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PHYSIOLOGIE-PHYSIOLOGY

Influence of the glycogen stores on de novo lipogenesis after ingestion of 500 g carbohydrate (CHO)

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Among 8 male subjects (22±1 years; 68±9 kg), 5 consumed a high CHO diet (82% CHO, 8% fat energy) for 6 days (group 1) and 3a high fat diet (13% CHO, 76% fat energy) for 3 days (group 2), to alter their body glycogen stores before ingesting a 500 g CHO test meal. Respiratory exchange measurements were made continuously for 24 h after the test meal. The fasting nonprotein respiratory quotient (NPRQ) was used as an index of the glycogen stores. Before the load, the NPRQ was 0.95±0.02 (group 1) and 0.76±0.003 (group 2). It exceeded unity 2.5 h after the meal in group 1 and remained greater than 1 for a further 10 h. In group 2 it rarely exceeded 1, maximum values being reached 10 h after the meal. During periods of NPRQ > 1, fat synthesis surpassed fat oxidation by 10.4±1.8 g (group 1) and 0.9±0.3 g (group 2). A correlation ($r = 0.868$, $p < 0.01$) was found between the fasting NPRQ and net lipogenesis. These results suggest that the state of the glycogen stores can influence the amount of net lipogenesis after a large CHO meal.

Load compensation in human ankle muscles: contributions of short and medium latency activity

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Load compensation in human ankle muscles was investigated by applying disturbances which rotated the foot while the subject was endeavouring to maintain a constant position against a preexisting force. 2 separate stages of the stretch reflex response were distinguished. 1. For the initial 100 msec a 15% increase in force (expressed as a percentage of the total force required to correct the disturbance) which was attributed to the viscoelastic resistance of muscles active prior to the disturbance. A short latency (SL) EMG response at 33 msec added force to the viscoelastic response and compensated for its nonlinear characteristics. 2. After approximately 130–150 msec a medium latency (ML) increase in force which was preceded by an increase in EMG activity at 120 msec. The ML force response also contributed a 15% increase in force. Both SL and ML components of the stretch provide insufficient force to correct for load disturbances in ankle muscles.

Activity of 'motor' thalamic neurons during control of isometric finger force

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The role of the 'motor' thalamus in the generation of precise controlled finger muscle contractions was investigated in 2 monkeys. They were trained to produce, between the thumb and index finger, a double step-and-hold increase in isometric force. The first step was lower (0 to ca.0.2 N) than the second step (0.2 to ca.0.7 N). 180 thalamic neurons were recorded extracellularly during the task and 77 were identified with microstimulation (15–40 μA) through the recording electrode. Our ongoing data analysis indicates that 3 populations of neurons exist. One population (31%) contains cells whose discharge rates decrease during the higher force step, while the other population (14%) contains cells exhibiting increased discharge rates during the same force phase. Oscillating discharge rates were observed in some neurons during and after the release of force at the end of the trial. Neurons in the third population (34%) fired phasically only when a large increase or decrease in force occurred. Based on these observations we postulate that thalamic neuron activity could serve as a command signal for PT neurons in addition to providing a route for afferent feedback to the motor cortex.

BAT thermogenesis in the hypothyroid rat: unmasked by phentolamine administration

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The nonshivering thermogenesis induced by i.m. noradrenaline administration, NA-NST, is practically nil in hypothyroid (Tx) rats, whereas brown adipose tissue preparations from the same animals do respond to NA and exhibit an in vitro thermogenic capacity equal to that of controls. Since BAT is regarded as the dominant site of NST, the question is raised as to whether O_2 and/or substrates delivery to BAT cells might be limiting in hypothyroidism. In order to test this possibility a vasodilator, phentolamine (PH), which per se doesn't affect resting metabolic rate, was administered together with NA. Under these conditions, an NA-NST developed in Tx rats, which was of the same magnitude as that of euthyroid ones. In vitro experiments showed that PH also potentiates NA-NST of brown adipocytes in the absence of O_2 or substrates limitation. It remains, however, that the potentiation is considerably higher in vivo than in vitro. One possible explanation is that NA-NST is dependent on an action of thyroid hormones on BAT vascular function.

Importance of pituitary GnRH receptor concentration for the regulation of gonadotrophins secretion in male rats

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Regulation of pituitary LH and FSH secretions is controlled both by the central nervous system through gonadotrophin releasing hormone (GnRH), and by direct action of sex steroids from gonadal origin. Castration of adult male Wistar rats produced a rapid increase of both plasma LH (10×) and FSH (2×), a decrease of hypothalamic GnRH content (2–3×) and a sustained increase of pituitary GnRH receptor concentration (3×). In acutely castrated rats (2 days post castration), a 7-day treatment with either testosterone, 17 β-estradiol or 5 α-dihydrotestosterone produced a complete, dose-dependent normalization of all parameters studied. When substitution was initiated 8 days after castration or later, only plasma LH and FSH but not pituitary GnRH receptor content, nor hypothalamic GnRH content were restored to normal levels by this 7-day treatment. These results indicate that obviously the marked increase of gonadotrophin secretion after castration is mediated at least in part by increased pituitary GnRH receptors. Whereas sex steroids always act rapidly to restrain gonadotrophins secretion of castrated rats despite

markedly increased pituitary gonadotrophins reserve and high GnRH receptor number, action at higher centers to restore hypothalamic GnRH synthesis and storage is either slower or ineffective after about 8 days of castration.

Vasopressin (AVP) and corticotropin (ACTH) in conscious rats are tightly coupled over a 100-fold change in plasma concentrations

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Plasma AVP and ACTH were measured by RIA in groups of 4–9 conscious rats with indwelling carotid catheters. To a 27% haemorrhage, AVP increased from 4±1 to 19±5 pg/ml (mean±SEM) and ACTH from 0.2±0.05 to 1.0±0.3 ng/ml (control). In rats pretreated (−30 min) with 10 µg naloxone, 60 µg β-endorphine or 10 µg enkephalin, AVP rose to 160±47, 121±42 and 61±17 pg/ml and ACTH to 2.1±0.3, 2.4±0.8 and 1.7±0.5 ng/ml, respectively. Pretreatment (−5 min) with 10 µg corticosterone or 0.16 mg dexamethasone potentiated AVP and ACTH release up to 7-fold. By including all groups above, log (ACTH) was linearly correlated (r=0.88) with log (AVP) over concentrations of 2–200 pg/ml for AVP and 0.1–4 ng/ml for ACTH. The results indicate that AVP and ACTH are tightly coupled even when hypothalamo-hypophysical function is altered by opiate agonists and antagonists or glucocorticoids.

The high sensitivity of primary spindle afferents to small stretches is not preserved during larger movements of physiological amplitude, unless they are very slow

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Primary spindle afferents deprived of fusimotor drive possess, at constant mean muscle length, a very high sensitivity to small stretches (P.B.C. Matthews and R.B. Stein, J. Physiol. *200,* 723, 1969). This is even higher than during dynamic fusimotor action (G.M. Goodwin, M. Hulliger and P.B.C. Matthews, J. Physiol. *253,* 175, 1975). This phenomenon has been attributed to the persistence of stuck cross bridges between intrafusal actin and myosin filaments. Thus the question arose whether this pronounced sensitivity persisted during larger movements, as they occur normally. The responses of primary afferents from cat soleus to sinusoidal stretching, synchronized with and superimposed on slow triangular movements (1.2 mm half peak to peak amplitude), were recorded and analyzed for different segments of the triangle cycle. The units responded to sinusoids of 50 and 100 µm during slow stretching but not normally during release. Small amplitude sensitivity was high at constant mean length (controls) and during very slow stretching (below 0.05 mm/s). At higher speeds the sensitivity dropped to 10–25% of the control values. Preliminary data further indicate that, under these conditions, dynamic fusimotor action raises the afferents' small amplitude sensitivity above the level of the passive spindle.

A proctolin-like peptide from cardiac ganglion increases heart muscle contraction in *Limulus*

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Proctolin is a pentapeptide (Arg-Tyr-Leu-Pro-Thr) first extracted from the viscera of the cockroach. Sullivan has shown that a proctolin-like peptide occurs in decapod crustacean pericardial organs. We find that proctolin has an excitatory action on the neurogenic heart of *Limulus polyphemus,* increasing the amplitude of contraction. Proctolin acts directly on the cardiac muscle, rather than on the neurones of the cardiac ganglion or on the amplitude of the EJP's at the cardiac neuromuscular junction. From the cardiac ganglion, we have extracted an active fraction which displays an apparent molecular weight, an enzymatic susceptibility and an action on isolated *Limulus* heart very similar to those of synthetic proctolin. These observations suggest that proctolin, or a family of closely related peptides, plays a physiological role in the modulation of muscle contraction in more than one subphylum of the Arthropoda.

Corticotropin releasing factor (CRF): relative importance of arginine vasopressin (AVP) and catecholamines

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Microdissected median eminences (110 µg wet weight) of rats were electrically stimulated in vitro. Incubation media were assayed for CRF with dispersed pituitary cells and AVP by RIA. Dose (AVP)-response (CRF) curves for media were not significantly different ($p > 0.4$) from those for synthetic AVP. In the presence of 1 mM ascorbic acid, AVP release was similar but accounted for only 55±7% (means±SEM, n=7 experiments) of released CRF. At a given AVP level, CRF activity was not significantly different ($p > 0.2$) in the presence or absence of 0.13 µM haloperidol or 1.3 µM propranolol in ascorbic acid rich media, but 17±8% ($p < 0.05$) lower in the presence of 0.35 µM phentolamine. The results suggest that AVP and an α-adrenergic agonist are the predominant CRF released from the rat median eminence in vitro.

GABA-ergic modulation of parasympathetic insulin release in nucleus ambiguus

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Nucleus ambiguus (NA) is one source of vagal monoteurons that innervate the pancreas (J. Auton. Nerv. Syst. *2,* 61, 1980) and modulates the release of insulin (IRI) (Diabetologia *19,* 257, 1980). The possibility that these NA-neurons are under tonic GABA-ergic inhibition (Science *204,* 1106, 1979) was tested in anesthetized male Wistar rats by bilateral microinjection of the GABA antagonist bicuculline and monitoring peripheral plasma levels of IRI and glucose (G.). Only in the presence of α-adrenergic blockade (by prior phentolamine treatment, which significantly elevated baseline IRI but did not change G) was bicuculline injection followed by a prompt rise of IRI levels (peak at 5 min+66%) without significant changes of G. Vehicle injection in NA or bicuculline injection outside NA did not

or significantly less affect IRI levels. We conclude that some vagal outflow to the pancreatic B-cell in under tonic GABA-ergic inhibition.

Neurally mediated insulin secretion in the sham-feeding rat

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To further characterize cephalic neural phase insulin secretion we monitored peripheral plasma levels of insulin (IRI) and glucose (G) during sham-feeding of a liquid diet in rats bearing chronic jugular catheters and gastric drainage fistulas. There was a prompt and sustained IRI increase (+100% of baseline) during sham-feeding (n=7), representing 28% of the total incremental IRI-surface produced by a 10-min meal with closed fistula. Only small and nonsignificant changes of G were observed. Prior i.v. injection of 2 mg/kg atropine-methylnitrate blocked 88% of the IRI response but did not significantly reduce sham-feeding. Prior i.v. injection of 1 mg/kg phentolamine-HCL (n=5) resulted in a doubling of IRI-baseline, the relative IRI response of approx. +100% being preserved, while G showed no change. We conclude that cephalic neural signals are able to significantly stimulate pancreatic IRI-secretion during feeding and that the sham-feeding rat is a valuable model.

In vitro phosphorylation of the brush border membrane from rat kidney proximal tubules

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Rat kidney cortex slices were incubated in a phosphate-free medium in the presence of carrier free [^{32}P] orthophosphate and afterwards brush border membranes were prepared by a Mg/EGTA precipitation method. The kinetics of phosphate incorporation into brush border membrane lipids and proteins, respectively, were different. After separation of the proteins from the lipids by chromatography (LH-60, HCOOH (80%):(EtOH=1:4) it could be shown that phosphorylation of the proteins equilibrates after 60 min whereas the incorporation of ^{32}P into the lipids increases linearly. Incorporation of ^{32}P into about 20 polypeptides was analyzed by SDS-polyacrylamidgel-electrophoresis and subsequent autoradiography. Most of the protein-bound phosphate is linked to serine and/or threonine residues and is sensitive to metabolic inhibitors. After incubation of the slices with 5 mM cAMP or 1 mM dBcAMP a slight increase of the phosphorylation pattern was observed in the region of 50,000 daltons.

Effect of catecholamines on plasma-free fatty acids in fed and fasted cattle

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Plasma-free fatty acids (FFA) increased during norepinephrine (NE), epinephrine (E), isoproterenol (ISO) and phentolamine infusions, whereas dopamine and phenylephrine (PH) had no effect. PHE did not modify FFA responses to ISO. Propranolol (PRO) inhibited the effect of NE, E and ISO on FFA. PRO alone had no effect on FFA in fed animals but lowered elevated FFA during starvation (for 4 days). The increase of FFA during E infusions was more marked in starved than in fed animals. Basal plasma E concentrations were similar in fed and starved animals but increased to higher levels during E infusions in starved than in fed animals. The data indicate that β-adrenergic agonists enhance lipolysis whereas α-adrenergic agonists have no effect on plasma FFA in cattle. Enhanced lipolysis during starvation may partially be mediated by increased β-adrenergic activity in adipose tissue. Enhanced FFA responses to E during starvation seems to be due to increased sensitivity of fat tissue and associated with altered metabolism of E.

Variation of uterine androgen receptors during the estrous cycle of the rat

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The female rat is known to produce testosterone and to contain androgen receptors in the uterus. In the present investigation we wondered if this androgen system is integrated into the estrous cycle. Rats were killed at a known stage of the estrous cycle. The concentration of testosterone was measured in the serum of trunk blood. The amount of receptors present in the 100,000×g supernatant of the uterus was determined by Scatchard-plot analysis. The results indicated that both of these parameters varied systematically during the estrous cycle, reaching a peak at proestus and a trough at metestrus. At these 2 days 1.13±0.32 and 0.41±0.13 pmoles of receptors were present in the uterus, respectively (m±SD n=5). This difference was significant ($p < 0.01$) and indicated that androgens could play a physiological role in the reproductive biology of the female rat.

Autoradiographic demonstration of muscarinic cholinergic receptor binding in the pigeon CNS

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The regional distribution of muscarinic cholinergic receptor binding in the visual system and other CNS regions of the pigeon CNS was studied autoradiographically with dry-mount autoradiography using scopolamine and quinuclidinyl benzilate as ligands. The results obtained with the 2 ligands were identical. Of the visual structures, the tectal layers 4,7,9 and 10 and DLL were highly labeled. Intermediate receptor density was found in layers 5a, 11 and 12 and HIS while the labeling was low or absent in layers 1-3, 8, 13-15, HA, HD, IHA, E, Rt and T. A high binding density was further observed in A, DMA, GLdp, GLv, GTv, LMmc, LPO, SLu and the molecular layer of the cerebellum. The binding was low or absent in Imc, ION, Ipc, N and the granular layer of the cerebellum. The binding pattern in the tectum and hyperstriatum does not overlap with the CAT and AChE distribution indicating that specific regions are predominatly cholinergic terminal fields while others are rich in cholinergic cell bodies.

In vivo release of aspartate and glutamate from the pigeon optic tectum induced by electrical stimulation of the optic nerve

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Previous investigations suggested glutamate and/or aspartate to be transmitters in pigeon optic nerve terminals. In order to test further his hypothesis, the effect of electrical

stimulation of the optic nerve on the release of these amino acids was investigated. The upper strata of the optic tectum were perfused with Ringer bicarbonate solution using a push-pull cannula. Amino acids released from the surrounding tissue were collected and determined by mass fragmentography of their N-pentafluoropropionylhexafluoroisopropyl ester. The mean resting release of aspartate and glutamate in the optic tectum was 250 fmoles/min and 1.5 pmoles/min respectively. Electrical stimulation of the optic nerve (40 Hz) induced an 18-fold and 6-fold increase of the tectal outflow of glutamate and aspartate respectively. These findings are consistent with the putative transmitter role of the 2 substances in the optic nerve.

The effects of stimulation of the dorsal spinal cord on the stretch reflex in the decerebrate cat

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It has been reported that stimulation of the dorsal spinal cord may, by an unknown mechanism, produce a dramatic and long lasting reduction in spasticity. The effects of dorsal cord stimulation (DCS) on stretch reflex activity in triceps brachii were investigated in 9 intercollicular decerebrate cats and 2 decorticate cats. During stimulation (0.2 msec, 50 Hz, 80–150 μA for 1–20 min at the C_1 level) the tonic stretch reflex (TSR) was often completely abolished whereas the phasic, monosynaptic reflex (MSR) was only partially inhibited. After cessation of stimulation, the MSR always recovered quickly whereas the TSR often remained depressed for up to 5–20 min, the time course for recovery being unrelated to the duration of the stimulation. Stimulation at more caudal levels (T_2) was much less effective. In the decorticate cat, DCS (C_1 level) had similar effects. Thus, the present result indicate that DCS produces a marked inhibition of stretch activity. In forebrain-lesioned cats, the inhibitory effects did not, however, outlast the period of stimulation for hours as found in spastic man.

Neuronal mediation of optokinetic reflexes in the frog

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Large field, visual movement induces compensatory motion of the eye and head. In *Rana temporaria* 2 midbrain centers receive this input from the contralateral retina. In the basal optic region, neurons are predominantly sensitive to vertical movements of large targets presented to the contralateral eye. In a 2nd visual center, ventral to the rostral tectum, cells increase their firing with horizontal, temporonasal movement of such a target. Recording sites are verified after ejection of horseradish peroxidase from the recording electrode. Neurons, antidromically activated from oculomotor nerve stimulation, also have these selective sensitivities found for the vertical and horizontal sensory cells, both in terms of their direction selectivity and of their velocity sensitivities. Anatomical findings indicating a direct projection of basal optic neurons to oculomotor neurons, coupled with the similarity of these sensory nuclei responses to the oculomotor responses, suggest the possibility of a 3-neuronal, retino-ocular reflex, which aids in coupling visual surround movement to compensatory, slow phase movement of the eye. Furthermore, selective lesions of these midbrain sensory to centers provoke selective impairments of slow phase movement of the head to visual large field movement as well.

Potassium diffusing through the retina of the honeybee drone enters glial cells but not sensory neurones

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When there is an accumulation of K^+ in the extracellular space of a nervous tissue, the K^+ disperses partly by diffusing through the extracellular clefts and partly by entering cells. It has been suggested that more K^+ temporarily enters the glial cells than the neurones: I present evidence that in the drone retina this is indeed the case. The cut head preparation (Baumann, J. gen. Physiol. *52*, 855, 1968) was superfused with Ringer solution containing 3.2 mM K^+ and intracellular recordings were made with a double barrelled K^+-sensitive microelectrode. When the K^+ concentration was increased to 17.9 mM the apparent K^+ concentration in the glial cells increased (e.g. by 3.6 mM/min) but in the photoreceptors the increase was barely detectable (less than 0.3 mM/min).

Endogenous and exogenous modulators of the hydrosmotic action of vasopressin (VP) in toad bladder

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Recent reports from this and other laboratories showed that somatostatin (a peptide present in toad bladder) and methohexital (a barbiturate) selectively inhibit the hydrosmotic effect of VP. A unique pattern characterizes this action: 1. The hormonal inhibition is partially surmountable by raising the concentration of VP. 2. The hydrosmotic actions of cAMP and of serosal hypertonicity are not inhibited. 3. The natriferic effect of VP is unaffected. A strikingly similar array of effects was reported in 1964 by Petersen and Edelman when serosal Ca^{++} was raised to 10 mM. Of necessity, these 3 agents must act at a step prior to cAMP generation. The following model is proposed: a) somatostatin and methohexital are endogenous and exogenous ligands, respectively, for a common site specifically involved in the modulation of the hydrosmotic action of VP; b) such a site is Ca^{++}-sensitive and could likely be a calmodulin-sensitive adenylyl cyclase.

Na-stimulated ATPase in basolateral membranes of renal proximal tubular cells

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It has been proposed that 2 different Na pumps are responsible for Na extrusion from renal proximal cells (Proverbio et al., BBA *211*, 327, 1970), one associated with the classical Na-K-ATPase and the other with a Na-stimulated ATPase which has been located in aged microsomal fractions (Proverbio et al., BBA *394*, 281, 1975). Differential centrifugation has been applied to localize the Na-ATPase to the basolateral membranes. The enzyme may be demonstrated in fresh preparations if the membrane fraction is resuspended in a medium of pH 7.8 or if suspensions at pH 7.2 are incubated with 25 μM Ca. Ouabain completely inhibits Na-K-ATPase but has no effect on Na-ATPase; 2 mM ethacrynic acid totally inhibits Na-ATPase but depresses Na-K-ATPase by only 60%; furosemide (1.5 mM) completely inhibits Na-ATPase without affecting Na-K-ATPase. There is a close correlation between the effects of different inhibitors on the Na-ATPase and their actions on the K-independent Na pump which extrudes Na and Cl from the cell.

Effects of hyperoxia, hypoxia and hypercapnia on the electroretinogram of the cat

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The aim of this study was to relate changes in the ERG induced by hyperoxia, hypoxia and hypercapnia, to autoregulation. Amplitudes of b- and c-waves of the ERG were measured during 10-min periods of ventilation with 100, 60, 10 or 5% O_2, with 5 or 10% CO_2 in room air and with 5% CO_2 in 95% O_2. - Hyperoxia left the b-wave unchanged; hypoxia of 10 and 5% O_2 induced decreases of the b-wave by 35% and 75% respectively. Hypercapnia reduced the b-wave by 20%. - Hyperoxia induced decrease of the c-wave by 35%. Hypoxia of 10% and 5% O_2 resulted in increases of the c-wave by 40% and 120% respectively, followed by a transient fall of 50% below the control. Hypercapnia increased the c-wave by 45%, also followed by a transient decrease. - Hypercapnia induced a decrease of arterial pH to 7.1-7.2; these and other results suggest that a decrease in pH may cause ERG changes. Furthermore, all experiments revealed changes in systemic blood pressure. All of these factors affect ocular blood flow. The reaction of the b-wave gives evidence for autoregulation of the retinal circulation. No such evidence is shown by the c-wave, the sources of which are supplied by the choroidal circulation.

Stabilization of gaze in the frog

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Passive displacement of the body provokes vestibular and optokinetic reflexes of neck and eyes. These reflexes cooperate, the evoked movements are compensatory in direction and tend to stabilize gaze. In the frog the stimulus response relationships of the reflexes were established by means of rotating either the animal in dark or light or the visual surround in a horizontal plane and recording head or eye movements with a magnetic field search coil technique. With the head free to move, collic reflexes compensate during combined stimulation for 80-90% of the imposed displacement (frequency: 0.025-0.5 Hz; amplitude: ±10-16°). The contributions of the optokinetic (OKCR) and vestibulo-collic (VCR) reflexes to the compensatory head movement seen during combined stimulation are frequency and velocity dependent. With an increase in frequency, the gain of the OKCR decreases and that of the VCR increases. With the head fixed, the maximal contribution of the eyes for gaze stabilization is about 15% during combined stimulation. The gain of both collic and ocular responses exhibited nonlinearities at lower stimulus amplitudes: the gain of collic responses decreased whereas the gain of ocular responses increased.

Genetic susceptibility vs resistance to labyrinthitis in a rat colony

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M. pulmonis, the primary pathogen in Murine Respiratory Mycoplasmosis (MRM), is found in all conventional rat and mouse colonies and even in a large percentage of 'germ-free' colonies. The most common manifestations of MRM are acute or chronic rhinitis and otitis media. An occasional complication of the latter is labyrinthitis, which produces a characteristic tilting of the head. The clinical diagnosis of labyrinthitis has been confirmed by histological and bacteriological examination in several animals from a colony of psychogenetically selected (RHA/Verh and RLA/Verh) rats. Although both groups of rats are constantly housed together, under identical conditions, it has been observed for at least the last 10 generations that, whereas about 3-7 clinical cases of labyrinthitis appear per generation in the RLA/Verh rats, the disease has become virtually nonexistent in the RHA/Verh rats, indicating a probable genetic resistance to labyrinthitis in that selected line.

Interaction of α-MSH and acetylcholine on an identified invertebrate dopamine neuron

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Peptide actions were studied in an in vitro preparation of the c.n.s. of the snail *Planorbis corneus*, especially with respect to possible effects on cholinergic input to the giant dopamine neuron (GDN) of the left pedal ganglion. Immunohistochemical findings point to the presence of neurons containing a compound related to α-melanotropin (α-MSH). α-MSH caused a marked, reversible depression of a) the excitation of GDN by iontophoretic acetylcholine and b) the excitation of GDN in response to electrical stimulation of another identified neuron of the same ganglion which is presumed to be cholinergic on the basis of electrophysiological data. The nature of the interaction between peptide and cholinergic input and effects of related peptides are being investigated. This gastropode nervous system appears to provide a convenient model system for the analysis of peptide effects at the cellular level.

Analysis of ion fluxes in rat colon mucosa in vitro

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Na and Cl fluxes across rat colon mucosa in vitro were studied under short-circuit conditions. Net Na flux was reduced but not abolished in absence of Cl ions, whilst net Cl flux was annulled by removal of Na. Diamox reduced m-s flux of Na without affecting short-circuit current (Isc); it abolished net Cl flux by reducing both unidirectional fluxes. Furosemide inhibited s-m flux of Cl and apparently unmasked a net secretion of bicarbonate. Theophylline raised Isc, inhibited m-s fluxes of both ions and slightly enhanced their s-m fluxes. Assuming the apical membrane to be permeable to Cl but not to Na, the results are interpreted in terms of interrelated Na/Cl symports and Cl/HCO_3 antiports in both membranes, together with an electrogenic Na entry across the brush border and a Na pump in the basolateral membrane. The symports would be driven by the Na gradients. Furosemide would inhibit the basolateral symport and theophylline the luminal one; both would be sensitive to diamox.

Permanent endocrine abnormalities after intrauterine exposure to antiandrogen

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Pregnant Wistar rats were treated with the antiandrogen cyproterone acetate (CA) (0.5 or 1.0 mg/day) and the offspring studied at adult age. In males, these doses caused no anogenital distance shortening, Wolffian regression, or changes in testicular weight, LH receptors or T response to hCG. Despite higher basal T, prostate was smaller and its

androgen receptors failed to rise after in vivo LHRH (controls, basal = 11±3, LHRH = 29±5 vs CA, basal = 8±2, LHRH = 9±1; fmoles/mg proteins), suggesting end-organ resistance to androgen. Basal PRL and LH-FSH pituitary content and secretion were normal. Adrenals were clearly reduced. Females had normal estrous cycle with appropriate changes in uterine weight and E2 or Pg receptors, suggesting normal end-organ sensitivity to estrogens. Pituitary PRL content and secretion was decreased and LH content and secretion was increased, in reverse correlation with lower basal PRL levels. Intrauterine exposure to CA resulted in permanent changes that may serve as a model of intersexual state in the male.

