluded that determination of M plasma concentrations may be important to develop a rational pharmacotherapy of echinococcosis.

Growth factor for parasympathetic ganglion

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Nerve growth factor does not induce neurite outgrowth in the parasympathetic ganglion. However, conditioned medium (CM) from cultures of striated muscle, glia and fibroblasts promotes the outgrowth of neurites in both explants and dissociated cells from ciliary ganglion cultures. A measure of cell growth and survival was made using the incorporation of ^{3}H-acetate into acetylcholine (ACh) in the dissociated cell cultures. With increasing concentrations of CM, there was an increase in neurite outgrowth and a concomitant increase in the formation of labelled ACh; when *no* CM was present in the dissociated cell cultures, there was almost no neurite outgrowth and no production of ACh. The muscle growth factor is a nondialyzable, heat-labile protein that is stable to freezing and thawing. CM from glial cells (generously supplied by D. Monard) was chromatographed on sephadex G-100 and 2 peaks of activity were found, one in the void volume and the other at mol. wt approx. 45,000.

In vivo ^{3}H-d-LSD-binding in punched out brain regions

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We improved the brain dissection technique of our in vivo ^{3}H-d-LSD-binding-method in the rat (Experientia *34/6*, 761, 1978). It has the advantage of being able to measure the ^{3}H-d-LSD-binding in specific brain regions and nuclei. Transverse parallel slices (⌀1 mm) were prepared on a slicing-apparatus at a temperatur of −20 °C. Different regions and nuclei were obtained with a cooled metallic punch (⌀1.5 mm). ^{3}H-d-LSD-binding is expressed as the ratio of cortex to cerebellum. As the most striking result, a gradual increase (2-fold) of d-LSD-binding-sites from occipital to frontal cortex is found. In displacement experiments (co-injection of cold d-LSD), the affinity of the

d-LSD-binding-site to its ligand is homogeneous in all cortical regions. We concluded that the cortex contains a uniform kind of a 'd-LSD-receptor' which is accumulated in the frontal region.

Stereospecific interactions of neuroleptics with ^{3}H-spiroperidol binding in bovine retina

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Optimal conditions for ^{3}H-spiroperidol binding were studied in bovine retinal homogenates. Tissue suspensions (450 µl, 50 mg w.w., ml^{-1}) were incubated with various doses of ^{3}H-spiroperidol (constant volume 25 µl) and drug solutions (25 µl) for 15 min at 37 °C and filtered under vacuum. Binding of ^{3}H-spiroperidol was saturable within the nmolar range and showed tissue linearity. The difference between the amount bound in presence of 10^{-6} M (−)-butaclamol (total binding, TB) and that bound in presence of 10^{-6} M (+)-butaclamol (nonspecific binding) yielded stereospecific binding (SSB) and accounted for 40–50% of TB. SSB was also found for thioxanthene isomers such as flupenthixol and clopenthixol. SSB was lowered to 26% of TB at 22 °C and abolished at 2 °C, as well as when tissue suspensions were preincubated for 5 min at 90 °C. Although basic criteria for receptor identification were fulfilled, search for the most suitable radioligand and/or displacing agent is needed to further characterize dopamine receptors in mammalian retina.

Extracellular hydrolysis of cAMP by neurohypophyseal tissue

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Isolated rat neurohypophyses, incubated in a low (5.6 mM) potassium-bicarboate buffer, hydrolyzed 52% of exogenously added [8-^{3}H]-cAMP (10^{-5} M). In the presence of excess potassium (56 mM) only 18% of the cAMP was hydrolyzed. Theophylline inhibited the hydrolysis of cAMP by both stimulated and unstimulated tissues. Small amounts of the radioactivity associated with the [8-^{3}H]-cAMP were taken up by the tissue. After a 10-min incubation the tissue/medium ratios were 1.20 ± 0.09 and 0.64 ± 0.11 in 5.6 and 56 mM potassium, respectively. The hydrolysis of exogenous cAMP was probably mediated by an extracellularly directed cAMP-phosphodiesterase. Further studies demonstrated that 56 mM potassium significantly reduced the amount of cAMP released into the medium by the tissue. With 5.6 mM potassium 2.99 pmoles cAMP/neural lobe/10 min were measured extracellularly, whereas only 0.18 pmoles were found in the presence of excess potassium. The results suggest that a cAMP extrusion mechanism and a cAMP ecto-phosphodiesterase may coexist in the rat neurohypophysis.

An ursodeoxycholic acid (UDCA) loading test for detection of liver disease

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Measurement of spillover of bile acids (BA) from the portal into the systemic circulation after oral administration of BA has been employed as a test for detecting liver disease. The optimal conditions (e.g. form of administration of BA) for such tests have yet to be defined. We have, therefore, performed BA loading tests with various doses and formulations of UDCA in healthy volunteers. This BA is present in human serum in very small concentrations only. In therapeutic doses used for dissolution of gallstones it is well-tolerated. When 0.1 g of UDCA together with 1 g $NaHCO_3$ in 125 ml H_2O was ingested by 6 healthy fasting volunteers, peak concentrations of UDCA in serum (8–15 µmoles/l; capillary gas liquid chromatography) were observed within 30 min. 60 min after administration, UDCA concentrations in serum were less than 30% of the peak concentration. Preliminary findings indicate that UDCA in serum peaks higher and remains elevated for a longer period of time in patients with liver disease.

Pharmacokinetics in the rat of the persistent model compound, 2,4,5,2′,4′,5′-hexachlorobiphenyl (6-CB)

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6-CB which is a major component of widely used polychlorinated biphenyls (PCB) has become an environmental contaminant. In rats a maximum of 15% of any dose is ever excreted; at least ½ as unchanged material in the feces. 70% of the absorbed dose was localized in liver and muscle after 1 h; 15% in skin and adipose tissues. After 42 days the figures for skin and adipose tissues were 10 and 80%. Equal values were obtained with single or multiple doses, i.v. or p.o. Hepatic extraction in isolated rat livers subjected to single-pass perfusion was 0.40, and concentrations per unit protein in microsomal/mitochondrial/cytosol fractions were 2.1/1.3/0.2 times concentration in homogenate. It is concluded that 6-CB shows a redistribution pattern liver → muscle → skin → adipose tissue, as well as extremely limited metabolism and excretion and therefore accumulation at any dose or dosage regimen. 6-CB belongs to the most persistent xenobiotics known.

Inhibition of calcium-dependent regulator (CDR) by neuroleptic drugs

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The CDR protein activates a variety of different enzymes when it binds calcium. Originally purified as an activator of phosphodiesterase, CDR has also been shown to be an activator of adenylcyclase, Ca^{++}-Mg^{++} ATPase, myosin light chain kinase and phosphorylase kinase in different tissues. It is therefore possible that CDR functions as a general intracellular Ca^{++} receptor and mediates the action of Ca^{++} on intracellular Ca^{++}-dependent events. Previous reports have shown that neuroleptic agents, major tranquilizers used in the control of schizophrenia, inhibit the CDR-dependent activation of phosphodiesterase by binding directly to CDR. In this study, the inhibition of CDR stimulated phosphodiesterase was reexamined with isomers of neuroleptic compounds. In each case, the clinically inactive isomer was equally as inhibitory as the clinically active isomer. It is concluded that the inhibition of CDR-stimulated phosphodiesterase by neuroleptic agents is nonstereospecific and unrelated to the clinical efficacy of these drugs.

Effects of guanylnucleotides (GN) on isoprenaline(IPN)-stimulated cAMP formation in rat reticulocyte ghosts

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Reticulocytes from acetic acid phenylhydrazide-treated rats were converted to ghosts by reversal of osmotic hemolysis at 0 °C. During a subsequent incubation period at 37 °C in an isotonic medium the cells recovered a low membrane permeability to ATP, GTP and Gpp(NH)p. If maintained at 0 °C the cells did not reseal to ATP and GN. With 2 mM ATP and 3 mM Mg the maximal rate of IPN-dependent cAMP formation was 2.4 nmoles/ml ghosts/min in leaky ghosts and increased to 4 nmoles in resealed ghosts. In leaky ghosts GTP and Gpp(NH)p stimulated cAMP formation maximally by 100%. If incorporated into resealed ghosts containing 1:100 or 1:10 dilutions of the original cytosol, GTP lost its stimulating effect and Gpp(NH)p was strongly inhibitory. However, if the cytosol was diluted more than 1000-fold intracellular GN retained a stimulatory effect on cAMP formation. It is concluded that an unknown cytoplasmic factor regulates the interaction between GN and adenylate cyclase in reticulocytes.

Pharmacokinetics of ethanol and 1,3-butanediol in dogs

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Both, 1,3-butanediol (BD), a synthetic calorie source and food additive, as well as ethanol (EtOH) are metabolized by alcohol dehydrogenase (ADH). Since in vivo the NADH-reoxidation is also rate limiting for alcohol metabolism, the in vivo maximal velocity (V_{max}) is expected to be lower than the V_{max} in vitro. Fructose accelerates NADH-reoxidation and increases the amount of NAD+ available for ADH. Hence, V_{max} in vivo with fructose should approach the in vitro values. In order to test this hypothesis BD and EtOH elimination rates from blood after i.v. administration were compared with the corresponding oxidation rates by liver ADH in vitro. The average V_{max} of 4 dogs in vivo is lower for BD (1.41±SD 0.46 μmoles min^{-1} g^{-1} liver) than for EtOH (1.78±0.31). The in vitro V_{max} for EtOH (1.79±0.36) correlates well with the one in vivo. For BD-oxidation the in vitro V_{max} (1.58±0.14) is slightly higher than in vivo. Infusion of fructose increases V_{max} in vivo for BD to 2.30±0.54 and for EtOH to 3.23±1.16. These high values suggest that acceleration of NADH-reoxidation alone may be insufficient to explain the extent of the fructose effect.

Mechanism of action of spirolactone in toad bladder

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The pharmacodynamic action of prorenone (a spirolactone, SC23133) on aldosterone-dependent Na^+ transport was studied in vitro by the short circuit current technique. Prorenone appears to fulfill the criteria of a specific competitive antagonist: 1. After preincubations of 2 or 12 h, prorenone had no significant agonist activity on base line Na^+ transport for as long as 8 h and at concentration as high as 20 μM. 2. Prorenone antagonized the aldosterone dependent Na^+ transport with an estimated inhibition constant (K_i) of 8 μM, a value which is 9-fold higher than that of spironolactone (SC9420). 3. The antagonist activity of prorenone was reversible upon further addition of aldosterone. As prorenone can be tritiated at the same specific activity as aldosterone, the interaction of this spirolactone with the mineralocorticoid receptor can now be studied and possibly, its binding activity correlated to its biological activity.

Effects of local anaesthetics on phosphate efflux in nerve

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The efflux of phosphate from rabbit vagus nerves was measured as described (Ferrero et al., J. Physiol., Lond. *282*, 507, 1978). Cinchocaine, tetracaine, lidocaine, mepivacaine and procaine were found to inhibit the efflux at low concentrations. The maximal inhibition in Locke with 1 mM phosphate did not exceed 55% of the normal efflux. The inhibition varied with the pH of the solution, and for various concentrations of the anaesthetics, a S-shaped relation between inhibition and calculated concentration of anaesthetic in oleyl alcohol could be established. The following order of decreasing potency was found: cinchocaine, tetracaine, procaine, lidocaine. With concentrations for maximal inhibition the transstimulation of phosphate efflux by external phosphate was abolished. With higher concentrations the anaesthetics produced a lowering of efflux followed by a large increase. Both effects were reversible. The results suggest that local anaesthetics act on phosphate efflux by binding to a hydrophobic receptor in the membrane.

Angiotensin II (ANGII), ANGII antagonist and ANGII fragments: Their influence on renin and cell growth in 3T3 and SV3T3 cells

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Renin was found to be synthesized in 3T3 and SV3T3 cells. With time after infection renin decreased in SV40 transformed cells, while it increased in mock-infected 3T3 cells. There was a reverse relationship between cell growth and renin content. ANGII stimulated cell proliferation and decreased renin content in 3T3 cells. The ANGII antagonist Saralasin inhibited cell growth in normal 3T3 fibroblasts and in SV3T3 cells. The ANGII fragments ANG(2-8) heptapeptide and ANG(4-8) pentapeptide did not affect cell growth. These results point to the possibility of the renin-angiotensin system being involved in the regulation of cell growth.

Effects of drugs and toxins upon *Xenopus laevis* oocyte meiosis in vitro

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Several nonsteroidal agents were shown to mimick progesterone to induce meiosis in *X. laevis* oocytes in vitro, possibly by displacing calcium from membrane stores (Differentiation *9*, 67, 1977). Recently cholera toxin (CHT) was found to inhibit meiosis (C.r. Acad. Sci. *286*, 685, 1978), due to cAMP increased measured in various conditions of CHT treatment (Cell *15*, 1269, 1978). Poorly hydrolyzable cAMP analogs (injected into oocytes) or cAMP generated by CHT

hampered the formation and/or amplification of a cytoplasmic maturational factor. However, no cAMP increases were found after exposure of oocytes to other meiosis inhibitors such as gammexane or cycloheximide. Conversely, cAMP decreases were not apparent after exposure of eggs to insulin, which can induce meiosis (El Etr et al., in press). *X. laevis* oocyte maturation seems thus to involve multiple structural and functional systems, such as steroid membrane sites, calcium translocation, phospholipid turnover, protein synthesis, cytoplasmic factors and/or cAMP metabolism.

Evaluation de l'intoxication hépatique chez le rat par la mesure de la vitesse de la déméthylation de l'aminopyrine-^{14}C et de l'activité des transaminases plasmatiques

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Pour l'étude des effets hépatoprotecteurs de certains agents thérapeutiques, il est important de disposer de tests permettant d'évaluer le degré de l'intoxication expérimentale d'une part et l'effet de la restauration à la suite d'un traitement d'autre part. La présente communication rapporte les résultats obtenus chez le rat avec un test basé sur la vitesse de déméthylation in vivo de l'aminopyrine-^{14}C, conjointement à la détermination des transaminases plasmatiques. La mesure du taux de $^{14}CO_2$ expiré après administration de l'aminopyrine-^{14}C chez le rat normal et chez le rat intoxiqué par le CCl_4 permet de rendre compte de l'altération de la capacité de métabolisation hépatique de l'aminopyrine-^{14}C. Lors de l'administration chez le rat adulte des doses de 0,05, 0,2 et 1,0 ml/kg de CCl_4, la réduction du taux de $^{14}CO_2$ expiré après administration de l'aminopyrine-^{14}C est de 9%, 50% et 97% respectivement, alors que les activités des GOT et GPT augmentent de manière significative. Le test décrit dans cette communication est actuellement utilisé pour l'étude des effets hépatoprotecteurs de nouveaux produits.

Potentiation of apomorphine-induced climbing in mice by d-LSD

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Climbing behaviour (CB) in mice was used for studying possible interaction(s) of d-LSD with dopamine receptors. Mice were injected with various dopamine-related drugs, pushed into cylindrical cages and scored for CB (Costentin et al., Nature *257*, 405, 1975). Doses of apomorphine ranging from 0.5 to 5.0 mg kg^{-1} increased the climbing scores as compared to mice injected with Ringer solution. In contrast, doses of d-LSD ranging from 0.25 to 2.5 mg kg^{-1} constantly inhibited the CB. However, when similar doses of d-LSD were injected 10 min before 5 mg kg^{-1} of apomorphine, a constant potentiation of apomorphine effect was observed. Subsequent experiments with 5-OH-tryptophane (a serotonin precursor) and/or haloperidol (compared to those of d-LSD with and without apomorphine) would indicate that d-LSD alone displays typical serotoninergic symptoms (including inhibition of CB), whereas in the presence of apomorphine, an interaction at pre- and/or postsynaptic receptors may possibly modulate dopaminergic activity.

Méthode rapide de dosage de la riboflavine urinaire et son application au contrôle de la discipline thérapeutique

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Afin de contrôler la régularité de la prise des médicaments à l'insu du patient, on dose dans l'urine la riboflavine administrée comme traceur (6 mg) en même temps que le médicament. 5 ml de l'urine du patient collectée entre 1 et 4 h après l'administration sont introduits dans une colonne de phase inverse liée sur gel de silice (SEP-PAK C_{18}, Waters, USA). On élue avec 5 ml d'eau distillée puis avec 1,5 ml d'éthanol 50% aq. La fluorescence à 365 nm de la riboflavine sélectivement extraite dans les derniers 0.75 ml d'éthanol est comparée visuellement avec celle d'urines étalons. La méthode permet d'examiner 30 échantillons à l'heure et de réutiliser au moins 20 fois les colonnes après régénération par du méthanol (5 ml) puis de l'eau (30 ml). La comparaison des résultats de cette méthode avec ceux obtenus par le dosage de la riboflavine par HPLC/fluorescence (colonne RP-8, éluant 20% aq. acétonitrile, excit. 472 nm, émiss. plus de 500 nm, $k' = 1,2$) montre que la méthode est très fiable (en moyenne 4,5 µg/ml avec traceur et 0,2 µg/ml sans).

Age-dependent differences in the metabolism of vitamin K_1-epoxide in rats following treatment with phenprocoumon

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Patients and rats in old age are more sensitive to oral anticoagulant drugs (OAD) than in young age, suggesting an altered receptor affinity for vitamin K_1 or OAD in the elderly (Sheperd et al., Br. J. clin. Pharmac. *4*, 315, 1977). The possible receptor for OAD is the vitamin K_1-epoxide reductase (ER) in the liver. An inhibition of this enzyme by OAD results in an increase of the vitamin K_1-epoxide/vitamin K_1 ratio (E/K_1 ratio). Therefore, we studied the disposition of vitamin K_1 in the liver of young adult (mean weight 280 g) and older (weight 465 g) Lewis rats following treatment with increasing doses (20–800 µg/kg) of phenprocoumon. In older rats the slope of the dose-response (E/K_1 ratio) curve was shifted to the left and the maximal response obtained was greater when compared to younger rats. These results suggest that the ER in older rats can be more effectively inhibited by OAD and furthermore, that the activity and/or amount of this enzyme is greater in older than in younger rats.

Increased sensitivity after repeated administration of ergot derivatives in rats

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In rats with unilateral degeneration of the nigrostriatal pathway, induced by intranigral 6-OHDA, bromocriptine (B) or hydergine (H) elicit contralateral turning. This effect starts after a latency of 2–3 h. Repeated daily administration of B or H (20 mg/kg/day p.o.) over 5 days lead to an increase in number of turns and to a shortening of onset latency. As compared to day 1 (1600 ± 350 turns) B elicited 2973 ± 285 turns on day 2. After H, 30 ± 17 and 233 ± 62 turns were counted on days 1 and 2, respectively. In intact rats, treated with the same dose of B, increased stereotypic responses were seen, whereas H did not elicit stereotypies.

In lesioned or intact animals, increases of responses to repeated drug administration were only observed when the initial dose was high enough to produce a visible effect. The results suggest that repeated administration of the 2 ergot derivatives leads to increased sensitivity of central receptor sites.

A peculiar dopamine receptor on striatal serotonin neurons

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Baclofen (B; 30 mg/kg i.p.) increased striatal 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). 5-HT was increased maximally after 2–3 h, and normal levels were reached after 6 h. The increase of 5-HIAA was slightly delayed. Apomorphine (AP) given twice at an interval of 105 min (2 mg/kg s.c.) had a similar, but shorter action. The effects of both drugs (B given 15 min after the first dose of AP) on 5-HT were additive; the combination affected 5-HIAA similar to B alone. However, AP tended to antagonize the effect of B in the early phase. Haloperidol (H; 1 mg/kg p.o.) reversed the effect of AP but not of B. H alone had no effect on striatal 5-HT or 5-HIAA levels. These results suggest the existence of a peculiar DA receptor located on 5-HT cells. A reduced DA concentration at this receptor seems to increase 5-HT and 5-HIAA levels. AP is thought to reduce DA release by stimulating DA autoreceptors and B by inhibiting the firing of DA neurons. This explains why H only reversed the effects of AP but not of B. Moreover, this DA receptor is relatively insensitive towards AP and H.

Tricyclic antidepressant drugs accelerate biological rhythms

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A number of antidepressant drugs induce rapid cycling between mania and depression in certain bipolar patients. This led us to investigate the effect of chronic antidepressants on the frequency of biological oscillations. The model chosen was the circadian rhythm of motor activity (as measured by running wheel activity in continuous darkness) in the hamster. Only very few drugs change the period of this rhythm (it is accelerated by oestrogen and testosterone, slowed down by lithium, heavy water and valinomycin). Preliminary results with silastic implants of desmethylimipramine or imipramine (releasing the drug as free base over 2–3 weeks with steady-state plasma levels similar to that used therapeutically in man) showed that: a) both drugs accelerated the cricadian rhythm, b) the effect was more marked and reproducible in female than in male hamsters, c) the latency of effect, 10–20 days, was similar to that of the time lag of clinical efficacy. This effect of antidepressant drugs on the 'biological clock' may provide new ways of understanding their mechanism of action.

Cardiotoxicity of carminomycin, a new anthracycline antibiotic, in rats

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Carminomycin (C) was considerably more toxic than doxorubicin (Adriamycin®, D) after repeated i.p. injections in rats. ECG changes, i.e. slowing of intraventricular conduction, coincided with sudden and rapid deterioration of general health. In rats treated with D, ECG changes developed gradually at doses that were well-tolerated. The morphological changes of the heart in C-treated rats were characterized by marked fibre atrophy due to serious loss of myofibrillar mass. The sarcoplasmatic reticulum and the mitochondria were only slightly altered. In D-treated rats there was severe dilatation of the sarcoplasmatic reticulum, fuzziness of the I-bands and Z-lines and degeneration of mitochondria. It is concluded that the cardiotoxic effect of C is less specific than that of D.

Ethanol increases the plasma concentration of orally administered mephenytoin (M)

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Drug elimination is known to be decreased by ethanol. In drugs with high presystemic elimination the influence of ethanol on systemic availability after oral administration, however, is as yet ill-defined. Studies were, therefore, carried out in 4 dogs, using the model drug M in oral doses of 12.5 to 100 μmoles/kg. M-concentrations were measured by GLC. During ethanol infusions to plasma concentrations of 0.8 to 1 g/l, M-peak concentrations were increased by 87±SEM 15.3% ($n=12$, $p < 0.001$) and systemic availability from 38.1±3.1% to 81.7±6.1% ($p < 0.001$). Ethanol reduced biliary M-clearance (measured by direct catheterization of the common duct) from 106±32 to 53±13 μl/min kg ($n=4$, $p < 0.025$) suggesting that the distribution of M between plasma and bile may be modified by ethanol. It is concluded that ethanol may diminish the first pass metabolism and alter the distribution of orally administered drugs. Thus ethanol induced reduction in presystemic elimination may potentially lead to drug toxicity.

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

4 major tRNA^{Phe} isoacceptors with the same basic sequence in *Drosophila melanogaster*

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Transfer RNA$^{Phe}_{Drosophila}$ (anticodon G^{m}AA) was isolated by means of anticodon-anticodon affinity chromatography with a tRNA^{Glu} column (*E. coli*, anticodon mam^{5}s^{2}UUC). This tRNA^{Phe} preparation can be separated into 2 spots on 2-dimensional polyacrylamide gels. Sequence data suggest that this separation is due to the presence of either a U or a D at the same position in the extra loop. Both tRNAs were eluted and further fractionated into 2 peaks on RPC-5 at pH 3.8 in the presence of EDTA. This separation is the result of either a C or a C^{m} at the same position in the anticodon loop. Thus the post-transcriptional modification of tRNA^{Phe} transcribed from one gene (or more genes with identical sequences) results in 4 isoacceptors with the same basic sequence.