Transepithelial osmosis and inhibitors of sodium transport in toad urinary bladder

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Uncoupling of Na and water fluxes is a distinctive feature of osmoregulatory, tight epithelia. We examined the hydrosmotic responses (Jw) to vasopressin (VP) and to cAMP in toad bladders pre-exposed to different Na transport inhibitors (0.1 mM). Amiloride, a blocker of Na-entry sites in the apical membrane did not inhibit Jw. Different patterns of responses were found with 3 Na-pump inhibitors: a) ouabain had no effect on Jw; b) vanadate significantly decreased, while quercetin significantly increased, Jw in response to VP or to cAMP. The results indicate that substances interacting with specific, extracellular sites for the entry (amiloride) or the exit (ouabain) of Na, do not inhibit the hydrosmotic response to VP. In contrast, inhibitors of the Na-pump which also affect other intracellular ATPases can either block (vanadate) or potentiate (quercetin) the hydrosmotic response to VP at a site beyond cAMP generation.

Angiotensin excitation in hippocampal slices of the rat

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Angiotensin II has been shown to be present in the hippocampus (Haas et al., Experientia, in press). In the present study the mechanism of action of angiotensin at the membrane level was investigated. Angiotensin II and III added to the perfusion fluid caused a dose-dependent increase of extracellularly recorded EPSP's and synaptically evoked population spikes. CA1 pyramidal cells were either depolarized and their firing rate increased or the membrane potential was unaffected. The effects were antagonized by angiotensin blockers. Saralasin decreased the firing of CA1 pyramidal neurones. Recurrent inhibition in double shock experiments with antidromically evoked population spikes and intracellularly recorded IPSP's were reduced, indicating a disinhibitory mechanism. Membrane conductance and the effects of locally applied GABA were unchanged.

Localization of kainate-binding sites in rat and pigeon cerebellum

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The pigeon cerebellum contains a unique kainate-binding site which exhibits cooperative binding and a 2nd binding site comparable to that in rat cerebellum. The localization of these binding sites was examined with drymount autoradiography. The cooperative binding site was exclusively localized in the molecular and Purkinje cell layer of the cerebellar cortex, suggesting that it is related to the Purkinje cell dendritic tree. The 2nd type of binding site was localized in the molecular layer and to a lesser extend in the granular layer in both pigeon an rat cerebellar cortex. This may implay that this 2nd type of binding site is related to other cellular elements, such as the granule cells. The autoradiographic procedure did not affect the principal kinetic and pharmacological properties of the binding.

High-frequency stimulation of hippocampal pyramidal cells in situ and in vitro

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Stimulation of the fornix at 0.5 Hz evoked long-lasting hyperpolarizations in cat pyramidal neurons in situ. Similar inhibitions were seen in rat pyramidal cells in organotypic cultures after field stimulations. Stimulus frequencies of 10–20 Hz in both situations elicited 2 different effects. At lower intensities, the IPSPs superimposed and clamped the membrane potential close to their reversal potential. At higher intensities, an initial hyperpolarization quickly reverted to a sustained depolarization followed by a marked hyperpolarization that was associated with a large increase of the input conductance and a shunting of all synaptic potentials. During stimulus-induced depolarizations, the evoked IPSP disappeared completely, iontophoretically applied GABA still abolished action potentials but hyperpolarized the membrane potential much less than during the prestimulus period. These results support the view that during strong tetanic stimulation, the effectiveness of inhibitory mechanisms is impaired.

Nerve growth factor (NGF) enhances the in vitro differentiation of telencephalic neurons

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The possible role of NGF in the central nervous system is still uncertain since many earlier clues have recently been questioned by relating the observed effects to traces of renin present in most NGF preparations (Avrith et al., Nature *285*, 248, 1980). Therefore, we investigated the possible influence of NGF on differentiating brain cells in rotation-mediated aggregating cell cultures. Cells of fetal rat telecephalon were grown for 12 days in a chemically defined medium. Daily additions of NGF (20 ng/ml) increased the activity of the neuronal enzyme choline acetyltransferase by 200%, without affecting the total protein and DNA content, nor the rate of tritiated thymidine incorporation into DNA. The responsiveness of the neurons to the NGF treatment decreased sharply with progressing differentiation. Angiotensin II (100–10,000 ng/ml) given twice daily didn't mimic the effect of NGF. Our results suggest that during a limited period in brain development, the cholinergic neuronal differentiation is influenced by NGF or NGF-like agents.

Estimation of the total volume of skeletal muscle mitochondria

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A new sampling scheme permits the estimation of the total volume of mitochondria of the entire skeletal muscle tissue in small mammals. By a weighted random sampling proce-

dure portions of muscle tissue are selected for morphometry with a probability proportional to their mass. By taking 6 samples from each of a group of 5–7 animals a $\leq 10\%$ difference in the mean volume density of mitochondria (between experimental groups) can be detected with 95% certainty. When multiplied by the total muscle mass, this parameter gives an estimate of the potential O_2 consuming capacity of an animal's skeletal muscle tissue. The relationships between total mitochondrial volume, mitochondrial volume in individual muscles and $V_{O2}max$ in the European woodmouse (*Apodemus sylvaticus*, 20 g) under varying conditions of activity are currently evaluated.

Autoradiographic localization of binding sites for ^{3}H-glycine, ^{3}H-β-alanine and ^{3}H-strychnine in CNS cultures

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Cultured rat CNS was used to visualize binding sites for ^{3}H-glycine, ^{3}H-β-alanine and ^{3}H-strychnine by light microscopic autoradiography. In spinal cord and brain stem cultures, ^{3}H-glycine and ^{3}H-strychnine were bound to a relatively large number of neurones, whereas in cerebellar cultures, no or only few binding sites were observed. This agrees with findings by DeFeudis (Int. Rev. Neurobiol. *21*, 129, 1979) demonstrating a high density of glycine receptors on synaptic membranes from spinal cord and brain stem but only a negligible amount in the cerebellum. The number of spinal cord and brain stem neurones labelled by ^{3}H-β-alanine was considerably smaller than that by ^{3}H-glycine and ^{3}H-strychnine. In contrast, glial cells did not show binding sites for these radioligands, suggesting that unlike neurones, glial cells may not possess receptors for glycine and β-alanine. This suggestion is also supported by electrophysiological studies (Hösli et al., Exp. Brain Res., in press).

Antioxidant enzymes in pig aorta and pulmonary artery endothelium. Effects of hyperoxia

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The known vulnerability of lung endothelial cells (EC) to hyperoxia and the association of lung oxygen tolerance with increased antioxidant enzyme levels prompted us to measure the activity of catalase (CAT), glutathione peroxidase (GP) and total and KCN-insensitive superoxide dismutase (SOD) in EC freshly isolated from pulmonary artery (PA) and aorta (AO) and cultured under control or hyperoxic conditions. The only marked activity changes affecting both types of cultured EC were the increase in both forms of SOD and the decrease in GP in postconfluent cells. AO EC differed from PA EC by their higher KCN-insensitive SOD and lower CAT. Hyperoxia resulted in a 2-fold increase in KCN-insensitive SOD in both types of EC, whereas total SOD increased only in AO EC, and in a reduction in PA EC CAT. Cell loss was the same in AO and PA EC. We can conclude: 1. Enzyme levels in cultured EC are in an unsteady state and do not always reflect in vivo conditions. 2. Since cytotoxicity was the same in both types of EC despite differences in enzyme changes, oxygen toxicity could not be related to one particular enzyme profile.

Single unit responses to interaural intensity differences in the medial geniculate body of the cat

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Single unit responses to 200 msec noise and tone bursts were recorded in the medial geniculate body of nitrous oxide anaesthetized cats. Units responding differently to monaural and binaural stimulation were further investigated by varying the intensity at one ear over a 40-dB range, while maintaining a constant moderate level in the other ear. 26 units were tested in this way with noise and 11 with tones. In both cases interaural intensity differences (IID) may induce a change either in the temporal response pattern or in the discharge rate. For 73% of the units these changes occur within the physiological range of IID (8 dB). In most cases the response is progressively suppressed as the intensity of the ipsilateral ear exceeds by a given amount that of the contralateral ear, whereas it reaches a maximum in the reverse case. None of the units were narrowly tuned to a certain IID.

Single unit responses to stimulation by complex tones of known harmonic content in the cat medial geniculate body

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Single units of the medial geniculate body of the unanesthetized cat were recorded while frequency-sweeped complex and pure tones of known harmonic content were applied. Poststimulus histograms of the activity of these cells were recorded and displayed on-line. A small number of cells responded consistently to the harmonic frequencies of the complex tones and to frequencies that are harmonically related to the fundamental frequency, but are not appreciably represented in the stimulus. For example, a cell which was stimulated with a square wave-shaped tone (composed of more than 99% odd harmonics) showed peaks of similar amplitude at the even and at the odd harmonics.

Cholinergic properties and electrophysiological parameters of ciliary ganglion cells in culture

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Dissociated ciliary ganglion cells were grown either alone, on striated muscle, heart fibroblasts or on glial cells. In all conditions the neurons express high levels of CAT and are capable of synthesizing ACh from either ^{3}H-choline or ^{3}H-acetate. Incorporation of ^{3}H-choline into ^{3}H-ACh was linear for a 1-h incubation period and a choline concentration of 100 µM was necessary for saturation of ACh synthesis. We are presently examining the synthesis of ACh in ciliary neurons to determine its dependence on muscle, fibroblasts and glial cells in culture. Electrophysiological studies have shown that the neurons exhibit an average RMP of -57 ± 9 mV ($n=65$) and an input resistance of 161 ± 64 MΩ ($n=16$). 2 h after having been placed in culture, the neurons elicit a single action potential following stimulation with an intracellular pipette; after 3–5 days in culture repetitive firing can be observed during a long lasting depolarization of the cell membrane. ACh sensitivity was examined by iontophoretically applying ACh to the neuron while recording from it intracellularly. As with the action potential, the ACh-evoked potential was already

present immediately after the cells were put into culture. ACh-evoked potentials were blocked by curare and hexamethonium.

Frontal and lateral visual field in pigeon: pattern discrimination interocular transfer and early deprivation

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Deficits in pattern discrimination learning and interocular transfer (IOT) after early monocular deprivation in pigeon are ascribed to effects in the thalamofugal visual system. Since the posterior part of the retina is better represented in the dorsolateral thalamus than the nasal part, the role of frontal (FF) and lateral (LF) visual field for pattern discrimination, IOT and the effect of early monocular deprivation has been investigated. 12 normal and 5 monocular deprived animals have been trained to discriminate 2 patterns, one in FF and one in LF. Acquisition in FF is faster than in LF for both patterns and equals that of animals with one eye totally free. Savings for the 2nd eye are from poor (21%) to good (65%), depending on pattern and visual field. Deprivation impairs acquisition in the 1st and savings for the 2nd trained eye in FF and LF. Retention tests under extinction conditions after 1st eye trained show similar transfer between FFs and LFs. Deprivation affects preferentially transfer between FFs.

Location of pulmonary stretch receptors (SR) in the rabbit

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Analysis of respiratory reactions and of the discharge pattern of single SR fibres following inhalation of histamine and/or ammonia led to the suggestion that in rabbit the majority of SR is located in the larger airways (Pflügers Arch. *386*, 231, 1980). - In order to verify this suggestion, the site of SR was investigated in anaesthetized, thoracotomized, artificially ventilated rabbits by local mechanical stimulation and local anaesthesia of small parts of lung parenchyma or of airways; further, in each receptor the response to increased postexpiratory pressure and to ammonia inhalation was tested. - The experiments showed that only about 50% of SR examined were located centrally, i.e. in the extrapulmonary and large intrapulmonary airways, but that 40% were located peripherally, i.e. were accessible only at the lung surface. The differences in the discharge pattern between these 2 populations were rather small. The peripheral SR fired spontaneously at higher rate and appeared to be more sensitive to both lung inflation and ammonia inhalation than SR located more centrally.

Intracerebral propagation of the rabies virus

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Contrary to the herpes virus the rabies virus inoculated in the eye of the rat infects predominantly the retinal ganglionic cells and is then transported along the visual pathways into the brain. Cultures as well as immunocytochemistry show the virus at first in the suprachiasmatic and pretectal neurons and in the terminal neurons of the accessory optic system. From these regions the infections spreads transsynaptically and progressively to more and more anatomically related neurons in the brain stem. Interestingly, the mesencephalic central gray and the midline thalamus seem to be infected well before the superior colliculus and the lateral geniculate nuclei. The evaluation of the observed clinical signs with respect to the lesions opens the possibility of understanding the function of the infected structures.

Environmental and genetic effects on stomach ulcer formation in food-deprived rats

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Roman high- and low-avoidance (RHA/Verh and RLA/Verh) rats had continuous access to water but were deprived of food for 4-5 days, after which they were sacrificed with chloroform. Their stomachs were removed and examined under magnification for petechiae and various stages of ulceration, using a gradated scoring system. Comparisons were made with a Mann-Whitney U-test (2-tailed). It was found that RHA/Verh food-deprived (FD) rats had more lesions than their controls, that RHA/Verh-FD rats had more lesions than RLA/Verh-FD rats did and that RHA/Ver-FD rats which were housed in the same room as the controls had more lesions than RHA/Verh-FD rats housed in a separate room where none of the rats had food. It was concluded that RHA/Verh rats were more sensitive to hunger than were RLA/Verh rats, probably due to their larger appetites and metabolic requirements, and that this sensitivity was aggravated by the psychological stress of being in the same room as rats which had access to food.

Effect of pre- and postparturient energy intake on blood plasma levels of hormones and metabolites in cows

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Possible relationships were investigated between the energy intake in the dry period and metabolic changes during early lactation in 25 Swiss Brown cows. Cows which were fed during late pregnancy on requirement level had 70 to 5 days ante partum (a.p.) lower levels of 3,5,3'-triiodothyronine ($p=0.01$), immunoreactive insulin ($p=0.003$) and elevated levels of growth hormone ($p=0.02$; determined by Dr J. Hart, NIRD Shinfield, Reading, Great Britain), free fatty acid (FFA) ($p=0.002$) and urea ($p=0.01$) than cows fed ad libitum. - After parturition the cows fed differently a.p. received the same ration and were fed roughage ad libitum and concentrates according to requirement for 125 days. Cows which were fed ad libitum a.p. showed during the first 60 days of lactation a tendency to elevated energy-yielding metabolites such as glucose, FFS and ketone bodies compared to cows fed p.a. on requirement level, whereas dry matter intake, milk yield and milk composition did not differ. However, the effect of energy intake a.p. in cows (with a milk yield of 3500 to 5000 kg/lactation) on metabolic changes was not significant. - Animals, fed only 75% according to requirement level for 60 days post partum exhibited more marked metabolic changes, typical for lack of energy, than animals fed during the same period with roughage ad libitum and concentrates according to requirement.

Commissural cerebellar cortical connections in turtle

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In contrast to the cerebral cortex there is so far little evidence for interhemispheric connections within the cerebellum. Such contralateral connections could now be demonstrated following injection of I^{125} labeled wheat germ agglutinin (WGA) and/or horseradish peroxidase into the cerebellar cortex of turtle. While there is little doubt about the origin of this projection within layer III (granule like cell) and the course and crossing of the fibres within the cerebellar cortex, the mode of termination has still to be established because of labeling of fibres of passage. In a few cases however the contralateral connection appears to terminate mainly within the homotopic region of the injection site and there is evidence that some fibres may terminate within the granular layer. This finding is based on the demonstration of radopactive material within the granule cell-free layer III of the contralateral homotopic region and on the fact that in 2 cases with injections restricted to layer I, no contralateral projection could be demonstrated.

Tissue distribution and subcellular location of melatonin receptors in the male rat

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Highest concentration of cytosolic receptors for melatonin was found in adrenals, hypothalamus and pituitary gland, whereas lower concentrations were observed in testes, eyes and kidney. Tissue concentration of endogenous or exogenous melatonin in these organs was proportional to receptor concentration. Saturation with ^{3}H-melatonin of plasma membranes and cytosol from rat hypothalamus revealed specific binding activity in both cell fractions with an optimal pH of 7.5. Binding specificity for ^{3}H-melatonin using different indolamines was much higher for plasma membranes than for the cytosol fraction. 6-hydroxy-melatonin, methoxy-indolacetic acid and 5-hydroxytryptophol readily inhibited ^{3}H-melatonin binding in the cytosol but had no effect in membrane preparations. Specific ^{3}H-melatonin binding was saturable at 5.10^{-7}M in the cytosol, whereas at 3.10^{-8}M with membranes. Receptor concentration was about 10-fold lower in plasma membranes than in the cytosol. These results seem to indicate 2 different binding sites within the cell, and suggest a rapid internalization and partial degradation of the hormone-receptor complex in different tissues.

Central neurotransmitters and prenatal gonadal function

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The anterior diencephalon of the rat is reached by ascending catecholamine fibres at embryonic day (ED) 15, i.e. shortly before the onset of increased activity in the male pituitary-gonadal axis, which suggests a functional relationship. Injection of 6-hydroxydopamine into the brain of fetuses at ED 16 was followed by a decrease of brain noradrenaline and by a reduction in the number of catecholamine fibres in the rostral forebrain at ED 18. At the same time, the rise in serum testosterone typical of ED 18 was absent in male fetuses. Nicotine which affects central catecholamine systems in adult and fetal rats, was also found to abolish the ED 18 peak of testosterone and to reduce steroid levels at ED 17 when administered from ED12 to ED 18 or 17, respectively. These results indicate that an alteration of adrenergic or cholinergic mechanisms can interfere with the control of the pituitary-gonadal axis in fetal life.

Behavioral 'monitoring' of aversively-motivated discrimination learning in genetically defined mice

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12 mice of the inbred strains C57B1/6 and DBA/2 were trained for discriminated avoidance in a tubular Y-maze (160 trials each). Preliminary data were available for the strains Balb/c and C3H.

Results. 1. The number of sensory modalities available for discrimination only marginally affects the speed of acquisition of the task. 2. Sequential recording ('monitoring') of all trials shows quantitatively that the choice behavior of each mouse is determined by the *dynamic interactions* of at least 3 tendencies, namely a) by responding to the discriminated stimulus, b) by remembering the result of the former trial (short-term spatial memory) and c) by hypothesizing (side preferences and alternations). 3. Specific tendencies are genetically determined. Those strains with a maximum of intra- and infrapyramidal mossy fibre terminals (see Schwegler and Lipp, this issue) appear to be more influenced by short-term spatial memory.

Optokinetic (OKN) and vestibuloocular (VOR) reflex modifications during compensation after vestibular neurotomy in the cat

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2 groups were used to investigate changes in OKN and VOR and their respective contributions to the retinal stabilization of the visual world after right neurotomy. In the first group, the EOG was recorded before the lesion right neurotomy. In the first group, the EOG was recorded before the lesion and at various times up to 1 month postoperative. The 2nd group recovered for more than 1 year before EOG was recorded. In the acute stage a dramatic fall of VOR and OKN gains was observed with stimuli to both directions. Both stimulus types resulted in asymmetrical eye movements; those to the intact side being more difficult. This was striking for OKN, which was virtually absent in this direction for several days. In the chronic stage the well-compensated behavior was reflected in the complete recovery of compensatory eye movements during rotation in the light and pure optokinetic stimulations to either directions. More variable were VOR responses in the dark among different animals, indicating the increased importance of vision for good sensorimotor performance. The biggest deficit was observed with low frequency sinusoidal stimulations, where a gain decrease and a larger phase lead was present when the eyes moved to the left. This observation, together with a curvilinear shape of the nystagmic slow phases and a gaze failure in the dark, suggests an impairment of the positional integrator.

Structural and functional characterization of mesial area 6 in the monkey (supplementary motor area, SMA)

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The supplementary motor area (SMA) is found in the mesial cortex of area 6, with the face representation anterior to the forelimb area. The hindlimb region is buried in the upper bank of the cingulate sulcus adjacent to the primary hindlimb area. Are the head and forelimb areas micro-excitable (< 30 µA) and does the forelimb area of the SMA contain corticospinal neurones? Cervical cord injections of HRP retrogradely labelled corticospinal cells in area 4 (paracentral lobule) and conspicuously small cells in area 6. The rostral extent of labelled cortex corresponded well with the most anterior region from which twitches of forelimb muscles were evoked by microstimulation in awake monkeys. In SMA, 142 neurones were activated antidromically by stimulating electrodes at various levels of the neuraxis. 133 were activated from the peduncle and 23 from the lower brainstem. 34 neurones projected to the cervical cord and 5 to the lumbosacral level. It is concluded that: a) the forelimb and possibly hindlimb areas of the SMA are micro-excitable; b) these areas include a region of cortex giving rise to corticospinal fibres.

Schedule induced ethanol and water consumption in psychogenetically selected lines of rats

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Schedule induced ethanol intake was established in male and female rats of 2 psychogenetic lines bidirectionally bred for extremes in shuttle box avoidance performance. The rats were maintained at 80% of their free-feeding body weight level and tested on a FT-1 min schedule with 3% (w/w) ethanol continuously available during each daily 45-min session. Baseline tests were given before and after this test series and involved the presentation of 45 Noyes pellets together at the start of the baseline test. Both sex and rat line significantly influenced ethanol intake. Roman High Avoidance (RHA/Verh) rats exhibited greater schedule induced ethanol intake than Roman Low Avoidance (RLA/Verh) rats and, furthermore, female rats exhibited greater schedule induced ethanol intake than male rats. Substitution of water for ethanol in this experimental design resulted in a similar pattern of results, thus, indicating that these effects are not specific to ethanol.

Role of the renin-angiotensin system in the stimulation of aldosterone biosynthesis by experimental edema

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Experimental edema, induced in potassium-depleted rats by s.c. polyethylene glycol, markedly enhanced the conversions of tritiated corticosterone and deoxycorticosterone to aldosterone and 18-hydroxycorticosterone by capsular portions of the adrenal glands. These effects of edema were prevented by bilateral nephrectomy or treatment with the converting-enzyme inhibitor Captopril. Infusion of high amounts of angiotensin II completely reversed the inhibition by Captopril, but only partly stimulated aldosterone biosynthesis in intact or nephrectomized rats without edema and was ineffective in nephrectomized rats with edema. According to these results, experimental edema stimulates late steps of aldosterone biosynthesis by a complex mechanism involving a) the renin-angiotensin system and b) an unknown kidney factor, which appears to be necessary for a full response of the adrenal cortex to the stimulatory action of angiotensin II.

Analysis of cigarette puffing behavior and inhalation

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Cigarette puffing behavior and inhalation (measured by expired CO) were measured in 110 unsuccessful abstainers in a single test session using cigarettes which the subjects regularly smoked. As the nicotine yield of their cigarettes increased, total smoke intake per cigarette generally decreased (0.5 mg increased nicotine yield produced a mean decrease of smoke intake of 93 ml). No relation was found between CO uptake and nicotine yield, which indicates that the smoke exposure across the different types of cigarettes was generally similar.

Biological importance of nerve growth factor (NGF) for the development of sensory neurons

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Previous studies have shown that NGF is internalized at nerve endings of sensory neurons and retrogradely transported to the cell bodies of dorsal root ganglia. Although retrograde transport of NGF in sensory neurons persists throughout life the physiological importance of NGF for sensory neurons was not known. We report here that NGF leads to an increase of substance P (SP) in dorsal root ganglia from newborn and adult rats. Conversely, administration of anti-NGF antibodies produces a marked reduction of the SP content in sensory ganglia, suggesting that NGF is important for the postnatal development of SP-containing neurons. Moreover, unilateral injection of NGF into the forepaw of rats leads to a specific increase of SP in sensory ganglia (C_6–C_7) of the injected side, which can be abolished by prior transection of the brachial plexus. These findings suggest that the function of SP-containing sensory neurons is dependent on NGF.

Variations of the oxygen consumption in the chick blastodisc during the early development

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The O_2 uptakes at given regions of the blastodisc were measured by scanning microspectrophotometry with a resolution of 100 µm (Kucera and Raddatz, Resp. Physiol. *39*, 1980). The values of O_2 fluxes (Q) and those corrected for the protein content (Q′) have been analyzed with respect to their position coordinates. The mean Q-values of the area pellucida (AP) and area opaca (AO) are 58 ± 14 and 38 ± 12 $nl \cdot h^{-1} \cdot mm^{-2}$ at the definitive primitive streak stage and 63 ± 15 and 53 ± 15 $nl \cdot h^{-1} \cdot mm^{-2}$ at the head fold stage respectively. The overall O_2 uptake increases as well during the considered period of development in both areas (from 0.4 to 0.6 $\mu l \cdot h^{-1}$ for the AP and from 1.6 to 5.5 $\mu l \cdot h^{-1}$ for the AO). On the other hand the average Q′ increases only in the AO (from 27 to 40 $nl \cdot h^{-1} \cdot \mu g^{-1}$) and it remains constant in the AP (17 $nl \cdot h^{-1} \cdot \mu g^{-1}$). However, there exist

within the AP axial and transversal variations of Q and Q', the most active regions being located at the laterocaudal margin of the AP.

Purine and serotonin uptakes are not sensitive indices of hyperoxic injury to vascular endothelial cells in vitro

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A decrease in serotonin (Block and Stalcup, 1979) and adenine (ADE) (DeBono et al., 1977) uptakes have been associated with vascular endothelial cell (EC) injury. Because a change in these uptakes might show subtle injury to vessels, we exposed postconfluent monolayers in primary cultures of EC from swine aorta to 95% O_2–5% CO_2 for 24 h and measured the following: uptakes after 60 min incubation with ADE, adenosine (ADO) (both at 10^{-4} and 10^{-6} M and serotonin (5×10^{-7} M); lactate dehydrogenase (LDH) and lactate release into media; cellular DNA, protein and ATP. Uptakes, when corrected for the significant loss of DNA and protein, were unchanged. The most sensitive index was LDH which significantly increased in the media. Cell function, as assessed by uptake measurements, appeared to be normal in the remaining cells.

Selective retrograde transport of D-aspartate in spinal interneurons and corticomedullary neurons

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Glutamate has been proposed as transmitter incorticofugal neurons and at spinal levels. This has been tested by the selective retrograde transport of D-aspartate, a metabolically inert aminoacid whose high-affinity uptake is similar to L-asp and L-glu. ^{3}H-D-asp was injected in rats dorsal column nn. (DCN) or in spinal dorsal horn; brain and cord were processed for autoradiography. Cortico-DCN fibres and layer-V pyramids in the contralateral cortex are labeled 12 h after DCN injection. Spinal injections label numerous small neurons insubstantia gelatinosa and sparse, larger neurons in medical dorsal horn but not other interneurons in the intermediate zone. This contrasts with absence of perikaryal labeling in the CNS after similar injections of ^{3}H-GABA. The results provide further evidence for asp or glu as transmitter of cortico-DCN neurons and suggest also a transmitter role of either aminoacid for some dorsal horn neurons.

Exploratory components of the movements of woodmice in a residential plus-maze: effects of entorhinal cortex lesions or amphetamine injection

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The sequence of the movements of isolated woodmice *(Apodemus sylvaticus)* in a residential plus-maze is continuously recorded on a PDP 11 03 computer. The alleys are 50 cm long plexiglass tubes crossed by IR light beams; any enclosure with a suitable opening can serve as a goal box. 2 different aspects of the exploratory behavior are investigated. The first reaction and adaptation processes following the introduction of the mouse into the maze is studied under various goal conditions (illumination, complexity, presence of congeneric scent, wheel). The knowledge thus acquired by the mouse is then tested 24 h later by modifying the structure of the maze or the content of the goals. Bilateral lesions of the entorhinal cortex decrease visit duration and affect the pattern of the visits during the initial phase and following the opening of a new goal. Amphetamine injection (2.0 mg/kg) affects duration and sequence of the visits, and selectively decreases the probability of entering a recently open goal.