Regulation of sterol synthesis in neuroblastoma cells by glia conditioned medium

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Serum-free medium conditioned by glioma cells, and its lipid extract, inhibit the incorporation of ^{14}C-acetate, but not of ^{3}H-mevalonate, into sterols in neuroblastoma cells. This effect is dose-dependent and in contrast to the macromolecular factor(s) inducing morphological differentiation of neuroblastoma cells, is lost upon dialysis of the medium. Similar results (as with glial conditioned medium) are obtained with dexamethasone. Cholesterol alone, abundant in the medium, has no effect. In addition to cholesterol, glioma cells synthesize and release other sterols which influence the sterol synthesis in neuroblastoma cells. These results indicate that glial cells could modulate the lipid metabolism of neuronal cells.

Interferon mediated expression of gene for myxovirus resistance in primary adult mouse hepatocyte cultures

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Interferon dependent inborn resistance to myxoviruses is expressed in cultured macrophages. Using primary monolayer cultures of adult mouse hepatocytes, we could show that freshly cultivated cells from both resistant A26 and susceptible A/J mice were equally permissive for infection with a hepatotropic avian influenza A virus. However, when cultivated 1-4 days before infection, A2G hepatocytes became protected, whereas A/J cells remained susceptible. Antibodies to mouse interferon type I could only abolish resistance when present from the very beginning of cultivation. 24-h supernatants of both types of liver cultures contained 40-80 units/ml of spontaneously released interferon. Thus, small amounts of interferon inadequate to protect A/J cells would seem to be sufficient for phenotypic expression of resistance gene in A2G hepatocytes.

Proteins activated by heat-shock in *Drosophila melanogaster*

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Exposure of *Drosophila melanogaster* to 37 °C produces the synthesis of a new set of proteins, while the synthesis of the 'normal' protein is strongly repressed. Kinetics of synthesis and cellular localization of these proteins have been studied. The most striking results show that the 'heat-shock' proteins of low mol.wt (27, 26, 23, 22 kdaltons) and at least a fraction of the 70- and 68-kdalton proteins, migrate to the nucleus just after their synthesis, where they behave as nonhistone proteins. If the temperature is shifted back to 25 °C, the synthesis of heat-shock induced proteins regresses and stops after several hours and the 'normal' proteins reappear. Then the low mol.wt 'heat-shock' induced proteins leave the nucleus and are predominantly localized in the cytoplasm, where they are found in aggregation.

Binding, internalization and lysosomal association of ^{125}I-glucagon in isolated rat hepatocytes (RH)

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Quantitative EM autoradiography was used to visualize the binding process of ^{125}I-glucagon to RH during a 60-min incubation in vitro at 20 °C and 37 °C. Under these conditions steady state binding is reached by 30 min at 20 °C and by 20 min at 37 °C. Morphologically, at 20 °C, autoradiographic grains predominantly localized to the plasma membrane during the entire observation period. At 37 °C there was a progressive intracellular translocation of autoradiographic grains detectable by 2 min of incubation. After 60 min, up to 35% of the grains were translocated intracellularly. The evaluation of internalized grains after 30-60 min incubation at 37 °C showed a preferential (6-fold) association of the grains with lysosomal structures. These data demonstrate that after initial binding to the cell surface radioactivity is translocated in a time- and temperature-dependent fashion to lysosomal structures.

Induction of fibre outgrowth and choline acetyltransferase in PC12 pheochromocytoma cells by conditioned media from glial cells and nervous tissue extracts

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The survival in culture of neurons derived from dissociated ganglia of the peripheral nervous system is dependent on the presence of specific protein factors. Recently, the abilities of factors other than nerve growth factor (the only well-characterized factor so far) to support the survival of neurons in culture have been described. In particular, it has been reported that a protease-sensitive component present in the medium conditioned by rat glioma cells (C6) can support the survival of sensory neurons. We report now that a cell line (PC12) derived from a rat pheochromocytoma responds to the glial conditioned medium (GCM) by fibre outgrowth and increase activity of the enzyme choline acetyltransferase. This enzyme induction has been used to quantify the effect of various tissue extracts. It has been found that a GCM-like activity is present in nervous tissue, whereas at comparable concentrations heart and liver are inactive. Further characterization of the factor(s) responsible for this GCM-like activity is now in progress.

Haemoglobin crystals in the midgut of *Rhodnius prolixus* Stal (Heteroptera, Reduviidae)

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The reduviid bug *Rhodnius prolixus*, vector of Chagas' disease, needs only blood for nutrition, development and egg production. The blood is stored in the capacious 'stomach' and discharged in small portions to the posterior, digestive part of the midgut. Haemoglobin from the haemolyzed guinea-pig erythrocytes crystallizes in the 'stomach'. Besides the typical tetrahedron other crystal forms are regularly found. The relative amount of different crystal types seems to depend on the developmental stage of the bug. 1 month after the blood meal, the surface of the

remaining crystals in the 'stomach' showed strong irregularities. Crystals have been investigated by light and electron microscopy and are currently being examined by X-ray diffraction and spectroscopy.

Migration and proliferation of vascular smooth muscle cells induce vasoconstriction

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Removal of arterial endothelium by balloon catheter induces platelet adhesion to the subendothelial surface, migration of smooth muscle cells (SMC) from the media (M) into the intima, proliferation of SMC and synthesis of connective tissue. Aorta and peripheral arteries of rabbits were fixed by perfusion of glutaraldehyde at 80 mm Hg up to one year after ballooning. Volume per unit vessel-length of lumen (VL), of neointima (NI) and of M and circumference of internal elastic lamina (IEL) were measured by semi-automated stereological techniques in the ballooned (BIA) and corresponding control (CIA) iliac arteries. In BIA maximal migration of SMC through fenestrations of IEL occurred on days 3-5, NI was maximal on weeks 2-8. VL of BIA and CIA were similar from days 1-4 (22 ± 2 mm^3/cm, mean$\pm$SE), then VL of BIA gradually decreased to 7 ± 2 mm^3/cm on week 2. This narrowing of the lumen was not only due to intimal thickening but predominantly to vasoconstriction, since NI accounted only for 1.7 ± 0.1 mm^3/cm and IEL became wavy and changed from 53 to 31 mm.

The DNA of a virus which inhibits mixed lymphocyte cultures

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We are characterizing the DNA of a virus which inhibits the generation of cytotoxic lymphocytes in mouse mixed lymphocyte cultures. The virus, first noticed by Bonnard et al., is of the parvovirus group. The major form of viral DNA extracted from infected cells is linear, mostly double-stranded, and migrates on agarose gel-electrophoresis as a molecule of 5 kb. It probably has a hairpin structure since it migrates in alkaline gels as a molecule of 10 kb and renatures immediately on cooling after heat denaturation. Using this form of the viral DNA we compared restriction enzyme digestion patterns of this virus and another mouse parvovirus, minute virus of mice (MVM). The DNAs of these 2 viruses are clearly closely related, but not identical. MVM has not been found to inhibit mixed lymphocyte cultures.

Transcription of simian virus 40 chromosomes

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We are interested in the question of what happens to nucleosome structure during transcription of DNA in chromatin. Purified simian virus 40 chromosomes can be transcribed in vitro by exogenous RNA polymerases. The rate of transcription in 0.5 M salt is about 10-fold lower than that seen with the corresponding naked DNA. Several lines of evidence indicate that, under certain conditions, nucleosomal histones can slide along DNA. To see whether histone sliding is involved in allowing transcription of chromatin DNA we are comparing the template properties of circular viral chromosomes with the template properties of EcoRI-cleaved linear chromosomes where histone sliding would be expected to be limited.

Cytochemical staining of glycocalix using ruthenium hexammine trichloride (RHT): Properties of an isolable intermediate

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The role of OsO_4 in preservation and characterization of extracellular mucosubstances with Ru-polyammines is still a matter of discussion. When RHT is mixed with OsO_4 in buffered solutions, an undialyzable black material is obtained: This intermediate contains 18.8% Ru (atomic absorption) in constant ratio with Os (X-ray microanalysis) and shows a characteristic UV-absorption at 277 nm ($E^{1\%}_{1cm}$: 267). In vitro, this reagent precipitates mucosubstances, acidic polyaminoacids and only a few proteins. If tissue pieces, fixed in glutaraldehyde, are immersed in the reagent solution before embedding, the glycocalix is well-preserved and lightly stained; its contrast is enhanced if OsO_4 is added to the reagent and suppressed if $SOCl_2$-CH_3OH methylation precedes the staining. Therefore we postulate that Ru-polyammines interfere ionically in Luft-type reactions through Ru,Os-intermediates.

Qualitative and quantitative immunocytochemistry of exocrine enzymes in the normal rat pancreas

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The use of the protein A-gold technique for demonstration of antigenic sites in thin sections of Epon-embedded tissue allowed to localize α-amylase (AMY), trypsinogen (TRY), chymotrypsinogen (CTR), carboxypeptidases A and B (CPA, CPB), DNase (DNS), RNase (RNS), lipase (LIP) and elastase (ELA) in the rough endoplasmic reticulum (RER), Golgi apparatus and zymogen granules (ZG) of rat acinar cells as well as in the acinar lumen. A quantitative evaluation showed a difference in the density of labelling among the enzymes. Moreover, an increasing gradient in the labelling from the RER to the Golgi and to the ZG was found for AMY, CTR, TRY, CPA, CPB and RNS, while a comparable low degree of labelling in the Golgi and in the ZG was observed for DNS, LIP and ELA. From these data we suggest that although the 9 enzymes are present in the same intracellular compartments, they may not be all concentrated to the same extent in the ZG.

Transfer RNA mediated suppression in *Drosophila melanogaster*

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Many suppressor mutations have been described in *Drosophila melanogaster* on a phenotypical level. The ability of tRNAs extracted from 6 different suppressor stocks to translate nonsense codons has been tested in several cell-free protein synthesizing systems with no positive result so far (Kiger, Nucl. Acids Res. *1*, 1269, 1974; Kubli, unpublished results). Therefore we decided to screen the following suppressor stocks for deviations from the wild type tRNA pattern on 2-d PAA gels: *su(f)*, *su(s)^2*, *su(t)*, *su(Hw)^2*, *Su(ss)^2*, *su(w^a)*, *Su(S)*, *su(pr^B)*, *Su(dx)*, *Su(er)-su(tu-bw)*. The only reproducible difference in comparison with the wild type was found in *Su(dx)*: one additional spot was found in the region of the larger tRNA species. 12 spots were obtained upon fingerprint analysis (Panc RNase). This suggests that only one tRNA isoacceptor is localized in this additional spot. Identification of this tRNA species by aminoacylation, genetical and cytological localization is in progress.

DNA polymerase γ from mouse P815 cells: Role of SH-groups for activity

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DNA polymerase γ from P815 mouse mastocytoma cells was partially purified by ion exchange and affinity chromatography; chromatographic behaviour as well as catalytic properties are in good agreement with those reported for DNA polymerases γ from other cells or tissues. This polymerase can be separated completely from DNA polymerase β by chromatography on DEAE-cellulose and from α on poly (rA)-CL-sepharose. It elutes as a single form irrespective of the subcellular origin and shows best activity using poly(rA).oligo(dT). The activities of eukaryotic DNA polymerase α and retroviral reverse transcriptase are inhibited by β-lapachone in the presence of dithiothreitol. The SH-groups involved may be protected to some extent by the template/primer (Experientia, in press, 1978). In the case of DNA polymerase γ, complete protection by template/primer is observed in preincubation experiments, whereas enzyme activity could never be inhibited to more than 40%. In conclusion it seems that an alteration of configuration of DNA polymerase γ leads to a level of reduced activity.

Plasmids containing excisable DNA corresponding to the entire genome of RNA phage Qβ

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A pCRI hybrid containing the complete Qβ DNA sequence flanked by AT-regions elicits Qβ formation in *E. coli* (Taniguchi et al., Nature *274*, 223, 1978). The Qβ segment was used to construct several plasmids allowing excision of Qβ DNA by restriction, and in vivo and in vitro expression was studied. Qβ DNA excised with nuclease S_1 was a) ligated with HindIII linkers and inserted in the HindIII site of pBR322 or b) (dA)-elongated and joined to pBR322 which had been cleaved with HindIII, filled up with polymerase I and (dT)-elongated. Only b) yielded plasmids that elicit phage, indicating that expression of phage DNA requires long flanking AT-regions. In vitro transcription of plasmids or excised Qβ DNA did not yield infectious Qβ RNA. Insertion of Qβ DNA into the PstI-site of pBR322 yielded among 1000 clones only 2 producing phage; both had acquired IS 1 (which terminates transcription) between the amp promotor and Qβ DNA, suggesting that Qβ DNA in an actively transcribed region is lethal.

Growth and characterization of endothelial cells on floating collagen gels

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Endothelial cells (EC) isolated from calf aortas were grown on collagen gels and on floating Nytex-100 mesh coated with collagen to analyze in vitro their proliferation and morphology. Aortas were treated with collagenase (200 U/ml) and the detached sheets of EC collected and cultures in DMEM/M199 supplemented with 20% foetal calf serum. Within the first day almost 80% of EC attached and cultures were confluent after 4–5 days. Electron micrographs showed the typical features of vascular EC including the vesicular transport system and cell-to-cell junctions. An extracellular material lining the EC basal side was observed. Scanning EM results demonstrated a continuous layer of EC with few microvillous projections. Experiments are in progress to study the transport of tracers and the passage of tumor cells through the reconstructed endothelium. In addition, kinetic studies of the attachment of EC to various surfaces were performed by reflexion-contrast microscopy.

Cloning of the thymidine kinase gene of herpes simplex I in pBR322

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Herpes simplex I DNA was a) cleaved with BamHI and the fragments ligated to BamHI-cleaved pBR322 or b) cleaved with KpnI, the fragments elongated with dA and joined to EcoRI-cleaved dT-elongated pBR322. The hybrid DNA was cloned in *E. coli* X1776 and screened by the Grunstein-Hogness procedure using a purified thymidine kinase (TK)-specific KpnI herpes DNA fragment. Several hybrid plasmids containing TK-specific DNA in both Kpn and Bam inserts were isolated and found to be capable of transforming TK^- L-cells, as assayed by determining their colony-forming capacity in HAT medium. The infectivities were about 40 colonies per 10^6 cells per 250 ng plasmid DNA. A restriction map of the TK fragment was prepared.

Peptide mapping of heterogeneous protein samples

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A simple 2-dimensional electrophoretic method for the peptide mapping of heterogeneous protein samples is presented. The reduced and denatured proteins of the mixture are separated in a first dimension by SDS-polyacrylamide slab gel-electrophoresis. After completion of the electrophoresis, the whole gel lane is equilibrated in stacking gel buffer and is transferred at right angle on a second slab gel. A partial proteolysis of the proteins to be analyzed is performed during the stacking phase of the second electrophoresis. The second electrophoresis resolves the characteristic pattern of peptides of each individual protein as a series of spots located below the original position of the undigested protein. In complex samples up to 20 individual proteins can be fingerprinted at once and a protein comprising only 1% of the total sample generates a clearly identifiable peptide pattern. Good reproducibility of the patterns obtained allows the comparison of samples of different origins.

The influenza virion-associated transcriptase: Its transcription products

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The influenza virus is a negative strand RNA virus with a segmented genome. An RNA-dependent transcriptase is associated with the virion. This transcriptase synthesises in vitro functional messenger RNAs as shown in a cell-free system for transcription and translation of the influenza virus genome (Content, De Wit and M.A. Horisberger, J. Virol. *22*, 247, 1977). The in vitro products of the transcriptase reaction have been further characterized since 2 classes of influenza transcripts were found in infected cells; the 2 classes are represented by complete and incomplete copies of each segment of the genome (Hay et al., Virology *83*, 337, 1977). Messenger RNAs correspond to the incomplete copies. Depending on the assay conditions, transcripts of the 2 classes have been obtained in vitro. Their characterization relies on the degree of protection against S_1

nuclease digestion of RNA-RNA hybrids obtained after annealing the in vitro transcripts with the genome RNAs.

Solubilization of Golgi apparatus glycosyltransferases by Triton X-100

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Combined light and intermediate rat liver Golgi fractions (Ehrenreich et al., J. Cell Biol. *59* 45, 1973) were mixed with Triton X-100 at ratios (R) from 0.8 to 6.5 mg/mg phospholipid. The distributions of sialyl-, galactosyl- and N-acetylglucosaminyltransferase were assayed (Bretz et al., J. Cell Biol. *79* 247, 1978) after centrifugation in linear sucrose gradients. Lysis and loss of VLDL content occurred at $R \approx 0.8$; the membrane-bound transferases cosedimented to a density of 1.09 g/ml and were purified 2-fold. Higher ratios ($R = 2$) led to extensive membrane damage, and 50% of the sialyl- and galactosyltransferase were solubilized. GlcNAc-transferase remained sedimentable, although membrane structures were no longer detectable. At $R \geq 4.8$, GlcNAc-transferase aggregated with other proteins at the bottom, whereas 80% of the other 2 enzymes were soluble. A separation of the transferases became possible only after the breakdown of the membranes.

A new procedure for the isolation of Rous Sarcoma virus specific mRNAs from infected cells

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DNA complementary to the 5′ end of Rous Sarcoma virus (RSV) genomic RNA ('strong stop' c-DNA) has been used to isolate from RSV-infected cells virus specific mRNAs of genomic and subgenomic sizes. 'Strong stop' RSV DNA was prepared using both endogenous and reconstructed viral DNA polymerase reactions. It was then mercurated and hybridized to various RNA fractions from infected cells in 0.3 M NaCl, 10 mM Tris buffer at 67 °C for up to 30 min. The hybrids were bound to SH-agarose columns and the columns eluted with β-mercaptoethanol and 99% formamide separately. Fingerprint analyses of the isolated RNAs showed that this method achieves a high degree of purification with excellent yields. The low complexity of the 'strong stop' c-DNA allows rapid and complete hybridization under conditions that prevent alteration of the RNAs as shown by the fact that the isolated RNAs are good templates for in vitro translation in the messenger dependent reticulocyte lysate system.

Activation of macrophages by soluble factors: III

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Murine splenic lymphocytes stimulated by Con A release an activating factor (AF) which can be detected by its capacity to induce mouse peritoneal macrophages (mØ) to kill intracellular Leishmania parasites (Experientia *33*, 814, 1977). MØ activation by a suboptimal dose of AF is potentiated by ng amounts of endotoxin, that are without effect on mØ in absence of AF. Incubation of mØ with AF enhances incorporation of ^{3}H-glucosamine, suggesting an increased turnover of the activated mØ membrane. AF stimulates superoxide production in mØ, as indicated by an increased NBT reduction in activated cells. At concentrations over 30 µg/ml, Con A inhibits activation induced by AF, presumably by binding to the factor, thus preventing its interaction with mØ membrane. In accordance, AF activity can be removed from supernatants by passage through Con A-sepharose.

Preparation of inner membrane surfaces of human erythrocytes

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Covalent attachment of human erythrocytes to glass or mica was achieved after derivatization of cover slip surfaces by incubation for 24 h at 45 °C with a 2% solution of 3-aminopropyltriethoxysilane in acetone followed by 60 min at 4 °C with an aqueous solution of 1% glutaraldehyde. The cells were then opened by squirting the monolayer with PBS, leaving behind fragments of membranes with their plasmatic face (PS) exposed. Antibody double-labelling techniques for immunofluorescence and freeze-drying preparation for electron microscopy were employed to characterize the orientation and accessibility of markers of the PS and external outer surface (ES), respectively. Thus, the attached membranes reacted with antispectrin whereas peripheral areas (representing curled up membranes) were reactive with Sendai virus particles, which bind to sialic acid containing receptor sites of the ES. Adsorption of virus prior to the attachment of the cells to ES allowed the monitoring, at the PS, of discrete penetration stages of virus particles through the membrane.

Murine leukemia virus antigens expressed on the surface of infected cells

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Viral antigen expression on the cell surface was studied by means of lactoperoxidase-catalyzed iodination of intact cells, followed by immune precipitation of the cell lysates with antisera specific for viral proteins gp 70 or p 30, and analysis on polyacrylamide gels. Anti-gp 70 serum precipitated surface-labelled gp 70 glycoprotein both in uninfected and in Rauscher MuLV-infected JLS-V9 cells. Anti-p 30 serum precipitated from infected cells 2 surface-labelled proteins of mol.wts about 85 K and 95 K, which could also be labelled metabolically with ^{3}H-mannose. Although present in reduced amounts, they seem analogous to the viral glycosylated polyproteins described by others on the surface of leukemia cells. Tryptic fingerprints on ion exchange columns were performed, after re-iodination with Chloramine T in the gel slice. 85 K and 95 K showed very similar patterns, which were different from that of gp 70. Comparisons with the fingerprints of viral structural proteins and of the polyproteins of AKR cells expressing endogenous virus will be presented.

Role of different cell types in MAF-mediated cell-cell interactions in the marine sponge *Microciona prolifera*

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Aggregation factor from the marine sponge *Microciona prolifera* (MAF) is a large protein-polysaccharide complex which promotes species-specific reaggregation of *Microciona* cells. Crude single cell suspensions from adult *Microciona* tissue were separated by velocity sedimentation into fractions enriched in distinct cell types. The response of these different fractions to MAF was tested. MAF promotes aggregation of the cells in all cases. In addition, in all fractions at least 95% of the cells bound to MAF-coated sepharose beads, indicating that most of the cells in these

fractions interact with MAF. Binding studies with I-125 labelled MAF suggest that the receptor density for MAF is similar for different cell types. Intact larvae are not affected by MAF; however, addition of MAF to dissociated larval cells causes their aggregation in a MAF-matrix. In calcium-magnesium-free seawater larval cells release a factor active in aggregating larval cells in the presence of calcium.

Organization of parental chromosomes in diploid mouse embryo cells

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The organization of parental haploid contributions following fertilization and establishment of a new diploid state in mouse eggs has been studied by 2 approaches both aiming at visualization of paternal chromosomes. a) ^{3}H-TdR labelling of sperm allows tracing of the paternal genome in eggs at early cleavage stages observed in semithin sections. Results confirm previous observations of a segregated arrangement of parental contributions in nuclei of 2-cell embryos and extend it to chromosomes observed in metaphases of the second cleavage division. b) Male mice carrying multiple metacentric chromosomes have been mated with females of a conventional laboratory strain (all chromosomes acrocentric). Metaphase spreads from F_1, embryos analyzed at various stages of development indicate a nonrandom location (topographic grouping) of marker chromosomes, thus confirming a segregated arrangement as in a. These findings suggest that individual chromosomes do not behave as units completely independent from each other or from their parental origin. Further experiments aim at elucidating how far this segregation extends in development.