Longtime ECG recording in rats by a biotelemetry system

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The experimental procedure required for the conventional recording of the electrocardiogram (ECG) in rats presents many difficulties: Anesthesia includes the risk of drug interactions, on the other hand restrainment of the rat is very stressful. In the present study preliminary results are presented from ECG data obtained by a telemetry system. The recordings were taken during a 3-month period from a unrestrained female rat. The FM transmitter (Dynamic Electronics: SNR 102F), weighting 3.25 g with batteries, was located in a socket attached on the rats skull. Stainless steel electrodes were implanted s.c. (Spoerry: leed D). The rats heart rate varied considerably over the day-night cycle and was much lower in comparison to those from restrained rats. The ECG time parameters could be calculated easily, whereas the voltage (amplitude) was dependent on the transmitters spatial location. The implanted socket and the electrodes were well tolerated by the rat.

Effect of a high carbohydrate low fat diet on diurnal and nocturnal catecholamine excretion in man

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Adaptive responses of noradrenaline (NA) and adrenaline (A) urinary excretion to a high carbohydrate low fat diet (HCHO) were studied in 5 young male subjects. After a 2-day balanced diet (45% CHO kcal and 40% fat kcal), a HCHO diet (82% CHO kcal and 8% fat kcal) was fed during 6 days followed by 2 days on the baseline diet. Within 48 h of HCHO, diurnal NA and A excretion increased and remained significantly elevated until the end of the HCHO diet. Diurnal NA excretion increased from 1.25±0.16 to 1.95±0.21 µg/h ($p < 0.05$), and diurnal A excretion from 0.28±0.05 to 0.46±0.09 µg/h ($p < 0.05$). These values dropped towards baseline when the balanced diet was resumed ($p < 0.05$). However, there was no significant change of NA and A excretion during the sleeping period: NA excretion was 0.52±0.04 and 0.63±0.11 µg/h, and A excretion was 0.10±0.02 and 0.09±0.03 µg/h for the balanced and HCHO diets respectively. The results suggest that the relative contribution of CHO and fat in the diet influences the diurnal sympatho-adrenal activity in man.

Inherited correlations of brain and behavior in genetically defined mice and rats: shuttle-box performance and hippocampal mossy fibre distribution

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By means of Timm's stain, the proportions of synaptic fields within regio inferior of the hippocampus were determined planimetrically at a selected sampling region. Analysis was carried out in 13 rats from 2 strains which have been

selectively bred for differential shuttle-box performance (Roman high avoidance and low avoidance) and in 34 mice from 7 strains known to differ genetically in 2-way avoidance (DBA/2, Balb/c, NMRI, C57B1/6, SM/J, C3H, ICR). The most distinct feature in both rats and mice, was a *negative* association between shuttle-box performance and the relative area covered by mossy fibre terminals synapsing on the *basal* dendrites of hippocampal pyramidal neurons. *Poorly* avoiding strains had far *more* of such terminals (strain differences in rats: $p < 0.001$; negative correlation in mice: $r_S = 0.98$, $p < 0.01$).

Correlations between 2-way avoidance and hippocampal terminal fields in individual mice

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It has been shown (Schwegler and Lipp, this issue) that the amount of hippocampal mossy fibres synapsing upon basal dendrites of pyramidal neurons is inversely correlated with the performance of 2-way avoidance in strains of rats and inbred mice. Those correlations were computed from the mean scores of 2 groups of animals from a given strain, one group tested for behavior, the other one for morphometry. Here we investigated individual mice bred systematically for randomization of their genotype. 12 mice (Albino, Füllinsdorf) were tested first for shuttle-box performance and subsequently for proportions of synaptic fields and layers within regio inferior. Evaluation was double-blind. Shuttle-box performance correlated negatively ($r = -0.80$, $p < 0.01$) with the presence of intra- and infrapyramidal mossy fibre terminals. Independently from this a 2nd negative correlation was found for avoidance and the relative area covered by pyramidal neurons ($r = -0.80$, $p < 0.01$).

Alterations of brown adipose tissue metabolic response in hypothalamic-lesioned rats

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The aim of this study was to investigate a possible involvement of brown adipose tissue (BAT) in the increased food efficiency observed in ventromedial hypothalamic (VMH) lesioned rats. The metabolic response to electrical stimulation of the BAT nerve supply was measured via continuous monitoring of NAD(P)H/NAD(P) redox state by measuring surface-emitted fluorescence. When BAT was excised from VMH-lesioned rats fed ad libitum the response was greatly reduced from 3 days post-lesioning onwards, indicating that BAT is functionally disconnected from its nerve control. When tissues were excised from lesioned rats immediately pair-fed with controls, the response to nerve stimulation was restored. In conclusion, the disconnection of BAT in VMH-lesioned rats can be reversed by food restriction indicating that the observed alterations do not result from interruption of a neural pathway but rather from altered patterns of metabolism secondary to a shift in autonomic balance.

Decreased metabolic response to nerve stimulation in brown adipose tissue of preobese ob/ob mice

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A decrease in thermogenic capacity has been shown to be the earliest detectable abnormality in the genetically (ob/ob) preobese mouse. In small mammals, brown adipose tissue (BAT) is the main thermogenic effector. In adult ob/ob, the metabolic response to nerve stimulation is greatly reduced. This could explain the weak thermoregulatory capacity of these mice. If this is true, the BAT response should be altered in preobese animals. Therefore, the metabolic response to nerve stimulation was studied on BAT of 33 mice 9-10 days old of the C57BL/6J strain. When responses were divided in classes according to their magnitude a bimodal distribution was found which was compared using χ^2 test to the Ho hypothesis: sum of 2 populations normally distributed with a probability of occurrence $\frac{1}{4}$ and $\frac{3}{4}$ (probability of expression of this recessive mutation). The test showed that Ho is acceptable at a high degree of probability. In conclusion: preobese ob/ob mice show a decreased metabolic response to nerve stimulation already at 9 days of age.

Psychophysiological effects of cigarette smoking and discrimination test performance

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The effects of smoking (no cigarette, 0.2 mg nicotine cigarette and 1.2 mg nicotine cigarette) were investigated in 28 subjects performing complex serial discrimination tasks (Stroop items) and in a group of 16 yoked subjects. Continouos recording during the 50-min sessions included heart rate, plethysmography, electromyography, respiration, skin conductance and EEG. Significant psychophysiological changes occurred in response to test performance and smoking conditions.

Endogenous and exogenous release of aminoacids from tectal slices of the pigeon

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Many evidences suggest that certain amino acids may be involved in neurotransmission in the optic tectum. Tectal slices, placed in a superfusion chamber, were exposed to 47 mM K^+ in the presence or absence of Ca^{++}. The amino acids released were extracted and separated by ion exchange chromatography. Out of 21 amino acids only asp, glu, gaba, β-ala and gly were released in a Ca^{++}-dependent manner. After preincubation with tritiated asp, glu or gly, release of these newly accumulated amino acids was also induced by 47 mM K^+ and was Ca^{++}-dependent. It is concluded that asp, glu, gly, β-ala and gaba may play a role in neurotransmission in this structure.

Activation of supraoptic endocrine neurones and neurohypophysial hormone release during stimulation of hepatic portal osmoreceptors

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40 supraoptic neurones in anaesthetized rats were antidromically activated from the neural lobe/stalk region. Superfusion of the hepatic portal vein with NaCl solutions (0.33-1.2 osmoles/kg, 37 °C, 5-120 sec) activated ($p < 0.05$) 7 out of 8 phasically firing neurones. Only 8 out of 28 continuously firing neurones were activated, and 4 silent cells were not affected. Plasma AVP, measured by RIA in 8 other rats, increased from 37±7 (mean±SEM) pg/ml to 111±17 pg/ml when the portal vein was superfused with 0.2 ml of a 1.2 osmolal NaCl solution. The concomitant oxytocin release was about 5 times less than vasopressin

release, as judged from the increase in mammary pressure of 5 lactating rats. The results confirm our previous suggestion that osmo- or NaCl receptors are located within the hepatic portal vein area and are consistent with the hypothesis that phasically and continuously firing neurones release vasopressin and oxytocin, respectively.

Effect of prolactin on LH/HCG receptors and steroidogenesis in the rat testis

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The effects of short (4 days) and long (4 weeks) term hypoprolactinemia, 3±0.4 ng/ml (bromocriptine treatment) and hyperprolactinemia (238±9 ng/ml (sulpiride treatment) were studied on gonad axis. Scatchard analysis of 125I-HCG binding to membrane preparations of these rat testes showed during hypoprolactinemia a 50% decrease in LH receptors and inversely during hyperprolactinemia a 130% increase as compared with controls (100±5 fmoles/mg prot.) without modification of the Ka. Plasma testosterone (T) was significantly higher in the hyper- than in the hypoprolactinemic group (T=4.6±0.7 vs 2.5±0.3 ng/ml) whose T is similar to that of C. Testes weights remained unaffected. - The in vitro basal and HCG stimulated steroidogenesis of the 2 types of Leydig cells isolated after centrifugation on a metrizamide gradient was not modified by short-term hypo-and hyperprolactinemia. - In conclusion, PRL has a dose-dependent trophic effect on LH receptors and for short-term no inhibiting effect on in vitro steroidogenesis of Leydig cells.

Adaptive changes in adult rat lung following bilobectomy

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By means of morphometric techniques we analyzed the late structural changes in the adult rat lung following the resection of the right upper and middle lobes (=25% of total lung volume). 45 days after surgery the lungs were fixed by standardized intratracheal instillation of glutaraldehyde, their volume determined by water displacement and the tissue processed for light and electron microscopic morphometry. Age matched controls and sham operated animals (=thoracotomy without resection) served as controls. At sacrifice lung volumes and body weights were almost identical in the 3 groups. Analysis of the lung compartments showed that airspaces, capillaries and parenchymal tissue were enlarged to control values in the lobectomized animals. Hence the tissue and capillary volumes were significantly increased when compared with those of the corresponding lobes inthe controls. More important, the gas exchange surface areas were completely restored, yielding a morphometrically determined pulmonary diffusion capacity in the normal range. This means that, as could be shown previously for the growing lung (Burri and Sehovic, Am. Rev. Resp. Dis. *119*, 769, 1979), the gas exchange apparatus of the adult rat is equally able to fully compensate for a major loss of tissue.

Food intake and growth of rats at constant and alternating temperature

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The absolute and relative food intake of rats increases with decreasing ambient temperature. Experiments were carried out with groups of female rats (n=10) which were alternately exposed to 2 temperatures (T_a 7:21 °C; 7:30 °C;; 21-30 °C) at intervals of either 1,2,4 and 8 days. Body weight, food intake (pellets), T_{re} (deep colon) and T_s °C (tail root) temperatures were measured daily. Each experiment lasted 28 days. - When taken from warmer to cooler T_a at all 4 interval lengths animals showed an energy intake deficit while there was an excess intake with change from cooler to warmer T_a. The temperature gradients T_{re}-T_s and T_s-T_a were greater in the former and smaller in the latter case, either to preserve, heat in the cold or to facilitate its dissipation in the heat. The maximum growth was found at T_a 30 °C, 35% RH and continuous exposure, the minimum growth at 7:30 °C alternating temperatures.

D-Aspartate retrograde labeling of the olivo-cerebellar climbing fibre pathway

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Selective retrograde labeling has been found a useful tool in identifying transmitter candidates with radioautography in neuronal connections of the brain (Streit, J. comp. Neurol. *191*, 429, 1980). We have applied this technique to cerebellar circuity. In rats, injections of ^{3}H-D-aspartate (50 nl of 10^{-2} M, 25 µCi) into cerebellar cortex, deep cerebellar nuclei and nc Deiter resulted after 6, 12 or 24 h survival in strong retrograde labeling of cell bodies in the appropriate regions of inferior olive, as well as nerve fires along the pathway followed by climing fibres. Similar, albeit weaker labeling was found after superfusing vermis with ^{3}H-D-asp (10^{-5} or 10^{-4} M, i.e. in range of K_m for high affinity uptake). Parallel fibres and granule cells were not labeled. These results are in line with the possibility of aspartate being a transmitter in climbing fibres.

Determination of amino acids and amines from brain perfusates using gas chromatography and nitrogen-selective detection

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Glass capillary gas chromatography in combination with thermoionic detection was developed for the measurement of trace amounts of nitrogenous compounds from brain perfusates collected in vivo by push-pull techniques. Amino acids, amines and α-methylphenylalanine as internal standard were extracted from the dried perfusate residues and converted to the corresponding N/O-pentafluoropropionyl/hexafluoroisopropyl esters by a 1-step procedure. The detection limit of protein amino acids and γ-aminobutyric acid ranged between 100 fmoles and 10 pmoles, the detection limit of catecholamines was in the subpicomole range. The assay was applied to the measurement of amino acids released in the pigeon optic tectum upon electrical stimulation of the optic nerve (40 Hz); a pronounced release of glycine, γ-aminobutyric acid, aspartic acid and glutamic acid was detected demonstrating the capability of the present method to study neurotransmitter release in vivo.

BIOCHEMIE-BIOCHIMIE-BIOCHEMISTRY

Subsets of human Ia molecules

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2 human Ia molecular subsets have been defined by their reactivity with monoclonal antibodies raised against Daudi cell surface proteins. The 2 subsets, called NG-1 and NG-2, are always co-expressed, independently of the DR phenotype. - Biochemical analysis of the different subsets was done by 2-dimensional peptide mapping. The results show that the β-chains of Ia molecules from NG-1 or NG-2 display a considerable degree of variability. Differences in the β-chain from the same subset are also observed in allotype different Ia molecules, thus showing that at least the β-chain carries the polymorphic determinants. - In contrast, the α-chains of a given subset are very similar, irrespective of the Ia allotype, but differ significantly from one subset to the other.

NBD-bacteriorhodopsin, a fluorescent derivative of the proton pump

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To delineate structural and functional properties of the membrane integrated protein, the hydrophobic probe 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) is used for modification of bacteriorhodopsin (BR). Labeling conditions were chosen which favored covalent binding of the reagent to hydrophobically located nucleophiles in the purple membrane. Upon reaction with nucleophiles NBD-derivatives become highly fluorescent (e.g. 7-butylamino-NBD: λ_{ex} 465 nm, λ_{em} 525 nm in ethanol). - The spectroscopic characteristics (absorption, fluorescence) of NBD-bacteriorhodopsin (binding ratio 0.8 moles NBD/mole BR) demonstrate the covalent binding of the label at a lysine ε-amino group. Modification of tyrosine can be excluded. - Collisional quenching of the NBD-BR fluorescence by iodide is not observed, indicating the location of the label in the hydrophobic part of the membrane. The binding site of the label is further characterized with respect to arylisothiocyanate binding. The observed impairment of H^+ pumping does not directly correlate with stoichiometric reagent binding.

Defence of food intake in the rat

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Rats feeding on an FR 1 schedule (Fixed Ratio 1, i.e. 1 lever press for each food pellet) eat a constant amount of food each day and show stable circadian patterns of food intake. - In this study we examined the effect of FR 1, 2, 5, 10, 20, 50 and 100 on food intake (27% casein). The amount of food eaten each day was inversely proportional to the number of lever presses per pellet ($r = -0.99$; $p < 0.01$) such that intake was halved at FR 75. - As the FR schedule increased from 1 to 20 the circadian rhythm of intake was accentuated so that progressively more food was eaten at night. Meal size increased and the number of meals eaten each day decreased. These results are analyzed in relation to current theories of food intake control.

Differences among UK, Swiss and German food composition tables

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The use of food composition tables are essential in therapeutic dietetics and in qualitative studies of human nutrition. The 3-day dietary records of 8 subjects were calculated using UK (Paul and Southgate, The Composition of Foods, 1978), Swiss (Sais, Tableaux de la valeur nutritionnelle des aliments, 1977; Migros, Manger correctement - mais comment?, 1977) or German (Cremer et al., Die grosse Nährwerttabelle, 1978) food composition tables. - The macronutrient content of foods when taken from the Swiss or German tables was generally similar but differed in many instances from those taken from UK tables. As a result there were large differences, in some cases up to 30%, in the energy, protein carbohydrate and fat contents of individual meals and of daily intake. These were largely accounted for by the differences amongst tables in foods classified as meats, vegetables and alcoholic beverages. These results underline the importance of using specific and appropriate food composition data in therapeutic dietetics and in qualitative studies on human nutrition.

Effect of dietary carbohydrate, protein and tryptophan on plasma tryptophan and neutral amino acids in humans

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Ingestion of a high carbohydrate (CHO) meal by hungry rats increases brain serotonin (5-HT) by altering the relative plasma concentrations of tryptophan (TRP) to the 5 neutral amino acids (NAA) which compete with TRP for entry into the brain (Fernstrom and Wurtman, 1972). - In this study, plasma TRP, NAA and insulin were measured at 0, 1 and 2 h postprandially in 8 healthy human male subjects on 4 evenings, at weekly intervals, after consuming at 20.00 h nothing or 500 kcal (25% fat) containing either 1.58% protein (CHO) or 20% protein (HP) or CHO + 0.4% TRP (CHO + TRP). - At 1 h and at 2 h changes in plasma TRP and NAA were such that plasma TRP/NAA ratios were altered only by CHO + TRP. Insulin attained maximum levels at 1 h but were 2-fold higher after CHO and CHO + TRP than after HP. At 2 h, levels had decreased and were similar in the 3 groups. It is concluded that the diet likely to influence brain 5-HT is one which has been increased in tryptophan content. The relationship of insulin to plasma amino acid changes will be discussed.

Extracellular expression of proteolytic activities in the rabbit V2-carcinoma

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Tumor specimens and controls were maintained in organ culture to study the release of proteinases into culture medium. 3 lines have been established: 1. Primary tumor from i.p. grown V2-carcinoma (T). 2. Normal s.c. tissue (S). 3. T and S together (TS). Enzyme activities tested were:

Elastase-like (E), chymotrypsin-like (C), cathepsin B-like (CB), plasminogen activator (PA), latent collagenase (LC), active collagenase (AC). Results were: 1. E and PA were not released by T, S and TS. 2. C was produced in equal amounts by T and TS, whereas S did not release this enzyme. 3. LC was secreted by all 3 cultures in nearly the same amounts. 4. AC was secreted in small amounts by T and S, whereas TS produced a significant AC activity. 5. CB was not secreted by S, was secreted by T and in significantly higher amounts by TS. The increased secretion of CB and the increase of AC when tumor specimens are in contact with host tissue suggests an interaction between T and S. This may have some relevance to the invasive potential of the tumor.

Cleavage of human IgG and IgM with neutral proteinases from human polymorphonuclear leucocytes

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Lysosomal elastase (EC 3.4.21.11) and cathepsin G (EC 3.4.21.20), 2 serine proteinases stored in the azurophil granules of human polymorphonuclear leucocytes, degrade human IgG and IgM. The detailed action of elastase has already been reported (A. Baici et al., Immun. Lett. *2*, 47, 1980; Scand. J. Immun. *12*, 41, 1980). A comparative analysis of the IgM cleavage products by the 2 enzymes revealed a close similarity between the 2 major fragments. An Fabμ and an $F(ab)_2\mu$-like fragment were produced both by elastase and cathepsin G, but at different rates. Some other fragments, produced in small concentrations and representing intermediate products, were different in the 2 cases. The relative susceptibilities of the 4 human IgG subclasses to proteolytic attack by elastase and cathepsin G were studied kinetically using monoclonal IgG preparations. The order of decreasing susceptibility was the following: IgG3 > IgG4 > IgG1 > IgG2 for cathepsin G, compared to IgG3 > IgG1 > IgG2 > IgG4 for lysosomal elastase.

Aspartate aminotransferase isoenzymes: intracellular localization and distribution in different chicken tissues measured by radioimmunoassay

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Radioimmunoassays for the independent determination of the anionic and cationic isoenzyme of aspartate aminotransferase with detection limits of 177 pg and 83 pg, respectively, were developed. The identity of both isoenzymes in different tissues with the corresponding isoenzymes isolated from heart was verified. Cell fractionation showed a strictly heterotopic localization of the 2 isoenzymes in chicken heart, the anionic form being located exclusively in the cytosol and the cationic form in the mitochondria. Determination of the cytosolic and mitochondrial isoenzyme in different tissues, cultured cells, and serum revealed marked differences in the content and the ratio of their concentrations.

Purification and properties of the *Myo*-inositol binding protein of *Pseudomonas putida*

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Myo-inositol induced cells of *P. putida* subjected to osmotic shock lose their ability to transport the cyclitol actively. A protein of 29,000 daltons has been purified to homogeneity from the shock fluid. The dissociation constant was found to be 1.1 μM while the K_m of the transport system is 5.6 μM. The protein lacks cystein and nearly 50% of the amino acid residues are hydrophobic.

Copper transfer from *Neurospora* Cu metallothionein to type 3 copper apoproteins

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The ability of *Neurospora* Cu metallothionein to reconstitute *Neurospora* apotyrosinase and *Carcinus* apohemocyanin has been studied. In the presence of Cu^{2+} the reconstitution of apotyrosinase is incomplete and proceeds very slowly. However, upon addition of Cu(I) as dithionite reduced Cu metallothionein to the apoprotein (4-fold excess, pH < 7), the activity is not only fully restored but the process is 10–20 times faster. Apohemocyanin cannot be reconstituted with Cu^{2+} but under the same conditions used for apotyrosinase the protein is fully reconstituted. Following the band at 335 nm typical of the oxygenated protein, a first order kinetic is observed ($k_{app.} = 5 \times 10^{-4}$ sec^{-1}). From these findings it is suggested that *Neurospora* Cu metallothionein can act not only as a copper storage protein but also as an in vivo copper donor to type 3 copper proteins.

Amino acid sequence of rat metallothioneins (MT) and of gene product of rat MT-mRNAs

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The N-terminal amino acid sequence of the isometallothionein MT-1 of rat liver was determined as acetyl-MDPNCSCSTGGSCTCTSSCACKNCKC ... It is identical with the corresponding portion of MT-1 of mouse liver (Huang et al., 1977) but differs in 7 positions from that of rat MT-2, i.e. acetyl-MDPNCSCATDGSCSCAGSCKCKQCKC ... (Kissling et al., 1979), suggesting an early gene duplication. Microsequence analysis of a [^{35}S]cysteine- and [^{3}H]serine-labeled in vitro translation product of MT-mRNAs contained in the 9S poly $(A)^+$ mRNA fraction from liver polyribosomes of $CdCl_2$-treated rats (Andersen and Weser, 1978) gave results compatible with that of a mixture of MT-1 and MT-2. The positions of Cys and Ser in the gene product synthesized in a wheat germ system is xxxx-CSCSxxxSCSC ... (x = unlabeled residue). It suggests that there is no precursor for metallothionein and that the N-terminal Met is the initiator Met.

Design, construction and applications of a process controller dedicated to the management of liquid chromatography (LC)

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A microprocessor controlled instrument, able to pilot pumps, solenoid valves, fraction collectors as well as any other electrical appliances was designed to improve the efficiency of our LC equipment. Facilities to select buffers, to load samples, to generate gradients and to modulate elution processes by positive feedback interaction with on-line detectors were included in the software. Task-oriented instructions may be programmed as simple coded mne-

monics and executed on the 16 parallele output lines with respect to actual time and/or external feedback signals. Sets of instructions may be stored on cartridges using a regular tape recorder. The main improvements brought to our LC systems are greater reproducibility of elution patterns and better resolution of the detected peaks. The fractionation of proteins from human serum upon DEAE-cellulose yields 13 peaks as compared to 7 obtained by conventional methods.

Immunohistochemical localization of galactosyltransferase in human fibroblasts and HeLa-cells

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Antihuman galactosyltransferase (EC 2.4.1.22) antibodies were elicited in rabbits and purified on a galactosyltransferase-agarose column. Purified antibodies were used to localize galactosyltransferase in acetone-fixed HeLa-cells and human lung fibroblasts. Both protein A-peroxidase developed with 3-amino 9-ethylcarbazole served to detect binding of antigalactosyltransferase antibodies. In cells of confluent cultures, antigalactosyltransferase staining appeared as a concise triangular structure in the juxtanuclear region with one angle oriented towards the bulk of the cytoplasm. The stained structure appeared as a dense cap on the nucleus in HeLa-cells and as a more extended granular structure in fibroblasts. In cells of sparse cultures, specific antigalactosyltransferase staining appeared in both HeLa cells and fibroblasts as granular, extended structure which was occasionally perinuclear. There was no evidence of cell surface localization of galactosyltransferase by light microscopy. The positively stained structures are interpreted as part of the Golgi complex.

A high yield preparation of brush border membranes from kidney proximal tubules

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A modification of the preparation originally published by A.G. Booth and A.J. Kenny (Biochem. J. *142*, 575, 1974) is described. By this method a high yield of brush border membranes was obtained (protein: 2.9±0.4%; alkaline phosphatase (AP): 48.3±7.9%; leucine aminopeptidase (LAP): 47±9%). In short, kidney cortex slices were homogenized with a polytron in an isoosmotic medium containing 5 mM EGTA. By 2 precipitations with $MgCl_2$ (12 mM) and differential centrifugation brush border membranes were purified. The enrichment for AP and LAP was 17.9±5.6 and 17.1±4.5 respectively, whereas no contamination of mitochondria, endoplasmic reticulum, lysosomes and basolateral membranes was observed. - The preparation was further characterized by transport experiments, SDS-polyacrylamidgel-electrophoresis and electron microscopy. Uptake studies with D-glucose and phosphate revealed that these membrane vesicles are very appropriate for transport experiments.

Different behavior of lysosomal enzymes during a plasma membrane preparation

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The acid phosphatase as a marker for the lysosomes is enriched 5-fold in brush border membranes prepared according to a Mg/EGTA precipitation method, whereas other lysosomal enzymes (glucosaminidase, glucuronidase, cathepsin) are enriched only 0.2-fold. This acid phosphatase activity which is inhibited by tatrate but not by theophylline, could not be washed out by various sugars known as competitive inhibitors for interactions of lysosomal proteins with plasma membranes. However it was possible to separate acid phosphatase activity from the ones of alkaline phosphatase and leucine aminopeptidase by means of free flow electrophoresis and sucrose density gradient centrifugation. - The data indicate that the acid phosphatase is not a constituent of the brush border membrane and secondly that the lysosomal marker enzymes may show a different behavior during a preparation of plasma membranes.

Influence of renal failure on the evaluation of parathyroid function by nephrogenous cAMP and plasma PTH

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Nephrogenous cAMP and plasma PTH (NcAMP) were determined in 10 cases of primary hyperparathyroidism (PHP), 10 cases of nonparathyroid hypercalcemia, and in 21 normal subjects. Plasma PTH was measured with 2 antisera, one predominantly anticarboxyterminal (C-PTH), the other predominantly antiaminoterminal (N-PTH), as demonstrated by chromatography of iPTH in renal failure plasma. NcAMP was expressed in absolute values, as well as corrected by glomerular filtration rate (GFR). The advocated advantage of C-PTH and NcAMP/GFR over N-PTH and NcAMP in the diagnosis of PHP was found to be due to the impairment of renal function, which correlates with the degree of hypercalcemia, increases carboxyterminal immunoreactive PTH-fragments and elevates the ratio NcAMP/GFR. Therefore, these advantages are valid only in comparison with controls having normal renal function. But when compared with themselves, the 2 hypercalcemic groups, both having comparable degrees of renal insufficiency, showed no overlap with none of the 4 parameters studied.

Mapping of the 5′-end of the primary ribosomal RNA-transcript from *Physarum polycephalum*

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The ribosomal genes of *Physarum polycephalum* are situated on an extrachromosomal palindrome of 60 kb length, repeated 100 times per nucleolus. The 19S, 5.8S and 26S rRNA molecules are first transcribed from this rDNA as a large precursor of 11.8 kb, and this is further processed in several steps to the mature rRNA's. - We have located the 5′-end of the primary transcript by using the method of Burk and Sharp: 5′-end-labeled restriction fragments of rDNA were hybridized to total cellular RNA. The resulting RNA-DNA hybrids were digested with the single-strand specific nuclease SI and resistant DNA was sized on denaturating gels. - Restriction analysis revealed multiple small Hpa I repeats immediately upstream from the initiation site. A similar situation has previously been found for rDNA from *Xenopus laevis*.

A matrix-localized mitochondrial protease processing cytoplasmically-made precursors to mitochondrial proteins

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The majority of cytoplasmically-made mitochondrial proteins are synthesized as larger precursors which are imported into the organelle in the absence of concomitant protein synthesis. This import is energy-dependent and accompanied by the proteolytic conversion of the precursors to their mature form. We have isolated a mitochondrial protease that appears to function in protein import for the following reasons: 1. It is present in the matrix of all mitochondria examined so far (yeast, rat liver and rat heart). 2. It cleaves mitochondrial precursors only to their mature size and no further. 3. Correct processing is only observed with precursors transported across the inner membrane, but not with precursors transported into the intermembrane space. 4. It fails to cleave any other protein tested. 5. It is different from all yeast proteases described so far. 6. Its inhibition in yeast cells causes accumulation of precursors. - The processing protease has been purified several 100-fold from yeast.

Phase separation of integral membrane proteins

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A solution of the nonionic detergent Triton X-114 is homogeneous at 0 °C but separates in an aqueous phase and a detergent phase above 20 °C. Triton X-114 is used to solubilize membranes and whole cells at 0 °C and the soluble material is submitted to phase separation at 30 °C. Hydrophilic proteins are found exclusively in the aqueous phase and integral membrane proteins with an amphiphilic nature are recovered in the detergent phase.

The ribosomal genes of *Physarum* are mosaics of coding sequences and satellite-like sequences

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The ribosomal genes of *Physarum polycephalum* are on a linear, palindromic rDNA of 60 kb, which is not integrated in chromosomal DNA. About 45% of the rDNA has a unique sequence and is transcribed into pre-rRNA. More than another 30% of the rDNA is satellite-like in sequence arrangement. This is concluded from observations on the mapping of rDNA with the restriction enzyme Kpn I. This enzyme cuts the coding region into large fragments of 6.2, 6.5 and 7.2 kb each, while the central noncoding region is cut into small fragments of 300, 350 and 400 basepairs in size. These small fragments are homogenous in size and presumably also in sequence. They are generated in several copies (12, 6 and 12) per rDNA molecule. 2 of these fragments are themselves inverted repeats.

Particulate occurrence of hexokinase in the culture form of *Trypanosoma brucei*

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Culture and bloodstream forms of *T. brucei* differ considerably in their energy metabolism. Their respiratory pathways are different, as is the relative importance of glycolysis as a source of energy. Opperdoes and Borst (FEBS Lett. *80*, 360, 1977) have postulated the existence in the bloodstream form of a particle, coined 'glycosome' and containing several glycolytic enzymes. Knowledge of the presence of such a particle in the culture form as well, could contribute to an understanding of its role. Nitrogen cavitation was used to disrupt cells of *T. brucei* obtained in culture. Fractionation of the homogenate was performed by differential and sucrose density gradient centrifugation. 47% of the hexokinase activity was concentrated in a 'small granule' fraction representing 7% of the total protein and sedimenting at a density of 1.225–1.245. The segregation of hexokinase in a particulate fraction, obtained from culture cells, is in agreement with the presence of 'glycosomes' in these cells also.

Localization of serine acetyltransferase from *Spinacia oleracea* L.

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Serine acetyltransferase (SAT) (E.C. 2.3.1.30) catalyses the formation of O-acetyl-L-serine (OAS) from L-serine and acetylCoA. OAS has been proposed as an acceptor of the sulfur reduced to the thiol level in assimilatory sulfate reduction, thus forming cysteine, and as a precursor in sulfolipid formation. Experiments using a combination of differential and density gradient centrifugation showed that about 30% of the total SAT activity of spinach leaves was localized in the chloroplasts. The K_m for L-serine (3 mM) was comparable to the concentration of this compound in chloroplasts. SAT activity was inhibited by low concentrations of L-cysteine. D-cysteine was much less effective. OAS up to 10 mM did not inhibit the enzyme activity.

Use of monoclonal antibodies against carcinoembryonic antigen (CEA) in immunoadsorbent and enzyme immunoassay

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8 different hybrids producing monoclonal antibodies (Mab) specific for CEA were obtained by fusion of P3-NSI/1-Ag4 cells with spleen cells from mice immunized with purified CEA. Antibodies from hybridoma 23, which had an affinity of 1.4×10^8 L/M were purified from ascitis by DE-52 chromatography. Immunoadsorbents with Mab 23 on sepharose 4B were capable of purifying CEA from crude saline extracts of human colon carcinoma in a single step. Purified Mab 23 were also used to develop a solid phase enzyme immunoassay (M-EIA) capable of detecting less than 1 ng of CEA per ml of serum. Polystyrene balls coated with goat anti-CEA antibodies were incubated first with heat extracted serum, then with purified Mab 23. The reaction was revealed by goat IgG antimouse IgG_1 coupled to alkaline phosphatase and by the enzyme substrate. There was a good correlation (0.88) between the M-EIA and conventional radioimmunoassay as determined on 200 sera from colon carcinoma patients.

Characterization of 3 human liver alcohol dehydrogenase isoenzymes

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3 human liver alcohol dehydrogenase isoenzymes were purified by ion exchange and ternary complex chromatography on pyrazole derivatized sepharose. The subunit composition was determined as $\beta_1\beta_1$, $\beta_1\gamma_1$ and $\gamma_1\gamma_1$ by

monomerization or hybridization with horse liver isoenzyme EE and subsequent starch gel-electrophoresis. With ethanol as substrate all 3 isoenzymes exhibit 2 pH optima around pH 10.5 and 7 but differ in their pH 10.5:7 ratio ($\beta_1\beta_1$:1.5, $\beta_1\beta_1$:3.4, γ_1:3.7). The specific activity at pH 10.5 of $\beta_1\gamma_1$ and $\gamma_1\gamma_1$ is 5-6 times that of $\beta_1\beta_1$. As observed previously with isoenzyme $\beta_1\beta_1$ (Dubied et al., J. biol. Chem. *152*, 1464, 1977) also $\beta_1\gamma_1$ and $\gamma_1\gamma_1$ exhibit nonlinear kinetics with ethanol as substrate when measured over a wide concentration range. Similar nonlinear kinetics were observed for all 3 isoenzymes with amylalcohol and cyclohexanol as substrates.

Purification and properties of human brain aldose reductase

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DEAE-cellulose chromatography of human brain extract yielded 2 peaks of aldose reductase (EC 1.1.1.21) activity. After rechromatography all the enzyme was present in one peak only. Subsequent gel filtration and chromatography on blue sepharose yielded a homogeneous enzyme preparation as judged by SDS-polyacrylamide gel electrophoresis. Molecular weight determination under protein denaturing and nondenaturing conditions gave values between 32,000 and 35,000, indicating the enzyme to be of monomeric structure. Isoelectric focusing gave a single peak of activity at pH 5.9. In contrast to the enzyme from bovine lens, which shows nonlinear kinetics, Lineweaver-Burk plots were linear over a wide range of concentration with respect to NADPH and glyceraldehyde. Comparison of the amino acid composition with the one of aldehyde reductase from human liver indicated little structural homology between the 2 otherwise similar enzymes.

Effect of cytochalasin D on the stimulation of aldosterone production by angiotensin II, ACTH and potassium in vitro

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The zona glomerulosa of the adrenal cortex produces aldosterone in response to 3 different stimuli: angiotensin II (AII), ACTH and potassium (K^+). We have used isolated rat adrenal glomerulosa cells to investigate the role of microfilaments in the stimulation of aldosterone production by AII, ACTH and K^+. Cells were preincubated for 10 min with cytochalasin D and aldosterone was determined in the medium at the end of a subsequent 2-h incubation in the presence of varying concentrations of AII, ACTH or K^+. Cytochalasin D (6 µM) inhibited maximal stimulation of aldosterone production by about 50% for AII and by 25% for ACTH and K^+, without affecting the ED_{50} of any of these stimuli. A 20% decrease was also observed for dibutyryl cAMP. The effect of cytochalasin D was dose-dependent from 1 nM to 100 µM, a concentration at which aldosterone stimulation by AII, ACTH and K^+ was almost completely abolished. In binding-inhibition experiments with ^{125}I-AII, the affinity of AII receptors in glomerulosa cells was not changed by cytochalasin D. We conclude that although AII, ACTH and K^+ act primarily by different mechanisms at the membrane level, microfilaments may represent a common step in the activation of aldosterone production by these stimuli.

Detection of mild sedative effects: valerian and sleep in man

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Extract of valerian root has long had a reputation as a mild sedative, but the evidence that it actually influences sleep is almost entirely anecdotal. Using a sleep questionnaire and a double-blind, random order design we have studied the effect of valerian on subjectively evaluated sleep quality in a total of 1141 nights (128 volunteers; 9 nights each). Valerian did produce a significant improvement in sleep quality and this effect was most marked in poor sleepers, smokers and habitual coffee drinkers. In contrast, sleep latency, night awakenings, dream recall and somnolence the next day were relatively unaffected. Thus the questionnaire technique, which is simple to use and relatively noninvasive, provides a sensitive means for detecting the effects of mild sedatives on different aspects of sleep in man. It also allows identification, within a population, of the subgroups most affected by the treatment.

Differential expression of a common tumor-associated antigen on 3 mouse tumor sublines with different behavior in vivo

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3 P-815 mouse mastocytoma sublines, Berne (BE), Sloan-Kettering (SK) and Bonn, separated for many years in different laboratories, which show characteristic differences in vivo with respect to malignancy and metastatic behavior were analyzed and compared by high-resolution, 2-dimensional gel-electrophoresis, in conjunction with staining, metabolic labeling, surface labeling and immunoprecipitation techniques, in order to find out if they express a common tumor antigen. All sublines expressed a membrane antigen mol.wt 70,000 and pI 7.4 not found on normal, syngeneic DBA/2 cells. On SK cells this antigen may be cryptic as it was not precipitated by antibodies against BE. However, antibodies against SK precipitate this antigen from BE cells. H-2 antigens are absent on Bonn cells which express large amounts of the 70,000 mol.wt antigen. Differences in in vivo behavior are clearly reflected by differences in surface-expressed antigens.

Derivation of glycocalicin from the von Willebrand factor receptor of human platelets, glycoprotein Ib

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Asialoglycoprotein Ib and asialoglycocalicin have been isolated from the membranes and from the supernatant after sonication, respectively, of neuraminidase-treated platelets, by lectin affinity-chromatography on peanut agglutinin. Both preparations contained only trace amounts of impurities. The asialoglycoprotein Ib and asialoglycocalicin in both the unreduced and reduced states were radioiodinated and digested with trypsin. The tryptic peptide maps showed great similarities between glycocalicin and glycoprotein Ib with the latter (both unreduced and reduced) containing additional peptides, supporting the idea that glycocalicin is derived from glycoprotein Ib. The unreduced glycoprotein Ib contained additional peptides

compared to the reduced due to the disulphide-bond linked β component. There were also slight differences between unreduced and reduced glycocalicin, indicating that at least one intramolecular disulphide bond is present.

A quantitative analysis of the calmodulin-mediated activation of phosphodiesterase and adenylate cyclase in bovine brain

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Comparison of Ca-binding to calmodulin with the activation of phosphodiesterase (PDE) and adenylate cyclase (AC) by Ca^{2+} allows a quantitative description of the sequence of reactions leading to the activation of both enzymes. Calmodulin possesses 3 high affinity ($K_d = 6$ µM) and 1 low affinity ($K_d = 200$ µM) sites for Ca^{2+}. The Ca^{2+} concentration at half maximal activation of PDE and AC depends on the concentration of calmodulin present. Activation expressed as a function of the various calmodulin-Ca species shows that CaM · Ca_4 are the only activating species. The interaction of the latter species with the enzymes obeys the mass action law with K_d's in the nM range. The activation of AC by CaM · Ca_3 (or CaM · Ca_4) is noncooperative; that of PDE shows positive cooperativity (n_H ca 2). Similar results were obtained with Sr^{2+} as the activating cation. Furthermore, the analysis of [^{125}I]calmodulin binding to synaptic membranes also indicates that only CaM · Ca_3 and CaM · Ca_4 have a strong affinity. These results support the idea that the activating species in calmodulin-mediated processes contain at least 3 Ca^{2+}.

Metabolism of a ketogenic substrate during exercise in man: a 4-fold approach

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The metabolism of medium-chain triglycerides (MCT) and maltodextrine (MD) during 1 h of bicycle ergometer exercise (60% $\dot{V}O_2max$) is compared in 12 healthy subjects aged 17-19. 2 tracers, 1-^{13}C-octanoate (MCT) and ^{13}C-glucose (MD), are added to the respective meal (870 kJ) administered 1 h before exercise. Differences in overall carbohydrate and lipid oxidation are followed by indirect calorimetry, the oxidation of exogenous substrate is evaluated by the enrichment of expired $^{13}CO_2$, the utilization of endogenous carbohydrate during exercise is determined from glycogen decrement in muscle biopsy samples of the vastus lateralis. Calorimetric data are corrected if required for retention of ketone bodies and losses in sweat and urine and for ventilatory losses of acetone. Analyses in progress show: 1. After MCT expired $^{13}CO_2$ appears already 10 min post administration, resulting in distinct kinetics to MD. 2. There is no differential sparing effect on muscle glycogen. 3. Ventilatory losses of acetone MCT are small.

Nutritional follow-up of a 100 km footrace

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Water/electrolyte status was studied in 14 runners aged 22-55 engaged in a 100-km race. Intake was measured directly during the run and by diary for 3 days after. 6 subjects (A) received a glucose-electrolyte flavored drink (glucose 9.5%, Na 25, K 16, Ca 5 and Mg 2.4 mM) exclusively for the whole run (8-16 h) and 8 subjects (B) fed themselves freely. There were no differences between A and B with respect to intake of energy (5.3 MJ, 99% CHO), liquid (3.1 l), electrolytes (Na 81, K 44, Ca 16, Mg 10 mmoles), to weight loss (2.9 kg) and self evaluation of performance. Glycemia was normal at finish. Blood changes included a rise in renin (20×), aldosterone (Ald, 8×), creatinine (Cr), urea, lactate, K and Ca. Persistent changes at 1 week were low blood Na (B only), Ca, Mg and Ald. Urinary changes up to 4 nights included low Ald (B only), high urea and Cr levels without increased N excretion. Energy intake during recovery was constant (3 days, 12.0 MJ/d), 15-18% as protein. Hence a simple balanced drink providing CHO, salts and water can cope with the drastic metabolic changes of extreme exercise.

Inhibition of reassociation and reactivation of lactate dehydrogenase isoenzymes by peptides isolated from human liver

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2 peptides have been isolated from human liver by TCA precipitation, ultrafiltration, Sephadex G-25 and Bio-Gel P-2 column chromatography, affinity chromatography on immobilized LDH-isoenzymes and HPLC (Schoenenberger et al. in: Regulatory Peptides *1*, 223-244, 1980). These peptides did not influence the catalytic activity of native, tetrameric LDH-isoenzymes. However when LDH was dissociated into monomers by low pH and thereby inactivated, reassociation and reactivation after adjusting the pH was inhibited by these peptides. It was found that 1 peptide with a mol.wt of ~2500 only inhibited H-LDH reactivation and the other peptide of a mol.wt of ~1800 only M-LDH reactivation. The effect of the peptides was strongly positive cooperative with Hill coefficients of 2.4 in the case of the H-specific and 2.9 in the case of the M-specific peptide. Half-maximal inhibition of the reactivation was observed at a concentration of 7×10^{-8}M M- and of 3×10^{-8} M H-specific peptide (LDH concentration = 2.5 µg/ml). Computer fittings which were based on different models led to the conclusion that the peptides react with monomers, dimers or a transition state during the tetramerization process. A kinetic which implies monomers scavengered by the peptides from the reassociation process seems to be less probable.

Neurotransmitter binding studies on normal and pathological senescent human brains

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A long-term study has been initiated on regional distribution and kinetic properties of neurotransmitter binding sites in senescent normal and pathological human brains (age range so far 75-102 years). Binding of QNB, spiperone, 5HT and naloxone to membranes from frontal pole, temporal lobe, thalamus, putamen, caudate and hippocampus is being determined in cases of senile dementia of Alzheimer Type (SDAT), other senile dementias and normal aged

brain. In pre- and postcentral gyrus of both hemispheres from cases of secondary degeneration (transsynaptic, retrograde and Wallerian) the unaffected hemisphere serves as internal control. Preliminary results on the first 14 cases of the dementia study show a trend towards higher specific binding per mg membrane protein, except for a decrease in spiperone binding in frontal lobe, in the SDAT brains.

Inactivation of aminopeptidase in extracts of bean cotyledons

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Crude extracts were incubated at 2 °C in a standard medium (50 mM acetate buffer, pH 5.4) containing 0.1% mercaptoethanol. During germination of bean seeds both the extractable activity and the stability of aminopeptidase (AP) decreased. A 'factor' inactivating AP was found to be present in cotyledons from germinated seeds. This 'factor' was heat sensitive, partially inhibited by 3 mM tosyl-lysine chloromethyl ketone (an inhibitor of some endopeptidases), excluded by sephadex G-25 and precipitable in 75% ammonium sulfate. Extracts of germinated seeds (low AP) were preincubated at various temperatures for 1 h, mixed with fresh extract from ungerminated seeds (high AP) and incubated afterwards at 2 °C. If the preincubation temperature was above 53 °C, then AP remained more stable and the acidic endopeptidase was inactivated. The similar properties of the 'factor' inactivating AP and of the endopeptidase suggest that AP could be inactivated by the acidic endopeptidase increasing in bean cotyledons during germination.

Characterization and regional distribution of calcitonin receptors in the rat brain

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Receptors for calcitonin (CT), as assayed by the displacable binding of [125-I]iodo salmon CT ([125-I]CT), were found, on a membrane fraction prepared from rat brain. The half times of association varied between 23 and 7.1 min as a function of the temperatures used in the incubation medium, ranging from 6 to 37 °C. 67% of specific binding of labeled hormone was not dissociable, even after 6 h of incubation with an excess of unlabeled hormone. [125-I]CT extracted from the membranes was not degraded, as judged by gel permeation chromatography, and retained binding activity. Specific binding was highest in the hypothalamus, followed by the brainstem. It was intermediate in the midbrain thalamus and the striatum, lower in the cortex and negligible in the hippocampus, and cerebellum and the spinal cord. Salmon CT in amounts of as low as 10^{-10} M inhibited the binding of [125-I]CT to the membranes, whereas the virtually biologically inactive free acid of human CT and human CT sulfone did not affect the binding. 130–8900 times higher amounts of porcine CT and human CT and analogues thereof (with potency ratios of 1.5 to 300 with respect to salmon CT in the hypocalcemic bioassay) were required to achieve an inhibition of binding equal to that produced by salmon CT. Interestingly, salmon CT-(10–32), which is inert in the hypocalcemic bioassay, was almost as potent as intact salmon CT-(1–32) in this system. Apparently the 1–7 ring structure, which is a constant feature of all the known calcitonins, is not important for binding to the brain membranes.

Electrophoretic variants of transcobalamin II in mouse serum

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Mouse serum contains only 1 type of vitamin B12 binding protein, which has been identified as transcobalamin II (TC II). Genetically determined electrophoretic variants of TC II have been described in man and in the rabbit. In search for polymorphic variants in mice we have investigated 25 different strains, using polyacrylamide gel-electrophoresis and autoradiography after saturating the serum with radioactive cobalamin (Fràter et al., Blood *53*, 193, 1979). 3 different patterns were observed. 18 inbred strains exhibited 1 strong band plus 2 weak bands. 5 strains (4 of them crossed with wild types) exhibited 2 strong bands and 2 weak bands. 2 further inbred strains exhibited a complicated pattern of at least 6 bands of variable intensity. The genetic consequence of these findings shall be evaluated in future breeding experiments between mice strains expressing the different variants.

Endocrinological and physiological reaction patterns to various stressful situations in male RLA/Verh and RHA/Verh rats

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RLA/Verh and RHA/Verh rats, selectively bred for poor or rapid acquisition of 2-way avoidance, respectively, have been found to differ in corticosterone excretion during a 10-min openfield test. In further experiments, corticosterone-, ACTH- and prolactin-plasma concentrations were determined after having exposed animals of both lines to various stressful situations (e.g. shuttle box, ether-stress, i.p. injection). Additionally, colon temperature, FFA, blood glucose and defecation score, 4 further indicators for the stress experienced by an animal, were determined. The results show, in part, different endocrinological and physiological reaction patterns for the 2 selected lines, depending on the situation. - As RLA/Verh rats were more like control rats in their reactions, these findings were interpreted in terms of a reduced 'emotional response' for RHA/Verh rats.

DSIP a circadian 'programming' substance?

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Mammalian sleep represents a separate state of vigilance embedded in the diurnal/circadian rhythm. Daily i.v. injections of 30 nmoles/kg Delta-Sleep-Inducing-Peptide (DSIP) to rats and constant recording of their locomotor activity showed after 3 days a reversal of the locomotor activities opposite to the persisting light:dark cycle as external impulse (synchronizer/'Zeitgeber'). DSIP-P, i.e. the phosphorylated derivative (serine pos. 7), exerted this phenomenon sooner and more pronounced *but* at a dose of 100 pmoles/kg b.wt. This suggests that the active principle might be the phosphorylated form. Additionally injections of DSIP at different times of the day (7.00 and 18.00) produced significant changes in the 24-h rhythmicity of 5HT, dopamine and noradrenaline in the rat brain and of

the serum proteins and corticosterone levels: a) Significant retardation and/or advancement of the circadian peaks were observed. b) Significant differences with respect to the morning or the evening injection occurred. Mathematical treatments of the values obtained revealed that the circadian shift of these parameters was not correlated. This strongly suggests that DSIP acts on a higher level of coordination as a 'programmer' for the circadian rhythmicity, the sleep inducing property appearing as a subset.

Distribution and specific binding of ^{3}H-DSIP

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The occurrence of endogenous DSIP and the permeability of the blood-brain barrier were shown by Kastin et al. (Brain Res. Bull. *3*, 691, 1978). We investigated the distribution of ^{3}H-DSIP and ^{3}H-tryptophane (= control) in 25 organs of rats, 5, 15 and 60 min after i.v. injections. Autoradiography, extraction and TLC were used. Serum concentrations levelled of from a peak of 97.2 ± 8.7 (DSIP) and 111.6 ± 4.5 (Trp) fmoles/mg after 5 min to a mean of 45 fmoles/mg after 60 min. With both compounds the highest peripheral radioactivity after 5 min was found in the pancreas. It increased after 15 min and by 100% after 60 min. In the brain generally lower activities by 80% were found. Significantly higher concentrations occurred in the pineal and pituitary when compared to 9 other different regions of the brain. Surprisingly after ^{3}H-Trp injection successively increasing amounts of ^{3}H-DSIP over time were found in the pinealis, pituitary and hypothalamus ($p \leq 0.01$). After ^{3}H-DSIP injections the DSIP levels in these regions decreased. This suggests that DSIP is an ubiquitous occurring peptide equilibrating rapidly with Trp. However with a protein fraction (5% w/w) of the pig pineal gland a Kd of $\leq 2.50 \times 10^{-9}$ moles/mg and a B_{max} of $\leq 2 \times 10^{-12}$ moles/mg suggested strong binding sites in this organ.

Effect of small dietary changes in aromatic amino acids on plasma amino acid levels in adult rats

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To study the influence of the dietary aromatic amino acids on plasma amino acid levels and the ratio tyrosine/large neutral amino acid (Tyr/LNAA, which is a measure of the availability of tyrosine to the brain). The following diets (12% prot., 10% fat, 62% CHO) were fed to adult rats (447 ± 37 g): B 13% casein; C, D and E 8.7% casein + 4.2% aminoacid (AA) mixture (C 3/3 Tyr + Phe, D 2/3 Tyr + Phe, E 4/3 Tyr + Phe of diet B). Blood was sampled at 9, 16 and 21 h after 7-11 weeks on the diets. The dietary treatments resulted in a significant change in plasma aromatic AA (Tyr and Phe) but not Val, Met, Ile, Leu and Trp. All those AA showed a circadian rhythm, except Met. There was a direct correlation between dietary Tyr and plasma Tyr, Tyr/LNAA for all collection times, whereas dietary Phe was significantly correlated to plasma Phe only at night. Hence even for small dietary changes in aromatic AA there is a change in plasma levels and the diet effect is more pronounced at night.

Occurrence of a higher molecular mass form of insulin-like growth factor II (big IGF-II) in cerebrospinal fluid and serum

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Insulin-like growth factors I and II (IGF I and II) are 2 somatomedins isolated from human serum. These polypeptide hormones have a molecular mass of 7500 daltons and are structurally homologous to proinsulin. As measured by radioimmunoassay normal human serum contains about 150 ng of IGF-I and about 600 ng of IGF-II/ml while spinal fluid contains only about 3 ng of IGF-I and approx. 55 ng of IGF-II/ml. Apparently 2 forms of IGF-II occur, one with an apparent Mr of 7500, the other with an apparent Mr of 9200. In spinal fluid 66% of IGF-II ($n = 6$) is of the higher molecular mass type. This 'big' IGF-II is active in 2 different biological assays (fat cell assay, thymidine assay), can be measured by radioimmunoassay for IGF-II and competes with IGF-II for binding to the IGF binding protein. In serum and plasma only $\frac{1}{6}$ of IGF-II is present as 'big' IGF ($n = 5$). Present data suggest that this 'big' IGF can be converted to IGF.

Evidence refuting the antithiamine effect of chlorogenic and caffeic acids

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Contrary to earlier literature reports, chlorogenic acid (I) is shown to have little or no deleterious effect on thiamine (II) when the 2 compounds are codissolved in aqueous solution. Evidence from nmr spectroscopy and from thiochrome determinations demonstrate that when II is heated at 60 °C and at pH 7.5 for 18 h, 15% is destroyed. This result is the same whether I is present or not. Incubating II with I or with caffeic acid (III) in a phosphate buffer at pH 7 or in thiamine assay medium at pH 6 for up to 24 h at 37 °C did not reduce the growth activity of II on *Lactobacillus viridescens*. We conclude that I and III are *not* antithiamine agents.