The molecular envelope of adenovirus type 2 hexon and its interactions in the viral capsid

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Hexon, a trimer with 3 subunits each of mol.wt 120,000, is the major coat protein of adenoviruses. The icosahedral viral shell is formed from 240 hexons at the faces and 12 pentons and fibres at the vertices. The molecular envelope of hexon has been traced from an electron density map determined by X-ray crystallography. Hexon has a solid pseudo-hexagonal 'base' and a less dense triangular 'top', with the apices of the triangle roughly above the midpoints of 3 basal edges. A small hole at the top of the molecule widens to form a cavity extending to a larger opening at the base. The hexagonal shape of the base permits packing of hexons to form the flat continuous surface seen on a face of the viral capsid. The imperfect hexagonal symmetry probably has implications for the dissimilar interactions at the capsid edges. The hole could be an example of structural economy. Electron micrographs of the intact virus showing a spiky appearance are consistent with an array of triangular hexon tops.

Deficiency of serum of atherogenic-fed swine to support in vitro endothelial regeneration

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On the hypothesis that a major factor in the initiation of atherosclerosis is a failure to properly heal wounds of the vascular endothelium, we have developed an objective, quantitative in vitro assay of wound-healing using endothelial-like cultures of 3T3-B mouse cells. We count the number of cells that migrate across a 1-mm starting line in 22 h. We find that sera from mini-pigs fed an atherogenic (hyperlipid) diet consistently have less migration activity than sera from control pigs. In certain cases the deficiency is due to an inhibitor that is nondialyzable and not associated with the serum lipoproteins. Further serum migration activity is not simply correlated with cholesterol or triglyceride content of serum. The appearance and disappearance of the deficiency follow rapidly changes in the diet.

Creatine kinases in cultures derived from embryonic chicken muscle: Degradation and modulation of synthesis

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Degradation of creatine kinase (CK) B-CK and muscle specific M-CK subunits were determined by pulse chase experiments in differentiated myogenic cultures (DMC), BrdUrd-suppressed myogenic cultures (BMC) and fibrogenic cultures (FB). The half-lives for B-CK, M-CK and total protein (TP) were: 63, 75, 51 h in DMC; 37, *, 39 h in BMC; 46, *, 47 in FB (* M-CK not detectable). Myosin and actin half-lives showed similar trends with enhanced stability in DMC. Differential turnover appears not to govern the differentiative transition of B-CK to M-CK but may affect levels of CK in various cells. Variations in culture conditions were shown to influence the synthesis of CK: fibroblast growth factor inhibited M-CK synthesis specifically; increasing concentrations of embryo extract enhanced TP synthesis and the ratio of M-CK to B-CK synthesis; horse serum affected TP alone but not the M-CK to B-CK ratio. Insulin and creatine were without effect under the conditions used.

Distribution and ultrastructural characterization of β-endorphin immunoreactive cells in the human pituitary; the same cells stains with ACTH-antisera

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An antiserum specific for human β-endorphin(β-E), was used to localize immunoreactive cells in pituitaries of human adults. In median sagittal paraffin sections such cells were distributed as follows: a) uniformly throughout the anterior lobe, b) lining the colloid cysts of the intermediate zone, and c) in the region of basophilic invasion of the posterior lobe. Post-embedding immuno-electron microscopy disclosed variations in cellular ultrastructure, including hormone granule size. β-E immunoreactive cells stained also with non-cross-reacting antisera to ACTH 1-39, ACTH 1-24, ACTH 17-39. Latter results demonstrate the simultaneous presence of β-E and ACTH in cells of the human adult pituitary and support therefore the theory of a common precursor for both peptides.

An actin destabilizing factor is present in plasma and serum of various animal species

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Immunofluorescent staining with human anti-actin antibodies of cultivated fibroblasts, epithelial cells and frozen

sections of various organs (e.g. liver and skin) results in different staining patterns according to whether the whole serum or the purified antibody is used. Thus, in nonconfluent mouse embryo fibroblasts, the purified antibody shows typical stress lines, whereas the whole serum shows few thin lines, in anotherwise unstained cytoplasm. In liver sections, the purified antibodies stain blood vessel and bile duct smooth muscle as well as hepatocytes with a polygonal pattern, whereas the whole serum does not stain hepatocytes. In vitro incubation of filamentous actin with normal plasma or serum from different species (man, rat, rabbit, guinea-pig) and with anti-actin antibody containing serum produces the dissociation of the filaments in small clusters. We conclude that plasma and serum contain a factor responsible for the destabilization of filamentous actin.

The translational repression of the free cytoplasmic globin messenger ribonucleoprotein complex

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In its native form mRNA is associated with proteins to form ribonucleoprotein complex. In the duck reticulocyte cytoplasm, the proteins linked to globin mRNA vary with its subcytoplasmic localization, i.e. in the polyribosomes or in the post-polyribosomal supernatant. In cell-free protein synthetizing systems, these 2 types of mRNP show translational abilities reflecting their natural state: the polyribosomal mRNP directs globin synthesis whereas the free cytoplasmic form remains untranslated. The globin mRNAs extracted from each complex have similar translational efficiencies. Research into the factor responsible for the translational repression within the free cytoplasmic mRNP reveals that this factor is tightly linked to globin mRNA. Deproteinization techniques which avoid loss of RNA yield fully translatable mRNA, leading is the conclusion that the repressor activity needs protein factors to be effective. Further characterization of the repressor will be discussed.

In vivo studies on endothelial regeneration

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Endothelial regeneration was studied in rabbits after the aortic endothelium was abraded by the balloon catheter technique. The extent of endothelial regeneration was quantitated various times after injury by i.v. injection of Evans-blue and determination of the amount of dye taken up by the aortic wall. Intimal healing took place immediately after the mechanical trauma. Most of the regenerated endothelium originated from existing branches which was assessed by the Evans-blue uptake pattern and confirmed by transmission and scanning electron microscopy. The process of re-endothelialization was enhanced by glucocorticoid treatment whereas inhibition of endothelial regeneration was observed in hyperlipaemic rabbits. These results support the concept that endothelial regeneration competes with the proliferative response of the vessel wall to injury.

Scanning electron microscopic observations of differently cross-linked polyacrylamide gels

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N,N'-Methylenebisacrylamide (BIS) and N,N'-Diallyltartardiamide (DATD) were used as cross-linker compounds in polyacrylamide gels. The amounts of both were varied and structures of equimolar gels were compared. The surfaces of DATD-cross-linked gels were different to that of BIS-cross-linked gels. This may explain the better adherence of DATD-cross-linked gels to glass. As the concentration of the cross-linker compound raised the BIS-cross-linked gels showed greater changes in their structure than the DATD-cross-linked gels did. The structures of DATD-gels remained practically unchanged. These observations coincide very well with those reported earlier (P. J. Späth and H. Koblet, Analyt. Biochem. *93*, 275, 1979).

Acetylcholinesterase (AcChE) activity in Friend erythroleukemia cells

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Several clones of Friend cells were obtained from separate cases of Friend leukemia and they differ in the extent to which they respond to dimethylsulfoxide (DMSO). However, when compared to a whole series of different, nonerythroid cell lines, they all display very high levels of AcChE activity, even in the absence of DMSO. This suggests that the enzyme is an early marker of erythroid differentiation which appears before hemoglobin (Hb) production is initiated. After treatment with DMSO, AcChE activity increases up to 4-fold, not only in all Friend cell clones which initiate Hb production, but also in some of those that do not. While significant Hb production cannot be detected before the cells have been exposed for 3 days to DMSO, 50% or more of the total AcChE increase is already present after 1 day. Thus, AcChE activity must be regulated by mechanisms which are different, at least in part, from those regulating Hb production.

Construction of recombinant plasmids containing avian vitellogenin structural gene derived from complementary DNA

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Part of vitellogenin structural gene was enzymatically synthesized and cloned using plasmid pBR322 and *E. coli* χ 1776. cDNA was synthesized using AMV reverse transcriptase. Double-stranded cDNA was synthesized from cDNA using *E. coli* DNA polymerase 1. Following S1 nuclease treatment, poly dA was added to 3-min termini with terminal transferase. dcDNA fragments were annealed with linear plasmid pBR322 tailed with poly dT. χ 1776 was transformed with the hybrid molecules and ampicillin-resistant colonies were screened by in situ colony hybridization. Southern blots were prepared from linearized plasmids containing cloned cDNA. These were hybridized with ^{125}I-mRNA vitellogenin and control mRNA from untreated chicks. The 4 largest plasmids which hybridized only with vitellogenin mRNA were selected for further characterization. These contained inserts of 1.0-2.5 kb as determined by restriction analysis and from heteroduplexes with parental plasmid pBR322. Restriction maps of at least 3 of the cDNA clones differ substantially from one another.

Characterization of a Rous Sarcoma virus mutant temperature sensitive for cell transformation

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We have found that the LA23 ts mutant of Rous Sarcoma virus (phenotype Prague B), even when passaged repeatedly at high multiplicity of infection, does not give rise to transformation defective deletion mutants comparable to those derived from RSV. In view of this fact and of the high rate of production of this mutant at 41 °C, we have undertaken a detailed analysis of this virus. The genome was characterized by ordering all large T_1 oligonucleotides and by determining their nucleotide sequences. The results indicate a high degree of mutation in the onc gene as compared to that of Pr-A, Pr-B or Pr-C. In addition the products of in vitro translation of the genomic RNA and of polyA$^+$ RNA fragments have been characterized.

Cycle lumineux et modifications cytoplasmiques dans l'épithélium pigmentaire rétinien

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L'influence du cycle lumineux sur le contenu en phagosomes (segments externes des photorécepteurs phagocytés), ainsi que quelques modifications cytoplasmiques intervenant lors du passage obscurité/lumière sont étudiées dans l'épithélium pigmentaire rétinien de *Rana ridibunda*. Des animaux sont soumis à un cycle de 12 h obscurité/12 h lumière. Leurs yeux sont prélevés à différents temps du cycle et préparés pour la microscopie électronique. Le nombre de phagosomes est fonction du cycle, un maximum étant atteint après 2 h d'illumination. Différentes techniques de fixation modifient ces résultats. Au début de la phase lumineuse, le volume des corps myéloïdes passe du simple au double; leur nombre reste constant. Dès l'enclenchement de la lumière, la densité de surface du réticulum endoplasmique lisse baisse, puis augmente à nouveau 1 h après. Le nombre de microperoxisomes par contre ne semble pas subir de modifications lors du passage obscurité/lumière. Il semble que les phagosomes soient finalement éliminés par exocytose dès 6 h de lumière.

Morphogenic haploid cultures of *Zea mays* L.

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Morphogenic haploid (n) and aneuploid (2n-?) tissue cultures are highly desirable for in vitro selection of recessive mutants and plant regeneration. Scutella from immature corn embryos give morphogenic tissue cultures. *Stock 6* From a cross involving alcohol dehydrogenase null *($Adh1^-$)* mutant in *Stock 6* background as female and *Stock 6* as male, immature embryos were screened using allyl alcohol. Diploid embryos *(Adh^+/Adh^-)* died due to ADH activity, but maternal haploid embryos *($Adh1^-$)* with no ADH activity survived.

The biosynthesis of an unglycosylated envelope glycoprotein of Rous Sarcoma virus in presence of tunicamycin

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Cells infected with RSV were treated with tunicamycin to prevent the glycosylation of the precursor (pr 92) to the 2 viral envelope glycoproteins gp 85 and gp 35. A new polypeptide made in the presence of the drug was identified by immunoprecipitation of pulse-labelled cell lysates with monospecific anti-gp 85 and anti-gp 35 sera. This polypeptide, migrating on SDS-polyacrylamide gels as a molecule of 62,000 daltons (pr 62), contained no mannose, was labelled with radioactive amino acids, could not be chased into the higher mol.wt glycosylated form and contained the same ^{3}H-arginine tryptic peptides as pr 92. The unglycosylated pr 62 was detectable 2 h after the pulse labelling of the cells. The lack of glycosylation did not seem to reduce its stability. No clear evidence for the incorporation of this molecule or its cleavage products into viral particles could be obtained. To code for an envelope polypeptide of 62,000 daltons only 1500 nucleotides or 15% of the coding capacity of the virus is needed.

Cobalt-reconstituted trout hemoglobins

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Cobalt Proto and Meso Porphyrin derivatives of trout *(Salmo irideus)*. Hemoglobin components I and IV have been reconstituted. The O_2-affinities of the CoHbs are much lower than the ones of the iron-containing proteins: Only the CoMeso-HbI can be fully oxygenated at room temperature under pure oxygen. Similarly to the native protein the O_2-binding of this derivative is cooperative and pH-independent (log $p^1/_2 = 2.4$; $n = 1.2$). EPR spectra of CoProto and CoMeso HbI in air show that, at −150 °C, they are completely saturated with oxygen. At higher pH the hyperfine structure of the signal is less resolved suggesting a greater spin transfer from Co to Oxygen. This mey be related to a hydrogen bonding between O_2 and the distal histidine (Ikeda-Saito et al., J.B.S. *252*, 48882, 1977). In the g region the deoxy CoMeso-HbIV shows a small change with pH which can be related to a structural change in the T-state when the Root effect is operative.

Multiply labelled α-MSH derivatives for degradation studies

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Several new analogues of α-MSH carrying 1-3 different labels (^{3}H, ^{125}I, rhodamine B) at defined sites have been synthesized in order to investigate the degradation of this tridecapeptide in plasma, and during or after the interaction with target cells. The radioactive compounds exhibited a high specific activity (34–110 Ci/mmole [^{3}H] and 1500 Ci/mmole [^{125}I]) and almost full biological potency (details: Eberle et al., Helv. chim. Acta *62*, 1979). In a first series of experiments, ^{125}I-containing α-MSH was incubated in rat plasma in rather low (physiological) concentrations (100 pg/ml) at 4 °C and 37 °C respectively. Analysis in intervals of 15 min revealed that α-MSH is degraded about 5–8 times more rapidly at 37 °C (15 min: 30%; 30 min: 55%; 60 min: 70%; 120 min: 85%; 180 min: > 95%) than at 4 °C (15 min: 5%; 30 min: 8%; 60 min: 15%; 120 min: 22%). These findings are an important basis for a successful and reliable radioimmunological determination of α-MSH following its partial purification from serum.

Purification of choline acetyltransferase from pig brain

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In a mixed population of nerve cells, it has not been possible so far to unambiguously identify cells synthesizing acetylcholine. This is mainly due to the lack of a specific marker for these cells. One way of solving this problem is to use specific antibodies raised against the purified enzyme responsible for the synthesis of acetyltransferase (ChAT). The main difficulties in the isolation of ChAT lie in the very low specific activity of mammalian tissues and in the instability of the enzyme. We report here on the purification by a factor of over 200,000-fold of ChAT from pig brain. The final preparation had a specific activity of 102 µmoles acetylcholine/min/mg prot. and appeared as the only major band on SDS-polyacrylamide gel-electrophoresis. Briefly, 20 kg pig brain were homogenized in 5 vol. of water, centrifuged and resuspended in 0.2 M NaCl. The supernatant was adsorbed on CM-sephadex, the eluate precipitated with 50% $(NH_4)_2SO_4$ and chromatographed on hexylamine sepharose. The active fractions were precipitated by $(NH_4)_2SO_4$ and adsorbed on Cibacronblue sepharose. ChAT was eluted with 500 µm coenzyme A and 5 mM choline.

Permeability of alveolar-capillary membrane in pulmonary edema

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The permeability of the alveolar-capillary membrane to horseradish peroxidase (HRP) - at a dose of 5 mg/100 g b.wt injected in 0.2 ml 0.9% NaCl - was investigated in normal rats and in rats with pulmonary edema produced by a single i.v. injection of adrenalin. In normal lungs HRP was transported through pinocytotic vesicles but not through tight junctions of capillary endothelium. Alveolar epithelium was not labelled. During edema, HRP-labelled pinocytotic vesicles and tight junctions of both capillary endothelium and alveolar epithelium and leaked in the alveolar space.

Aortic permeability and endothelial cell replication during different types of hypertension

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Hypertension was produced in rats by: a) ligature of the aorta, b) uninephrectomy and Na rich diet, c) uninephrectomy, 1% NaCl to drink and desoxycorticosterone acetate (DOCA) s.c. Permeability of aortic endothelium to horseradish peroxidase, rate of replication and/or cell density of endothelial cells were correlated with plasma renin concentration (PRC) and aldosterone level (PAL) at early and late stage of hypertension. Increased permeability and mitotic rate and/or cell density were seen: a) early after aorta ligature (PRC and PAL were increased compared to controls), b) early and late after DOCA (PRC and PAL were low but exogenous DOCA was high). No permeability change and normal mitotic rate were seen: a) late after aorta ligature, b) uninephrectomy and Na-rich diet (PRC and PAL were low). We conclude that permeability and mitotic rate of aortic endothelium are not affected by hypertension per se; they may be related to mineralocorticoid levels.

Mass determination by quantitative scanning transmission electron microscopy

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Due to the linear relationship between the mass of a small particle and its total elastic scatterin cross-section, the mass of proteineous macromolecules can be determined by measuring the number of scattered electrons. The quantitative capability of our scanning transmission electron microscope (STEM) which is interfaced to a minicomputer allows the number of scattered electrons to be measured at high precision. The factor relating this number to the mass of the investigated particle can be derived from scattering theory taking into account the geometry of the darkfield detector. Alternatively, it can be determined by measuring particles of known masses. A good correlation between the 2 values was found and measurements on different structures (T-even phage polyheads, fd-phages, P23 monomers) prove the method to be reproducible. Mass determination can be accomplished over a broad range of specimens and requires very small amounts of material. The purity of the sample is not very critical provided it can be discriminated from impurities by its particular shape and impurities do not interact with the specimen of interest. Since the mass of a particular structure sets severe limits on the possible molecular arrangement, the method allows structural information to be extracted where conventional techniques fail to work. At present several filamentous protein agglomerates (P22 ribbons, RNA-polymerase cables, different types of T-even phage polyheads) are being investigated and preliminary results allow some conclusions on their structures to be drawn.

Which component of the Friend virus (FV) induces erythropoietin-independent erythropoiesis?

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In mice infected with FV, erythropoiesis becomes independent of normal regulation by erythropoietin (EPO) and animals develop erythroblastic leukemia. FV comprises a replication defective spleen focus-forming virus (SFFV) and a helper virus, usually lymphatic leukemia virus (LLV). In this study, an LLV-nonproducer, SFFV-positive fibroblast cell line was rescued with the helper viruses LLV-F, LLV-$Gross_F$ and LLV-Moloney and the viruses produced were injected into mice. Bone marrow cells cultured from these animals formed large numbers of EPO-independent erythroid colonies after 2 days (CFU-E) and 3-5 days (intermediate BFU-E), regardless of the helper virus present with SFFV. In contrast, normal cells form CFU-E (day 2) and BFU-E (day 10) only in the presence of EPO. The specific Friend helper virus (LLV-F) was not required to induce EPO-independent erythropoiesis (several unrelated helper viruses were as effective) indicating that SFFV itself causes erythroid precursor cells to become EPO-independent.

Isolation and characterization of the 4 mRNAs coding for vitellogenin in *Xenopus laevis*

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Cloning of vitellogenin cDNA of *Xenopus* has revealed that vitellogenin is coded for by a family of 4 related genes

which represent 2 groups each comprising 2 more closely related genes. To characterize the proteins encoded in these 4 genes, we have isolated the corresponding mRNA's by filter hybridization to different cDNA clones under stringent conditions. From hybridization with nick-translated cDNA clones cross-contamination of the isolated RNA by that of the opposite group is estimated to be less than 1%. Melting curves of hybrids formed by the isolated RNA's and the cDNA clones under nonstringent conditions, reveal the presence of the mRNA's corresponding to all 4 genes. Electron microscopy of R-loops prepared with the cDNA clones specific for each gene indicates that cross-contamination of the isolated RNA's by the RNA corresponding to the closely related gene is not greater than 10%. In a reticulocyte lysate translation system the 4 isolated mRNA's code for 200,000 daltons proteins which are precipitated by vitellogenin antibody. Further characterization of the in vitro translation products should disclose divergence of primary structure of the vitellogenins synthesized in vitro and their relationship to the naturally occurring vitellogenins.

Investigation of biosynthetic pathways using permeabilized *Cephalosporium acremonium* cells

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Permeabilizing cells by various methods makes it possible to analyze enzymes which cannot be assayed in crude cell extracts or in intact cells. Permeabilized bacteria have been used in many studies. Much less is known about the permeabilization of eukaryotic cells. In order to analyze the biosynthesis of the β-lactam antibiotics we have worked out a method to permeabilize the fungus *Cephalosporium acremonium*. With 2 enzyme systems, RNA polymerases and hexokinase/glucose-6-phosphate dehydrogenase, we could show that, after ether-treatment, these fungal cells are permeable to substrates which do not enter an intact cell. The DNA transcription requires the 4 nucleoside triphosphates as substrates and can be stopped by actinomycin D. The 2 glycolytic reactions need ATP and $NADP^+$ as substrates. Having shown that 2 enzyme systems function in such cells, we will try to find enzymes active in the biosynthesis of β-lactam antibiotics.

Effects of changed source/sink relations on proteolytic enzymes in wheat leaves (*Triticum aestivum* L.)

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During wheat leaf senescence aminopeptidase and carboxypeptidase activities decrease and neutral endopeptidase shows a peak. If the ears are removed about 2 weeks after anthesis, the exopeptidases (aminopeptidase and carboxypeptidase) remain more active in flag leaves. The neutral endopeptidase, which is believed to be a major factor in mobilization of foliar proteins during senescence, is less active than in control plants. The total nitrogen remains high in flag leaves of earless plants and free amino groups accumulate. In the second leaf from the top only minor differences were detected between treated and control plants. Senescence was already initiated in the second leaf when the ear was removed, while the flag leaf was still fully active. It remains open, whether hormonal effects or accumulated metabolites are responsible for the delayed changes in the pattern of proteolytic enzymes in the flag leaf of earless plants.

Molecular properties of mouse complement component C4: Glycosylation of the subunits

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An antiserum has been prepared from rabbits against partially purified mouse complement component C4 (SS-protein). After absorption using mouse plasma from SS-low strains the serum is highly specific. Using this serum for indirect immunoprecipitations it was shown that mouse peritoneal macrophages in primary tissue culture continue to synthesize C4 and to secrete it into the tissue culture medium. The secreted molecule has an approximate mol. wt of 205 kdaltons and dissociates upon reduction into 3 subunits: $\alpha = 98$ kdaltons, $\beta = 75$ kdaltons and $\gamma = 33.5$ kdaltons. It is derived from a single chain intracellular precursor molecule. Using pulse-chase experiments it was shown that the intracellular precursor gets cleaved within approx. 4 h after its synthesis, whereas it takes approx. 10 h unti all newly made material has been secreted into the extracellular space. The α- and β-subunits can be metabolically labelled with mannose and glucosamine, the γ-subunit not.

Glial factor activity: Behavior on Con A sepharose

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Glial cells in culture release a macromolecular factor(s) which induces morphological differentiation of neuroblastoma cells. This macromolecule has been partially purified using 2 simple chromatographic procedures: diethylaminoethyl cellulose and Con A sepharose. Its behavior on Con A sepharose is unusual its elution cannot be accomplished by α-methylglycoside alone. It is, however, eluted by 2 M NaCl in Con A buffer. The elution pattern, the recovery data and the electrophoretic behavior of glial factor activity are presented.