A rapid measure of ε-deoxy-lactulosyl-lysine in milk powders using ferricyanide

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The furosine technique (E. Bujard and P.A. Finot, Ann. Nutr. Alim. *32*, 291, 1978), which was developed specifically to control the level of lactulosyl-lysine in milk products, is a too sophisticated technique for routine use. We now suggest that the reducing power of milk powders as measured by ferricyanide (R.A. Chapman and W.D. McFarlane, Can. J. Res. *23B*, 91, 1945) can be used as a much simpler and more rapid indicator of lactulosyl-lysine. In this test, ferricyanide appears to be reduced mainly by lactulosyl-lysine but also to a lesser extent by protein-bound cysteine and such soluble reducing compounds as vitamin C. We stored 12 different types of milk powder and infant formula at 37 °C and 5-10% moisture. We then compared their protein-bound reducing power (measured as the difference between total reducing power and non-protein reducing power) with their content of lactulosyl-lysine as measured by the furosine technique. There was a

good correlation (r = 0.90) between the 2 methods when all products were considered together, but even better correlations when the products were considered individually.

Interactions of fibronectin (FN) with Clq, a subcomponent of the first component of complement (Cl)

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Human plasma fibronectin (FN), known to react with collagen, has been shown to interact also with human Clq. Using ELISA and radioimmunoassay, fixation of Clq to solid phase FN, as well as FN to solid phase Clq has been demonstrated. Cl, containing in addition to Clq, Clr and Cls, displays 25–30% of the reactivity of the free Clq for FN. - Clq contains a triple-helical collagen-like region which can be uncoiled by heating to 51 °C for 15 min. After cooling, such Clq will bind 40–50% more FN than native Clq. Increased binding is also observed with Clq deprived of its globular parts by pepsin digestion, whereas collagenase treatment of Clq abrogates its FN-binding capacity. - Thus, the collagen-like regions of Clq are involved in FN-binding, although the affinity for FN is considerably lower than that of collagen or gelatin. The binding sites on Clq for FN appear to be related to those for Clr and Cls.

Reduced brain enzyme activities in Pick's disease

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Pick's disease (PD) is a rare form of senile dementia particularly characterized by atrophy of the temporal lobe. The activities of some glycolytic enzymes, ATPases, carbonic anhydrase (CA), acetylcholinesterase (AChE) and protein kinase (PK) were determined in human autoptic temporal lobes from patients with senile dementia of Alzheimer's type (SDAT) and 3 cases of PD. In comparison to age-matched controls, some key enzymes of glycolysis, CA and AChE were significantly reduced in cases of SDAT; in PD, the reduction of many glycolytic enzymes and CA was more pronounced. Other enzymes like ATPases and PK (±cAMP), which were not significantly affected in SDAT, were considerably reduced in PD. In contrast, soluble hexokinase and AChE were not changed in PD, but were affected in SDAT. The results of this preliminary study point to different degenerative processes in the brain under conditions of SDAT and PD.

Effect of germination on the proteinase activities detected in soybean cotyledons

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Soybeans (*Glycine max.* var. Altona) were germinated for up to 4 days. Radicles, teguments and epicotyls were then removed and aqueous extracts were obtained from freeze-dried, ground and defatted cotyledons. The proteolytic activity of the soymeal extracts was investigated using 16 different synthetic and natural substrates. Neutral and acid proteinases were detected, as well as aminopeptidases and carboxypeptidases. Comparison between germinated (1, 2, 3 and 4 days) and ungerminated cotyledons showed a dramatic increase of neutral proteinase and carboxypeptidase C activities with germination, whereas acid proteinase and aminopeptidase activities decreased significantly from the first day of germination. Preliminary results demonstrated that a minimum of 3 molecular species were responsible for the aminopeptidase activity, whereas molecular weight monodispersity characterized the neutral proteinases.

Effect of insulin on the disposal of i.v. glucose

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Euglycemic insulin clamp technique and indirect calorimetry measurement were performed to examine the effect of insulin on the disposal of i.v. glucose. When insulinemia was raised by 100 μU/ml, total glucose metabolism (M) was 5.4 mg/kg/min (insulin clamp technique), glucose oxidation rose from 1.2 to 2.3 mg/kg/min and glucose storage to 3.4 mg/kg/min (indirect calorimetry). At higher hyperinsulinemia (163 μU/ml) M (8.0) and glucose storage (5.3) increased significantly ($p < 0.01$) but glucose oxidation (2.7) remained unchanged. DeFronzo et al. have shown (in press) that in the same experimental conditions 85% of glucose infused is metabolized by muscle tissue. It is concluded that the primary effect of insulin on muscle tissue is to enhance glucose storage. The ability of insulin to stimulate glucose oxidation is saturated at relatively low plasma insulin level and further increase in glucose metabolism seen with higher insulin level is the result of increased glucose storage in muscle.

Hepatocellular (Na-K)-transport and α-adrenergic activation of glycogenolysis

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α-Adrenergic activation of hepatic glycogenolysis is paralleled by a net release of Ca^{2+} and by a transient uptake of K^+. Hypothetically, the K^+ uptake may be linked to the generation of a 2nd messenger promoting the release of Ca^{2+} from intracellular stores, or α-stimulation may directly release Ca^{2+} from the plasma membrane. In perfused livers from fed rats ouabain inhibited the K^+ uptake following α-receptor stimulation but did not prevent the activation of glycogenolysis. This indicates that K^+ uptake is not necessary for the metabolic response but may be a consequence of the Ca^{2+} release. Possible interactions between Ca^{2+} and (Na-K)-ATPase were further investigated with purified liver plasma membranes. Ca^{2+} (0.03–3 mM) inhibited (Na-K)-ATPase but did not affect the ouabain-insensitive ATPase. In the intact liver, α-adrenergic activation of the ouabain-sensitive (Na-K)-ATPase may thus reflect the release of Ca^{2+} from a membrane-associated pool.

Demonstration of a precursor of mitochondrial aspartate aminotransferase (mAATase) in cultured chicken embryo fibroblasts (CEF)

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In CEF, shortly pulsed with [^{35}S]methionine, a precursor of mAATase with higher mol.wt (Δmol.wt ~3000) was detected by immunoprecipitation and SDS-polyacrylamide gel-electrophoresis. Similarity of peptide maps of pre-mAATase and mAATase indicated partial identity of their amino acid sequences. The radioactively labeled pre-mAATase disappears when the pulsed cells are chased with

unlabeled methionine. Uncoupling of oxidative phosphorylation with the proton translocator CCCP results in accumulation of pre-mAATase and absence of labeled mature enzyme indicating that the import into the mitochondria and/or the processing of pre-mAATase is an energy-dependent process. No precursor of the homologous cytosolic AATase could be detected.

4-N,N-dimethyl-aminoazobenzene-4'-isothiocyanate, a colored, hydrophobic label for membrane proteins

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The apolar and intensively colored 4-N,N-dimethyl-aminoazobenzene-4'-isothiocyanate (DABITC $\varepsilon = 36{,}000$, in 1 M acetic acid, 1% SDS) has been used for hydrophobic labeling of human erythrocyte band 3. Characteristic differences in the absorption spectrum between DABITC and the reacted probe (e.g. butylamine) are observed. Additionally the absorption characteristics are highly pH dependent. Therewith the partition of the probe into the lipid bilayer can be demonstrated by spectroscopic analysis of labeled membranes in slightly acidified media. Covalent modification of erythrocyte band 3 is demonstrated by specific reagent-binding to membrane integrated segments. Following thermolytic and peptic digestion of labeled ghost membranes, the 17,000- and 10,000-dalton fragments are isolated. Label incorporation is spectroscopically determined. Under defined conditions 2.3 moles DABITC are bound per mole 10,000 daltons peptide. An insignificant amount of label is recovered in the 17,000-dalton fragment.

Catalytic properties of crystalline mitochondrial aspartate aminotransferase, lattice-induced functional asymmetry of the 2 subunits

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The enzymic activity of noncrosslinked microcrystals without diffusional rate limitation was measured in 30% (w/v) polyethyleneglycol. Determination of the Michaelis-Menten parameters showed that the packing of the enzyme dimer into the crystal lattice not only decreases its activity but also induces a functional nonequivalence of the 2 subunits which in solution behave identically. Apparently, the crystalline enzyme possesses a high and a low affinity site with K_m differing by one order of magnitude and k_{cat} being 4% and 15% of that in solution, respectively. The notion of 2 different active sites in the crystalline enzyme is supported by selective mechanism-based inactivation of the high or low affinity subunit.

Analysis of the antiphosphorylcholine repertoire of BALB/c mice by N-terminal amino acid sequence determination of monoclonal antibodies

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To study the phosphorylcholine (PC) repertoire in BALB/c mice, immune spleen cells of normal and idiotypically suppressed mice were fused with a myeloma cell line. IgG and IgM producing hybridoma lines were obtained. The antibodies were isolated, the heavy (H) and light (L) chains separated, and the N-terminal amino acid sequences of the variable (V) regions were determined. Most H chains had N-terminal sequences of isotype V_{H-4}, as all other PC-binding myeloma proteins (PCBMP). Only 2 IgM antibodies belonged to a new V_H isotype. The V regions of the L chains all contained V_K isotypes known in PCBMP. The majority of the hybridoma antibodies expressed V_H-V_L association pairs known from PCBMP, i.e. T 15-like, MOPC 167-like and McPC 603-like. The data suggest that the anti-PC repertoire in BALB/c mice is narrow and that a limited number of V_H and V_L germ-line genes, possibly together with somatic variants thereof are being expressed.

Development of dietary obesity: effect of litter size and high sucrose diet

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Effects of early over and under nutrition (litters of 4, 10 and 16) together with high sucrose diet (from weaning) on the development of dietary-induced obesity was investigated in Sprague-Dawley rats up to 13 weeks of age. Overnutrition during lactation markedly increased quantitative development of adipose tissue (inguinal, epididymal and retroperitonal) and to a lesser extent body weight. In the high sucrose group this effect on adipose tissue was less evident due to sucrose stimulation of obesity. This influence of high sucrose was significant in all the litters. The effect of mild malnutrition is less pronounced than that of overnutrition but becomes significant in comparaison with other high sucrose groups. Development of adipose tissue (cells) in all group is predominantly hypertrophic. Resting insulin levels at 13 weeks is positively correlated with group differences due to pre- and postweaning nutritional experience (especially with percentage body fat and the mean adipose cell volume).

The IgA dimer receptor from rabbit liver and mammary gland is a transmembrane precursor of the secreted secretory component

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Secretory component (SC), a biochemically and genetically heterogenous glycoprotein, bound to IgA dimer in exosecretions, is synthesized by liver and mammary epithelial cells. Antibodies against milk SC interact with epithelial basolateral cell surfaces and prevent the interaction of IgA dimer with liver and mammary membrane receptors. Deoxycholate solubilized liver and mammary membrane proteins, adsorbed on anti-SC- or IgA dimer-sepharose yield molecules with Mr ~116-122 and 93-100 Kd on SDS-PAGE. Partial proteolysis of these proteins show peptide maps similar to those of secreted SC. In contrast to secreted SC the solubilized membrane SC are amphiphilic as assessed by charge shift electrophoresis. Their transmembrane orientation has been documented recently (Mostov et al., PNAS, Nov. 1980). These results indicate that high Mr SC precursors inserted into the cell surface membrane function as IgA dimer receptors for the transepithelial translocation of polymeric immunoglobulins.

Serotonin-sensitive protein phosphorylation measured within a single living nerve cell

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There has been much speculation but little evidence relating protein phosphorylation to the physiological effects of neurotransmitters. We have examined this by injecting γ-[^{32}P] ATP directly into identified *Aplysia* neuron R15, and

separating the resulting phosphorylated proteins on polyacrylamide gels. The results indicate that phosphorylation of individual protein bands can indeed be measured within a single living neuron. Serotonin, which causes a K^+ channel in the R15 membrane to open (Drummond, Benson and Levitan, 1980), appears to selectively stimulate the phosphorylation of one or several high molecular weight proteins. The possibility that these phosphoproteins may play a role in regulation of membrane K^+ channels is under investigation.

Surface labeling pattern of human lymphocyte membrane proteins after mitogen pulses

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Human peripheral blood lymphocytes were treated with mitogenic concentrations of phytohemagglutinin (pha) for 1 h at 4 °C, washed, incubated in MEM at 37 °C and surface-labeled with lactoperoxydase and ^{125}I. The labeling pattern was studied by 2-dimensional gel-electrophoresis and autoradiography. It is observed that pha remains the mainly labeled species for a long time and that the labeling pattern of the endogeneous membrane proteins returns slowly. In order to determine whether pha at the cell surface becomes linked to cytoskeletal structures, detergent lysates of cells were equilibrated with sepharose-DNAase I columns. It is observed that pha may be retained together with the specifically adsorbed actin. Actin in control cells cannot be surface-iodinated; however, no evidence for a bridging protein between actin and pha is as yet obtained. Further experiments shall establish whether the actin-pha linkage is unspecific or functionally relevant.

Transport of newly synthesized cellular glycoproteins via coated vesicles

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Chicken embryo fibroblasts were labeled by short pulses of ^3H-mannose or ^{35}S-methionine. Clathrin-coated vesicles were purified from these cells by a modified procedure of Pearse (J. molec. Biol. *97*, 93, 1975). It was shown that these vesicles contained a variety of newly synthesized glycoproteins. A major component thus found was identified as fibronectin (mol wt 240,000), a protein secreted to be part of the extracellular matrix. It is further investigated whether coated vesicles mediate this transport in 2 waves, i.e. from the endoplasmic reticulum to the Golgi apparatus and from the Golgi apparatus to the plasma membrane, a mechanism suggested for the transport of a viral glycoprotein (Rothman and Fine, Proc. nat. Acad. Sci. USA *77*, 780, 1980).

An integrated system (including HPLC) for accurate measurements of endogenous neuropeptides

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Brain tissue is extracted in a small volume of 0.1 N HCl. The homogenate is filtered through a microfilter (BAS) directly into an Eppendorf reaction vial which is transferred to the automatic sample injection device ASI 45 (Kontron). A modification of ASI 45 allows the injection of 50 out of 80 µl of the sample, which is then developed through an RP-18 column (4.6×250 mm) with a linear gradient formed from 10 to 50% n-propanol in 5% AcOH/0.2% TFA during 20 min (modified according to A. Dell et al. in: Molecular Endocrinology, Ed. McIntyre and Szelke, 1979). The appropriate collected fraction is evaporated to dryness at 50 °C in a Speed Vac Concentrator (Savant). To the dry tubes the RIA-buffer is added and the concentration of peptide is determined by radioimmunoassay. The entire chromatographic procedure is under the control of an Altex Programmer, model 420. - This integrated system gives high resolution of a number of neuropeptides and reproducible recoveries in the ng-range (at least 80% for Met-enkephalin, FK 33-824 und LHRH, which have been tested so far). The combination of HPLC with RIA provides a maximum of specificity, the crucial point in quantitative measurements of endogenous neuropeptides.

In vitro killing of intracellular parasites by macrophages exposed to redox compounds

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Mouse peritoneal macrophages were infected with amastigotes of the protozoan parasite *Leishmania enriettii*, then exposed to various concentrations of the redox compounds methylene blue (MB), toluidine blue or phenazine methosulphate. This resulted in intracellular parasite killing within 24–48 h, at drug concentrations far below those inducing direct toxicity to the microorganism. MB-induced macrophage toxicity was inhibited by cytochrome c, whereas catalase and superoxide dismutase alone or in combination had little effect. MB also stimulated O_2 consumption by macrophage suspensions, suggesting that MB-induced toxicity to parasites might be related to the production of active metabolites of oxygen.

Selective solubilization and modulation of human erythrocyte membrane acetylcholinesterase by bee venom components

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Treatment of leaky ghosts with either melittin (ME) up to 0.5 mM or bee venom phospholipase A_2 (PLA) 0.7 IU/ml alone does not solubilize membrane components. However ME (1 µM to 1 mM) in combination with PLA (0.7 IU/ml) extracts, in a concentration dependent way, up to 65% of acetylcholinesterase (AchE). In these conditions, about 70% of the phospholipids are degraded and up to 40% of lipid phosphorus is released from the membrane. Removal of lysoproducts from PLA treated ghosts with defatted serum albumin does not lead to any release of AchE. SDS-PAGE shows that most of the other membrane proteins remain sedimentable. Sucrose density gradient centrifugation and preliminary kinetic experiments reveal that ME preserves AchE in an enzymatically active dimeric form, indicating a hydrophobic interaction between ME and AchE.

Creatine kinase (CK): localization of a tryptophan residue at adenine binding site by fluorescence quenching titration

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^{1}H-NMR and fluorescence studies of rabbit skeletal muscle CK and its complexes with nucleoside phosphates suggest the occurrence of tryptophan at or near the coenzyme binding pocket (Vašák et al., Biochemistry *18*, 5050, 1979). This conjecture is now further supported by dynamical fluorescence quenching studies with iodide and acrylamide which indicate that binding of ADP or GDP completely shields one of the 4 tryptophan residues from contact with these reagents. The close proximity of an indole group to the active center is also strongly indicated by efficient static quenching of tryptophan fluorescence by AgI bound stoichiometrically to the cysteine residue of the active site.

Characterization of rat capsular adrenal (zona glomerulosa) Na/K-ATPase activity

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The mineralocorticoid aldosterone is synthesized in capsular adrenal (zona glomerulosa). The biosynthesis of this hormone is directly stimulated by angiotensin II, ACTH, serotonin and by a high extracellular potassium concentration. Quabain, a specific inhibitor of Na/K-ATPase, was also shown to affect aldosterone biosynthesis. Therefore, a possible role of Na/K-ATPase in the regulation of aldosterone biosynthesis might be envisaged. - Na/K-ATPase activity was measured with 32-P-ATP. A higher enzyme activity was found in capsular than in decapsulated adrenal (zona fasciculata). In capsular adrenal the K 0.5 for Na^+ and K^+ were 20 mM and 1 mM, respectively and the I 0.5 for ouabain was 50 µM. No unusual characteristics of capsular adrenal Na/K-ATPase were found. Angiotensin II, ACTH and serotonin, all potent stimulators of aldosterone biosynthesis, did not affect the enzyme. - Conclusion: Capsular adrenal Na/K-ATPase is not the direct location where aldosterone stimulators might act. But an active role of this enzyme in mediating a regulatory signal cannot be ruled out.

Glycerides and fatty acids enable a specific α-actinin membrane interaction

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Little is known about the interaction of membrane associated proteins with lipids and lipid membranes. Alpha-actinin has been proposed to be such a protein anchoring the microfilament bundles within the cell membrane (Nature *264*, 272, 1976). Experiments performed in a Langmuir trough showed that α-actinin spontaneously penetrates into a lipid monolayer and specifically forms a rigid protein-lipid layer at the air-water interface, provided nonphosphorylated glycerides and palmitate or palmitoleate were present in the lipid monolayer. Removal of the protein-lipid layer and analysis revealed a ratio of glycerides to palmitoleate of 1.04±0.05. The ratio of fatty acids to α-actinin dimers was 1.3±0.4. All other lipid components and particularly the phospholipids were excluded and displaced. Short-time digestion studies with trypsin or α-chymotrypsin showed that at least one cleavage site of the α-actinin is protected in the presence of liposomes containing the mixture of glycerides and fatty acids.

Proteolysis of thylakoids from *Chlamydomonas reinhardii*

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After treatment of EDTA-washed thylakoids of *Chlamydomonas reinhardii* with proteases, the resistant, intrinsic polypeptides and fragments were analyzed by SDS-PAGE. In spite of their different specificities pronase and trypsin lead to similar protein patterns. Above the 20-kD region both kinds of polypeptides are found, those not altered by proteolysis (75 kD from CP I; 25 kD; 20 kD) and those containing a large intrinsic part (29 kD; 24 kD; 34 kD; with fragments of 27.5 kD; 23 kD and 19 kD, resp.). The correlation of the intrinsic fragments to the undegraded polypeptides was achieved by 1-dimensional fingerprints or by comparison of the intensities of the protein bands during proteolysis. The polypeptides of 29, 25 and 24 kD are part of the LHCP-complex. After proteolysis of the membrane this complex can be separated into 2 green subcomplexes. One contains the intrinsic 25-kD polypeptide, the other the 23- and 27.5-kD fragments of the 24- and 29-kD polypeptides. We conclude that the LHCP-complex is composed of at least 2 nonidentical subcomplexes.

Isolation of a cell specific binding fragment from the sponge aggregation proteoglycan complex

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3 components are required for specific cell aggregation of the sponge *Microciona prolifera:* a) a surface proteoglycan aggregation factor (AF) of mol.wt 21×10^6, b) plasma membrane AF receptor, c) Ca^{++}. AF has 2 functional domains: a Ca^{++}-independent cell binding site and a Ca^{++}-dependent interaction site. Treatment with 5 M urea, 40 mM EDTA at 80 °C, alone or followed by trypsin, were used to obtain the cell binding site from the AF complex. After fragmentation, 7 glycoproteins of apparent mol.wt 7.5×10^6, 2.5×10^5, 1.2×10^5, 7×10^4, 2.7×10^4, 5×10^3, 3×10^3 were isolated. Each had all 3 properties expected for the cell binding site: a) specific binding to homotypic AF free cells, b) inhibition of AF promoted aggregation and c) no binding to AF agarose beads irrespective of the presence of Ca^{++}. Association constants decreased linearly with the size of the fragments. This supports the concept that high polyvalency of AF contributes to the force and specific affinity of the recognition aggregation processes.

Nonenzymatic browning in vivo: an aging process for long-lived proteins

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The nonenzymatic browning or Maillard reaction is an aging process in stored foods. Reducing sugars react with proteins to form pigmented, fluorescent and cross-linked adducts. The initial stage of this reaction - the nonenzymatic glycosylation - has been shown to occur in vivo. The occurrence in vivo of browning products involving lysine and glucose has been investigated. - Lens proteins from human cataracts were alkylated, reduced with ^{3}H-borohydride and acid hydrolyzed. A fluorescent, yellow and radioactive amino acid was isolated by G15 sephadex, cation exchange and HPLC chromatography. It co-chromatographed in all 3 systems with a lysine derivative isolated from the brown reaction mixture of α-t-boc lysine with glucose which was processed as described above. Control experiments show that new amino acid is not an artefact

due to acid hydrolysis of glucitolyl lysine. These and other results suggest that nonenzymatic browning may be involved in pigmentation and cross-linking of lens proteins.

Effect of in vivo application of glucagon on the conversion of glyceraldehyde-3-P to glucose-6-P by isolated rat liver cytosol

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Whether glucagon activates fructose-1,6-bisphosphatase is a matter of debate. In our experiments, fed male rats anesthetized with methoxyflurane received glucagon i.p. (500 µg/kg b.wt); controls received vehicle only. The hormone treatment accelerated the conversion of glyceraldehyde-3-P to glucose-6-P catalyzed by subsequently isolated liver cytosols. The glucagon effect is time dependent and is maximal at 15–20 min after injection. At 20 min, the activation is 47±8% as compared to the controls (mean±SEM, n=12). Experiments with fructose-1,6-bisphosphate showed that the increased rate is due to an activation of fructose-1,6-bisphosphatase. Glucose-6-P formation can also be increased by addition of 10 µM EGTA to the assay medium. This activation is smaller with cytosol from glucagon-treated than from control animals. The glucagon effect is seen only when cytosols are prepared with 100 µM FMN which is a protein kinase inhibitor (J.F. Kuo et al., Biochim. biophys. Acta *212*, 79, 1970).

Isolation and characterization of a rabbit platelet secretory protein with antiheparin activity different from PF4

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A protein was isolated from the soluble release products of thrombin-aggregated washed rabbit platelets. It eluted from heparin-agarose with 0.9 M NaCl, migrated as a single band in SDS PAGE with a mol.wt < 12,000 dalton and had a heparin-neutralizing activity of 0.16 mg polybrene/mg. The corresponding values for rabbit PF4 were: 1.6 M NaCl, 16,000 daltons and 1.22 mg polybrene/mg. Gel filtration pointed to a mol.wt of about 20,000 daltons. - Antisera against this protein were produced in goats and a radioimmunoassay was developed. Plasma and serum levels ($\bar{x} \pm SD$) were 26±6 ng/ml and 2900±960 ng/ml, respectively. Washed platelets contained 2.0 ng of the protein per 10^6 platelets. After injection of collagen into rabbits plasma levels rose from 26±8 ng/ml (n=6) to 72±29 ng/ml after 30 sec. Evidence for the secretory nature of the protein was the increase of plasma levels in parallel to platelet aggregation induced by collagen.

Binding of autoantibodies (IgG) to human red blood cells

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Recognition of senescent human red blood cells (RBC) by phagocytes appears to occur on the amount of bound autoantibodies (IgG) directed against senescent RBC (M.M.B. Kay, PNAS *72*, 3521, 1975). Binding of autologous IgG to RBC was studied by measuring bound ^{125}I-labeled protein A on fresh, density fractionated and ATP-depleted RBC and the released vesicles. Dense RBC fractions (senescent) showed a significantly increased binding of protein A as compared to light RBC fractions (young) (total binding 0.1 ng protein A/µg bande 3). ATP-depleted RBC bound only a third of the amount of protein A detected on fresh RBC. However protein A binding increased with addition of autologous IgG up to the level detected on fresh RBC. These RBC depleted of ATP for 40 h have lost some surface as spectrin-free vesicles (H.U. Lutz, JBC *73*, 548, 1977). Protein A binding on these vesicles increased up to 4-fold by adding 5 mg/ml autologous IgG. These data suggest that binding of autologous IgG to RBC in situ is almost irreversible. Only a prolonged incubation of the RBC during ATP-depletion removes the IgG. Furthermore the IgG receptor becomes more exposed in spectrin-free vesicles as compared with the cells.

Calmodulin-stimulated protein kinases in the nervous system of *Aplysia*

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Evidence is accumulating that in mammalian brain cAMP and Ca^{2+}-regulated phosphorylation may play an important role in synaptic function (O'Callaghan, Dunn and Lovenberg, PNAS *77*, 1980). Phosphoproteins also may be involved in the regulation of invertebrate nervous system function. We are investigating protein kinases from the nervous system of the mollusc *Aplysia*. Cytosol proteins are separated by isoelectric focusing and bands with protein kinase activity are localized on the polyacrylamide gels. Several of the protein kinases focusing in the pH 5–9 range are stimulated in a Ca^{2+}-dependent manner by the addition of bovine brain calmodulin to the gel assay. Our aim is to characterize calmodulin- and cAMP-dependent protein kinases and their substrates in *Aplysia* neurons, and to examine their possible role in neuronal function.

The C-terminal sequence of human liver aldehyde reductase

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Aldehyde reductase (EC 1.1.1.2) has a mol.wt of about 36,000, contains about 313 amino acids, a blocked N-terminal and 4 methionines. The enzyme was cleaved with CNBr and by acid hydrolysis using 50% formic acid. The peptides obtained were purified by gel and ion exchange chromatography. Automatic sequence analyses of the isolated peptides were performed on a liquid and solid phase sequencer. 103 amino acids of the C-terminal could be sequenced including 2 methionine overlaps, which corresponds to a third of the total enzyme.

Assay of natural protease inhibitors by fibrinogen-agarose electrophoresis

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Fibrinogen-agarose electrophoresis has been modified by us to become a method of high resolution and sensitivity for the analysis of natural protease inhibitors (PI). It has the advantage over the currently used methods that the PI are visualized through their biologic function. In principle bovine fibrinogen is incorporated into buffered 1% agarose and heat-precipitated. After electrophoresis of PI the plates are incubated in a solution containing the protease of choice at its appropriate concentration for 15 min and then

rinsed with distilled water. Undigested fibrinogen, which remains where PI are located, is stained with amidoblack. Since every protease can be used for the fibrinogen digestion provided that it is active between pH 7 and 9 the method is ideally suited for the determination of protease specificity of the PI in addition to electrophoretic migration. - In this way PI of blood, bronchial secretion, colostrum and granulocytes of different animal species are being studied by us in relation to various physiological and pathophysiological conditions.

Functional involvement of phenylisothiocyanate in the anion exchange process of human erythrocytes

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In human erythrocytes phenylisothiocyanate binds covalently to membrane-integrated proteins, particularly to the anion transport protein. Modified erythrocytes remain discoid and enzymatic activities of membrane-bound hydrolases (acetylcholinesterase, phospholipase) are scarcely affected (±12%). Phenylisothiocyanate inhibition of sulfate efflux ($ID_{50} = 1.5 \pm 0.5$ mM) indicates interaction of the probe with the anion exchange process. However, upon modification of cells with phenylisothiocyanate in the presence of 4,4′-dinitro-2,2′-stilbene disulfonic acid (DNDS, a specific reversible inhibitor of the anion transport) and removal of the reversible probe, the anion-exchanging properties of the system are preserved. A commensurate action of both reagents, phenylisothiocyanate and DNDS, on the transmembrane process is thereby implied.

Problems encountered in purification of low molecular weight intestinal polypeptides

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In order to purify a new insulinotropic peptide from the gut, a crude extract of porcine duodenal mucosa was divided into fractions using usual techniques like ion-exchange chromatography and gel filtration. Quality of all the fractions were controlled by analytical isoelectric focusing and it appeared that these classical methods were not able to achieve full purification. - To separate these peptides of apparent low mol.wts (5000 daltons), the use of high performance techniques was imperative. Preparative electrofocusing and HPLC appeared to be suitable but there use introduced new difficulties which we attempt to overcome. We tried therefore to find original ways to separate peptides from ampholines after focusing and to treat HPLC fractions in order to make them suitable for further purification and for bioassay. - Preliminary results indicate that a high degree of purification can be obtained in separating low mol.wt polypeptides.

Specific transfer and hydrolysis of lecithins with longchain, unsaturated fatty acid by sheep red cell membranes

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In contrast to other membranes, sheep red cells contain only traces of lecithin. This is due to a phospholipase A_2 at the outside of the membrane, which preferentially splits lecithin. It is possible to transfer lecithin into sheep ghosts by incubation with human lipoproteins or liposomes. In presence of EDTA, where the enzyme is inactive, the lecithin is accumulated in the membrane. If calcium is present, most of the substrate is hydrolyzed. The lysolecithin remains in the membrane, whereas the fatty acid is transferred to the lipoprotein. Lecithins with longchain, unsaturated fatty acids are preferentially transferred.

Effects of insulin on the release and turnover of dopamine (DA), noradrenaline (NA) and adrenaline (A) in rat brain

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Recent observations that specific insulin receptors as well as insulin itself are present in rat brain (J. Havrankova et al., Nature *272*, 827, 1978, J. Havrankova et al., Proc. nat. Acad. Sci. USA *75*, 5737, 1978) prompted us to investigate the effects of insulin on DA, NA and A release and turnover in rat hypothalamus and the C1/C2 regions of the medulla oblongata. The 3 catecholamines were assayed using HPLC with electrochemical detection. Parenteral administration of insulin (20 IU/kg) causes an increase in turnover of central NA and A. Intraventricular administration of insulin causes a dose-dependent increase in turnover of central NA and A from a starting dose as low as 0.1 IU/kg. Exposure of hypothalamic slices to insulin (0.1, 0.4 and 4.0 IU/ml incubation medium) results in an increased, dose-dependent release of NA, A and DA. A high potassium concentration (30 mM) in the incubation medium further enhances the insulin induced release of catecholamines.

Synergistic effect of insulin-like growth factor (IGF) and platelet lysate on growth of cultured fibroblasts

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Passaged human skin fibroblasts were kept stationary for 2 days prior to stimulation by different media and concomitant binding experiments with ^{125}I-IGF. Primary cultures of chick embryo fibroblasts were seeded in 5% FCS. After 1 day, the cells were grown in test media. - Serum from hypox rats, deficient in IGF, stimulated DNA-synthesis and replication of normal fibroblasts much less than normal rat serum. After addition of IGF to hypox serum, cell growth was normalized. However, IGF alone in defined medium had little effect on DNA-synthesis and did not stimulate cell multiplication. - Plasma-derived serum, poor in platelet material, is much less potent in stimulating DNA-synthesis than clotted serum. We found a dose-dependent stimulation of DNA-synthesis in confluent cultures, but no effect on cell replication by crude platelet lysate. - By combining pure IGF with crude platelet preparations in defined medium, we found an additive effect on DNA-synthesis and a synergistic one on cell replication. Fibroblasts have specific binding sites for IGF.

Determination of ATP-sulfurylase in crude extracts of *Phaseolus vulgaris* L. using the luciferin-luciferase-system

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ATP-sulfurylase (EC 2.7.7.4) catalyses the first step in assimilatory sulfate reduction, forming adenosine 5′-phosphosulfate (APS) and pyrophosphate from ATP and SO_4^{2-}. The extractable activity of ATP-sulfurylase was determined in crude extracts from *Phaseolus vulgaris* by

measuring the production of ATP from APS and pyrophosphate produced in the reverse reaction, using a Lumac Celltester M 1060 (Fakola, Basel). The assay contained, in a final volume of 260 µl: 100 µl 50 mM tris-acetate, pH 7.75, containing 60 nmoles luciferin and 10,000 relative units luciferase (Boehringer, Mannheim, BRD); 20 µl 0.1 mM APS; 100 µl 1.75 mM pyrophosphate and 20 µl extract. The reaction was started by addition of the pyrophosphate solution. The rates of ATP-sulfurylase activity determined by this method were 10 times higher than the ones measured in the forward reaction as $AP^{35}S$ formed from ATP and $^{35}SO_4^{2-}$.

Assimilatory sulfate reduction during ontogenesis of primary leaves of *Phaseolus vulgaris* L.

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The extractable activities of ATP-sulfurylase (ATPSTase) (E.C. 2.7.7.4), adenosine 5'-phosphosulfate sulfotransferase (APSSTase) and O-acetyl-L-serine sulfhydrylase (OASSase) (E.C. 4.2.99.8) of primary leaves of *Phaseolus vulgaris* during ontogenesis were determined. The specific activity of ATPSTase reached a peak in leaves of 7-day-old seedlings, then declined. The specific activity of APSSTase had a maximum of 300 nmoles · (mg protein)$^{-1}$ · h^{-1} 10 days after germination. Both ATPSTase and ATSSTase were no longer detectable after 10–16 days. Specific OASSase activity did not change appreciably during this period of time. - Tracer experiments using $^{35}SO_4^{2-}$ showed that in vivo the maximal flux of sulfur into amino acids and proteins was in 10-day-old seedlings, while the flux into sulfolipids was maximal 7 days after germination, indicating a correlation between the extractable activity of ATPSase and APSSTase and assimilatory sulfate reduction in intact leaves.

Photosynthetic activities of isolated chloroplasts from *Euglena gracilis*

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It is possible to isolate chloroplasts from photoheterotrophically grown *Euglena gracilis* in isosmotic gradients of 10–80% Percoll (Ortiz and Stutz, FEBS Lett. *116*, 298, 1980). We began recently a study of the photosynthetic capacity of our preparations. Isolated chloroplasts from *Euglena* display rates of CO_2-dependent O_2 evolution of 30–50 µmoles · mg^{-1} chl · h^{-1} or 25–35% of the net O_2 evolution by the whole cells. In some ways the *Euglena* chloroplasts appear to be strikingly different from spinach chloroplasts. 1. Whereas concentrations of orthophosphate higher than 0.5 mM inhibit the CO_2-dependent O_2 evolution of spinach chloroplasts, in *Euglena* chloroplasts orthophosphate concentrations as high as 20 mM sustain near maximal rates of O_2 evolution. This observation argues against the presence in *Euglena* of a phosphate translocator on the terms described by Heldt and Rapley (FEBS Lett. *10*, 143, 1970) for spinach. 2. *Euglena* chloroplasts do not sustain OAA-dependent of PGA-dependent O_2 evolution, both of which have been implicated in a shuttle mechanism of reducing equivalents and metabolites between the chloroplast and the cytoplasm. Results pertaining to intactness, purity and storage-life of isolated *Euglena* chloroplasts will also be discussed.

Prediction of energy requirement in young and elderly men studied in a metabolic unit

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The energy requirement of 13 elderly men ($\bar{X}$ age±SD: 68±4 years) was compared to that of 12 younger control subjects ($\bar{X}$ age: 25±2 years) of similar weight. Both groups were confined in the same metabolic unit for at least 6 weeks. The energy requirement for the elderly was found to be 10,060± 1066 kJ/d (i.e. 134 kJ/kg or 1.68×BMR) whereas that of the younger subjects was 11,750±1296 kJ/d (i.e. 165 kJ/kg or 1.81×BMR). There was a significant reduction with age in energy requirement ($p < 0.005$), in BMR ($p < 0.05$), and in 24 h urinary creatinine excretion (index of muscle mass) ($p < 0.005$). The 2 single variables showing the strongest correlation with energy reqirement were found to be creatinine excretion ($r = 0.805$, $p < 0.001$) and total body potassium (TBK) derived from ^{40}K ($r = 0.791$, $p < 0.001$). The best prediction of energy requirement (multiple $r = 0.934$) was obtained when a combination of urinary creatinine + TBK + age + weight (order of importance) were used as independent variables in a multiple regression model.

Modified α-bungarotoxin as a probe for acetylcholine receptor quaternary structure in situ

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Acetylcholine receptor from *Torpedo* electric organ consist of 4 species of subunits named α, β, γ and δ, whose spatial organization in the membrane is not yet known. Only the function of the α-subunit is known. It contains the site for acetylcholine binding as well as the main neurotoxin binding site. - Use of ^{125}I-α-bungarotoxin, in which a photolabile cross-linker group is introduced via specific opening of 1 disulfide bridge, has proven a useful tool for understanding subunits assembling. The γ and δ subunits rather than the α have been labeled. The latter was labeled by the toxin when the same photolabile group was added to the thiols of the receptor after moderate reduction.

Comparison of the interaction of soya bean protease inhibitors with rat pancreatic enzymes and with human trypsin

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Feeding rats on raw soya flour diet results in pancreatic hypertrophy, development of hyperplastic nodules and hypersensibility to carcinogens. There is also strong evidence that these detrimental effects are due to soya bean trypsin inhibitors (T.I.). - We have purified rat acidic trypsin (Tr_1) the isoelectric point (Ip) = 4.5, basic trypsin (Tr_2) Ip = 11.5 and acidic chymotrypsin (Chtr) Ip = 4.8. We have also tested a sample of human basic trypsin which represents a major part, about 65% of the potential trypsin activity of the whole human pancreatic juice. - The inhibition spectra have been determined and show that: 1. T.I. (Kunitz type) inhibits 100% Tr_1, Tr_2 and Chtr activities in molar ratios inhibitor/enzyme 2:1.3:1.4:1, respectively, and 30% of human trypsin in a molar ratio inhibitor/enzyme 2:1. 2. T.I. (Bowman-Birk type) inhibits 50% Tr_1,

66% Tr_2, 90% Chtr and 30% human trypsin in a molar ratio inhibitor/enzyme 2:1. - It would appear that human basic trypsin is less sensitive to soya bean T.I. than each of the rat proteases investigated.

Inhibition of gluconeogenesis by ascorbate, isoascorbate and dehydroascorbate in rat liver hepatocytes

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In hepatocytes from fasted rats, ascorbate (Asc), isoascorbate (IA) and dehydroascorbate (DA) inhibited gluconeogenesis from alanine, lactate/pyruvate and serine but not from glycerol, dihydroxyacetone, fructose, and glutamine. DA was about twice as effective as IA and Asc. With alanine as substrate, 0.1, 1.0 and 10 mM Asc inhibited gluconeogenesis by 16, 25 and 45% respectively; gluconeogenesis was similarly inhibited in hepatocytes from fed rats. The inhibitory pattern with various substrates points to pyruvate carboxylase as a possible target for inhibition. Oxalate is a metabolite of all 3 inhibitors and is also a strong inhibitor of pyruvate carboxylase and consequently of gluconeogenesis. It is therefore postulated that oxalate mediates the effect of Asc, IA and DA on gluconeogenesis by inhibiting pyruvate carboxylase. The Asc content of fresh rat liver is about 3 mM, and the inhibitory Asc concentrations are therefore in the physiological range. This action of Asc may explain the hypoglycemic effect of Asc observed in guinea pigs and humans.

Hypocholesterolemic effect of milk constituents in growing swine

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Our previous study demonstrated a lipid lowering effect of whey fed in large amounts to growing swine. This study reports the effect of milk constituents on serum lipids in 4 groups of swine on a comparable fat feed. Each group comprised 12 animals with a starting weight of 35 kg. During the observation period of 9 weeks the animals were fed 25 energy percent (E%) butterfat. After a 4-week adaptation period on control feed (butterfat, barley and corn) group A received skimmed milk (50E%), B yogurt (50 E%), C control feed and D casein (10 E%) with barley and corn. The average serum cholesterol (mmoles/l) was 3.3 in A, 3.7 in B, 3.8 in C and 3.9 in D. HDL cholesterol (mmoles/l) was 1.38 in A, 1.42 in B, 1.64 in C and 1.55 in D. Serumtriglycerides varied little in all groups. The differences in serumcholesterol between group A and C/D are statistically significant ($p < 0.005$). Cholesterol was lowered by 13% in A and 5% in B in comparison to C. As previously shown for whey skimmed milk lowers significantly serumcholesterol in hypercholesterolemic swine. Fermentation to yogurt does not appear to enhance the lipid lowering effectiveness. Casein, the main constituent contained in skimmed milk but not in whey does not influence serumcholesterol.

Effects of coffee consumption on the thiamine status of rats

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Coffee and its orthophenolic constituents were reported to interact with dietary thiamine (J.C. Somogyi, Wld Rev. Nutr. Diet. *29*, 42, 1968). In earlier studies, however, we showed that coffee intake does not interfere with the thiamine status of experimental animals and human volunteers. To investigate further the possible effects of coffee on thiamine, we fed male rats with a thiamine deficient diet and gave 2% instant coffee as the only liquid. Thiamine deficiency symptoms did not appear earlier in animals consuming coffee as compared to the controls. Blood thiamine levels, urinary thiamine excretion and erythrocyte transketolase activity were not influenced by coffee intake. Incubation of chlorogenic acid ($2.8 \cdot 10^{-3}$ M) or caffeic acid ($5.6 \cdot 10^{-3}$ M) with thiamine ($1.2 \cdot 10^{-7}$ M) in a buffer at pH 7 for 24 h, did not reduce the growth activity of the vitamin in the *Lactobacillus viridescens* growth assay. - Based on our earlier findings and this experiment, we conclude that under realistic and practical coffee intake conditions, there is no disturbance of thiamine bioavailability.

Continuous monitoring of membrane potential changes in rat brush border vesicles isolated from small intestine (IBBV) and renal proximal tubule (RBBV)

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Vesicles were isolated by a Mg/EGTA precipitation method. The fluorescence of 3 µM 3,3'-diethylthiadicarbocyanine iodide in 74 mM Na_2SO_4 and 1 mM K_2SO_4 drops to about 15% after addition of 250 µg IBBV-protein but only to 30% with RBBV. Addition of D-glucose (20 mM) leads to a transient fluorescence increase, which is 4–5 times higher with RBBV. $LaCl_3$ in µmolar concentrations leads a) to a smaller initial fluorescence quenching and b) to an increase of the D-glucose signal. The effect of $LaCl_3$ is smaller with RBBV. Fractionation of IBBV by free flow electrophoresis leads to a decreased initial fluorescence quenching and an increased D-glucose signal with increased distance of the vesicles from the anode. Neither $LaCl_3$ nor electrophoretic separation produced altered tracer flux rates. These data suggest that the cyanine dye measures alterations in membrane potential as a function of transport rates. The size of the signal can be influenced by membrane surface charges (IBBV).

Isolation and characterization of photosynthetic mutants of *Chlamydomonas reinhardii*

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After mutagenesis with UV-light or with chemical mutagens we isolated new mutants of the eukaryotic alga *Chlamydomonas reinhardii* mt^+arg2^- with altered photosynthetic properties. All the mutants, like the initial strain, grow photoautotrophically. A first group of mutants shows a lower content of photosynthetic pigments, different photosynthetic activities and an altered PAGE-pattern of the membrane proteins as compared with the initial strain. Of special interest are mutants with a low content of chlorophyll b and a reduced amount of chlorophyll-protein complex II. Chlorophyll b is known to be associated with photosystem II and the light-harvesting chlorophyll-protein

complex. A 2nd group of mutants has an increased resistance against the electron-transport-inhibiting herbicide Diuron. The doubling time of exponentially growing mutant cultures and the photosynthetic activities of these mutants are less inhibited by Diuron than with the initial strain.

Crystallographic studies of conformational changes of mitochondrial aspartate aminotransferase (mAATase) during catalysis

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The 2.8 Å resolution structure of chicken heart mAATase has been determined from catalytically competent crystals, space group P1. Introducing the coenzyme-substrate complex N-5′-phosphopyridoxyl aspartate (PLP-Asp) into apoenzyme crystals of this form leads to a shift of the small domain towards the active site and tends to shatter the crystals. Upon cocrystallization a different form, space group $P2_1$, is obtained which is identical to holoenzyme cocrystallized with the inhibitor D,L-2-methyl aspartate. A comparison of a 4.4 Å resolution electron density map of the latter with the Pl structure at the same resolution has revealed the extent to which the small domain has moved. A difference map with PLP-Asp in $P2_1$ is featureless, proving that both derivatives have the same spatial structure which we believe is characteristic of covalent intermediates with specific dicarboxylic acid substrates. Higher resolution studies of these derivatives are underway.

Biological and immunological properties of human calcitonin in tissue extracts and plasma

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Immunoreactive components of calcitonin (CT) in tissue extracts and plasma of normal subjects and patients with tumors (medullary carcinoma of the thyroid [MTC] and a pancreatic tumor) producing CT were characterized by gel-filtration on Bio-Gel P-150. A peak coeluting with synthetic monomeric hCT-(1-32) was predominantly recognized in tissue extracts and further analyzed by reversed phase high performance liquid chromatography (HPLC). The peak obtained on gel-filtration could be resolved into at least 2 components: In normal thyroids and MTC, hCT-(1-32) and its sulfoxide were seen. In the pancreatic tumor, hCT-sulfoxide and an additional component eluting before hCT-(1-32) was found. The hypocalcemic activity of all components was tested in an in vivo assay. In plasma additional immunoreactive peaks with apparent mol.wt varying from 4500 to 13,000 were recognized. - In conclusion, tissue extracts from normal subjects and MTC patients predominantly contained CT with biological and biochemical properties similar to synthetic hCT-(1-32) and its sulfoxide, whereas in a pancreatic tumor producing CT ectopically an additional CT form was detected for the first time.

A solid-phase immunoassay for human galactosyltransferase

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A competitive solid-phase enzyme-linked immunosorbent assay (ELISA) for quantitative assessment of galactosyltarnsferase protein in human body fluids was developed. Monospecific rabbit antibodies against the soluble human milk enzyme were used. The ELISA was based upon competition with a galactosyltransferase-alkaline phosphatase conjugate. The standard curve covered a range of 30 to 1000 ng enzyme/ml. Within-assay and between-assay precision was 2.5-6-3 and 4.8-7.1%, respectively. Curves similar to the standard curve were obtained when purified antigen was replaced by appropriate dilutions of pooled human milk. Human milk samples covering a lactation period of 2 months were tested for galactosyltransferase activity and correlated with immunoreactive enzyme protein. A linear correlation between both parameters was found. Galactosyltransferase concentration was within 41-136 µg/ml and showed a decreasing tendency after 1 month of lactation.

Olfactory threshold: comparative study of nasal and retronasal perception

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Appetite is elicited by 2 types of stimulations: caloric needs and sensory needs such as odor, taste or tectile perception. The modalities of olfactory perception were studied by different methods. To examine the olfactory events that take place during food-intake, we have measured olfactory thresholds by direct and retronasal pathways. Aqueous solutions of odorous compounds were presented to a panel of 15 trained subjects. The sensitivity threshold was measured by signal detection, with sets of 3 sniff bottles (1 with and 2 without odorant). 3 methods of stimulation were used: sniffing odor by nose, sniffing odor by mouth and sipping the solution. The sensitivity thresholds obtained by these methods will be compared.

The 3 types of correlations encountered in nutritional studies

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Nutritional studies often follow a multivariate one-way analysis of variance layout. Consequently, relationships between variables can be expressed as correlations within the groups, correlations between the groups and total correlations. The correlations within the groups are calculated over experimental units receiving the same treatment. Accordingly, they express the intrinsic behavior of the experimental units. On the other hand, the correlations between the groups are calculated over the group means. Accordingly, they express the responses of the experimental units to the treatments. A total correlation is a resultant of the 2 others. One important point is that in the absence of any treatment effect, both the between and within correlations are estimates of the same parameter. As a result of this, between groups correlations should never be considered without the corresponding within groups correlations. This methodology will be illustrated by several examples.

Mechanism of insulin action on glucose transport in rat diaphragm: translocation of transport systems from an intracellular pool to the plasma membrane

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A method has recently been developed for quantitating the number of glucose transport systems in subcellular membrane fractions from rat adipose cells, based on the binding of ^3H cytochalasin B and its specific inhibition by D-

glucose. To examine the number of glucose transport systems in muscle cells, plasma membranes and microsomes have been prepared from rat diaphragm. Using cytochalasin B binding to quantitate the number of glucose transport systems in these subcellular muscle membrane fractions, the following observations have been made: insulin stimulation of glucose transport in the diaphragm increases the number of glucose transport systems in the plasma membranes from 16 to 28 pmoles transport system/mg protein and decrease the number of transport systems in the intracellular pool from 21 to 12 pmoles/ng protein. These results suggest that insulin stimulates glucose transport in the rat diaphragm through a translocation of glucose transport systems from an intracellular membrane pool to the plasma membrane.

Symmetry and asymmetry in the contractile protein myosin

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The duplex molecule myosin is composed of 3 identical pairs (2 heavy and 4 light chains) of polypeptides giving it the appearance of having a symmetrical structure. However its quarternary structure must be asymmetric resulting from natural chain folding and build-up of subunits. In addition heterodimeric forms occur. Enzyme kinetics, nucleotide and divalent cation binding are characterized by homogeneous processes in studies on proteolytically produced active monomeric myosin subfragments. With the intact duplex molecular species, enzyme kinetics, nucleotide and divalent cation binding exhibit negative cooperativity. Hence information passes between the subunits. The resulting differences in subunit conformation can be followed by chemical probing of certain reactive thiols in the heavy chains. It is suggested that this functional asymmetry between the subunits plays a central role during muscle contraction.

Intracellular precursor forms for α_1-antitrypsin (AT) and albumin (ALB) in human liver

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Recently an immediate intracellular percursor for rat albumin has been described. We demonstrate that human albumin is synthesized in the same way. Moreover, we found that AT, another liver produced serum protein, is also synthesized via an intracellular precursor form. - From human serum and liver specimens acetone dry powders were prepared, proteins extracted and chromatographed on DEAE-cellulose. After chromatography of the serum proteins single peaks for AT and Alb were detected with specific antibodies. In contrast, the chromatograms of the extracted liver homogenates showed, in addition to the serum peak, a 2nd early peak and a distinct shoulder in the ascending part of the curve for AT and Alb, respectively. - Liver slices were incubated for 20 min with ^{14}C-labeled amino acids. Proteins were extracted and separated as described. After precipitation with specific antibodies, radioactivity was found in the liver specific peaks of at and Alb only. The results strongly suggest that human AT and Alb are synthesized via intracellular precursors which are immunologically indistinguishable from their serum forms but differ in charge.

Effect of coffee grounds on the energy metabolism of the rat

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Coffee grounds were fed in several metabolism experiments to growing rats at various levels in the diet. Coffee grounds as a component of the feed for rats had a detrimental effect on the digestibility and metabolizability of the energy of the whole diet and of growth performance, mainly based on a lower fat deposition. Digestible energy of the coffee grounds amounted to about 6 MJ/kg. This value is higher than that found in other monogastric animals (growing pig: 1 MJ DE/kg) and in ruminants (sheep: 4,5 MJ DE/kg.). In the ad libitum fed rat the lower energy content of the diets with coffee grounds compared with diets without coffee grounds was partly compensated by a higher feed intake. The efficiency of the utilization of digestible energy for growth from diets with coffee grounds was lower than from rations without coffee grounds. This effect is partly based on a higher physical activity of the rats with coffee grounds in the feed. The rats used more than 50% of total heat production for activity compared to the rats without coffee grounds in the diet. They spent about 20% less energy for activity.

Regulation of T_4 synthesis by inorganic iodide in concentrations within the physiological range

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Native thyroglobulins (Tgb) contain between 10 and 60% of their iodine as thyroxine (T_4). In an attempt to define the mechanisms which regulate the efficiency of T_4 synthesis, we have iodinated in vitro 20 human goiter Tgb's using varying concentrations of NaI (0.25-50 µg/ml) and lactoperoxidase (LP) (0.01-0.6 IU/ml). Iodide in the medium did not exceed amounts which were entirely organified within 2-3 h. Kinetic studies have demonstrated that synthesis of T_4 was independent of time and LP activity, but was strongly correlated to free iodide in the medium ($r = -0.988$, $p < 0.001$). Thus, optimal T_4 synthesis does not occur before all available iodide is bound to Tgb. If so, the in vitro coupling efficiency is similar to that in vivo. We conclude that the coupling reaction is governed by inorganic iodide at concentrations far below those inducing a Wolff-Chaikoff effect.