The role of *dnaA* in the replication of various plasmids

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We have used pulse labelling with ^{3}H-thymidine and DNA:DNA hybridization to follow the effect of a shift in temperature, in a *dnaAts* strain, on the replication of the plasmids colE1, pSC101, R100.1 (a drug-resistance plasmid) and RTF-TC (its derivative). In each case the replication of the chromosome was also monitored. The results demonstrate that, after a shift from 30 °C to 42 °C, R100 and RTF plasmids continue to replicate, while colE1 and pSC101 stop rapidly. As expected, chromosome initiation is inhibited. To differentiate between direct and indirect effects, experiments were repeated in strains in which chromosome replication was restored at 42 °C by integrative suppression, i.e. in strains in which chromosome replication was under the control of an integrated R100 plasmid. We found that, in such strains, colE1 could replicate at 42 °C and does not, therefore, need the *dnaA* product directly. In contrast, pSC101 needs *dnaA* for its replication. It is, so far, the only known plasmid to have this requirement.

Effects of an enriched environment on synaptosomal density from homogenates of young and adult mice brain

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1- and 3-month-old mice were subjected to enriched or to deprived sensory environments for 60 days. The mice were then subjected to psychological tests. The mice from the enriched groups displayed a greater degree of exploratory activity and had a better performance in a complex maze. The electron microscopic morphometric analysis of brain homogenates showed that the density of mitochondria were the same from all groups. However the density of synaptosomes in brain homogenates from the enriched groups was significantly higher. Similar results were obtained with adult mice. These results show that behaviour as well as neuronal structure can be affected by the environment and that these environmental-related changes are not restricted to young-age animals.

The nicking-closing enzyme assembles chromatin in vitro

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Purified nicking-closing enzyme can mediate the assembly of nucleosomes from the 4 core histones (H2A, H2B, H3 and H4) and DNA under physiological conditions of ionic strength and temperature. When histone-DNA complexes are assembled in vitro from relaxed circular DNA, nearly physiological numbers of superhelical turns are induced in the DNA molecule. Electron microscopy of the complexes reveals a beaded structure and a reduction of the contour length compared to the DNA. Micrococcal nuclease digestion of the histone-DNA complexes yields 145 bp DNA fragments typical of nucleosome core particles and shorter subnucleosomal DNA fragments of discrete length. The rate of chromatin assembly in vitro is consistent with physiological rates.

Chemostat studies on the cytochrome P-455 content in the n-hexadecane assimilating yeast *Candida tropicalis*

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C. tropicalis hydroxylizes n-hexadecane presumably via a microsomal monooxygenase system, involving cytochrome P-455. We investigated the influence of 3 parameters (oxygen limitation, growth rate and limiting substrate) on the concentration of cytochrome P-455 in growing cells. The yeast microsomal cytochrome P-455 was compared to the rat liver microsomal cytochrome P-450 on the basis of substrate- and CO-binding spectrums. NH_4^+- or n-hexadecane as the growth limiting factor showed no significant effect on the level of cytochrome P-455 (at constant growth rate and unlimiting O_2-saturation). When the growth rate was increased from 0.07 h^{-1} to 0.24 h^{-1} (at unlimiting O_2-saturation), the cytochrome P-455 content increased significantly from 0.015 to 0.026 nmoles/mg protein. When the O_2-saturation was decreased from 18% (unlimiting) to 4% (limiting O_2-saturation, at constant growth rate), the cytochrome P-455 concentration increased significantly from 0.020 to 0.072 nmoles/mg protein, indicating that the concentration of the yeast cytochrome P-455 is very sensitive to O_2-limitation. In contrast, the influence of the substrate flux on the cytochrome P-455 was less expressed.

Genes activated by heat shock in *Drosophila melanogaster*

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Exposure of *Drosophila* to 37 °C produces the activation of a small set of specific genes, previously silent, while most of the other genes, active before the heat shock, are strongly repressed. DNA segments containing such heat activated genes have been cloned and analyzed by restriction mapping, heteroduplex and nucleotide sequence determination, and also in relation to the total *Drosophila* genome. Such results will be presented.

2 antiproliferative principles (chalones?) present in rabbit epidermal extracts

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The antiproliferative activity of extracts was measured in vivo on intact and wounded mouse ear epidermis using mitotic index and/or ^{3}H-thymidine incorporation. Wounded epidermis only reacted to the G1 and G2 inhibitors present in a sephadex G-75 fraction comprising a range of mol.wts of about 10,000 to 45,000 daltons. Intact epidermis was probably more sensitive, showing greater inhibition and reacting to a wider range of fractions. After boiling the extracts for 10 min, heatstable antiproliferative activity could be concentrated by sephadex G-75 and DEAE chromatography. Active DEAE fractions contained proteins with mol.wts mainly between 10,000 and 20,000 daltons. The proliferation of colon epithelial cells was inhibited by unfractionated extracts as well as by high and low mol.wt fractions of boiled extracts. A marked leukopenia was induced by the DEAE fractions. Thus, tissue-specificity could not be demonstrated unambiguously.

A cloned mouse DNA fragment comprising the β-globin gene contains sequences complementary to a 'small nuclear RNA'

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Labelled RNA from L-cells or Friend cells was hybridized to cloned mouse DNA containing a β-globin major gene (Tilghman et al., PNAS *75*, 1309, 1978). Analysis of the bound RNA by PAGE revealed 4 prominent bands with slightly different mobilities, corresponding to about 84–87 nucleotides. Fingerprint analyses showed that these RNAs were identical except for a heterogeneous 3′ terminal region (... (AC)(AU)$(U)_{1-4}$N). The 5′ terminal T_1 oligonucleotide was pppGp. The fingerprints were indistinguishable from those published by Peters et al. (J. Virol. *21*, 1031, 1977) for a set of RNAs (16a–e), associated with MuLV RNA. At least part of RNA 16 was found associated with the poly(A) RNA fraction and could be released by heating. The DNA segment hybridizing to RNA 16 is located about 1400–2500 b.p. downstream from the β-globin gene. It seems that this DNA segment is not the gene for RNA 16, but is closely related to it.

Quantitation of genes coding for egg shell protein in germ line and soma of *Ascaris lumbricoides*

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Messenger RNA coding for the egg shell protein of *Ascaris lumbricoides* has been isolated from polyploid uterine epithelial cells and used for cDNA synthesis. Studies on the kinetics of hybridization and reannealing of egg shell cDNA indicate that there exist about 7 different species of egg shell mRNA in the uterine epithelial cells and that each egg shell gene is composed of unique and interspersed moderately repetitive sequences. Kinetic analysis of the annealing reaction of egg shell cDNA to germ line and somatic DNA reveal that the number of egg shell genes per haploid genome remains constant during development of the DNA eliminating nematode *A. lumbricoides.*

Development of *Myxovirus* resistance during differentiation of mouse brain in aggregating cell cultures

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The genetically determined resistance of A2G mice towards i.c. infection with neurotropic influenza A virus (causing fatal encephalitis in susceptible A/J mice) develops shortly after birth. Rotation-mediated aggregating cell cultures of fetal mouse brain are known to mature from a undeveloped to a morphologically and biochemically well-differentiated state. Here, we report that A2G brain cells in such cultures express antiviral resistance as a consequence of age-dependent maturation in vitro. Whereas 10-day-old A2G cultures were as susceptible to NWS virus (Ho, Nl) as A/J cultures of various developmental ages, highly organized A2G aggregates cultured for 30 days proved resistant. In vitro resistance appeared to depend on interferon type I in agreement with recent results indicating intimate cooperation of interferon and host genes in mediating in vivo resistance (Haller et al., J. exp. Med., in press).

Toward an experimental system for transformation in plants

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Agrobacterium tumefaciens induces tumors in numerous plant species by transferring Ti-plasmid DNA into conditioned cells. Our aim is to develop, on the basis of this naturally occurring genetic transformation, an experimental system for genetic engineering of higher plants. We intend to use the transferable DNA-stretch as vector for other genes, and to approach transformation at the single cell level in vitro. We have selected a model plant which expresses intensive tumor formation upon infection in situ. We have established the necessary single-cell system by isolating, culturing and regenerating protoplasts from this model plant (see also poster H. Lörz). And we have isolated the Ti-plasmid in sufficient quantities from a nopaline producing, morphogenetic *A. tumefaciens* strain. We will approach transformation on 3 experimental lines: protoplasts + Ti-plasmid/young (conditioned?) cells + Ti-plasmid/young cells + *Agrobacterium.* Selection will be via auxinautotrophy, verification through DNA-hybridization and through opine synthesis.

Intergeneric somatic hybridization studies with *Citrus* and *Nicotiana*

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The establishment of hybrid cell lines is the first step in the production of somatic hybrid plants. Mesophyll protoplasts from a streptomycin resistant mutant of *Nicotiana tabacum* (auxin auxotrophic) were fused with protoplasts from suspension cultures of an embryogenic auxin autotrophic line (L5 E^+) of *Citrus.* Fusion products were easily recognized microscopically by visual markers and differential nuclear staining. Single hybrid colonies, hand-picked according to visible morphological characteristics, can be cultured in appropriate media, thus providing selection and cloning in one step. From 5 fusion experiments 1102 colonies were obtained and were tested simultaneously on $auxin^+$ and $auxin^-$ media. 70 colonies are able to grow on both media. For further confirmation colonies are also tested for the expression of streptomycin resistance, embryogenesis and growth on lactose and galactose. Chromosomal analysis, isozyme patterns and characteristic secondary products are checked to establish the presumed somatic hybrid nature of the selected colonies.

Internalization of lactogenic hormones in mammary cells

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Internalization of lactogenic hormones (oPRL) was followed in mammary cells in order to characterize the cellular events which allow these hormones to exert their effect on gene expression. Cells (5×10^6) were incubated at 4 °C for 6 days with 25 pM ^{125}I-oPRL, washed, brought to 37 °C and processed for light and EM autoradiography. At 4 °C, all the grains were at the cell surface. At 37 °C, 30%, 50% and 65% of the grains at 5, 15 and 30 min, respectively, were associated with intracellular membranes, and 8%, 18% and 25% with nuclei. Upon elution from cells or from purified nuclei, ^{125}I-material comigrated in SDS-PAGE with native oPRL, except in cells incubated 15 and 30 min at 37 °C, in which some lower mol. wt hormone was found, indicating intracellular degradation. The correlation of rapid uptake of oPRL and nuclear localization with fast increase in rate of casein transcription suggest that oPRL, alone or associated with its receptor, acts intracellularly on nuclear structures.

Loss of nuclei from spermatids in *Drosophila*

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The lateral attachment of nuclei and axoneme in sperm of *Drosophila subobscura* may promote shifting of nuclei in the tail region of elongating spermatid bundles as found in 'sex ratio' males. In *D. melanogaster* nuclei and axoneme are arranged longitudinally. In disfunctional sperm of SD nuclei remain in proper position.

Electrophoretic analysis of the salivary proteins of *Chironomus* larvae

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The salivary glands of *Chironomus* larvae produce large amounts of secretory proteins thought to be coded for by an RNA of unusually high mol.wt (14×10^6). These proteins were analyzed by gradient acrylamide gel-electrophoresis in the presence of 8 M urea, 6 mM Triton (pH 2.4) or 0.1% SDS (pH 8). Whole glands or isolated secretion were solubilized and proteins reduced in 0.1 M DTT, 8 M urea, 10 mM EDTA or 0.1 M DTT, 2% SDS, 10 mM EDTA (pH 8) for various times, alkylated for 30 min and immediately electrophoresed. On urea-Triton gels the secretory proteins showed 3 major and 3 minor bands. With prolonged treatment of glands or secretion the major bands became faint while a series of new, faster migrating bands appeared. This change could also be followed on SDS gels: A specific pattern of decomposition products (mol.wt 10,000 or multiples) was shown, suggesting reductive cleavage at specific sites. Immunological analysis of these cleavage products is in progress.

Granulocyte/macrophage colony stimulating activity (G/M CSA) release by human peripheral blood leucocytes exposed to agar extract

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Human peripheral blood leucocytes (10^6/ml) release small amounts of G/M CSA in the culture medium upon incubation. The addition of a 37 °C aqueous extract of crude agar greatly increases the amount of G/M CSA released. PHA (Difco PHA-P) has a similar effect. However, agar extract demonstrates a much weaker mitogenic activity than PHA whereas induction of G/M CSA release is comparable for both agents. Mitogenesis is not inhibited when agar extract is added with PHA to the culture. These results suggest that mitogenesis and G/M CSA release may occur by independent mechanisms.

α-Actinin is associated with mouse lymphocyte plasma membrane and cocaps with surface immunoglobulins and Thy-1 antigen

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α-Actinin has been identified as a biosynthetic product of mouse spleen lymphocytes. This protein focuses at the same isoelectric point and migrates with the same apparent mol.wt as pig skeletal muscle α-actinin. ^{35}S-methionine labelled lysates were immunoprecipitated with antimembrane and antisurface antibodies. Glycoproteins were selected by concanavalin A and immunoprecipitated with anti-Con A antibody. 2-dimensional polyacrylamide gel analysis of these immunoprecipitates showed that α-actinin is neither a glycoprotein nor an externally exposed membrane component. Immunoprecipitation with an antimembrane antibody showed that α-actinin is an integral membrane protein or a cytoplasmic protein associated with the plasma membrane. Cocapping experiments showed that α-actinin follows the surface distribution of immunoglobulins and Thy-1 antigen. This supports the view that α-actinin is involved in surface receptor movement.

Cloning and expression of the *Drosophila melanogaster* H2B histone gene in CV-1 monkey cells

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A 1.05-kb DNA fragment containing an H2B histone gene and its putative promotor and terminator signals was excised from the cloned histone gene repeat unit of *Drosophila melanogaster* (Lifton et al., 1977) and joined to the SV40 early region (coordinates 0.14–0.82) by cohesive-end ligation. CV-1 monkey cells were transfected with the ligated DNA, together with tsA58 helper DNA, and 9 independent hybrid viruses containing the *Drosophila* H2B histone gene were isolated. CV-1 cells were infected at high multiplicity with the hybrid virus and the *Drosophila*-specific RNAs and proteins analyzed. The entire cloned *Drosophila* sequence was found to be transcribed in these cells as the internal part of a 1.65-kb poly(A)-plus, 19S-type late SV40 mRNA. No *Drosophila*-specific RNA with mature histone mRNA length could be detected at any time during infection. The 1.65-kb hybrid RNA directed the synthesis of *Drosophila* H2B histone protein. This heterologous H2B protein associated with the viral minichromosome in the mature virions, without impairing their infectivity.

Monoclonal markers for neurons

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Neurons in culture can be identified by surface staining with tetanus toxin or anti-Thy-1 or by cytoplasmic staining with anti-neuron-specific enolase. We have also identified a group of monoclonal reagents which bind selectively to neurons; the phosphoryl-choline binding myeloma proteins (PC-BMP) (TEPC 15, MOPC 167, McPC 603, McPC 870). Cultures of dissociated cells from mouse spinal cord were exposed to either a) ^{125}I-PC-BMP or b) PC-BMP followed by FITC-goat antimouse α-chain. As judged from light microscope autoradiographs and immunofluorescent preparations, only neurons were marked. No labelling was observed in the presence of 10^{-2} M phosphoryl-choline. Other IgA MP of different specificities did not stain any cell type in the cultures. PC-BMP can be used as surface markers for living or fixed neurons.

Quantitative and qualitative differences in samples of various muscles in 2 African mammals, Wildebeest and Dik-Dik

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Muscle samples of 12 different muscles were studied in a Wildebeest (W) 102 kg and a Dik-Dik (D) 4.3 kg. Origin of muscles: hindlimb 8, forelimb 1, trunc 2 and the diaphragma. Diaphragma was clearly separated from the other muscles with respect to the volume density of mitochondria (V_{VM}) in muscle fibres. V_{VM} in diaphragmatic fibres was 12.3% in D and 10% in W, while in all the other muscles it ranged between 1.5 and 5.6%. Higher values were usually found in D. The highest values for the number of capillaries per mm^2 were also found in diaphragma. Values between 697 and 1788 were found in D and between 355 and 1194 in W. The area equivalent circular diameter of the

fibres was between 35 and 51 µm in D and 33 and 56 µm in W. In this respect diaphragma was not different from the other muscles. We found a general tendency for mitochondria in most of the muscle fibres of both animals to be more numerous near the periphery of the fibres. Subsarcolemmal aggregates of mitochondria were especially prominent in the fibres of the diaphragma of the 2 animals.

Intracellular glycosylation of proteins: Evidence for an en bloc transfer of the carbohydrate chains onto the influenza virus hemagglutinin

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The hemagglutinin (HA) of the influenza virus is an envelope glycoprotein. It is coded by the viral genome and is glycosylated by cell-specified enzymes. After infection of cells in culture with the influenza virus A NWS (HON1), host specific protein synthesis is suppressed and replaced by the synthesis of the virus-specified proteins; the synthesis of the HA polypeptide is traced using ^{35}S-methionine. Inhibition of glycosylation is obtained by adding glucosamine to the culture medium. The heterogeneity of under-glycosylated HA polypeptides is analyzed by polyacrylamide gel-electrophoresis followed by autoradiography. Under-glycosylated HA polypeptides are distributed in discrete molecules of increasing mobility, separated by a constant mol.wt. These results allowed the mol.wt and the number of the oligosaccharide side chains of the HA polypeptide to be determined; they support the view that the core sugars of glycoproteins are transferred to the protein acceptors as a bloc.

Identification of visual cortical neurons receiving thalamic axons in cat and monkey

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Lateral geniculate (LGN) axons terminate mainly in layer IV of the visual cortex (area 17). Most end on dendritic spines, the rest on dendritic shafts or neuronal somata (Garey and Powell, Proc. R. Soc. B *179*, 41, 1971). It is not known in detail which neuronal types receive thalamic afferents, as layer IV contains spines from both stellate and pyramidal cells. In order to determine the identity of neurons postsynaptic to thalamic axons, lesions were made in the LGN of cats and monkeys which were sacrificed 3-4 days later. Area 17 was prepared with a Golgi technique (Fairén et al., J. Neurocyt. *6*, 311, 1977). Individual neurons were then excised and embedded for electron microscopy, which allows the demonstration of degenerating thalamic terminals synapsing with cells of known identity.

Thyroid hormone induced competence for estrogen dependent vitellogenin synthesis in *Xenopus* liver

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In the liver of adult *Xenopus laevis* estrogen induces vitellogenin synthesis, but larval liver has been found to be refractory to estrogen and to establish competence to respond to this hormone after metamorphosis. Since amphibian metamorphosis is dependent upon thyroid hormone, we investigated whether the onset of competence is dependent upon thyroid hormone or reflects age-dependent maturation of the liver. Using rocket immunoelectrophoresis of blood samples, it was found that in normally developing tadpoles spontaneous synthesis of vitellogenin starts during late metamorphosis, whereas in estrogen treated tadpoles competence to respond to estrogen was already established earlier. Thyrostatic tadpoles, in which metamorphosis was suppressed, failed to respond to estrogen, even at an age when normal animals already synthesized vitellogenin. In contrast thyrostatic tadpoles, after induction of metamorphosis by thyroxine, were found to be fully responsive to estrogen, showing that thyroxine is needed to establish competence of the liver to estrogen.

DNA of the heterotrich ciliate *Climacostomum virens*

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DNA was isolated from 3 strains (s) of *C. virens:* s1, a wild type, containing endosymbiontic algae (genus *Chlorella*); s2, without endosymbionts obtained by treatment with DCMU; and s3, free of endosymbionts and amicronucleate, obtained by UV-irradiation. Density determination and G+C content of the total DNA of each of the 3 strains give different values. Densities measured by CsCl analytical ultracentrifugation vary between 1.7082 and 1.7090 g/cm^3. These data indicate a G+C content of 49.5-50.0%. Thermal denaturation studies give a G+C value of 47.8-50.3%. This percentage is higher than that obtained for other heterotrichs, such as Stentor, with a G+C of 32% (Pelvat, de Haller, Genet. Res. *27*, 277, 1976). We have determined the relative proportions of DNA, RNA and proteins for s2. Electron microscopy reveals that the DNA molecules are long (more than 20 µm), as has been shown for other heterotrichs. The cell cycle lasts ca. 24 h. Based upon ^{3}H-methyl-thymidine incorporation, the D-phase represents 8% of the cycle, the G1-phase 40% and the S-phase 52%.

Transcription complexes in early mammalian embryos - an electron microscopic study

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Mouse embryos at 2, 4-8 cell and morula/early blastocyst stages were obtained from superovulated CBA/CaJ females. The zona pellucida was thinned by brief pronase treatment and finally removed by aspirating the embryos in a solution of PMSF (pronase inhibitor), with a fine pipette. The zona-free embryos were lysed using NP40 detergent and the lysate was spread according to Miller and Bakken (1972). Transcription complexes of pre-mRNA type were observed at all developmental stages. Quantitative analysis of nascent pre-mRNP chain lengths showed a shift in length distribution between subsequent developmental stages. pre-rRNA transcription complexes were difficult to find in well-dispersed form, probably due to a rather high degree of compaction of the nucleolus associated chromatin in early embryos. We have observed structures resembling DNA replication loops in all stages. We have also examined spread mitotic chromosomes and occasionally found isolated transcription complexes of pre-mRNA type. On a few occasions we have found replication loop-like structures on these chromosomes.

Integration and expression of mouse mammary tumor virus genes in mice strains exhibiting high and low tumor incidence

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A representative cDNA to MMTV RNA was used to identify restriction fragments of C3H, Balb/c and GR

mouse DNA which contained viral sequences. Similar patterns were found in liver DNA of C3H and Balb/c mice with additional bands in C3H DNA. A totally different pattern was found in GR liver DNA. Tumors in C3H and GR mice contained extrabands hinting at their clonal origin. These extrabands vary in different tumors. Moderate cDNA excess hybridizations confirmed the increased copy number of integrated proviral DNA in tumors. Viral RNA was not detected in liver but in mammary gland and tumor RNA at 0.02–0.1%. Gel-electrophoresis and hybridization of RNA bound to DMB paper revealed virus-specific mRNA of 10 kb, 8.8 kb and 4.4 kb.

An ISl-mediated inversion observed in the genome of plaque forming bacteriophage P1

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Temperate coliphage P1 carries one ISl element in its genome, which also contains a 3-kb invertible segment known as C-region. In the course of work with P1Cm transposition derivatives of P1, we isolated a number of Cm^s subclones from a particular strain of P1Cm. These subclones carried 2 ISl in opposite orientation at a distance of 24 kb. While the gene order in most subclones studied was normal, one subclone had the entire 24-kb segment flanked by the 2 ISl elements inverted. This 24-kb segment which represents about one fourth of the P1 genome englobes the C-region mentioned above. These conclusions are based on restriction cleavage and electron microscopical analysis. The inversion is thought to result from reciprocal recombination between the 2 ISl elements. Despite of the extensive inversion the phage in question grows normally both in its vegetative propagation as a phage and in the lysogenic condition as a prophage.