Properties of phospholipase A in yeast plasma membranes

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Plasma-membrane vesicles of *Sacharomyces cerevisiae* prepared by mechanical disruption and aggregation of the mitochondrial fraction at pH 4.5 (G.F. Fuhrmann et al., BBA *433*, 583, 1976) contained a high amount of free fatty acids (FFA). The FFA could be removed to a great extent by incubation with bovine serum albumine. This treatment caused a 2.5-fold activation of the membrane bound Mg^{++}-dependent ATPase and a decrease in sugar transport capacity. - In order to test the hypothesis that the FFA were generated during the vesicle preparation procedure by the hydrolysis of membrane phospholipids, the phospholipase A-activity of the vesicles was analyzed. With dipalmitoylphosphatidylcholine as substrate, a broad pH-optimum

between 3 and 4.5 was determined. Anionic detergents (cholate, deoxycholate, SDS) had a concentration-dependent activating effect. The enzyme could be dissolved by 0.05% SDS and 5% cholate. After purification by acetone precipitation and ammoniumsulfate fractionation a single band (mol.wt 140,000) was obtained by SDS slab gel-electrophoresis, which was also detectable in the protein pattern of the plasma membrane.

Side-directed fusion of red cell membranes

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Sheep erythrocyte membranes contain a phospholipase A_2 which is localized at the outside of the membrane. As a consequence this membrane only include minute amounts of lecithin. A method is developed to incorporate this enzyme into other membranes by fusion. If inside-out vesicles of sheep red cell membranes are fused with sealed human erythrocyte ghosts, the enzyme is incorporated in the inner side of the membrane. Due to the asymmetric disposition of lecithin in human red cell membranes, little substrate is present. This can be verified by the low splitting of lecithin by the enzyme. In contrast, if the acceptor membrane is used in form of inside-out vesicles, the incorporated enzyme finds large quantities of substrate, which results in high splitting rates of lecithin. The method demonstrates the possibility of side specific incorporation of phospholipase A_2 into other cell membranes.

Inhibition of salivary α-amylase by maltitol and maltotriitol and their influences on acid production in human dental plaque

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Hydrogenated glucose syrups (Lyca sin®) contain sorbitol, maltitol, maltotriitol and hydrogenated dextrins. One of the brands is used in Switzerland as a sugar substitute in candies and labeled 'safe for teeth'. The aim of the present study was to evaluate the contribution of each constituant of the hydrogenated glucose syrup to its in-vitro fermentation by the human dental plaque. - Sorbitol, maltitol and maltotriitol are little or not fermented by the dental plaque bacteria; low α-amylolysis of the dextrin fraction which release fermentable maltose and maltotriose. - The low α-amylase activity is caused by the high level of branching in the dextrin and by competitive inhibition of the α-amylase by maltitol and especially maltotriitol.

In vitro phosphorylation of intact optic nerve

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The phosphorylation of proteins by endogenous protein kinases was studied in intact optic nerves. Freshly dissected rat optic nerves were incubated in physiological solution in presence of ^{32}P orthophosphate. After extensive washing the nerves were homogenized in 3% perchloric acid, the total nerve associated ^{32}P, as well as the synthesized ^{32}P-ATP was measured after separation of $^{32}PO_4^{3-}$ and ^{32}P-ATP on TLC-plates. A large number of protein bands including both peptides corresponding to the myelin basic protein were phosphorylated. When NaCl (130 mM) was replaced by KCl in the physiological solution, the PO_4^{3-}-transport system was inhibited (5-fold) resulting in a corresponding lowering of ^{32}P-incorporation into proteins, whereas the ATP/PO_4^{3-}-ratio remained unchanged, suggesting that neither the ATP synthesis nor the overall ATPase-activity was affected by high external KCl-concentration. This model provides a useful system to investigate the regulation and the functional role of nerve protein phosphorylation.

Preliminary characterization of 2 different binding proteins for insulin-like growth factor

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Insulin-like growth factors (IGF) in human serum are noncovalently bound to specific binding proteins (BP). 2 BP can be separated by gel-filtration, one of them (BP I) with an apparent molecular mass of approx. 125 kdaltons is acid-labile. BP II (approx. 50 kdaltons) is acid-stable. BP I cross-linked covalently to ^{125}I-IGF yields upon acid treatment 2 radioactive peaks of approx. 125 and 65 kdaltons, whereas the apparent size of BP II under these conditions remains unchanged. Similarly, the apparent size of BP I, but not of BP II, is reduced upon denaturation-renaturation. BP I, but not BP II, is bound by Con A-sepharose. These results suggest that human serum contains at least 2 distinct IGF-binding proteins. BP I is likely to be a glycoprotein composed of at least 2 probably nonidentical subunits, whereas the acid-stable BP II is apparently a monomer not identical to either of the 2 subunits of BP I.

A rapid radiochemical microassay for catechol-O-methyl transferase (COMT) activity using pyrocatechol as the substrate

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An improved radiochemical assay for COMT is described which is easy to perform and particularly suitable for the measurement of large numbers of symples. Pyrocatechol (1,2-dihydroxy-benzene) and S-adenosyl-L[methyl-^3H] methionine are used as cosubstrates. A phosphate buffer-substrate mixture (pH 7.6) is incubated at 37 °C for 15 min directly in counter tubes in the presence of $MgCl_2$, dithiotheitol and purified adenosine-deaminase. At the end of the incubation, ^3H-guajacol (the product of the reaction with a low polarity) is easily extracted into 10 ml of scintillation fluid (butyl-PBD, toluene, n-hexane) with added unlabeled guajacol by vortex mixing the counter tubes. Using an assay time of 15 min, the rate of reaction was shown to be linear using different amounts of purified COMT enzyme preparations, rat liver extracts as well as lysates of rat and human erythrocytes. COMT activity was easily measured in 50 µg protein from rat whole brain, cerbellum and striatum. The assay procedure described here is accurate and is particularly valuable for the rapid assessment of COMT activity in column chromatography eluates for enzyme purification. It is now being applied in clinical studies for the assay of COMT in erythrocytes of parkinsonian and psychiatric patients.

ZELL- UND MOLEKULARBIOLOGIE/GENETIK
BIOLOGIE CELLULAIRE ET MOLÉCULAIRE/GÉNÉTIQUES
CELL AND MOLECULAR BIOLOGY/GENETICS

Recombination between redundant serine tRNA genes of *Schizosaccharomyces pombe*

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When a prototrophic strain carrying the UGA mutation *ade6-704* and the UGA suppressor allele *sup3-e* of a $tRNA^{Ser}_{UCA}$ gene is selfed, adenine dependent progeny spores which have lost the active suppressor due to local alterations in the tRNA gene are formed with high frequency (10^{-5}). This suggests that meiotic recombination with related tRNA genes (Munz and Leupold, A. Benzon Symp. *16*, 70, 1980) may contribute to their formation. 22 out of 106 isolates studied carry an alteration at the site of the original anticodon mutation and have reverted to the functional wild-type allele $sup3^+$. They may have arisen by recombination with the 2nd known $tRNA^{Ser}_{UCA}$ gene, $sup9^+$. 84 isolates carry inactivating secondary alterations which do not restore the wild-type function and which in their majority map at 3 selected sites distinguishable by recombination. They may have been formed by recombination with closely related tRNA genes, such as a knwon $tRNA^{Ser}_{UCG}$ gene which differs from *sup3-e* in only 3 base pairs of the boding sequence (J. Mao et al., Cell *21*, 509, 1980).

Repetitive sequences common to rDNA, chromosomal DNA and poly(A)-containing RNA of *Physarum polycephalum*

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The ribosomal genes of *Physarum polycephalum* are located on extrachromosomal, linear and palindromic molecules of 60 kb length and are transcribed into large precursor molecules of about 12 kb. Various hybridization experiments with cloned rDNA fragments, originating from the external transcribed spacer, the internal transcribed spacer and the region of the introns of the 26S gene, show that they contain at least 3 different classes of middle repetitive sequences which are also present on the chromosomal DNA. One of these sequences is present twice per half palindrome: one copy is located near the 5'-start of the primary transcript and it is repeated a second time at a site about 2 kb beyond the 3'-end of the 26S gene. The same sequence is also found in poly(A)-containing RNA isolated from whole plasmodia.

Stimulation of hepatic lipogenesis by insulin and vasopressin

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The effects of vasopressin and insulin on short term regulation of lipogenesis have been studied in isolated hepatocytes from fed rats. The 2 hormones increase fatty acid synthesis by 40-100% in a dose-dependent manner. A significant increase in fatty acid synthesis is measurable 20 min after addition of either hormone. When lactate and pyruvate are added to the incubation medium the effect of vasopressin and insulin are abolished suggesting that these hormones act at least partly before or at the level of pyruvate dehydrogenase. Vasopressin also stimulates acetyl CoA carboxylase, considered as the rate limiting step in fatty acid synthesis. The action of vasopressin on the enzyme is still measurable after freezing of the hepatocytes whereas the reported action of insulin is measurable only when the tissue is kept at room temperature (J. biol. Chem. *254*, 6644, 1979). Vasopressin does not antagonize the inhibitory action of glucagon on lipogenesis whereas insulin does. These 2 observations suggest that insulin and vasopressin act on lipogenesis and on acetyl CoA carboxylase by different mechanisms.

Molecular cloning and structural analysis of 2 different size classes of 18S and 28S rDNA repeats of *Ascaris lumbricoides*

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DNA from oocytes and 10-day-old larvae of *A. lumbricoides* has been isolated, digested with different restriction enzymes and the resulting DNA fragments separated by agarose gel-electrophoresis. The 18S and 28S rDNA fragments were localized on the gels by Southern blot analysis. Since Bam HI cuts the rDNA repeat only once, whole rDNA repeats could be isolated and cloned in *Escherichia coli* HB 101, using pBR 322 as a vector. Restriction enzyme analysis demonstrated that the rDNA repeats of *A. lumbricoides* exist in at least 2 different forms, one containing an additional DNA insertion of about 400 bp in the spacer region. Moreover, these rDNA repeats show another restriction pattern within the 28S coding region.

Expression of a genomic rabbit β-globin gene is enhanced by a small segment of simian virus 40 DNA

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Various fragments of SV40 DNA were joined to a 4800 basepair DNA fragment containing the rabbit β-globin gene cloned in pBR322. These DNAs were transfected into human HeLa cells by a modified calcium phosphate technique. 2 days later, RNA from the whole cell population was extracted. S_1 mapping revealed about a 100-fold increase of globin gene transcripts after linkage to SV40 DNA. This was not due to readthough from an SV40 promoter, since 90% of the transcripts (about 1000 per cell) had the same 5'-end as authentic rabbit globin mRNA. Production of globin protein could be detected in a fraction of the transfected cells by immunofluorescent staining. This modulation of globin gene expression is a *cis*-acting effect. It is not dependent on expression of large or small SV40 tumor antigen, nor does it require a functional SV40 origin of replication. The viral modulator activity resides in a DNA segment near the origin of replication. This segment, inserted 1500 basepairs upstream of the globin gene, can act in either orientation.

The carcinogen N-acetoxy-acetylaminofluorene reacts preferentially with a control region of the intracellular simian virus 40 chromosome

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We have investigated the binding of the ultimate carcinogen N-acetoxy-acetylaminofluorene (AAAF) to specific regions of the SV40 chromosome in situ in the intact infected cell. SV40-infected cells late in the lytic cycle were incubated with ^{3}H-AAAF. SV40 DNA was extracted, digested with restriction enzymes HaeIII and KpnI, and radioactivity in each DNA fragment determined. The results indicated that a stretch of DNA near the origin of replication of the intracellular SV40 chromosome was more susceptible to attack by AAAF than the rest of the SV40 genome. When naked DNA was labeled with ^{3}H-AAAF in vitro no hyperreactive region was seen. The hyperreactive region may represent a stretch of DNA which is nucleosome-free or has another unusual chromosomal structure.

Histone genes injected into *Xenopus* eggs persist and are expressed during early development

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With the intent of designing an in vivo assay system for studying developmental regulation, fertilized eggs of *Xenopus laevis* were injected with a cloned repeat of sea urchin histone genes. The injected DNA sequences persist at least up to the swimming tadpole stage of development and, during the early stages of development, some of the injected genes are faithfully transcribed into RNA species with the correct 5′- and 3′-termini. It would be best to study developmental regulation in a homologous system. I am presently cloning *Xenopus* H1 and H5 histone genes for this purpose.

Calcium binding proteins from rat brain and muscle

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Many functions of calcium ions are mediated by specific receptor proteins. One of them, calmodulin, is present in most nucleated cells, regulating some forms of motility and modulating important metabolic pathways. In contrast, distribution and function(s) of parvalbumin, a calcium receptor protein structurally related to calmodulin, are unknown. In an attempt to study its distribution, parvalbumin was isolated from rat leg muscles. The heat stable protein had 2 Ca^{2+} binding sites and a characteristic amino-acid composition (no trp and tyr; 8 phe). Immunoglobulins (IgG) were isolated from antisera induced in rabbits and linked to CNBr-activated sepharose. Extracts from total brain were applied to columns and specifically bound proteins eluted with 3 M KI. These fractions contained proteins with pI and Mr similar to parvalbumin (2 D O'Farrell gels).

Inhibition of lymphoblastogenesis by pregnancy-associated plasma protein-A (PAPP-A)

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PAPP-A is a recently described pregnancy protein of decidual origin. Its possible role as immunomodulator during human pregnancy was examined using the phytohemagglutinin (PHA) induced lymphocyte transformation test. As a source of PAPP-A, pregnancy plasma and pure protein were used. Pregnancy plasma or pure PAPP-A was not cytotoxic to lymphocytes but markedly inhibited their transformation into lymphoblasts when stimulated with PHA. This inhibition is present even if PAPP-A preincubated with the cells before PHA is added. PAPP-A incubated with prestimulated cells in absence of PHA, inhibits also their transformation. According to our results, PAPP-A is thus immunosuppressive. It inhibits lymphocyte transformation probably by action on the secretory products of the transformed lymphocytes.

Radioreceptor assay for muscarinic agonists using ^{3}H-cis-methyldioxolane

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Muscarinic agonists are difficult to characterize in radioreceptor assays which use antagonists as radioligands because they only weakly inhibit the binding of antagonists and show inhibition profiles which deviate from the law of mass action. To overcome these difficulties we developed a radioreceptor assay using the muscarinic agonist ^{3}H-cis-methyldioxolane (^{3}H-MD, NEN 36 Ci/mmole) with membranes from rat cerebral cortex. Bound and free ligand were separated by centrifugation. Binding of ^{3}H-MD was saturable, but highly dependent on the buffer used. The values of K_D and B_{max} in K,Na phosphate, HEPES and tricine buffers were 4.3 nM and 226 fmoles/mg protein, 2.32 nM and 375 fmoles/mg protein and 2.7 nM and 600 fmoles/mg protein respectively. IC_{50} values for muscarinic agonists, measured at 1 nM ^{3}H-MD, were between 1 and 20 nM, as compared to 1000 and 50,000 nM in ^{3}H-QNB binding. Antagonists showed similar IC_{50}'s in ^{3}H-MD and ^{3}H-QNB binding. The ^{3}H-MD radioreceptor assay appears to be a useful tool to test muscarinic agonists.

Nonhistone proteins in mouse teratocarcinomas

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Mouse teratocarcinomas with restricted capacities for differentiation are especially suitable to investigate at the molecular level the developmental processes from a stem cell to a particular cellular phenotype. The gradual acquisition of cells for specialized functions is presumably achieved by differential regulation of gene activities at the chromosomal level. There is convincing evidence that nonhistone proteins (NHPs) are involved in this process. We have isolated them from 5 unidifferentiated teratocarcinomas as well as from a multidifferentiated and an undifferentiated type. Separation of the NHPs by differential salt extraction and hydroxyapatite chromatography gives 3 fractions: tris-soluble 0.35 M NaCl-extractable, and 2 M NaCl/5 M urea-extractable NHPs. Their comparison by gel-electrophoresis reveals that there are differentiation-specific NHP-patterns which are characteristic for a special cellular phenotype. We intend to test the biological activity and specificity of the NHP-fractions via microinjection into teratocarcinoma cells.

Effect of rat hypothalamic extract administration on insulin secretion in vivo

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Extracts from both the ventromedial and the ventrolateral hypothalamus from normal rats have an insulin secretion

promoting activity when, injected in vivo to recipient normal rats: the insulin levels are increased, within 2 min, by 2.56±0.40 and 1.84±0.27 ng/ml respectively. A partial purification of these extracts reveals that the factor(s) responsible for this effect are low molecular weight compounds (i.e. smaller than 1200 mol.wt). Catecholamines, acetylcholine and enkephalines seem excluded as potential candidates as pretreatment of recipient rats with the specific blockers (phentolamine, propranolol, atropine and naloxone) cannot prevent the increase in insulin plasma levels observed after the injection of lateral hypothalamic extracts. However, atropine and propranolol can partially reduce the rise in plasma insulin levels that follows the injection of ventromedial hypothalamic extracts. The presence of hypothalamic factor(s) possibly involved in the physiological regulation of insulin secretion, and that retains at least part of its insulin promoting properties in vivo, is therefore suggested.

Large molecular weight RNA transcripts with immunoglobulin V-gene sequences

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It is believed that mouse kappa L-chain genes are expressed into RNA transcripts which extend from the 5′-end of the V-gene to the 3′-end of the C-gene, covering therefore a large J-C intron. The length of these transcripts varies from 4.1 to 5.3 kb depending on which of the 4 J segments is expressed. Polyadenylated RNA from mouse myeloma tissue was fractionated by electrophoresis and probed for the presence of immunoglobulin L-chain sequences using Northern blot hybridization. Specific probes for the C- and the V-region expressed in this myeloma were used. This myeloma has both of its C-genes rearranged. Large mol.wt RNA was detected (of about 9 kb) corresponding to RNA transcripts clearly greater in size than the expected precursor. These large RNA transcripts were identified with a specific probe for the V-region, which implies that they contain sequences complementary to the expressed V-gene. These transcripts may result from the read through of normal termination signals of L-chain genes. Alternatively, these molecules could result from initiation of transcription upstream from the normal promoter, possibly at promoters of neighboring V-genes.

Development of oligodendrocytes in mouse brain cell cultures

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Both galactocerebroside and myelin basic protein are markers for oligodendrocytes (Raff et al., Nature *274*, 813, 1978; Sternberger et al., Proc. nat. Acad. Sci. USA *75*, 2521, 1978). We studied the expression of these 2 markers by immunocytochemical methods during the development of oligodendrocytes in fetal and newborn mouse brain cell cultures. Results showed that the early stage of development of oligodendrocytes in culture is characterized by the presence of galactocerebroside only, while oligodendrocytes which are more differenciated express both galactocerebroside and myelin basic protein.

Preferentially micrococcal nuclease-sensitive polynucleosomes are repeats of heterotypic core histone tetramers

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Conditions are described under which the putative transcriptionally active chromatin fraction, derived by brief micrococcal nuclease digestion of mouse nuclei shows a cross-linking pattern characteristic of half nucleosomes. It is demonstrated, using methyl 4-mercaptobutyrimidate cross-linking, that the polynucleosome chains of this chromatin fraction are repeats of heterotypic, H2A-H2B-H3-H4, core histone 4-mers as opposed to the H1-(H2A-H2B-H3-H4)$_2$ histone 9-mer found previously as the building block of bulk chromatin polynucleosomes (T.L. Reudelhuber, T. Boulikas and W.T. Garrard, J. biol. Chem. *255*, 4511, 1980).

Isolation and characterization of mouse α-amylase genes

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The genes encoding pancreas, salivary gland and liver α-amylases of mouse have been isolated by molecular cloning in phage λ. The structure of these genes was elucidated by a combination of restriction enzyme analysis, electron microscopy and direct DNA sequencing. The gene which is expressed in the pancreas has a size of 13.5 kb and is interrupted by at least 9 introns of variable sizes. The DNA sequence which is transcribed in the salivary gland has a size of 23 kb and contains 10 introns. The DNA sequence which is expressed in the liver contains 10 introns and has a size of 18.5 kb. The liver and salivary gland sequences are identical except for the first exon and part of the first intron. Introns occur at analogous positions in the pancreas and the salivary gland/liver genes while their size can vary considerably. No rearrangement of α-amylase has been found in various mouse tissues examined.

A single DNA sequence specifies 2 distinct α-amylase mRNAs in different mouse tissues

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The major α-amylase mRNAs which accumulate in the mouse salivary gland and liver are identical, except for their 5′ nontranslated sequences. Genomic DNA which specifies the 5′ terminal one-quarter of these mRNAs has been cloned; these DNA sequences occur only once in the mouse haploid genome. Sequence analysis of this cloned DNA and of the mRNA 5′-ends demonstrates that different 5′ leader sequences are spliced tissue-specifically onto the same mRNA body.

Heteroduplexes comparison between different *Xenopus* species of ribosomal gene fragments containing the origin of transcription

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The EcoR1 fragments from *Xenopus laevis, borealis, clivii, bunyoniensis, tropicalis* ribosomal genes containing the start

of transcription have been cloned in pBR 313 and 322 plasmid vectors. The fragments were isolated from their vector and hybridized in pairs. The 28S and the 18S sequences present at both ends of the fragments as well as sequences around the 40S start were seen as duplexes in the electron microscope. Moreover the *X. laevis* 'Bam islands' which are reduplications of the 40S start were found to hybridize to some sequences in the nontranscribed spacer of the other species. Computer comparison of the known sequences from *X. laevis* (T. Moss et al., NAR *8*, 1980), *borealis* and *clivii* show that the hybridizing zones observed correspond to partly homologous sequences. The functional and/or evolutional significance of the conservation, in the nontranscribed spacer, of some sequences homologuous to the 40S start can be discussed.

Untranslated sequences of genomic and intracellular Rous Sarcoma Virus RNAs

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We have determined the nucleotide sequence of the untranslated region preceding the 5'-end proximal gene ('gag') of Rous Sarcoma Virus (RSV) 35S genomic RNA. The relatively long leader sequence (370 nucleotides) possesses unique features that suggest a novel mechanism for the initiation of the 'gag' gene translation. S_1 nuclease mapping using DNA fragments complementary to the leader of RSV genomic RNA indicate that this sequence is conserved in the crude 35S intracellular RSV polysomal RNA. The purification of the various intracellular RSV specific RNA using affinity chromatography and the characterization of the 35S species will be described. The complete nucleotide sequence of the junction between the gene 'gag' and 'pol' has also been determined and its implication for the existence of a separate mRNA for the 'pol' gene will be discussed. An analysis by UV irradiation of RSV indicates that the intercistronic region 'gag'-'pol' interacts with the virion protein p 19.

Stretch mimics vasopressin-induced membrane modifications in freeze-fractured toad urinary bladder

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The vasopressin-induced permeability increase of the toad bladder is accompanied by modifications of the luminal plasma membrane of the granular cells. These include exo- and endocytosis and the appearance of aggregates of intramembrane particles. We now report that simply stretching the bladder induces morphological changes reminiscent of those induced by vasopressin, although its permeability to water is low in the absence of the hormone (Kachadorian and Levine, 1979). Granular cells from stretched bladders have many sites of exocytosis (1.8 vs 0.1 per μm^2 on nonstretched bladders) and they loose 50% of their granules during a 5-min stretch prior to fixation. Furthermore, typical aggregates of intramembrane particles are frequently found in the luminal plasma membrane, as well as numerous looser clusters of particles, often in shallow depressions on the membrane. These stretch-induced events may help to understand how membrane structural changes are involved in the permeability increase of vasopressin-sensitive epithelia.

Nucleosome phasing on tRNA genes of *Xenopus laevis*

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We have mapped the positioning of nucleosomes on a cluster of 8 tRNA genes from *Xenopus laevis*. This cluster is 3.2 kb in length and is repeated about 100 times per haploid genome. By mapping distances from known restriction enzyme sites to micrococcal nuclease cutting sites (micrococcal nuclease cuts preferentially in nucleosome spacer regions) we have been able to determine the position of nucleosomes throughout this DNA repeat. Nucleosomes are phased in nuclei from both mature erythrocytes and cultured kidney cells, although they occupy a different set of positions in each tissue. Since the specific position of nucleosomes with respect to a tRNA gene probably influences the binding of regulatory molecules to control regions of DNA, the different phases of nucleosomes may be partly responsible for differences in transcriptional activity.

Cloned mouse mammary tumor virus DNA is biologically active in transfected mouse cells and its expression is stimulated by glucocorticoid hormones

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We have cloned circular unintegrated mouse mammary tumor virus (MMTV) DNA from infected rat hepatoma cells in bacteriophage lambda. 7 independent clones containing MMTV DNA of homogeneous length of 9 kb (5) or 10 kb (2) were identified. The 5 9-kb clones had identical restriction maps consistent with that of 9 kb unintegrated DNA, the other 2 were aberrant. MMTV DNA inserts were purified, ligated and used for cotransfection of Ltk^- cells together with a plasmid containing the thymidine kinase gene of herpes simplex virus. All Tk^+ cell clones acquired new MMTV sequences and those transfected with the 9 kb MMTV DNA synthesized normal viral RNA and proteins. Viral gene expression was increased by the addition of dexamethasone.

Agonist and antagonist binding to the muscarinic cholinergic receptor is modulated by guanine nucleotides

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The ability of guanine nucleotide to decrease the binding affinity of agonists but not antagonists has been well-documented in a number of hormone and neurotransmitter receptor systems. We now present evidence for a novel form of modulation of receptor binding by guanine nucleotides. For the muscarinic cholinergic receptor ((−)[^{3}H]quinuclidinyl benzylate binding sites) in membrane preparations of frog and rat heart and rat brain both agonist and antagonist binding appear to be regulated in a reciprocal fashion by guanine nucleotides. 2 forms of the receptor appear to be present in comparable proportions: one displaying high agonist-low antagonist affinites and the other low agonist-high antagonist affinities. GTP and Gpp(NH)p convert the former type of sites into the latter. Conventional 'normalized' plots of antagonist competition curves tend to mask the findings. A nonlinear least squares computer curve fitting method according to mass action law has been applied to determine the binding parameters.

Organization of mouse immunoglobulin V-genes studied with recombinant cosmids

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The diversity of antibodies relies to a great extent on the existence of a large number of V-genes in the genome. On the basis of sequence homology, the genes coding for the different V-regions can be assigned to distinct 'families'. The study of the chromosomal organization of these V-genes will determine if related V-genes are physically grouped and may contribute to an understanding of the expansion and diversification of V-genes. In order to study large segments of the mouse genome, we have cloned partial EcoR1 digest of embryo DNA in a cosmid vector. The V-genes studied will therefore reflect the germline organization. 12 clones hybridizing to a mouse L-chain V-gene probe were obtained and found to contain inserts of 35 to 40 kb. As expected, no L-chain C-gene was identified in these clones. By analysis with restriction enzymes, 3 clones overlap to a great extent and 3 other overlap over only a short segment. These clones correspond therefore to about 250-300 kb of mouse DNA. 9 distinct V-genes, all of the same 'family', were identified in these clones by Southern blotting. These different V-genes give a range of signal strengths in these hybridizations that is compatible with the various degree of sequence homology expected within a V-gene 'family'.

Monoclonal antibodies against renin

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The immunohistochemical localization of the enzyme renin in extrarenal tissue has been hampered by the impurity of the antigens used to raise antisera. Because a complete purification is virtually impossible, we used hybridoma techniques which enables to sort out a peculiar antibody secreting cell from a population expressing various specificities. We used this method to raise monoclonal antibodies against partially purified mouse submaxillary gland renin. Mouse FO-myeloma cells were fused with spleen cells of hyperimmune mice or rats and distributed in microtiter plates. Hybrid supernatants were tested by enzyme-linked immunoassay or solid phase radioimmunoassay. Positive hybrids were cloned 2 times and the supernatants were tested on kidney tissue sections using immunohistochemical methods. Supernatants of selected, antibody secreting cultures reactive in all 3 tests were characterized immunochemically and in an enzyme-inhibition test. 3 clones secreting IgG antibodies which recognize renin in the kidney, were choosen for immunohistochemical localization of renin in the brain. Preliminary observations confirm an intraneuronal storage of renin.