Bgl II: A restriction enzyme from *Bacillus globigii*

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The restriction enzyme, *Bgl* II, which cleaves the sequence AGATCT has been purified to homogeneity and its properties studied with the aid of a small plasmid containing a single site for the enzyme. The enzyme binds to substrate DNA in the absence of Mg^{+2} to form a complex that is insensitive to heparin (the free enzyme is irreversibly inhibited by heparin). When Mg^{+2} is added to these complexes in the presence of heparin the DNA is cleaved in both strands without accumulation of a stable nicked intermediate. Thus the same enzyme molecule cuts both strands without dissociating from the DNA. Antibodies prepared against the enzyme do not cross-react or inhibit any other enzyme we have tested including *Bgl* I from the same organism and *Bam*H I from a related organism and cutting a similar sequence. The possibility that these enzymes are closely related genetically is thus unlikely.

Euglena gracilis chloroplast DNA contains a fourth 16S rDNA sequence

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It was shown (i.e. Jenni and Stutz, Eur. J. Biochem. *88*, 127, 1978) that *E. gracilis* (Z-strain) chloroplast DNA contains 3 rRNA genes (16S+23S) being located in 3 clustered repeated DNA segments of 5600 bp. We show by DNA: DNA and DNA:RNA hybridizations, and by fine structural mapping using the restriction enzymes HaeIII, HpaI, HindIII that a DNA segment of approx. 1500 bp which is part of the Eco. B (19,000 bp) fragment carries complementary sequences for the 16S rRNA. This rDNA sequence is located about 2000 bp away from the next complete rRNA gene set.

Involvement of microfilaments and microtubules in rod outer segment (ROS) phagocytosis by pigment epithelium (RPE)

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In order to investigate the involvement of microfilaments and microtubules in the phagocytosis of shedded ROS disks (Experientia *33*, 816, 1977), frog retinas are fixed at various time-intervals of a 12 h light/12 h dark cycle. Moreover distribution and estimation of Ca^{++} content was undertaken with pyroantimonate-osmium technique and X-ray probe. Ultrastructural studies revealed a rich stubble of short microfilaments under the apical plasma membrane shortly after onset of light. This assembly of microfilaments was preceded by an increase of Ca^{++} concentration in RPE cells, and occurred before invagination of RPE plasma membrane. Newly formed phagosomes migrate in the RPE cytoplasm basically and show acPase activity. These organelles are associated with 7-nm and 10-nm filaments and surrounded by numerous microtubules. Our data suggest: a) microfilament assembly and/or endocytosis of ROS disks is Ca^{++} mediated, b) phagosome translocation is related to microfilaments whereas microtubules may be involved in direction of movements.

SV40 and polyoma virus exert hormone-like impact on host cells

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SV40 and polyoma virus, small DNA containing tumor viruses, induce in permissive cells a lytic infection leading to production of progeny virus and cell death; in nonpermissive cells infection remains abortive and no viral progeny DNA is synthesized; abortive infection leads, however, to virus-induced mitosis and 'cell transformation'. Lytic and abortive infection begin with the expression of the early viral genes, i.e. synthesis of early 19S viral in RNA's and of the tumor T-antigens; this is followed by a mitogenic reaction of the host cell which comprises duplication of the host cell chromatin. Our recent results have shown that lytic and abortive infection with SV40 and polyoma virus trigger, shortly after onset of T-antigen synthesis, a switch in the expression of the host cell genome. This leads to stimulation of synthesis of the major species of cellular RNA (ribosomal 45S, 28S, 18S and 5.8S RNA, nuclear heterogeneous premessenger RNA, poly A^+ mRNA's, 4S transfer and 5S RNA) and of most host cell proteins; results from analyses in 2-dimensional gels suggest that infection leads to a reprogramming of cellular protein synthesis. Stimulated synthesis leads to a marked increase (30–60%) in total cellular RNA and protein. The observed effects of infection are reminescent of those induced by certain growth promoting hormones.

Purification of metaphase chromosomes and scaffold proteins from tissue culture cells of *Drosophila melanogaster*

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Metaphase chromosomes of *Drosophila* tissue culture cells were purified by sedimentation through a sucrose gradient containing NP-40. The stained preparation was examined in a microscope and found to be free of nuclei and cell debris. After depletion of the metaphase chromosomes in 2 M NaCl, the dissociated proteins were removed from those that remained bound to the DNA ('scaffold proteins') by differential centrifugation. The scaffold proteins of metaphase chromosomes were analyzed by SDS-polyacrylamide gel-electrophoresis and compared to proteins purified similarly from interphase chromatin.

Organization of light-harvesting polypeptide in monomolecular layers

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Light-harvesting polypeptide (LHP) was found to be part of a red-shifted bacteriochlorophyll-protein complex presumed to represent a subunit of the light-harvesting antenna of the photobacterium *R. rubrum*. Monolayers of lipid- and pigment-free LHP were studied in order to get information basic for the investigation of protein-lipid and protein-pigment interactions in model membranes. Monolayers were transferred onto solid supports for electron microscopic and IR spectroscopic investigations. These layers were found to be built up of a mosaic of domains with regular texture when transferred at 20 dynes/cm compression. The smallest repetitive feature obtained in the optically reconstructed image is assigned to one LHP molecule since its average area of 13.5 nm^2 is in good agreement with the area obtained from film balance measurements. IR spectra indicate extensive α-helical conformation in the spread protein.

Polyamine-mediated phosphorylation of a nucleolar protein from *Physarum polycephalum* which stimulates rRNA synthesis

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An acidic phosphoprotein with a subunit M_r of 70,000 is purified as an apparent dimer of 139,000 from nuclei of the true slime mold *Physarum polycephalum*. This protein is a component of the nucleolar deoxyribonucleoprotein complex (rDNP) containing the ribosomal genes, and it strongly stimulates rRNA synthesis by the endogenous RNA polymerase I of the rDNP. The phosphoprotein binds specifically and with high affinity (10^{-10} m) to rDNA (M_r 38×10^6) and also to 3 fragments of rDNA prepared by cleavage with HindIII, PstI and BamHI respectively. These fragments of M_r 21.1×10^6, 17.1×10^6 and 8.1×10^6 all include the symmetry axis of the palindromic rDNA. The protein in its dephosphorylated stage does not stimulate transcription and does not bind to rDNA or to the fragments indicated. This is the first isolation of a specific phosphoprotein with the capacity to stimulate transcription of a specific set of genes in a eukariote.

Incorporation of ^{3}H-thymidine (^{3}H-T) in rabbit arteries after endothelial denudation: Site of smooth muscle cell (SMC) proliferation and migration

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Endothelial denudation of arteries induced proliferation of SMC in the media (M), their migration through the internal elastic membrane and formation of a neointima (NI). Rabbits were injected with ^{3}H-T and sacrificed at different time intervals after removal of the endothelium in one iliac artery and in the abdominal aorta. The site of cell proliferation was determined autoradiographically on arterial semithin cross-sections. In the M, ^{3}H-T labelling increased to 20 cells/cross-section on day 4, decreased to 6 on day 7 and was virtually nil on day 14. Up to day 4, ^{3}H-T labelling in NI was less than in M, was maximal on day 7 (35 labelled cells/cross-section) and decreased slowly to 15 and 2.5 on days 14 and 28, respectively. Correspondigly, the total number of NI-cells increased from 36 on day 4 to 450 and 1060 on days 7 and 14. Thus, early SMC proliferation takes place predominantly in the M. From day 7 proliferation of NI-cells vastly exceeds that of M. Maximal migration of SMC takes place before day 7.

CRABP, a possible marker for proliferation in human mammary tissue?

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A multiphasic polyacrylamide gel-electrophoresis system was adapted to separate free, unspecifically and specifically bound retinoic acid. This method was used to analyze small amounts of crude $100{,}000 \times g$ cytosol of normal and dysplastic mammary tissue as well as of carcinomas and fibroadenomas. CRABP, which has a specific affinity for retinoic acid, was found in all tissue categories examined. Compared to methods applied earlier (J. natl. Cancer Inst. *61*, 1375, 1978) a markedly lower detection limit is achieved. The results show great individual differences in CRABP content in each tissue category. The content (related to soluble proteins in cytosol) is increasing from normal tissues to carcinomas. Since CRABP levels are often elevated in fast dividing fetal tissue (PNAS *73*, 3976, 1976), it is possible that in adult tissue the occurrence of this binding protein is a useful marker for cellular proliferation and preneoplasia.

Immunofluorescence localization of arginine kinase (AK) in *Drosophila* muscles

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AK, a monomeric protein of mol.wt. 40,000 was purified from adult *D. melanogaster*. Antiserum to AK was raised in rabbits; its specificity was established by immunodiffusion tests and by immunoreplicas of electropherograms. After electrophoresis in nondenaturing conditions, the immunoreactive material was shown to possess enzymatic activity. Localization of AK by indirect immunofluorescence on washed myofibrils showed that at least a fraction of the total cellular AK is bound to the contractile apparatus. In muscle fibres of third instar larvae and in isolated adult fibrillar flight muscle myofibrils, AK was localized within the Z-line region. Adult tubular muscle fibres (tergal depressor of the trochanter) showed fluorescence in the A-band. In all cases, the localization was independent of

the state of contraction. Vertebrate B and M type creatine kinases have previously been localized at the Z-line and A-band (M-line) regions, respectively. Isoenzymic forms of AK have not been detected in *Drosophila*, however.

Growth of multicellular tumor spheroids of human origin

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Multicellular tumor spheroids (MTS) provide a useful model system for studies of the regulation of growth in 3 dimensions. We have grown MTS in vitro from human colon carcinoma, choriocarcinoma, malignant glioma and melanoma cell lines. Growth was usually initiated in liquid medium over a base layer of nutrient agar. For colon carcinoma cells (Co-115 cell line), MTS could also be grown in spinner flasks using Cytodex® (Pharmacia) beads as a carrier. Growth rates of MTS were variable and a wide range of maximal diameters was observed (200–1500 µm). Analysis of MTS sections by light and electron microscopy revealed a heterogeneity of cellular contacts including relatively loose associations as well as tight junctions and desmosomes. The types of contacts observed in MTS were frequently similar to those observed in solid tumors of the same histological type.

Sequence determination of RNA bacteriophage Qβ by DNA sequencing

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Plasmids containing the whole DNA sequence corresponding to Qβ RNA in a biologically active form (Billeter et al., these abstracts) were used to determine the phage genome sequence by the Maxam-Gilbert method. Close to 90% is tentatively established. Only minor discrepancies with the RNA sequences previously determined were found, reflecting in part sequencing errors and in part heterogeneity of the phage RNA population from which the Qβ DNA was cloned. Comparison with phage MS2 showed no extensive homology in the primary and secondary structure as far as they could be compared. Nevertheless a common ancestor of both phages seems now very likely: Whereas the relatedness of the coat proteins is questionable and the maturation proteins show only slight homology, the phage-coded replicase subunits possess long stretches of identical amino acid sequences (presumably required for interaction with the host proteins Tu, Ts and S_1) coded for by very different sets of codons.

Plasma membrane enzymes in human cancer cells

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Plasma membrane-associated enzymes involved in purine metabolism, transport and response to physiological signals were determined on normal human lymphocytes and malignant lymphoproliferative cells. Acute lymphoblastic leukemia cells (ALL) display a significant lower activity for γ-glutamyltranspeptidase (GLUPA), adenosine triphosphatase (ATPase), ouabain sensitive (Na-K)-ATPase, basal and NaF adenylate cyclase but not for 5′-nucleotidase (5′-AMPase) and alcaline phosphomonoesterase when compared to normal lymphocytes. In ALL cells and homogenates no alcaline phosphodiesterase activity (PDAase) could be detected. In contrast, in acute nonlymphoblastic cells (AML) PDAase was significantly increased whereas 5′-AMPase was decreased. Low activity of the latter enzyme and GLUPA characterized chronic lymphatic leukemia cells (CLL). 5′-AMPase, however, raised in CLL and CML blast crisis. Furthermore, ratios of the enzymatic activities related to the GLUPA activity show discrete distribution patterns for these cell populations.

From isolated protoplasts to plants – regeneration and differentiation studies in the genus *Hyoscyamus*

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Various species of *Hyoscyamus* (henbane) contain high amounts of tropane alkaloids and the genus is of special interest now as model plant for somatic cell genetics. Mesophyll protoplasts have been isolated from axenic shoot cultures of *H. albus* and *H. muticus*, and from cultured cells of *H. muticus*. Sustained cell divisions of mesophyll protoplasts and suspension cell-derived protoplasts have been induced with up to 50% plating efficiency in modified NT- and DPD-medium. *H. albus* responded to morphogenesis experiments with root formation only. *H. muticus*, in contrast, maintains a high regenerative capacity following protoplast culture. Shoot and root formation could easily be induced, even when 3–4-week-old calli were directly transferred from the original protoplast culture medium onto solidified differentiation medium. Formation of somatic embryoids was observed by culturing protoplast derived cells on MS-medium supplemented with kinetin (1 mg/l), NAA (0.1 mg/l) and 0.5% activated charcoal.

Fast and slow myosin coexist in type II A and II C fibres of an adult rabbit muscle

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Using serial sections of shock-frozen rabbit tibialis anterior muscle, fibres were typed according to classical staining reactions for myosin-ATPase, diaphorase and phosphorylase. In addition the sections were incubated with antisera raised in guinea-pigs against fast and slow rabbit myosin. The immune-complexes formed were visualized with the staphylococcal protein A immunoperoxidase technique. Type I fibres only reacted with the antislow-myosin serum and type II B fibres only with the antifast-myosin serum. In contrast, type II A and II C fibres reacted with both antisera thus indicating the presence of fast and slow myosin within the same fibre.

Induction of 6-thioguanine resistant mutants in granuloma pouch fibroblasts of rats

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The granuloma pouch assay was developed in an attempt to detect potential carcinogens in vivo in mammals. A rapidly proliferating granulation tissue was initiated with croton oil at the inside of a s.c. air pouch on the back of rats. 4 days after initiation, the newly grown tissue was excised and dissociated enzymatically. Subsequently fibroblast-like cells were cultured for 3 days, respread and tested for clone formation in a medium containing 10 µM 6-thio-

guanine (6-TG). Resistant clones were considered to be formed out of HGPRT$^-$ mutants. Experimental conditions were varied to allow recovery of a maximal number of induced mutants. From untreated animals, 1-4 resistant mutants per 10^6 granuloma fibroblasts were able to form clones. Rats were treated with the 2 carcinogens N'-methyl-N'-nitro-nitrosoguanidine (MNNG) and benz(a)pyrene (BP), injected directly into the pouch 2 days after initiation. A linear dose-related increase in mutation frequencies was found with both compounds in a range of 0.05–0.2 mg/granuloma pouch (GP) for MNNG and 0.03–0.125 mg/GP applied twice for BP. Mutation frequencies were approximately 10-fold lower with BP than with MNNG. This is in good agreement with the carcinogenic potential of the 2 compounds.

Localization of structural genes on the chloroplast DNA of *Chlamydomonas reinhardii*

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Hybrid plasmids carrying different chloroplast DNA restriction fragments have been used as templates in an in vitro coupled transcription-translation system. The in vitro products have been immunoprecipitated with antibodies against the large subunit of Ribulose 1,5 diphosphate carboxylase (LS) and against 4 different purified chloroplast membrane polypeptides. Hybrid plasmids which gave a positive response in this test were further examined by hybrid arrested translation, i.e. the DNA was hybridized to cellular polyA$^-$ RNA of *C. reinhardii* prior to in vitro translation in the reticulocyte system. Specific inhibition under these conditions of the synthesis of LS and 2 membrane polypeptides with mol.wts of 30,000 and 35,000 daltons has allowed to localize the corresponding genes on the chloroplast DNA map.

Introduction and maintenance of a rabbit β-globin gene in mouse L-cells

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Plasmid Z/pCRI/RchrβG-1 (van den Berg et al., Nature *276*, 37, 1978), a hybrid consisting of pCRI and a rabbit DNA segment containing a β-globin gene, was linearized by cleaving with SalI in the pCRI moiety and ligated to SalI-linearized plasmids Z/pBR322/HSV-TK/-1, 2 or 4, which contain the herpes virus thymidine kinase (TK) gene. TK negative L-cells were treated with the multimeric DNA by the calcium phosphate method and TK$^+$ colonies were selected and propagated in HAT medium. 19 of 21 TK$^+$ colonies were shown to contain rabbit β-globin DNA by restriction cleavage, Southern transfer and hybridization to a β-globin specific probe. 12 of 16 of the rabbit gene-containing colonies produced rabbit β-globin RNA of the mature (spliced) type, as detected by the assay of Weaver et al. (these abstracts).

Comparative analysis of skeletal muscles in various African mammals

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A correlated physiological and morphometric study of various African mammals (b.wt 0.42–251 kg) was undertaken, on which oxygen consumption at rest and at maximum work ($\dot{V}_{O_2}$max) had been measured (by C.R. Taylor and collaborators). Tissue samples were taken from the midbellies of m. semitendinosus and longissimus dorsi and from the lateroventral part of diaphragm. They were processed for electron microscopy according to standard procedures. Capillary counts, average size of fibre and volume fraction of mitochondria per fibre were estimated by morphometry. In semitendinosus and longissimus dorsi the volume fraction of mitochondria was found to correlate with body size and $\dot{V}_{O_2}$max. In diaphragmatic muscle fibres the volume fraction of mitochondria correlated more closely to $\dot{V}_{O_2}$max than to b.wt. No distinct relation was found in each muscle between capillary counts and $\dot{V}_{O_2}$max or b.wt. On the other hand, there were about 3 times as many capillaries per mm^2 of muscle cross-section in the diaphragm than in semitendinosus or longissimus dorsi. At the same time the volume density of mitochondria in diaphragmatic muscle fibres was also about 3 times that of semitendinosus and longissimus dorsi.

Ultrastructural analysis of the retinoid induced reversal of epithelial hyperplasia and metaplasia

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The DMBA-induced skin papilloma of the mouse and the MCA-induced hyperplasia and metaplasia in prostate organ cultures were studied in electron microscopy. The 2 types of tissues both showed a reversal of hyperplasia and metaplasia when treated with retinoids (= vitamin A and analogs), but this reversal was reached by means that are quite characteristic for a given type of tissue: a) in the skin, there were many necroses and an impressive mucous metaplasia (Matter and Bollag, Eur. J. Cancer *13*, 831, 1977). The latter might be at least partly responsible for the cell loss, probably through a loss of anchorage in the prickle-cell layer. b) In the prostate, no mucous metaplasia was observed, but an important depression of DNA-synthetic activity (Lasnitzki and Goodman, Cancer Res. *34*, 1564, 1974). The secretory apparatus reappeared, together with the microvilli, possibly induced by the slowing down of cell division (Müller-Salamin et al., JNCI, in press).

Pattern of gap junctions between resting and stimulated insulin-containing cells of the islet of Langerhans

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The distribution and spatial arrangement of gap junctions between insulin-containing cells (B-cells) under different conditions of insulin secretion were quantitatively analyzed in freeze-fracture replicas of isolated rat islets of Langerhans. The results obtained show that B-cells located at the periphery of the islet have twice as many gap junctions per unit membrane area as B-cells situated in the islet center ($p < 0.05$). In both locations, gap junctions assumed a nonrandom clustering on the B-cell membranes and were significantly increased during stimulation of insulin secretion. The degree of clustering was modified ($p < 0.01$) by the type of stimulation used (high glucose or glibenclamide) and depended on the location (central or peripheral) of the gap junctions in the islet. These data establish a quantitative pattern of gap junctions between pancreatic B-cells and demonstrate a modulation of these junctions during stimulated insulin release.

Transposition of AT-linked, cloned DNA from one vector to another

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Plasmid PβG consists of rabbit β-globin cDNA joined to PMB9 by AT stretches. The insert was excised by nuclease S_1 in formamide (Hofstetter et al., BBA *454*, 587, 1976), purified by velocity centrifugation, treated with T_4 DNA polymerase and deoxytriphosphates to generate flush ends and ligated to HindIII linkers. After HindIII digestion, the fragment was joined to HindIII-cleaved pBR322 and cloned. Several clones containing HindIII-excisable fragments with the same β-globin sequence as PβG, however lacking most of the flanking AT stretches were isolated. In parallel, samples of S_1 excised fragments were digested with exonuclease III for various times and treated with S_1. The fragments were cloned via HindIII linkers as above. A collection of clones containing β-globin inserts lacking defined segments at the 5′ and 3′ end, was recovered and characterized by sequence analysis. These fragments are to be used as primers in site-directed mutagenesis of the β-globin gene.

Chromatid breaks induced in the G_2-phase of Chinese hamster cell-cultures by low doses (12–100 rad) of pions

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Monolayer cultures of the fibroblast-like Chinese hamster cell-line 19/1 were irradiated in the G_2-phase of the cell cycle by low doses of negative pi-mesons (12–100 rad peak-dose, 6 and 10 rad/min). Proton energy 590 MeV, Target-material: beryllium, momentum of π^-: 176 MeV/c, beam contamination with electrons $\cong 10\%$, field size: 2.5 cm diameter $= 85\%$ isodose. Frequencies of induced chromatid-aberrations (mostly single-and isochromatid breaks, acentrics) were compared with data obtained from 200 and 140 kV X-rays. (Picker-machine, 10 rad/min: 200 kV, 1.0 mm Al + 0.25 mm Cu filters, HVL-0.70 mm Cu. Lower dose rates: 140 kV, 1.0 mm Al + 0.25 mm Cu + 0.4 mm Sn filters, HVL = 0.96 mm Cu.) The calculated values of RBE are for the pion-peak 0.82–1.2 and for the pion-plateau 0.52–0.86. 100 rad peak-pions produced relatively more isochromatid breaks than their corresponding X-ray irradiation; 6 rad/min: $p < 0.5\%$; 10 rad/min: $p = 2$–5%.

The effect of DRB on polyoma virus transcription

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Fraser et al. (Nature *272*, 590, 1978) have shown that the drug 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) causes premature RNA chain termination close to the promoter for the very long late transcriptional unit of adenovirus. Accumulation of RNA transcribed from sequences near promoter sites would be useful for mapping these sites. Polyoma infected mouse cells were treated with DRB during the late phase of infection and then pulse-labelled with ^{3}H-uridine in the presence of the drug; total RNA was extracted and analyzed by sedimentation. Polyoma-specific RNA sediments as a peak at 4–5 S and as a heterogeneous population between 20 and 50 S. In untreated cells, most polyoma-specific RNA sediments between 20 and 50 S. The 4–5 S viral RNA from DRB-treated cells hybridizes more efficiently with the L-strand of polyoma DNA fragment Hpa_{II}-3 than with the L-strand of fragments Hpa_{II}-1, 2 or 4. Fragment 3 lies at the 5′ end of the late region of polyoma DNA.

Development of tight and gap junctions in a liver cell line

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To study intramembranous events during tight and gap junction development between epithelial cells in vitro, we examined monolayers of a rat liver cell line (kindly provided by Dr G. Vergani, Ulm, Federal Republic of Germany) by an in situ freeze-fracturing technique. Our observations indicate that an early step in junction formation consists in the differentiation of a poorly-particulated membrane region showing a honeycomb pattern of slight depressions on P-fracture faces and bulges on complementary E-faces. This change in membrane organization precedes and accompanies the subsequent aggregation of junctional particles. The latter process results in the formation of irregular particle islands with peripheral branchings which tend to encompass the depressions in the membrane. The linear branchings grow and interconnect in a network of beaded strands, which gradually transform into smooth tight junctional fibrils as previously described in fetal liver in vivo (Montesano et al., J. Cell Biol. *67*, 310, 1975), while the particle islands become gap junctions.

Sequence analysis suggests a possible function for the ribosomal spacer DNA of *Xenopus laevis*

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With the aid of a technique of PolydA tailing and partial restriction digestion, the primary structure of the spacer DNA of a ribosomal gene unit from *Xenopus laevis* has been almost completely resolved. The spacer is found to consist of 7 distinct regions, 3 of which are internally repetitive. Using the polydA tailing technique, the 5′ end of the 40 S precursor RNA has been mapped. With this data and the 5′ trinucleotide sequence of the primary transcript (Reeder et al., PNAS *74*, 5402), it has been possible to identify the site(s) from which transcription is probably initiated. A sequence of 200 bp immediately preceding the probable site of initiation and thus itself a possible promotor sequence, is found to occur twice further in the spacer DNA. These 'pseudo-promotor' and 'putative promotor' sequences are separated by repeated 60–80 bp units, each of which itself contains a sequence of 40 bp homologous to the 'putative promotor' sequence. Thus a large segment of the nontranscribed spacer might function as a multiple binding site for RNA polymerase.

The transformation of the granulosa of the human Graafian follicle into the corpus luteum: A morphometric study

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The quantitative morphology of granulosa cells of 11 preovulatory and 2 just ruptured Graafian follicles as well as 20 corpora lutea at different times of the menstrual cycle has been examined. All patients had a regular period

of 28–30 days and underwent surgical treatment for various gynecological indications. In order to correlate cell morphology with serum hormone levels, LH, FSH, estrogene and progesterone have been measured at intervalls of 4–8 h up to 4 days prior to laparotomy. Luteinization starts before the mid-cycle LH peak and comprises an increase in volume of cells (10×), nuclei (3×), nucleoli (12×), mitochondria and lipid droplets (10×) and lysosomes (15×) as well as increase in surface of RER (4×), SER (22×), Golgi (12×), lipid drp. (15×), plasmamembrane (6×) and gap junction (28×). Progesterone increases 100-fold. Despite of the drop in serum-progesterone by the end of the cycle, the lutein cell morphology remains relatively unchanged for the present. It later shows a further increase in lipid and lysosomes and autophagic vacuoles and a decrease in size.

Site-directed mutations in the 3′ extracistronic region of Qβ RNA

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We are exploring the function of the 3′ terminal segment of Qβ RNA by site-directed mutagenesis. Stepwise in vitro synthesis was used to incorporate N^4-hydroxyCMP between positions 24 and 33 from the 5′ end of the minus strand. Plus strands synthesized on the substituted minus strands were transfected into spheroplasts and the RNA of individual phage was analyzed. 2 different mutants were found, one had an A → G substitution at position 29 from the 3′ end ($A_{-29} \rightarrow G$), the other a G → A change at position −25 ($G_{-25} \rightarrow A$). Both mutants had a decreased growth rate. The specific infectivity of $G_{-25} \rightarrow A$ particles was only 40% that of wild type or mutant $A_{-29} \rightarrow G$ phage.

Stimulation of presynaptic adenylate cyclase by an endogenous calcium-dependent regulator protein (CDR) in bovine brain synaptosomes

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Lysis of synaptosomes (SP) from bovine cerebellum releases ca. 0.5 µg CDR/mg SP protein in a hypotonic medium without EGTA and up to 5 µg in the presence of 2 mM EGTA. Moreover the specific high-affinity binding of [^{125}I]-labelled CDR to synaptic membranes increases 2-fold in lysed SP as compared to intact SP. Lysis also affects SP adenylate cyclase (AC) activity, which doubles in lysed SP where presynaptic sites are exposed, suggesting that ca. half of the synaptosomal AC has a presynaptic location, in agreement with the results of Weller (Biochim. biophys. Acta *469*, 350, 1977). CDR appears to influence presynaptic AC activity: Purified CDR (1 µM) increases AC activity 30% in intact SP, 40% in lysed and washed SP and 100% in SP lysed in the presence of 2 mM EGTA. Our results suggest that in addition to its multiple postsynaptic regulatory functions, endogenous CDR is also involved in the regulation of presynaptic AC activity.

X-ray microanalysis of crystalline surface concretions on basidiomycete cystidia

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Cystidia (excreting end cells) are present at the surface of the gills and sometimes at the stipe of some Agaricales (gill funghi). The cystidia of the genera *Inocybe* and *Melanoleuca* are frequently capped by numerous tiny crystals as shown by light microscopy; however, in view of the small dimensions of these crystals (average 5 µm), their shape can be studied with accuracy only by scanning electron microscopy. Calcium is the only detectable element found in the crystals by energy dispersive X-ray microanalysis. In the cell wall of the cystidia, potassium is found in large amounts whereas phosphorus, sulphur and chlorine are present in small quantities. On the other hand, calcium is not detected. These results suggest that Ca is excreted in the form of a salt which may be Ca-oxalate. Unfortunately, as energy dispersive microanalysis gives no data on light elements this hypothesis has to be verified by chemical methods.

Nerve growth factor-mediated induction of tyrosine hydroxylase in a pheochromocytoma cell line (PC12): Permissive action of glucocorticoids

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PC12 cells synthesize, store and release catecholamines and respond (like sympathetic neurons) to nerve growth factor (NGF) with fibre outgrowth. However, they do not show a further characteristic response to NGF, namely the induction of tyrosine hydroxylase (TH). After previous experiments had shown that glucocorticoids potentiate NGF-mediated TH increase in sympathetic ganglia in organ culture, the interaction of glucocorticoids and NGF in the induction of TH in PC12 cells was studied. A reproducible induction of TH (100% increase within 24 h) by NGF (10 nM) is achieved when adequate dexamethasone (optimally 10 nM) is present. The NGF-mediated TH increase can be abolished by cycloheximide but not by actinomycin D or α-amanitin indicating regulation of induction at the posttranscriptional level.

Isolation of a hybrid plasmid with homologous sequences to a transposing element of *Drosophila melanogaster*

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In *Drosophila* several transposing elements of the *white* locus are known. Transpositions of one such element, which carries both the *white-apricot* and the *roughest* genes, have been isolated at more than 100 sites scattered over the entire genome. (Ising and Ramel, in: The Genetics and Biology of Drosophila, vol. 1b, p. 947. Ed. M. Ashburner and E. Novitski. Academic Press, London 1976.) We have isolated a hybrid plasmid (61F4) containing some DNA sequences that are present on this transposing element. 61F4 hybridizes in situ to approximately 30 sites in the giant chromosomes including the nucleolus. Hybridization to chromosome band 3C3, the *white* region, was observed in *white-apricot*, but not in *white*$^+$ stocks. In 5 different transpositions tested, the genetic map position of the transposing element corresponds to a site of in situ hybridization of 61F4. 61F4 carries complementary sequences to a poly A^+ RNA that is abundant in K_C tissues culture cells.

Patterns of protein synthesis in differentiating, BrdUrd arrested myogenic cell cultures and fibroblasts

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Analysis of ^{35}S-methionine labelled proteins was carried out by 2-dimensional polyacrylamide gel-electrophoresis in order to characterize biochemical phenotypes of various types of cultured chicken cells. Some spots were identified by comparison with published data or others by immunological procedures (see Rosenberg et al., Experientia *35*, 976, 1979). In the course of myogenic differentiation enhanced synthesis of muscle specific proteins was observed like e.g. α-actin (α-A.), muscle forms of tropomyosin (TM), myosin light chains (LC), α,β-Desmin (α, β-D.) and each of the 2 forms of B-CK and in later stages of M-CK, as well as not yet identified spots. Patterns of fibroblasts lack most of these muscle specific spots but exhibit spots common to many cell types like e.g. β-, γ-A. and a 55,000 mol. wt protein. BrdUrd arrested myogenic cells gave similar overall patterns however the presence of some spots like B-CK, α-, β-D. and possibly muscle type TM was more conspicious and could be part of phenotype distinct from fibroblasts.

Microdissection and SEM study of human embryonic and fetal hearts

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The absence of an adequate knowledge of the normal organogenesis of the human heart troubles the understanding of pathogenesis of the congenital heart disease. The species differences only increase the general confusion. We have therefore studied the hearts of human embryos and fetuses from legal abortions (28–88 days of gestation) using new methods. The hearts were perfusion fixed at the operating theatre. They were microdissected, macrophotographed and finally examined using a scanning electron microscope. Preliminary evaluation of this material shows that at the cellular level there are only minor if any differences between human, dog, mouse or chick embryo hearts. At the organ level we were able to follow unequivocally the 3-dimensional relationships of great vessels and AV-ostia as well as the closure of the foramen interventriculare. Further detailed studies are necessary to identify the best animal model of human heart organogenesis.

Genetic control of the response of T-lymphocytes to a mitogen

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We have identified 2 genetic variants affecting the response of chicken lymphocytes to concanavalin A (Con A), a T-cell mitogen. The variants are carried by 2 inbred strains (C and G-B1) whose cells respond poorly to Con A. A number of possible causes for low response have been ruled out: low and high responder strains do not differ in a) the ability to respond to other mitogens (e.g. phytohemagglutinin) or to allogeneic cells, b) the proportion of 'T'-lymphocytes (lymphocytes without surface immunoglobulin) in blood, or c) the ability of peripheral blood leukocytes to bind or cap fluorescent Con A. In Con A-stimulated cultures of low responder cells, the numbers of mitogen-activated cells are 5–20 times lower than in high responder cultures at the peak of the response. Thus, low responder Con A-sensitive cells are either fewer in number or divide more slowly than high responder mitogen-sensitive precursors. Estimates of the numbers of mitogen-sensitive precursors in cultures where cell division has been blocked suggest that the former alternative is correct.

Is the histone H1 gene a transposable element?

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It is well-known, that the H1 protein is the most variable histone protein and that it may be developmentally controlled and is tissue specific. The secondary structure of the single stranded DNA of 3 variant histone clones of *P. miliaris* was analyzed by EM. A 1500-bp loop structure was found at one end. Knowing the arrangement of the genes on these 3 variant histone clones, it was possible to locate the H1 gene within this loop. Sequence analysis upstream and downstream of the H1 gene revealed an inverted repeat of $\binom{GA}{CT}_{16-27}$, which gives rise to the observed loop structure. This type of sequence appears to be ubiquitous inthat it also occurs in histone genes of different species, such as *Strongylocentrotus* and *Drosophila.* Near the inverted repeat, deletions of 100 bp and 60 bp are found, when various histone gene clusters of sea urchins are compared. This inverted repeat may serve to increase the variability of the histone gene cluster by site specific exchange of H1 genes.

Why do cereal protoplasts not express totipotency?

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What is known about the causes in the complex chain of events which leads from an isolated protoplast to a complete plant? Important parameters are: an unknown genetic basis (1), intercellular relationship (2), mechanism of differentiation (3), physiological and gene regulatory situation (4) before isolation, unknown effects of the isolation procedure (5) and the isolated single cell state (6), unsatisfactorily defined nutritional (7), hormonal (8), physical (9), culture conditions, presumptive inhibitors (10), unknown cell population effects (11), poor knowledge of the regulation of cell wall synthesis (12), of dedifferentiation (13), of nuclear (14), and cellular division (15), of cellular differentiation (16), of intercellular influences in cell cultures (17), lack of knowledge of morphogenetic pattern formation (18), of embryo or organ formation (19) in cell cultures. As long as we do not know why model plant protoplasts, e.g. tobacco, do express totipotency, it will be difficult to find out, why cereal cells do not express totipotency.

Nuclear injection into *Xenopus laevis* oocytes as a test system for the expression of eukaryotic genes

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By studying the expression of cloned and sequence manipulated eukaryotic genes injected into the oocyte nucleus it may be possible to identify transcription, translation and processing signals. Heterologous histone DNA transcription is dependent of RNA polymerase II, homologous tDNA transcription of RNA polymerase III. RNAs of expected

size and composition are formed. Specific sea urchin histone proteins are synthesized. We are currently attempting to characterize the transcription units of these DNAs by testing the genetic expression of smaller DNA fragments and manipulated gene sequences. Since it is possible to separate manually nuclei from cytoplasms the *Xenopus* oocyte is a system to study the transport of RNA through the nuclear membrane. Although a part of sea urchin histone RNAs is of heterodisperse length only RNA of distinct size is in the cytoplasm. Investigation of histone variant genes may further help to identify special regulatory sequences of these genes.

A restriction enzyme with a novel recognition site, GGTNACC, from a strain of *B. stearothermophilus*

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A restriction endonuclease was purified from a strain of *B. stearothermophilus* by a procedure involving PEI fractionation, $(NH_4)_2SO_4$ precipitation and phosphocellulose chromatography. The enzyme BstPI cleaves ∅29 and PβG DNA once, PCRI twice, λS711 times, and does not cleave fd, ∅X174RF, PM2, pBR322, SV40 and T7 DNA. The sequence around the cleavage site was established by a) cleaving DNA with BstPI, labelling the 5′ termini with ^{32}P phosphate, isolating and sequencing the termini individually by the Maxam-Gilbert method and b) isolating several DNA fragments carrying a BstPI cleavage site and determining the nucleotide sequence in the region of the cleavage site. The recognition site common to all cleaved regions is GGTNACC where N can be any nucleotide. Cleaved ends can be rejoined by DNA ligase.

Anchorage-independent muscle cell differentiation

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Suspended chicken myogenic cells (E.C. Puri and D.C. Turner, Exp. Cell Res. *115*, 159, 1978) were subcultured in a medium consisting of 56% Leibovitz L-15, 20% Ham's F-12, 10.5% 150 mM $NaHCO_3$, 3.5% chick embryo extract and 10% horse serum. In bacteriological petri dishes the cells fused to form suspended 'myoballs', in gelatinized tissue culture dishes they attached and formed elongated myotubes. Suspended and attached cells showed: a) similar extents of fusion (ca. 80% of nuclei in syncytia), b) qualitatively indistinguishable patterns of protein synthesis as revealed by 20 gel-electrophoresis, c) similar changes in synthesis of creatine kinases (increase in M subunits, decrease in B subunits), d) well-organized sarcomeres in electron micrographs. Thus, despite anchorage-dependent differences in cytoplasmic organization, the program of terminal muscle differentiation appears largely unaffected.

Intermediary structures in DNA replication in *Escherichia coli*

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3 classes of newly replicated single-stranded DNA molecules can be defined: neutral fragments, isolated under nondenaturing conditions, Okazaki fragments and long DNA, both isolated under denaturing conditions. Neutral fragments, a subclass of Okazaki fragments, have a comparable mean size (1000-2000 nucleotides). By cross-linking, in vivo, the 2 strands of DNA, we have shown that neutral fragments form duplexes with complementary long DNA. The kinetics of labelling shows that they behave as intermediary structures in DNA synthesis. A fraction of newly synthesized DNA is thus unstably bounded to the complementary strand. DNA:DNA hybridization shows that all 3 classes are produced at the chromosomal replication fork. The smallest Okazaki fragments are synthesized preferentially in the 3′→ 5′ direction. The polarity of neutral fragments is less pronounced, with the same directional preference. Long DNA has the opposite polarity. Neutral fragments could be the product of postreplication repairs, but study of the *ung*$^-$ mutant shows that the source of instability is not the excision of uracil incorporated by error in to DNA.

Characterization of the neural crest cells and of some autonomic ganglia of avian embryos with fluorescent lectins

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The migration pathway and the ultimate fate of neural crest cells depend on their original location along the closing neural tube. Since cell-cell interactions during migration and at the final location are also thought to play a role in the ultimate differentiation, and since these interactions are mediated by the cell surface, it is of interest to characterize the membranes of these cells. Neural crest cells isolated from different levels of the neural tube and put into culture were all stained to the same degree by fluorescent lectins. Con A, WGA and RCA_{120} were well-bound, whereas N-acetyl galactosamine-specific lectins (SBA and RCA_{60}) did not stain the cells. In young embryos adrenergic and cholinergic ganglia show the same lectin pattern as crest cells, but during later development their membranes are so altered that lectin-binding capacity increases: they then become able to bind SBA and RCA_{60}.

Isolation of subviral particles with different methods from Semliki Forest virus (SFV) infected chick embryo fibroblasts (CEF) and Vero cells

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Intracellular postpolysomal ribonucleoproteins (RNP's) of viral origin were isolated from SFV infected CEF and Vero cells with 2 different methods using detergents (method A) or no detergents (method B). The postpolysomal particles sedimented in sucrose gradients at 40 S, 60 S, 80 S and 110 S (methods A and B). An additional peak appeared at 26 S using method A. The 26 S mRNA of SFV was isolated from the 26 S, 40 S and 60 S particles, the genomic 42 S RNA from the 80 S and 110 S particles. The RNP's at 80 S and 110 S contain the viral core-protein. The particles are not attached to ribosomes or ribosomal subunits. No difference with respect to the sedimentation behaviour of the particles from CEF or Vero cells was observed. We assume that at least some of these particles are free in the cytoplasm and may represent precursors to polysomal mRNP's or nucleocapsids.

Incorporation of [^{3}H]dUTP into DNA of CHO cells

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Cells partially lysed with Brij-58 incorporated [^{3}H]dTTP into DNA of more than 25 S (as determined in alkaline sucrose gradients), while DNA synthesized with [^{3}H]dUTP as substrate sedimented with only 4 S. This difference in mol.wt was, at least in part, attributable to degradation of dUMP-containing DNA. Addition of 2-5 mM uracil to the reaction mixture inhibited degradation, whereas pyrimidine, dihydrouracil, thymine, fluorouracil and dUrd were much less effective. [^{3}H]dTTP incorporation into DNA was stimulated by the addition of cell extract, whereas [^{3}H]dUT was rapidly converted to [^{3}H]dUMP in the presence of cell extract. In intact cells incubated with 10^{-6} M aminopterin, [5-^{3}H]dUrd was inefficiently incorporated into DNA, and 5 mM uracil had no detectable effect.

Estrogen receptors in GH_3-cells

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GH_3-cells are rat pituitary tumor cells, which synthesize and secrete prolactin and growth hormone. Prolactin synthesis is stimulated by estrogens. Our finding suggests that this synthesis might be correlated with the presence of estrogen-receptors in cytosol and nuclei. The amount of receptor measured in cytosol and nuclear 0.4 M KCl-extract, respectively, was 121 fmoles/mg protein (n=5) and 89 fmoles/mg protein (n=2). GH_3-cells which had been grown in charcoal-treated serum showed a strong decrease of the receptor concentration in both particulate fractions. The dissociation constant of the cytosol-receptor was 1.8×10^{-10} M/l (n=3). As detected by sucrose density centrifugation the predominant species was the 4 S-receptor. By isoelectric focussing after trypsin treatment a single component with a isoelectric point of about 6.5 could be demonstrated. In competition experiments the affinities of the receptor protein for estrogen and diethylstilbestrol were similar, whereas the affinity for estriol was 10-fold lower. Progesteron, dehydrotestosteron, hydrocortison and dexamethason didn't compete for estrogen binding sites.

Heterogeneity of chicken creatine kinases revealed by immunoreplicates of 20-gels

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Homomeric, highly purified creatine kinase (CK) isoenzymes MM-CK and BB-CK were subjected to 2-dimensional polyacrylamide gel-electrophoresis (2D-PAGE). Both types of subunits, M-CK as well as B-CK, were resolved each into at least 2 major spots migrating with the same MW but exhibiting different apparent isoelectric points. Immunoreplicates of 2D-PAGE gels demonstrated that both the B-CK and M-CK reacted with antibody specific for the appropriate CK subunit. The 2 types of B-CK were shown to be present in myogenic cell cultures, heart and brain. M-CK was present as 2 species in myogenic cell cultures, embryonic or adult skeletal muscle. Both types of CK subunits synthesized in vitro by a cell free system primed by polysomal RNA from myogenic cells or embryonic muscle exhibited the same heterogeneities which are thus unlikely to arise from protein modification but could possibly indicate the existence of multiple mRNA species and thus also multiple genes for CK.

Crypt size of lectin receptors on the red blood cell membrane

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Lectin-labelled gold markers were prepared from gold granules of increasing sizes (5-75 nm in size) with concanavalin A (ConA), soya bean agglutinin (SBA), *Ricinus communis* lectin (RCA_I) and wheat germ agglutinin (WGA) (Horisberger and Rosset, J. Histochem. Cytochem. *25*, 295, 1977). The binding characteristics of the gold markers were studied using human and rabbit red blood cells (h and rRBC). Binding to hRBC occurred only when the ConA, SBA and RCA_I-labelled markers had a size below 12, 50 and 65 nm, respectively. However WGA-labelled markers of all sizes reacted with hRBC. It is therefore concluded that the ConA, SBA and RCA_I receptors are located on hRBC membrane between glycoprotein brushes of increasing spacing while WGA receptors are found at the periphery of the brushes. Contrary to hRBC, rRBC bound SBA-labelled markers of all sizes. The estimation of the crypt size of various membrane receptors by the gold method is under investigation using other type of cells.

Role of the Tn10 transposon in the integration of the drug resistance plasmid R100.1 into the bacterial chromosome

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The multiple drug resistance plasmid R100.1 contains tetracycline resistance genes located on a transposon: Tn10. This transposon is limited by 2 IS10 sequences, 1500 bp long, in the inverted repeat configuration. The extremities of these sequences which are distal to the resistance genes (outer extremities) have been implicated in transposition (Kleckner et al., J. molec. Biol. *97*, 561, 1975), while both the inner and outer extremities of the IS10 seem to be involved in deletion formation and inversion (N. Kleckner, pers. comm.). We have obtained evidence by electron microscopy and by DNA:DNA hybridization, using the transfer technique of Southern, which indicates that the ends of IS10 proximal to the resistance genes are also active in transposition and promote the integration of R100.1 into the bacterial chromosome.

Escherichia coli DNA binding protein HU forms nucleosome-like structure with circular double-stranded DNA

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A low mol.wt (10 K) DNA binding protein composed of 2 closely related species was isolated from enterobacteria and cyanobacteria. By several of its properties (amino acid composition, sequence conservation, presence on the bacterial nucleoid isolated at low ionic strength) this protein resembles the eukaryotic histones. In an attempt to elucidate the possible role of this protein, we studied its association with double-stranded circular DNA. In a reconstitution system analogous to that of histones and DNA, HU can

introduce negative superhelical turns in relaxed DNA in the presence of a chromatin extract rich in nicking-closing activity. Up to 16 negative turns are obtained per SV 40 or plasmid DNA in the presence of an equimolar amount of HU, compared with the 20-24 obtained during histone-DNA assembly in the same conditions. Electron microscopy studies reveal that HU compacts DNA by a factor of 2.0-2.4 which is similar to the action of histones in vitro or in vivo (2.6).

Specificity of transcription initiation on rDNA, genes and gene fragments, injected into *Xenopus* oocytes

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Cloned *Xenopus* rDNA, genes and gene fragments, injected into *Xenopus* oocyte nuclei, are transcribed. Hybridization of the RNA synthesized in injected oocytes with rDNA restriction fragments showed that the plasmids carrying rDNA inserts with the known initiation site are more actively transcribed than plasmids containing other gene fragments. Mapping of the sequences transcribed after injection of the entire ribosomal gene, showed preferential transcription of the sequences situated downstream of the known 40 S pre-rRNA initiation site. From the hybridization data it appears that there might be weak initiation site(s) also within the external 'nontranscribed' spacer of rDNA, but, in this case, contribution of random transcription cannot be excluded. These results indicate that injected rDNA is transcribed with enough specificity to allow the approximate localization of a transcription initiation site. This opens interesting possibilities for the study of structural eukaryotic genes.

Distribution of actin and myosin in normal and transformed cultured cells

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We studied by double label immunofluorescence the distribution of actin and myosin in normal and transformed cell lines from mouse, rat and monkey as well as in secondary cultures of mouse embryo fibroblasts. Cells moving in nonconfluent cultures and migrating over a 'wound' were compared with contact inhibited cells in confluent cultures. The patterns of actin and myosin are superimposed in confluent and nonconfluent cultures; myosin is more diffusely distributed in the cytoplasm and in several instances is absent in leading lamellae. Moving cells display distinct arrangements of microfilament bundles (MB) which are almost as large as in growth arrested cells. In transformed cells, MB are thin and short, and their arrangement is disorganized. They are more prominent in transformed lines where cells are flattened on the substrate than in spindle shaped cells. We suggest that the expression of MB is correlated with cellular adhesiveness rather than locomotion.

Influence of nerve growth factor (NGF) on the ontogenesis of the presynaptic cholinergic nerve fibres in the rat superior cervical ganglion (SCG)

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Pre- and postsynaptic changes occurring after NGF-treatment were investigated in the SCG of newborn rats. Daily injections of 10 µg NGF/g for 10 days led to increases in the total protein content from 113 µg/ganglion in control to 593 µg/ganglion in treated animals and in tyrosine hydroxylase (a biochemical marker for the maturation of the postsynaptic neurons) from 0.015 nM DOPA/min/mg protein to 0.056 nM DOPA/min/mg protein. The activity of choline acetyltransferase (a biochemical marker for the development of presynaptic nerve terminals) increased from 0.30 nM Ach/min/ganglion to 0.48 nM Ach/min/ganglion. This corresponds to a 1.9-fold increase in the number of synapses. Presynaptic axons were also counted. The naturally occurring loss, from 12,500 at birth to 10,000 at day 10, was not only prevented by NGF, but axon numbers even reached 37,000 after 10 days of treatment. These results demonstrate important - probably indirect - effects of NGF on the presynaptic cholinergic nerve terminals.

Characteristics of heat- and cold-sensitive mammalian cell-cycle variants

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A series of heat-sensitive (hs, arrested at 39.5 °C) and cold-sensitive (cs, arrested at 33 °C) clonal variants was selected from the same clone of the murine P-815 cell line. After shift to the nonpermissive temperature, all variants were reversibly arrested in G_1 phase, and cells remained viable for many days. Of some hs and cs clones, less than 1% of cells entered the S period per 24 h at the nonpermissive temperature. After shift to 39.5 °C, hs variants accumulated rapidly in G_1; labelling kinetics suggest that some hs clones were blocked near the G_1/S boundary, while others were blocked earlier in G_1. Accumulation of cs variants after shift to 33 °C occurred more slowly, and 1-4 days were required for near-complete cell cycle arrest. If cs variants arrested at 33 °C for 2-6 days were reincubated at 39.5 °C, cells began to enter the S-period after a lag period of 16-20 h.

Simian virus 40 used as a transforming vector for the insertion of foreign DNA into rat cell chromosomes

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Rat embryo fibroblast cells were transformed with a recombinant virus in which the Simian virus 40 (SV40) 'early' region was linked to a segment of sea urchin histone DNA. The recombinant DNA became covalently integrated into the rat genome in the transformed cells. In the majority of these cell lines, the integrated recombinant DNA was present as a greater than unit length array containing head-to-tail duplications with at least one intact histone DNA moiety present. If the transformed cells were fused with monkey cells which are permissive for SV40 growth, the sea urchin histone DNA was excised and amplified together with the SV40 DNA segment. In several cell lines this rescued recombinant DNA turned out to be indistinguishable from the SV40-histone DNA recombinant originally used for transformation.

Analysis of cloned cDNA copies of the 15 S precursor of mouse β-globin mRNA

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Double-stranded cDNA prepared from purified 15 S β-globin mRNA with reverse transcriptase was elongated with dA, joined to dT-elongated PCRI and used to transform *E. coli* X1776. 300 of 3000 kanamycin-resistent colonies were β-globin-specific, as identified using β-globin mRNA as a probe. The β-globin-specific colonies were rescreened using DNA from the large β-globin intron as probe. Several hybrid DNAs from positive colonies were characterized by restriction analysis. One hybrid, with an insert of about 1200 bp contained both the large and the small introns identified by van den Berg et al. (Nature *276*, 37, 1978) in the mouse β-globin gene Mβ-2 cloned by Tilghman et al. (PNAS *75*, 725, 1978). This conclusively proves that not only the large (Tilghman et al., PNAS *75*, 1309, 1978) but also the small β-globin introns are transcribed into the 15 S mRNA precursor.

Quantitative evaluation of gap junctions during the development of the brown adipose tissue in the rat

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Developmental changes in gap junctions of rat brown adipose tissue (an effector organ in regulatory heat production in neonatal mammals) were studied with freeze fracture technique. Junctional frequency and mean junctional area were evaluated; both values increase after birth, reach a maximum during the functional phase of the tissue (from birth to 3-4 weeks) and decrease during involution (from 3-4 weeks to death). Gap junctional area per cell volume, which has been considered as a measure of intercellular communication, was found to be maximal during the functional phase of the tissue, and closely parallels physiological parameters linked to heat production. The analysis of gap junction frequency and area provides information about gap junction growth and decay and suggests an involvement of these junctions during functioning of brown adipose tissue.

Transcriptional properties of cloned EcoRI fragments of *Euglena gracilis* chloroplast DNA

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Previously we had reported the cloning and analysis of the rDNA region of *Euglena* chloroplast DNA (U.C. Knopf and E. Stutz, Molec. Gen. Genet. *163*, 1, 1978). We have now cloned, using the vector pBR322, the EcoRI fragments G, H, I, J′, K, L, N, O, P, Q+M, T′, U′ and V. Total chloroplast DNA as well as some of these cloned fragments were used as templates in a coupled transcription translation system. The fidelity of this system was tested by analysis of the translation products on gels (SDS-PAGE). The coding site for the large subunit of the ribulosediphosphate carboxylase was mapped with respect to restriction enzyme cleavage sites.

Are hexose monophosphate (HMP) shunt-related reactions involved in the activation of macrophages?

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Resting macrophages were obtained by peritoneal lavage from untreated mice and put in culture (J. exp. Med. *148*, 435, 1978). 3 h after harvesting adherent cells were exposed during 1 h to phagocytosable particles, i.e. zymosan, formaldehyde-treated sheep erythrocytes (SE) or latex beads, or to nonparticulate stimuli. Phagocytosis of zymosan or SE activated the macrophages, as shown by the release of lysosomal glycosidases and the secretion of plasminogen activator. Phagocytosis of latex, however, did not affect the cells. Since the 2 activating particles, in contrast to latex, also stimulated the HMP-shunt, we tested the effects of electrochemical HMP-shunt stimulation. The macrophages were exposed for 1 h to methylene blue which oxidizes NADPH and drives the shunt. This treatment induced the secretion of plasminogen activator. We conclude that a burst of shunt activity or shunt-related biochemical reactions could be a triggering event in macrophage activation.

Glial factor activity is sensitive to delipidation

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Glial cells release a macromolecular factor(s) into their culture medium which can induce morphological differentiation of neuroblastoma cells. Delipidation of the concentrated serum-free glia conditioned medium with butanol-diisopropylether causes loss of glial factor activity. Addition of cholesterol or lecithine to the delipidated material restores the activity, whereas addition of oleate or phosphatidylserine shows no effect. Cholesterol and lecithine alone have no effect on the morphological differentiation of neuroblastoma cells.

Posttranslational control of acid phosphatase in *Saccharomyces cerevisiae*

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Acid phosphatase (a.Pase) of *S. cerevisiae* is derepressed upon inorganic phosphate starvation. Genetic data suggest operator-repressor mechanisms for the regulation of a.Pase. (Toh-E et al., 1978). We measured no significant difference in the amount of translatable mRNA of repressed and derepressed cells in the reticulocyte in vitro translation system. There is a 25-fold difference in specific enzyme activity in repressed versus derepressed cells whereas the amount of ^{35}S-methionine labelled enzyme protein as measured by antibody precipitation varies only 2-3-fold. This argues for posttranslational regulation of preexisting inactive a.Pase. Minor regulatory effects at the transcriptional or translational level cannot be excluded.

Acid phosphatase of yeast is a cell surface marker for differentiation

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Acid phosphatase (a.Pase) of yeast is a mannoprotein and located at the surface of the cell (Linnemans et al., 1977). Mannoproteins on the cell surface of yeast may contribute species-specific information for the organization

of the cell wall and for cell-cell recognition (Ballon, 1976). A.Pase of growing cells is inhomogeneous as measured by its heat sensitivity. In contrast the enzyme of stationary phase cells (G1) is homogeneous. Mutants of *S. cerevisiae* blocked in the cell division cycle and mutants of *S. pombe* altered in the control coordinating cell division with cell growth differ from wild types in activity and stability of a.Pase. This indicates that a.Pase is a cell surface marker signalling the state of differentiation of the cell during the cell cycle.

Chromatid breaks induced by small doses of X-rays in somatic cells of *Drosophila*

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The neuroblasts in the larval ganglia of *D. melanogaster* represent a system of cells in vivo highly suitable for studies of induced chromosome aberrations (Gatti et al., Genetics *77*, 701, 1974). Wild type larvae (96 h) were exposed to various doses (5–100 rad) of 200 kV X-rays and metaphase chromosomes from neuroblasts irradiated in late S/G_2-phase were screened for chromatid breaks. It was found that already the lowest dose of 5 rads induced chromatid breaks in 2.9% of the tested cells (0.0304 breaks/metaphase) which is significantly higher than the 0.87% metaphases with chromatid breaks (0.0087 breaks/metaphase) in a corresponding cell population in nonirradiated ganglions.

Localization of cloned histone genes in the polytene karyotype of *Drosophila subobscura* and *Drosophila affinis*

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Tritiated *E. coli* plasmid DNA including one histone gene repeat unit from *Drosophila melanogaster* was hybridized in situ to polytene chromosomes of *D. subobscura* and *D. affinis*. Autoradiographic label was found over one region in the karyotype of *D. subobscura* and over 2 unlinked regions in *D. affinis*.

Sequence analysis of fragments generated by partial proteolysis from SV 40 T-antigen

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T-antigen was labelled with radioactive amino acids, extracted from SV 40-infected CV-1 cells, immobilized as immune complex on staphylococcal protein A-sepharose and subjected to limited enzymatic proteolysis. The resulting T-antigen fragments were eluted from the adsorbent with sodium dodecyl sulfate, separated by preparative gel-electrophoresis into species ranging from 17 to 71 kdaltons and characterized by peptide mapping. Primary structures near the newly generated N-termini were determined in an automatic sequenator, using high pressure liquid chromatography and scintillation counting for PTH-amino acid identification. Experimental data for 2 fragments matched an amino acid sequence predicted from a nucleotide sequence at about 0.51 map units of the viral genome. We have thus identified the reading frame of T-antigen translation beyond the noncoding sequence at 0.60–0.53 map units.

Assay of intercellular adhesive specificity in transformed and mutant cell lines

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Cell-cell recognition and adhesion are thought to play important roles in development, immune responses and tumor metastasis. A possible approach for identifying the relevant cell surface components is to study the adhesive behavior of mixed cell populations. A method was thus devised for labelling cells with fluorescamine which did not appear to affect cell viability, morphology or adhesive properties. These cells were mixed with unlabelled cells of the same (control) or another type, and the resulting aggregates assayed quantitatively by fluorescence microscopy. Mixtures of lectin-resistant CHO cells (Stanley et al., Cell *6*, 121, 1975) with normal CHO cells showed only minimal segregation. Interestingly, a glucosamine-6-P acetylase deficient mutant 3T3 line (AD6; Pouysségur and Pastan, PNAS *73*, 544, 1976) adhered randomly to the parental cell type, whereas SV40-3T3 and 3T3 cells showed marked segregation. These studies indicate the applicability of this technique for determining adhesive specificity.

Metastatic behavior of human colon carcinoma in nude mice

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Solid colorectal tumors of human origin have been grown in vivo in BALB/c nude mice. S.c. and i.p. routes were tested with Co115, a poorly differentiated carcinoma, compared with Co111, 112 and 125 more differentiated adenocarcinomas. Occasional tumor emboli were seen in lymph nodes regional to Co115 SC grafts only, rarely in lungs. Trypsin-dispersed Co115 cells injected i.p. ($\geq 10^6$) resulted after 3–4 weeks in an aggregate form with peritoneal and diaphragm invasion. The ascitic growth could be maintained by i.p. passages. Co115 aggregates gained access to the mediastinal lymph nodes. Lung metastases developed with time starting from intravascular embolized tumor foci. In contrast, i.p.-injected dispersed Co111, 112 and 125 cells induced peritoneal solid masses, not ascitic and without detectable mediastinal or lung metastases after 2–3 months. The results show that both the degree of differentiation and the route of inoculation participate for the expression of an ascitic, invasive and metastatic type of tumor growth.

Serum-free cultivation of *Aedes albopictus* (mosquito) cells

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In preliminary experiments, the serum-free cultivation of Singh's *Aedes albopictus* cells was examined. The fetal bovine serum (FBS) in the medium (normal formula: 8 parts of Mitsuhashi-Maramorosch basal medium [M. and M., Contr. Boyce Thompson Inst. *22*, 435, 1964] and 2 parts of FBS) was replaced by different concentrations (0.5–10%) of bovine albumin fraction V. A step by step adaptation of cells to serum-free medium was not done. In serum-free

medium cultures grew slower but became nearly as confluent as in normal medium. The cell morphology of the cultures became slightly altered but remained heterogeneous. In older cultures cell foci were observed as in normal cultures. Cultures grown in serum-free medium could be infected with Semliki Forest virus and a persistent infection, as shown with the plaque test, resulted.

Immunological characterization of glial cells from mammalian brain

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The development of new methodology to purify oligodendrocytes from mammalian brain and to maintain them in culture has made possible the characterization of surface markers of these cells by immunological methods. We have produced in the rabbit 2 antisera, one to bovine oligodendrocytes, the other to bovine myelin. The antisera were assayed for evidence of antibody activity by indirect immunofluorescence (FITC-Protein A) or by a binding test (^{125}I-Protein A) with the use of target cells maintained in culture. Rabbit antiserum to oligodendrocytes and rabbit antiserum to myelin did bind to oligodendrocytes. Antibodies to oligodendrocytes were not absorbed by myelin. In addition binding of both antisera was also obtained with clone G 26-24, a glial cell line synthesizing large amounts of myelin lipids. These results suggest that oligodendrocytes share antigenic determinants with the myelin membrane and extend previous observations indicating that the G 26-24 cell line has oligodendroglial features.

Modulation of type I and type III collagen synthesis in normal human fibroblasts in culture

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Fibroblasts in culture are reported to synthesize type I and type III collagen in rather constant proportions and thus do not reflect processes that occur in vivo. However, we have found that cell density, prostaglandin E_2 (PGE_2) and epidermal growth factor influence the amount and ratio of type I and type III collagen synthesized in vitro. The relative amount of type I collagen produced per cell in dense cultures is much lower (36%) than in sparse cultures (100%) whereas the reduction in type III collagen synthesis is less marked (49%). Therefore, in dense cultures, the ratio of types III/I collagen is higher. Treatment of sparse cultures with PGE_2 increased this ratio. In contrast, in dense cultures treated with EGF the ratio of types III/I collagen dropped. Since cAMP levels are low in rapidly growing cells (sparse cultures or EGF-treated dense cultures) and high in dense cultures or in PGE_2-treated sparse cultures, our results are consistent with a role for cAMP in modulating the amount of the 2 collagen types synthesized by fibroblasts. This in vitro system may be a useful model for the study of regulatory processes in vivo during development and repair.

Migration factor is a growth factor that acts differently from FGF and EGF

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Migration factor (MF) is isolated and purified from the conditioned medium of a transformed cell line and induces migration, growth in low serum and glucose uptake in normal 3T3 cells. We compared the mitogenic response of MF to that of fibroblast growth factor (FGF) and epidermal growth factor (EGF) and found that: 1. Saturating amounts of MF give a response quantitatively similar to that of 10% Foetal Calf Serum (FCS). The response to either FGF or EGF is lower. 2. In serum-free conditions the response to FGF and especially to EGF decreases markedly whereas MF does not appear to have a serum requirement. 3. Short time exposure to MF (down to 10 min) can produce a response equivalent to 75-100% of the 24-h exposure response; with EGF at least 7-8 h are necessary to give a full response, FGF falling in between these 2 extremes. - We conclude that MF acts through a different mechanism than either EGF or FGF.

The sensory projection of ectopic leg neurons in a homoeotic antennal mutant of *Drosophila*

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The central projection pattern of sensory cells from the wild-type antennal arista of *D. melanogaster* was established by filling their axons with cobalt. The pattern consists of a main component in the lower posterior part of the ipsilateral antennal glomerulus (AG), a small number of terminals in the contralateral AG, and a tract of a few fibres projecting into the suboesophageal ganglion (SOG). In the homoeotic mutant *spineless-aristapedia* which transforms the arista into an ectopic tarsus, sensory axons rarely cross the midline. They project predominantly into the ventral half of the ipsilateral AG without showing any evident pattern. Some fibres extend into the SOG via a tract comparable to that occupied by the aristal axons. Ectopic leg fibres projecting into normal, thoracic leg centers have not been observed. It is concluded that the homoeotic projection pattern is influenced both by intrinsic (the identity of the sensory cells) and extrinsic (the properties of the c.n.s.) factors.

Agents affecting the differentiation of mouse lymphoid cells alter endogenous virus expression

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B-cell mitogens stimulating resting spleen cells to proliferate and differentiate into antibody secreting cells induce endogenous C-type virus release. The finding that an antisera against endogenous virus can block immune responses (Nature *269*, 600) suggests a functional role for virus in lymphoid cells. Both proliferation, shown by inhibitors of DNA synthesis and hot thymidine suicide experiments, and maturation, in experiments with antisera that block differentiation, appear necessary for virus expression. However, another drug, bromodeoxyuridine which increases virus expression blocks final differentiation of B-cells. On the basis of these experiments we hypothesize that virus is expressed by B-cells in an intermediate stage of differentiation, generated by certain mitogens and amplified by bromodeoxyuridine.

Application of the disc method to plant cell cultures for drug sensitivity testing

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In bacteriology, the disc method (or agar diffusion method) is frequently used for determination of the susceptibility of

bacteria to antibiotics. Under certain conditions it is possible to apply this method to plant cell cultures. If a densely plated cell suspension of *Rosa* or *Zea mays* is exposed to a disc containing mercury compounds or amino acid analogs a clear halo with more or less sharp edges is formed after incubation. In some cases the diameter of the halo can be used as measure for the concentration of the drug or the sensitivity of the cells. The method was successfully applied to dividing protoplasts of *Rosa*. Perhaps, it presents a new, more efficient way of isolating drug-resistant variants from plant cell cultures and of comparing these to the wild type.

Morphometric evaluation of the process of neurosecretory axon degeneration in implanted posterior pituitaries

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Posterior pituitaries of albino and Brattleboro rats were implanted under the kidney capsule for several time periods (Süss and Pliška, Experientia *34*, 955, 1978). 3 subcellular particles associated with the phagocytic activity of pituicytes - lipid bodies, multilamellate bodies and lysosome-like bodies - were investigated stereologically in the implanted tissue (Weibel, Lab. Invest. *12*, 131). The volume ratio subcellular particle:tissue was measured in various implantation stages. It varies significantly for the individual particles. The ratios for lipid bodies as well as for multilamellate bodies display a high maximum at the days 6 and 7, whereas the ratios for the lysosome-like bodies are roughly constant over the whole implantation period. These results are in agreement with the recently published data about the decay of neuronal markers in equal conditions (see ref. above). This correlation indicate that the desintegration of the axons is associated with an enhanced phagocytic activity of pituicytes.

Hydra head regeneration: Stimulation by 2 neuropeptides

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Chromatography of aqueous extracts of sonicated *Hydra attenuata* and *Actinia equina* on sephadex G-10 or G-25 eluted with H_2O allowed the separation of a fraction A migrating as the synthetic substance P (sP) and of a fraction B corresponding to the hydra head activator (HHA) (H.C. Schaller, J. Embryol. exp. Morph. *29*, 27, 1973). On 2-dimensional thin layer chromatography (silica gel 60, n-butanol, acetic acid, H_2O 40:10:90 and n-butanol, acetic acid, pyridine, H_2O 30:6:20:14) fraction A gave spots revealed by fluorescamin. One spot had the same R_f values (0.26 and 0.35) than that of synthetic sP. Furthermore both fractions exhibited an unequivocal stimulation of hydra head regeneration. Thus at least 2 natural neuropeptides present in hydra stimulated hydra head regeneration: sP and HHA. Current experiments are carried out in order to determine their apparently different modes of action.

T-even polyhead structure as revealed by different preparation methods and STEM darkfield

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The high contrast of the STEM darkfield mode allows the influence of nonconventional preparation methods on the appearance of protein structures to be investigated. A study of preparation procedures aiming for the omission of negative staining with heavy salts is at present being carried out, using T2 phage-A-type polyheads as a test system. To this end 3 approaches are followed. 1. Various negative stains are gradually diluted and partially replaced by glucose in order to maintain the sustaining activity of the stain during dehydration. 2. Polyheads are incubated in heavy metal salts and air-dried in glucose after elimination of nonbound heavy ions by dialysis. 3. Unfixed, completely unstained and thoroughly washed polyheads are freeze-dried, transferred to the STEM in a dry nitrogen atmosphere and observed without previous metal or carbon shadowing. While the first 2 approaches do not bring about any progress, a combination of positive staining with sodiumsolicotungstate followed by negative staining with uranylacetate allows the polyhead structure to be imaged to a resolution beyond 15 Å as determined by differential filtering. Micrographs of unstained freeze-dried polyheads recorded in STEM darkfield mode at moderate doses (10–30 $e^-/Å^2$) produce indexable diffraction patterns with considerable reproducibility. However, the optical reconstructions so far obtained still remain to be correctly interpreted.

Effect of nitrogen transport compounds on leaf nitrogen assimilating enzymes in beans

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In bushbeans the major nitrogenous compounds involved in long distance transport via the transpiration stream were nitrate, allantoic acid, asparagine and glutamine. When these compounds were fed via the transpiration stream to primary leaves of nitrate-grown plants for 3-6 h in an artificial sap solution, equivalent in composition to bleeding sap N collected from nitrate-grown plants, leaf nitrate reductase in vitro activity decreased only when nitrate was absent from the feeding solution. Increasing the concentrations of allantoic acid, asparagine or glutamine in the feeding solution up to the levels measured in the bleeding sap of NH_4^+ or NH_4NO_3-grown plants had either no effect or slightly increased nitrate reductase activity provided nitrate was present. Under the same conditions glutamine synthetase activity was slightly decreased but with all treatments there remained a high in vitro activity. These results suggest that the activities of enzymes involved in nitrogen assimilation are not greatly affected by changes in the flux of the major reduced nitrogen transport compounds into bean leaves.

The effect of Mitomycin C on the bristle organ forming cells of the Dipteran flies *Drosophila melanogaster* and *Phormia regina*

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The bristle organ of Dipteran flies consists of the tormogen, trichogen, sense and neurilemma cells. Injection of Mitomycin C (MC) into larvae of *D. melanogaster* or *P. regina* results in the formation of bristle organs without sockets (Tobler, Experientia *25*, 213, 1969; Schaerer, Wilh. Roux Arch. *179*, 145, 1976). This defect might be due to an inhibition of the differential cell division after MC-treatment or to a repression of polytenization. Ultrastructural analysis of differentiating bristle organs in MC-treated larvae of *D. melanogaster* (Walt and Tobler, Biol. Cell. *32*, 291, 1978) and cytophotometric investigations of bristle

organ forming cells in *P. regina* (Schaerer, Wilh. Roux Arch. *179*, 145, 1976) indicate that differentiative cell divisions leading to the tormogen and trichogen cells do occur after application of MC, but that polytenization of the tormogen and trichogen cells is considerably retarded.

Freeze-fracture study on the presynaptic membrane during transmitter release

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Vesicle attachment sites (vas) at spinal cord nerve endings in rat ranging from 5 to 39 nm in diameter were classified into: type 1 without particles, type 2 with particles around vas, type 3 with particles within vas. Application (i.v.) of 1 mg/kg 4-aminopyridine (4-AP) known to enhance transmitter release increased the frequency of vas in the active zone as compared with the nembutalized controls ($p < 0.001$) but not the proportion of the 3 types. The active zone contained predominantly type 1 and 2 (exocytosis?), while type 3 prevailed in the surrounding zone (endocytosis?). Large intramembranous particles (> 10.5 nm) adhering to the cytoplasmic membrane leaflet were aggregated more densely within the active zone than without in test and control groups ($p < 0.001$). Their density increased ca. 100% under the influence of 4-AP. These findings suggest that the large intramembranous particles may participate in the mechanism of transmitter release possibly by controlling transmembranous Ca^{++} fluxes.

Micropuff-like structures in heat-shocked *Neurospora*

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46 °C heat-shock determines premature conidiogenesis in *N. crassa* macroconidia (microcyclic conidiation). After 2 h or more exposure to high temperature, large 'dense spots' frequently located around the nucleolus are observed in a majority of nuclei of macroconidia (Ton-That and Turian, 1978). Cytochemical reactions were applied to thin sections of these cells: 'dense spots' react negatively to DNA-specific staining and positively to preferential staining for RNproteins. These 'dense spots' are partially digested with pronase; when RNase is applied on whole fixed cells, they appear strongly altered. Therefore, 'dense spots' of *N. crassa* seem to react similarly to micropuffs in *Allium cepa* (Risueño et al., 1978). Numerous 'dense spots' are also present in the cytoplasm of *N. crassa* macroconidia, some of them being in contact with the nuclear membrane thus suggesting transport and storage of macromolecules from nucleus to cytoplasm.

Cell survival over the depth profile after irradiation with a negative pion beam

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The proliferation- and colony-forming ability of Chinese hamster fibroblast cells in vitro have been investigated after irradiation with a negative pion beam. These experiments are being carried out in connection with the preclinical evaluation of pions for radiation therapy at the Swiss Institute for Nuclear Research (SIN). We applied the technique described by Skarsgard et al., 1974. In order to measure cell survival along with depth of penetration, cultured cells were uniformely suspended in a gel-medium mixture. This mixture turns fluid at 37 °C whereas at the radiation temperature of 17 °C it remains solid. After irradiation, 2 mm thick slices are cut off. This allows to observe the biological effect of pion irradiation at various points of the depth dose curve. The resulting cell survival profiles show a peak which corresponds with the peak in the physical depth dose curve. The relative biological effectiveness (RBE), calculated to 140 KeV X-rays at surviving fractions of 0.5 and 0.1, were 1.3 in the peak region and 0.8-1.0 in the plateau region.

Growth and regeneration of amphibian optic axons in the absence of a contralateral optic tract

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Some conditions affecting decussation at the chiasma, as well as the diencephalic course of optic axons (OA), were studied in *Xenopus*. One optic vesicle was removed in 25 embryos before OA grow into the brain. The path followed by the remaining OA was traced at midlarval stages (15 tadpoles) by the HRP method (anterograde transport). All these OA decussated at the chiasma; their path and terminations in the brain were as in control tadpoles. The optic nerve of the remaining 10 tadpoles was cut and after 18 days the regenerated OA were traced. These OA had an abnormal diencephalic trajectory in that they followed the lateral margin of the white matter. These results suggest that a) decussation of growing OA is independent of interactions among OA concomitantly reaching the chiasma from the 2 eyes, b) axons regenerating toward their proper target use paths different from those of the homologous axons growing de novo.

Transcription of rabbit β-globin DNA in yeast cells

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A cloned DNA fragment containing the rabbit β-globin chromosomal gene and its flanking regions (the large HhaI fragment of plasmid Z/pCRI/RchrβG-1; van den Berg et al., Nature *276*, 37, 1978) was joined at the HpaI site to the PMB9 moiety of hybrid plasmid pJDB219 (Beggs, Nature *275*, 104, 1978), which consists of the 2 μm yeast plasmid, the yeast leu-2 gene and PMB9. The new hybrid was cloned in *E. coli* X1776 and characterized by restriction analysis. *Saccharomyces cerevisiae* MC16 (leu^-) was transformed with the hybrid and leu^+ colonies screened for the presence of globin DNA. RNA from a positive yeast clone was analyzed by a modification of the Berk-Sharp method. So far only plus strand globin-specific RNA containing the small intron was identified; there was no indication of spliced RNA species.

The sequence of a cloned rabbit DNA segment containing a β-globin gene and its flanking regions

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The sequence of a segment of cloned rabbit DNA extending from 222 bp before the β-globin transcription unit to 106 nucleotides beyond it was determined and compared

with that of a corresponding mouse DNA fragment. In both, the exons are in 3 blocks, separated by a small and a large intron. The mouse and rabbit DNA show strong similarities everywhere except for the introns and part of the mRNA trailer sequence. Corresponding rabbit and mouse introns differ greatly and show homologies mainly at the exon junctions. If mouse and rabbit introns derive from a common sequence, then they have been subject to unexpectedly high genetic drift, suggesting that no specific function is associated with most of their length. A variant of the 'Hogness-box' (T-A-T-A-A-A-T) is found 22–23 nucleotides preceding the begin of the mRNA, namely C-A-T-A-A-A-A-G-G (rabbit) and C-A-T-A-T-A-A-G-G (mouse).

Adhesion of human red blood cells to polystyrene

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The adhesion on polystyrene of glutaraldehyde-fixed human red blood cells (RBC) was found to increase with the concentration of the chlorides of mono- and divalent cations. RBC were sedimented by gravity in polystyrene tubes and the adhesion was calculated by measuring spectrophotometrically the number of resuspended unadsorbed cells. Half of the maximum of irreversible adhesion (Ad_{50}) was obtained at 22 °C in 2.2 mM NaCl for neuraminidase-treated cells (nRBC) and in 5.5 mM NaCl for the untreated cells. Ad_{50} of RBC was similar for all the monovalent cations. With the divalent cations, Ad_{50} occurred between 0.4 and 0.5 mM. By lowering the temperature to 0 °C, Ad_{50} was not affected in NaCl. Between pH 6.5 and 8.5, adhesion of RBC to polystyrene decreased by 30% in 140 mM NaCl. Polyethylene glycols (PEG, mol.wt 10^4–3×10^5) decreased the adhesion of RBC to the plastic surface. Ad_{50} of RBC occurred at PEG's concentration between 3.3 and 6.5×10^{-5} M. However adhesion of nRBC was much less affected by PEG's having a low mol.wt. At present it is not known if this type of nonspecific adhesion has any biological implication.

Characterization of transcription in nuclei isolated from concanavalin A stimulated lymphocytes

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Isolated cell nuclei provide a simplified system for studying the mechanism controlling transcription and maturation of defined RNA species. In our study we isolated nuclei 50 h after stimulation with concanavalin A. RNA synthesis measured by incorporation of [^{3}H]UTP into TCA precipitable material, was shown to be dependent on the presence of all 4 nucleoside triphosphates. Substrate inhibition was found at concentrations of precursors above 0.5 mM. In most systems describing RNA synthesis by isolated nuclei the rate of RNA synthesis remarkably decreases within 1 h incubation time. We found that the decrease is independent of the presence of inorganic pyrophosphatase, indicating that it is not due to the accumulation of pyrophosphate. On the other hand, RNA synthesis is increased by the presence of sodium fluoride, an inhibitor of nucleotide hydrolysis. This shows that the depletion of nucleoside triphosphates occurs during RNA synthesis.

Immunoelectron microscope localization of epoxide hydratase in various subcellular fractions of rat liver cells

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The localization of epoxide hydratase (EH) in rat liver microsomal, nuclear and mitochondrial fractions was studied using ferritin conjugates of goat monospecific antibodies to this enzyme. Microsomal vesicles from which the ribosomes had previously been detached were labelled with the ferritin-antibody conjugate exclusively on their outer surfaces. Proteolytic solubilization experiments suggested that EH may be burried deeply in the hydrophobic phase of the membrane (J. Seidegård et al., Biochim. biophys. Acta *543*, 29, 1978). However, our immunocytochemical experiments show that antigenic sites of EH exposed at the cytoplasmic surface of the membrane are responsible for the specific binding of antibody-ferritin conjugates. Pretreatment of rats with either phenobarbital or 2-acetylaminofluorene increased the labelling of microsomal vesicles. The outer envelope of nuclei isolated from liver of phenobarbital-induced animals was moderately labelled with the ferritin-antibody conjugate, whereas intact mitochondria showed virtually no binding.

A hybridization procedure allowing full discrimination between closely related RNAs

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To distinguish unambiguously between rabbit and mouse β-globin mRNA, which cross-hybridize under nonstringent conditions, we devised a modification of the Berk-Sharp procedure. The 2 mRNA sequences differ immediately following the termination triplet, where the rabbit RNA has a BglII recognition sequence but the mouse not. The rabbit β-globin cDNA plasmid PβG was cleaved with BglII and ^{32}P-labelled at the 5′ termini. Mouse and rabbit mRNA mixed in different ratios were hybridized to excess denatured probe in 80% formamide. After S_1 digestion, the DNA was analyzed by PAGE and autoradiography. Less than 30 pg of rabbit mRNA sufficed to give the expected 500-bp band. 100 ng mouse globin mRNA gave no such band and did not interfere with the detection of the rabbit mRNA. Using a 5′-labelled AluI site in the small mouse β-globin intron, the location of the 5′ end of 15 S β-globin mRNA was determined in the presence of 10 S β-globin mRNA. The 5′ ends of precursor and mature RNA were found to coincide.

Site-directed mutagenesis in the 5′ noncoding region of cloned rabbit β-globin cDNA

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Site-directed mutagenesis, involving the in vitro incorporation of mutagenic N^4-hydroxydCMP residues, was previously limited to regions close to restriction sites (Müller et al., JMB *124*, 343, 1978). We have improved the method to allow introduction of transition mutations at any preselected site of a DNA. To obtain mutations in the 5′ noncoding region of rabbit β-globin cDNA, a collection of

cloned terminally shortened globin cDNA fragments was prepared and characterized by sequence analysis for use as primers (communication by Meyer et al.). Primers of appropriate length were hybridized to full-length globin cDNA providing a primer template for dHOCMP incorporation by polymerase I into selected sites preceding the coding region. A strand complementary to the elongated primer was synthesized in vitro, the mutagenized duplex segment was cloned and the progeny characterized by sequence analysis. 1 of 9 clones examined so far contained mutations within the expected region.

The architecture of the photosynthetic membrane of *Rhodopseudomonas viridis*

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The photosynthetic membrane of *Rhodopseudomonas viridis* (gift of Prof. Pfennig) forms large stacks of flattened vesicles and exhibits a highly ordered structure (Giesbrecht and Drews, Arch. Mikrobiol. *54*, 297, 1966). Because of its periodicity this membrane is suitable for a structural analysis by high resolution electron microscopy. In a first step we studied the morphology of the isolated membrane by freeze-etching: Both membrane surfaces showed a hexagonal arrangement of subunits with a lattice constant of 130 Å, however with a different appearance on the 2 sides. When the membranes were fractured again a hexagonal arrangement of the unit cell was seen, but only when the samples were frozen without glycerol. Image processing of micrographs obtained from negatively, positively and unstained specimen revealed a hexagonal structure of the unit cell itself. It seems to consist of a central globular part with a diameter of 50 Å surrounded by 12 hexagonally arranged cylinders of 30 Å diameter which are perpendicular to the membrane plane.

Purification of the *Drosophila* DNA-binding protein 2 (DB-2) and demonstration of its sequence specificity

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DB-2, originally purified from unfertilized eggs of *Drosophila* by conventional protein purification procedures, was used to select hybrid plasmids carrying the presumptive DNA-sequence recognized by DB-2. Hybrid plasmid A17 selected by DB-2 was shown to hybridize to a single site on salivary gland polytene chromosomes. More recently, DB-2 was purified by affinity chromatography using a column of plasmid A17 coupled to cellulose. In order to demonstrate the sequence specific interaction of DB-2 with plasmid A17 DNA, DB-2 was bound to labelled A17 DNA followed by DNase I digestion. A DNA fragment, protected from DNase I by protein DB-2, was isolated and hybridized to various restriction digests of plasmid A17 separated on agarose gels and transferred to millipore filters. In each restriction digest the protected piece hybridized to only one restriction fragment. When aligned on a restriction map, hybridization occurred always on overlapping sequences suggesting that DB-2 binds to and protects a unique DNA-sequence.

Degenerating oocytes in juvenile mouse ovary

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Along with oocytes containing an intact germinal vesicle a considerable number (up to 40% depending on age) of different types of degenerating oocytes can be isolated from the juvenile mouse ovary by mechanical disruption of the tissue: a) 'Atretic' oocytes having a shrinking vitellus, a dissolved zona pellucida and no stainable nucleus were found in all age groups investigated (on days 8, 15, 18, 21, 24 and 28 p.p.). b) Oocytes resembling those after germinal vesicle breakdown in vitro were first observed in ovaries of 15-day-old mice. At this stage oocytes actually start to become competent for resumption of meiosis in culture. Cytogenetic and morphological observations indicate that these oocytes have resumed meiosis in situ and that also the zona pellucida and the vitelline membrane surface show signs of precocious maturation as was visualized by scanning electron microscopy. These precociously matured oocytes seem to originate from nonatretic follicles. c) Fragmented oocytes were first observed in mice aged 18 days. Their cytoplasm and nuclei are dissociated into unequally sized fragments and furthermore there is evidence to suggest that the stage of precocious maturation is followed by fragmentation.

The freeze preservation of cultured plant cells

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Suspension cultures of *Zea mays* and other species can now be routinely freeze preserved thanks to refinements in 2 stages of the freezing process: 1. The use of a step-wise freezing programme, holding the specimen at −30 °C before storing in liquid N_2. 2. The use of new cryoprotectants. Proline (a compound produced by plant cells subjected to cold or drying stresses) demonstrates pronounced cryoprotectant properties when applied exogenously to plant cells, either alone or in combination with glycerol and DMSO. Pregrowth in proline gives a culture which, uniquely, needs no further treatment before freezing.

Drosophila cell hybrids: Resistance to growth inhibition by molting hormone is dominant

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Cell hybrids between 2 established cell lines, MDR3 and Schneider's line 3 (S3), can be selected and identified (C. Wyss, Somatic Cell Genetics *5*, 29, 1979). Both parent cell lines respond to ecdysone with morphological changes and with cessation of growth. Of 15 independent hybrid clones between S3 and MDR3, all proved to be about as sensitive to ecdysone as the parental cells. Several independent mutant clones of MDR3 resistant to 1 µg/ml ecdysone (β-ecdysone, Rohto) were isolated. These were then fused to (wildtype) S3 cells using polyethylene glycol. Hybrids were selected in TAM cloning medium in the absence of ecdysone. For each mutant at least 5 hybrids with S3 were tested for sensitivity of proliferation to ecdysone. All hybrids between S3 and ecdysone resistant mutants of MDR3 proved to be ecdysone resistant.

The role of cell cycles in cell-type conversion

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When iris epithelial cells (IECs) of adult newts, which are fully differentiated and normally nondividing, are activated to proliferate, they either become lens cells, or regain the original differentiation. From studies of pathways in situ after lentectomy and in vitro, a working hypothesis has been proposed, according to which a passage of more than 6 cell cycles in sequence is required for the conversion to occur. Estimates of the minimum number of cell cycles passed by progenies of IECs before they express the lens phenotype were made by cell cycle analysis combined with immunofluorescence for crystallins. The results of in situ and in vitro studies support the above idea. A cytochemical EM test has shown that lysosomes are activated together with proliferation in IECs and initiate autophagy of melanosomes, which is followed by exocytosis of the organelles. The cell cycle requirement discussed above may be related to the loss of melanosomes caused by autophagy.

Migration factors induce growth and transformed morphology in quiescent cultures

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2 growth factors were purified from conditioned medium of SV28 cells (BHK 21/13 cells transformed by SV40 virus) for their ability to induce cell migration. Both factors can stimulate the growth of cultures of Balb/c 3T3 cells to above their saturation density. Within hours of addition of factor to culture medium confluent quiescent 3T3 cells elongated and exhibited pleomorphic forms typical of fibroblasts. Factors A and B induced criss-crossing at different concentrations and A further caused clumping cf cells rather like the culture morphology seen when 3T3 cells are transformed. SEM revealed a strikingly diminished number of microvilli on the surface of factor treated cells. Low magnification TEM of negatively stained Triton X-100 extracted cytoskeletons revealed contacts between confluent quiescent 3T3 cells with fibres orientated towards them. After factor treatment the cytoskeleton had changed and contacts between cells had disappeared. The meshwork of fibres and filaments seemed to be arranged in parallel with the extended pseudopodia and the cytoskeletons passed over adjacent cells without interaction. These factors also stimulated thymidine incorporation and glucose uptake in quiescent confluent cultures of 3T3 cells.

In vitro synthesis of a putative precursor of mitochondrial aspartate aminotransferase

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This study was initiated as a first step towards the elucidation of the mechanism which selectively translocates mitochondrial aspartate aminotransferase (mAAT, a dimer of mol. wt $2 \times 45{,}000$) from its site of synthesis in the cytosol into the mitochondrial matrix. Polysomal RNA from chicken liver was translated in a rabbit reticulocyte lysate containing ^{35}S-methionine. The translation product was purified using antiserum prepared against mAAT from chicken heart and on SDS-PAGE gave 1 major band with mol. wt 48,000. In controls using preimmune serum, this band was absent. Peptide maps of the in vitro translation product and the mature mAAT were similar, suggesting that the 48,000 protein is a precursor of mature mAAT. Excess of mature mAAT did not compete with the precursor in the immunological extraction. This finding might indicate that the precursor is a monomer which exposes antigenic determinants not accessible to antibodies in mature dimeric mAAT.

A structural skeleton of nonhistone proteins in interphase chromatin

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Interphase chromatin can be isolated as a discrete structure after removal of the nuclear membrane and pore network (R. Hancock et al., Meth. Cell Biol. *15*, 127, 1977). We are studying the elements responsible for maintaining the folded form of the DNA in chromatin structures from mouse cells. Histones are completely dissociated using 2.5 M NaCl, without unfolding the DNA. Digestion of RNA by RNAase does not unfold; however, proteolytic enzymes, SDS and urea result in rapid and complete unfolding. We conclude that a skeleton of nonhistone proteins is responsible for the folding of interphase chromatin. - We have visualized this skeleton and the attached DNA by Kleinschmidt spreading after removing histones. In favorable spreading conditions loops of DNA, 20 Kb or longer, are found attached to the skeleton. Digestion with the restriction enzyme Eco R1 leaves about 2.5% of the chromosomal DNA associated with the skeleton. Agarose gels of this DNA digested with Eco R2 indicate that it contains predominantly repetitive mouse satellite DNA sequences. Between 20 and 30 proteins are found in skeleton preparations with a predominant group of bands of mol. wt 65,000–70,000.

CONGRESSUS

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1st AMES-YISSUM virology conference on: Latent and persistent virus infections in man

Jerusalem, 14–18 October 1979

The conference will cover aspects of: 1. The role of viruses in disorders of the central nervous system: measles and related viruses, 2. Herpes viruses, 3. viral hepatitis. Information by the 'Virology conference secretariat', P.O. Box 983, Jerusalem, Israel.

CONGRESSUS

Italy

International congress of neurotoxicology

Varese, 27-30 September 1979

The Italian Society of Toxicology plans the following sessions: Toxic reactions of the nervous system to environmental and industrial chemicals; Advances in the biology of alcoholism; Therapeutic agents as CNS toxicants; Selected aspects of clinical and experimental neurotoxicology.
Further information by Prof. L. Manzo, University of Pavia, Department of Medical Pharmacology, Piazza Botta 10, I-27100 Pavia, Italy.

1st international congress on hormones and cancer

Rome, 3-6 October 1979

Several satellite symposia are organized: 1. Induced uterine proteins, 2. Perspectives in steroid receptors studies, 3. Adrenal androgens, 4. Central and peripheral regulation of prolactin function, 5. Steroids and their mechanism of action in non-mammalian vertebrates.
Information by the scientific secretariat: Prof. S. Iacobelli, Laboratorio de Endocrinologia Moleculare, Istituto di Clinica Ostetrica e Ginecologica, Università Cattolica, via Pineta Sacchetti 644, I-00168 Roma, Italy.

Czechoslovakia

International congress of polarography, in memoriam Jaroslav Heyrovsky

Prague, 25-29 August 1980

Information by Dr J. Kûta, secretary of the congress, Vlašska 9, 118 40 Praha 1, Malá Strana, Czechoslovakia.

Switzerland

Problems in nutrition research today

Berne, 18/19 October 1979

On the occasion of 10 years activities of the 'Swiss nutrition foundation' a symposium is organized with the following scientists: Prof. J.G.A.J. Hautvast, Wageningen/The Netherlands; Prof. J. Solms, Zürich/Switzerland; Prof. B. Hallgren, Uppsala/Sweden; Prof. R. Paoletti, Milano/Italy; Prof. G. Schlierf, Heidelberg/BRD; Dr M.R. Turner, London/England; Prof. R. Buzina, Zagreb/Jugoslawia; Sir K. Blaxter, Buchsburn/Aberdeen/Great Britain; Prof. R.G. Whitehead, Cambridge/England; Prof. E. Mrak, Davis, Cal./USA.
Information by: Swiss nutrition foundation, c/o Med.-chem. Institut, Universität Bern, CH-3000 Berne 9, Switzerland.

Federal Republic of Germany

4th international symposium of the European weed research society

Mainz, 8-12 October 1979

General information by: Prof. Dr. G. Schumann, Deutsche Pflanzenschutztagung, Messeweg 11/12, D-3300 Braunschweig/BRD.

Austria

1st world biomaterials congress

Baden, near Vienna, 8-12 April 1980

Information by: World biomaterials congress secretariat, Mrs E. Maurer, c/o Wiener Medizinische Akademie, Alser Strasse 4, A-1090 Wien, Austria.

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