Coexistence of angiotensin II and renin immunoreactivities in the epithelioid granular cells of the juxtaglomerular apparatus

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The effects of angiotensin II (AII) are generally supposed to be mediated by systemic, blood-borne, peptide. The intrarenal regulatory functions may however be performed by AII synthesized locally in the region of the juxtaglomerular apparatus. In this immunohistochemical study we have detected the site of intrarenal production of AII. Rats were perfused with fixative and paraffin sections of the kidney were processed with a palette of antisera against renin and angiotensins. Renin immunoreactivity was observed in the juxtaglomerular granular cells (JG cells) in the media of the afferent vessel of the glomerulus. AII immunoreactivity was found to coexist within the same cells. These results suggest that renin produces AII intracellularly in the JG-cells. AII may then be released concomitantly with renin in the lumen of the afferent arteriole or in the interstitial space. The 'paracrine' secretion of AII could exert a local regulatory influence on the tonus of the glomerular vessels and on the Na^+-transport in kidney tubules.

Myosin isoenzymes in rat muscle spindles

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We have studied the myosin isoenzyme composition of intrafusal muscle fibres in the rat extensor digitorum longus using enzyme-histochemical and immunohistochemical methods. Serial cryostat sections were either stained for myofibrillar ATP-ase activity or reacted with antisera against fast myosin (FM), slow myosin (SM) and the light chains of heart myosin (HCl), the latter known to cross-react with the light chain of SM. Nuclear chain fibres only stained with anti-FM and their ATP-ase activity was not affected by alcaline preincubation. Nuclear bag-1 fibres reacted with anti-SM and anti-HCL and showed an acid-stabile, alcali-labile ATP-ase activity. Sometime FM and SM/HCL immunoreactivities coexisted segmentally in the same bag-1 fibre. Nuclear bag-2 fibres only reacted with anti-SM and anti-HCL, although exhibiting a strong alcali-stabile ATP-ase activity, otherwise characteristic for FM containing muscle fibres. This apparent contradiction could be explained by the presence of a special isoform of myosin.

Biochemical and biological features of the actin depolymerizing factor present in human plasma

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An actin depolymerizing factor (ADF) has been purified from human serum by ammonium sulphate fractionation, DEAE-cellulose and blue sepharose chromatography. The activity of ADF has been tested on F-actin in vitro, by preincubation on fixed cultured cells and cryostat tissue sections before immunofluorescent staining with an antiactin antibody. ADF is a single polypeptide of about 90,000 mol.wt with a pI between 6.0 and 6.5. It is acting very rapidly and there is no change in actin mobility on SDS-PAGE after incubation of F-actin with ADF. ADF is heat- and trypsin-sensitive, not stained by PAS on a SDS-PAGE, not retained on a Con A-sepharose column and inactivated by EGTA. An antibody against ADF has been raised in rabbits. As suggested by immunofluorescence and immunotransfer techniques, white blood cells and platelets appear to contain ADF. ADF may play a role in the organization of cellular actin and could be useful for studying the stability of actin filaments in various physiologic and pathologic processes.

Newly isolated Friend cells display a differentiated state similar to that of long-established clones

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We reported previously (J.-F. Conscience and W. Meier, Exp. Cell Res. *125*, 111, 1980) that different clones of Friend erythroleukemia cells (FLC) express similar levels of differentiation prior to treatment with an inducer. All the clones tested, however, had been in continuous culture for several years, so that their constitutive properties might simply reflect a certain, stable, epigenetic state that prevails in long-term culture. To resolve this problem, we isolated 13 new cell lines from the hemopoietic organs of DBA/2 mice infected with Friend virus (FV). As soon as technically feasible, these lines were analyzed immunocytochemically for carbonic anhydrase (CA) II and spectrin, as well as biochemically for hemoglobin and enzymatically for CA, acetylcholinesterase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase and catalase. All 13 cell lines exhibited levels of these markers which were similar to those detected in the 'old' FLC. Thus, the differentiated state of these cells most probably reflects that of the cell type which outgrows in vivo during FV-induced tumorigenesis, rather than that of a cell type selected in long-term culture.

Preservation of DNA-plasms in dinoflagellates: influence of preparation techniques

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Preparative procedures modify differently the ultrastructural pattern of DNA-plasms in eucaryotes and procaryotes: these differences may be related to DNA itself (presence or absence of a nucleosome-like protection) or to its proteinic partners (histones or other basic proteins). *Prorocentrum micans* E. is a primitive free-living dinoflagellate: recent analysis has shown that its chromatin lacks nucleosomes but contains low amount of basic proteins (mol.wt 12,000 and 13,000, Herzog and Soyer, 1980): it is therefore a specially convenient model for comparative studies of the fine structure of protocaryotic DNA-fibrils by use of different preparation procedures. Preliminary results indicate that low denaturing Lowicryl embedding, after pure aldehyde fixation, prevents the DNA-fibrils collapse generally observed in case of usual embedding procedure.

A differentiation marker of mouse bone marrow cells, defined by a rat monoclonal antibody

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In an attempt to identify new hemopoietic differentiation markers, monoclonal antibodies have been raised against mouse bone marrow. One of these antibodies recognizes an antigen present on about 50% of bone marrow cells. Whilst it is most strongly represented on these cells, it can also be detected on subpopulations of spleen and thymus cells. The distribution of this antigen has been investigated in terms of the mature, functional cells and the progenitor cells on which it is present. Initial studies suggest that this antigen may be useful for defining subpopulations of precursor cells of various hemopoietic lineages.

A new and simple technique for the isolation of large numbers of hybridoma clones for monoclonal antibody production

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We have developed a new approach for isolating hybridoma clones for the production of monoclonal antibodies. The method involves direct cloning of the fusion mixture in a semi-solid medium containing H.A.T. It is simple and largely obviates the need for repeated recloning of hybrids, as well as the constant feeding of cells during cloning. The technique also makes it possible to obtain and handle with ease large numbers of hybridoma clones from a single fusion. This means that it may be particularly useful for investigators attempting to produce monoclonal antibodies against a wide range of antigens present in an impure immunogen, including those antigens which may only be poorly represented in the immunogen. This approach has been successfully used to obtain monoclonal antibodies against differentiation antigens present in mouse bone marrow.

A polyoma vector system for the expression of cloned genes in vivo

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The efficiency of polyoma virus DNA in enhancing the expression of a genomic rabbit β-globin gene was compared to that of simian virus 40 DNA (cf. Banerji, Rusconi and Schaffner, these abstracts). The results can be summarized as follows: 1. The stimulation of the globin gene expression in mouse cells is not dependent on polyoma DNA replication nor on the expression of large tumor antigen, similar to the findings with SV40 DNA. 2. The globin gene can be inserted into the polyoma 'early' or into the 'late' region, in either orientation. In all cases transcripts which initiate at the globin gene cap-site are found. 3. Polyoma DNA enhances globin gene expression only in mouse (3T3, 3T6) cells but not in human (HeLa) cells. SV40 DNA on the other hand enhances globin gene expression in monkey (CV-1), human (HeLa) and mouse (3T3, 3T6) cells but not in cells of *Xenopus laevis* embryos. We note that this 'host range' for the stimulation of globin gene expression seems to parallel the host range of the 2 viruses in inducing tumorigenic cell transformation.

Ultrastructural study of corticotectal neurons in cat visual cortex

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Corticotectal neurons have been investigated either by the rapid Golgi method or by retrograde transport of horseradish peroxidase (HRP). The neuron is morphologically defined by light microscopy and the same cell embedded for ultrastructural investigation. HRP-labeled neurons are reconstructed from ultrathin serial sections by means of a computer. The spatial distribution of axodendritic and axospinous synapses is calculated at several distances from the cell body. Axosomatic synapses are also plotted. These ultrastructural features are compared to those of other layer V pyramids to determine whether corticotectal neurons show specific structural features.

DNA sequences preceding the rabbit β-globin gene required for the formation of β-globin RNA with the correct 5′-terminus

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Mouse TK^- L-cells were transformed with cloned herpes simplex virus I TK DNA linked to rabbit β-globin DNA, in which the globin gene was preceded by a flanking sequence of 14–1500 nucleotides. Selection for TK^+ cells led to lines producing 5–1500 globin mRNA strands per cell. The 5′-termini mapped (a) to the 'cap' nucleotide, (b) to positions 42–48 nucleotides downstream or (c) to a position in the vector DNA. With only 14 bp of 5′-flanking sequence high levels of globin RNA were produced, however no transcripts had the correct 5′-end; most originated in the vector. With 66 bp of 5′-sequence, 5%, and with 76 bp or more 30–85% of the 5′-termini were correct. The region between 14 and 66 bp, comprising the Hogness box, is essential for correct initiation. The region between 66 and 76 bp before the cap site comprises a variant of the sequence $GG^C_TCAATCT$ (Benoist et al., NAR *8*, 127, 1980), and may modulate the efficiency of transcription.

Production of a specific antiserum to choline acetyltransferase and immunohistochemical localization of the enzyme

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Characterization of neurones with respect to the neurotransmitter they synthesize is of central importance in neurobiology. One way to solve this problem is to demonstrate with immunohistochemical methods - involving the use of a specific antiserum - the presence of a specific transmitter synthesizing enzyme within a neuron. We report here the production of a specific antiserum to choline acetyltransferase, the transmitter synthesizing enzyme of cholinergic neurones. The enzyme was purified from pig brain to a final specific activity of 135 μmoles acetylcholine · min^{-1} · mg^{-1} protein, and subjected to electrophoresis in the presence of SDS. The band corresponding to choline acetyltransferase was used for immunization. The antiserum shows no precipitin line when tested against brain homogenates (this is because there is too little enzyme present in crude homogenates), but a single line was seen against partially purified ChAT. This antiserum was used to demonstrate histochemically cholinergic cells in the nervous system of the rat.

Studies on the yeast movable element Ty1

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The movable element Ty1 was discovered in *Saccharomyces cerevisiae* by Cameron, Loh and Davis (Cell *16*, 739, 1979). Sequence studies of its target sites and terminal repetitions revealed similarities to procaryotic and other eucaryotic elements. The use of the Ty1 element for transposition mutagenesis is presently examined. Hybridization experiments showed that Ty1 specific sequences are present with varying copy numbers in *Saccharomyces* species but not in other yeast genera. Sequence studies of 2 tandem inverted repeats found in *S. cerevisiae* reveals a plausible mechanism for their generation including Ty1 transposition and excision.

Effect of Ambroxol on pulmonary surfactant production

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Ambroxol, metabolite VIII of Bisolvon was administered to adult rats (per os, at a dose of 200 mg/kg/day, during 3 days) and the incorporation of ^3H palmitate into lung and surfactant phospholipids was examined by means of a) autoradiography of lungs and b) biochemical analysis of alveolar lavages. Autoradiography showed a significant increase in grain density over type II epithelial cells, particularly over lamellated bodies in animals treated with Ambroxol when compared to controls. For alveolar lavages a significant difference was found between Ambroxol-treated and control rats in the radioactivity of total lipids, of total and separated phospholipids. Our findings indicate that administration of Ambroxol is followed by an increase in surfactant production in rats.

The significance of the cAMP-dependent protein kinase system as mediator during prolactin release in GH3 cells

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The activation of cAMP-dependent protein kinases in GH3 cells has been demonstrated under a variety of conditions. The activity ratio of the cAMP-dependent protein kinases is an accurate measure of cAMP-mediated events. Kinetic studies revealed that TRH or its analogue Methyl-TRH (5 nM to 5 μM) exhibited no significant effect on the protein kinase activity ratio. Similar results were obtained using the Ca^{+2} ionophore A23187 and chloropromazine, a dopamine antagonist. Both compounds are able to stimulate the rPRL-release in GH3 cells. - In contrast to these observations it was found that choleratoxin and DBcAMP exhibited a time and dose-dependent activation of the protein kinases with a subsequent stimulation of the rPRL-release. At maximum doses DBcAMP (1 mM) and choleratoxin (10^{-8} M) the cAMP-dependent protein kinases were fully activated after 5 sec and 30 min respectively. The cAMP-response clearly preceded the rPRL-release response. - These results give strong evidence that 2 independent mechanisms for the rPRL-release do exist which seem to influence the Ca^{+2} fluxes across the plasma membranes of GH3 cells by different ways.

Visualization of a transcriptionally active chromatin fraction by spreading

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We have developed a modification of the Miller spreading technique which allows visualization of a chromatin fraction enriched in transcribing regions (Wurtz, unpublished). We find individual transcription complexes of nonnucleolar type exhibiting lateral RNP chains composed of granules irregular in size and shape. - The morphology of the chromatin fibres is heterogeneous, regardless of the presence of transcription complexes: a) Smooth fibres as well as beaded fibres are observed. b) In beaded fibres the distance between nucleosomes can be variable. c) The nucleosomes can appear heterogeneous in size and shape. - In beaded fibres nucleosomes are often observed on both sides of the transcription complex. Using this technique we plan to study structural features of transcribing chromatin after exposure to different chemical conditions in vitro.

DNA sequence analysis of the large terminal repeat of mouse mammary tumor virus (MMTV) DNA

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Large terminal repeats (LTR) of MMTV DNA might play an important role in the integration of viral DNA into the host genome. The restriction enzyme fragment containing the LTR might also harbor the sequences necessary for the glucocorticoid sensitivity of viral gene expression. Therefore we decided to sequence this 1.5-kb fragment derived from circular MMTV DNA cloned in phage. For this purpose, we subcloned all of the Pst I fragments in the plasmid pBR 322 prior to sequence analysis.

Interactions of human tumor cell aggregates with normal human tissue

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The morphological events occurring after confrontation of human amnion with aggregates of human tongue squamous cell carcinoma under in vitro conditions have been studied by means of electron microscopy. In a first stage the spherical tumor aggregates establish contact to the epithelial layer of the amnion. Immediately beneath the aggregates, the epithelial cells appear slightly retracted. At the edges of the aggregates some tumor cells have inserted processes into fissures, that open up as intercellular junctions of epithelial cells were disintegrated. In a later stage the tumor cells still remain in spherical aggregates. Some of the lower tumor cells have squeezed through between epithelial cells and make contacts to the basement membrane. In such areas the basement membrane appears thin and defective. These preliminary results show, that this organ culture model allows morphological analysis of the various steps of human tumor cell invasion.

Topographical distribution of (Na^+, K^+)-ATPase on various amphibian cells

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The topographical distribution and the stoichiometric relationship of (Na^+, K^+)-ATPase subunits were investigated in various toad cells using specific antisera reacting with each subunit. Surface exposed sites of the enzymes were revealed using a 3-stage immunocytochemical method. Viable cells were incubated with rabbit antisubunit sera, then with biotinylated sheep IgG antirabbit IgG, and finally with avidin coupled to FITC, TRITC or colloidal gold. The distribution of the label was homogenous on nonpolarized cells (erythrocytes, leucocytes) and restricted to one pole of polarized cells (toad bladder [TBM] and A6 cell lines from *Bufo marinus* and *Xenopus laevis* respectively.) Sites were also revealed on the cytoplasmic face of these cells suggesting a transmembrane orientation of both subunits.

Comparison of nucleotide sequences of mouse V kappa genes indicates a highly permissive pattern of sequence divergence

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More or less pressure is exercised on structural genes during evolution depending on how the absolute integrity of the amino acid sequence affects function. A stringent requirement for structure conservation manifests itself in a bias against replacement mutations and towards a relative accumulation of silent mutations. The Ig V-genes represent an interesting case where one might expect less pressure for the conservation of amino acid sequence. We have determined the nucleotide sequence of an expressed kappa V-gene and compared it with the 2 other sequences published for expressed kappa genes. The number of replacement and silent substitutions observed were compared with the possible substitutions in each category. The ratio of these figures ('permissivity index') reflects the degree of bias against replacement mutations. The results indicate that V-gene divergence involves an unusually high permissivity. Compared with other genes with known DNA sequence, V-genes are 5 times more permissive than rat insulin genes and even more than the β-globin gene and pseudogene. The sequence comparison also shows that not only base substitution but also insertions/deletions account for V-gene diversity. It also suggests that only a very limited pressure is acting directly on the nucleotide sequence itself.

Cell growth stimulation by chemicals in a s.c. tissue of rats

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Growth kinetic of cells from the s.c. loose connective tissue in adult rats was investigated. Cell growth was initiated by the formation of a dorsal, s.c. air pocket (physical growth stimulation). The influence of known tumor promoters or compounds which stimulate cell proliferation in vitro was determined. 12-O-Tetradecanoylphorbol-13-acetate, vasopressin, putrescine and prostaglandin E_2 were injected into the pouch. The time-dependent interference of these chemicals with the physical growth stimulation was measured with the following method: Histopathological examination of the intact tissue, incorporation of ^{3}H-thymidine, plating and cloning efficiency in vitro of cells from dissected and dissociated tissues. - For all chemicals tested a dose-dependent stimulation of ^{3}H-thymidine incorporation was found. The ability of cells to grow in vitro (cloning efficiency) was not affected. However all test compounds enhanced the proliferation capacity (clone size) of cells compared to untreated cells. Labeling index were highest 24-48 h after pouch formation.

Xenobiotic-metabolizing enzymes in *Drosophila*

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In *Drosophila* like in mammals many compounds including indirect mutagens and carcinogens are metabolized by the enzymes of the mixed function oxydases (MFO). In microsomal preparations of *Drosophila* several key enzymes of the MFO could be demonstrated and were found to correspond to the analogous enzymes in mammals. This is particularly important with respect to the worldwide use of *Drosophila* as a test system for mutagenicity. For a long

time the presence of the MFO was only suspected based on the mutagenic activity of model compounds which need bioactivation. The recent biochemical studies improve the usefulness of *Drosophila* as a test organism for a wide spectrum of genetic damages because a) the relative activity of different MFO activities can be measured, b) strain differences can be detected and c), possibly, tester strains with particularly high MFO activities can be developed and will help to increase the detectability of indirect mutagens with low mutagenicity.

Transcriptional signals of a $tRNA^{leu}$ gene from *Xenopus laevis*

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We have used homologous in vitro transcription to examine the nucleotide sequences required for activity of a cloned *X. laevis* $tRNA^{leu}$ gene (Clarkson et al., Cell *14*, 713, 1978). We have compared the transcriptional properties of the wild type gene with different mutant genes. The mutants were generated by enzymatic resection from each end separately with Bal-31 (a double-strand exonuclease) and subsequent rejoining, which results in deletions, insertions and substitutions. We found that only sequence elements within the structural gene are required for transcriptional activity as it was previously shown for a *Xenopus* $tRNA^{Met}$ gene in homologous in vitro transcription systems and by injecting into frog oocytes.

Molecular cloning of the *Drosophila* homoeotic gene complex *Antennapedia*

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Mutations at homoeotic loci in *Drosophila* result in the replacement of one body region by another normal region. Thus in *Antennapedia* and *Nasobemia* the adult fly antennae are replaced by mesothoracic legs. These 2 mutations are closely grouped with others forming a cluster of homoeotic genes on chromosome 3R. We would like to characterize the *Antennapedia* complex at the molecular level, in particular asking if these are DNA sequences encoding proteins. Recombinant plasmids have been mapped by in situ hybridization near to this chromosomal region. From each an overlapping series of clones is being constructed which will allow either a direct 'walk' to the gene complex or a 'jump' there using an inversion with one breakpoint near the recombinant sequences and the other within the homoeotic complex.

A transneuronal influence on membrane morphology of Purkinje cells: a freeze-fracture study

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Climbing fibre deafferentiation of Purkinje cells was obtained in the rat after a selective destruction of the inferior olivary nucleus by 3-acetylpyridine (3-AP). Quantitative analysis of Purkinje cells plasma membrane reveals changes in the number and distribution of intramembranous particle (IMP) of the Purkinje cells dendrites, the target of climbing fibres. The main significative differences ($p < 0.001$) in 3-AP treated versus untreated rats were: a) a decrease in the IMP content in the E-face of dendritic trunks (241 ± 11 vs 375 ± 15 IMP/μm^2); b) an increase in the IMP content in the P-face of dendritic spines (983 ± 46 vs 682 ± 49); c) an increase in the percentage of dendrite P-faces with IMP aggregation (20% vs 6%). These results suggest a transsynaptic effect, mediated by climbing fibres, on Purkinje cells membrane organization.

Role of triiodothyronine (T_3)nuclear binding sites in the antimineralocorticoid action of thyroid hormones in the toad bladder

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In the urinary bladder of the toad *Bufo marinus*, T_3 inhibits the effect of aldosterone on Na^+ transport in vitro. In the same tissue, high affinity ($K_d = 57\pm19$ pM, $n=3$), low capacity ($N_{max} = 56$ fmoles/mg protein, $n=3$) nuclear binding sites for T_3 can be demonstrated by incubation of both intact tissue and nuclear extracts with hormone in physiological concentrations. Further studies show: 1. Aldosterone-dependent Na^+ transport measured in the intact bladder at 25 °C (by the short-circuit current technique) is inhibited in a dose-dependent manner by T_3 (0.06-6 nM). The dose-response curve correlates well with the occupation curve of the solubilized nuclear binding sites at 0 °C. 2. A good correlation exists between the biological potency of T_3 analogues such as isopropyldiiodothyronine (iso-T_2) and reverse T_3 and their respective ability to displace [^{125}I] T_3 from solubilized nuclear binding sites at 0°C (iso $T_2 \geq T_3 \gg$ rev T_3). We conclude that the T_3 nuclear binding sites can be considered as 'receptors' involved in the antimineralocorticoid action of thyroid hormones.

Estrogen-induced change in chromatin conformation of the vitellogenin genes in *Xenopus laevis* revealed by DNase I digestion

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The vitellogenin genes are preferentially digested by DNase I in hepatocytes stimulated by estrogen to express these genes. In contrast in unstimulated hepatocytes the vitellogenin genes are as insensitive to DNase I as in blood cells where vitellogenin synthesis cannot be induced. Apparently estrogen treatment alters the chromatin conformation of the vitellogenin genes to a state which is more accessible to DNase I. Analysis of the β1-globin gene revealed that this gene is preferentially digested in erythrocytes but is extremely resistant to DNase I in its inactive form in hepatocytes. Comparing the different DNase I sensitivity of the β-globin and the vitellogenin genes in their inactive state we conclude that at least 2 different chromatin conformation exist. Further experiments have revealed a tissue-specific pattern of DNase I hypersensitive regions in the β-globin and vitellogenin genes.

Biochemical comparison of CNS myelin between 2 myelin-deficient mutant mice

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In the brain of shiverer and *mld* mutant mice, myelin is poorly compacted and the major dense line of the myelin is practically missing. Major biochemical differences were detected between mutations. In *mld* myelin, myelin basic proteins are mainly affected and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) exhibits a very high specific activity. In shiverer myelin, in addition to basic proteins, all

major myelin proteins are also decreased while CNP specific activity is moderately increased. In spite of similar morphological alterations, the biochemical differences observed between the 2 mutants could be explained a) by different thresholds of expression of a single mutation in 2 different strains, b) by 2 different mutations occurring in a single genetic locus (alleles), and c) 2 unrelated mutations expressing a similar phenotype.

Molecular analysis of a large transposing element in *Drosophila*

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Several strains of *Drosophila melanogaster* have been isolated in which very large segments of DNA (up to an estimated 1000 kb) can transpose to many different chromosomal locations. We have been studying one such strain, *TE 98*, in which some 3 chromosomal bands (3C2-3C5(6)), containing the *white* and *roughest* genes, have transposed from their normal position on the X-chromosome to a new location on chromosome 3R, very near to a large heat induced puff at 87A7 encoding the major 70 kdalton heat shock protein. We have 'walked' down the chromosome from these 70 k hsp genes to the site of the transposon insertion and are currently studying the sequences located at the ends of this large transposing element. At least one of these ends is a composite of 2 different moderately repetitive gene families.

Structure of an aberrant rearrangement of an immunoglobulin light chain gene resulting in allelic exclusion

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Allelic exclusion refers to the observation that immunoglobulins (Ig) produced by lymphocytes or plasmocytes derive from only one of the 2 alleles. It has been proposed that the basis for allelic exclusion is the absence, in one of the chromosomes, of the V/C-gene rearrangement that is required for Ig gene expression. We have shown however that both chromosomes can undergo rearrangement and proposed that somehow one of these does not result in Ig production. Various types of such nonproductive rearrangements can indeed be envisaged. We have cloned and studied a 10.5 kb EcoR1 fragment from myeloma DNA which hybridizes to a L-chain C-gene probe. This fragment was found to be identical, over 9 kb, to the DNA immediately downstream from the mouse kappa C-gene. Nucleotide sequence analysis showed that the identity between the 2 fragments extended within the C gene but only within the 3′ untranslated region. The exact point of divergence between the rearranged and the nonrearranged genes was located 40 nucleotides 3′ from the termination codon. Although the origin of the 1.5 kb of DNA upstream from the rearrangement point is not clear, it contains stop codons in all 3 reading frames. This type of rearrangement is an example of a mechanism allowing one of the 2 C-gene alleles, in Ig producing tissue, to be made nonfunctional.

Structural studies of a β-globin gene cloned from a β° thallassemic

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β° thallassemics with no gross deletion and with no mRNA synthesis represent interesting transcriptional mutants. A 5.2 kb EcoR1 fragment containing the β-globin transcriptional unit (except for the 3′-end), as well as 3 kb of upstream sequence, has been cloned from both normal human DNA and a β° thallassemic. The latter was chosen because of the absence of globin mRNA and of detectable deletions by Southern blot analysis. The overall restriction map of the normal and thallassemic DNA fragments are identical. Heteroduplex analysis using S1 digestion also showed no differences. A fine restriction analysis revealed an additional Hinf site in the thallassemic DNA 1 kb upstream from the CATAA box. The nucleotide sequence of selected regions of the 2 clones were compared. No nucleotide differences were observed at the boundaries of the 2 introns and in the upstream sequence extending through the CATAA box and up to the CAAT box. This result indicates that the molecular basis for this β° thallassemia is either a defect in the termination of transcription or a change in a regulatory sequence located outside the transcriptional unit.

Complete nucleotide sequence of a 16S ribosomal RNA gene from *Euglena gracilis* chloroplasts

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The recombinant clone pEgcll carries the fragment BamHI · D which contains a set of rRNA genes from the *Euglena gracilis* chloroplast genome. In extension of earlier work (Graf, Kössel and Stutz, Nature *286*, 908, 1980) this clone was used for mapping and sequencing of the 16S rDNA according to the method of Maxam and Gilbert (PNAS *74*, 560, 1977). Extensive homology is observed between the primary structure of *E. gracilis* chloroplast, *Zea mays* chloroplast and *Escherichia coli* 16S rDNA. Screening of the sequences for possible hairpin structures confirms secondary structure models proposed for bacterial 16S rRNA (e.g. Glotz and Brimacombe, N.A.R. *8*, 2377, 1980) in which 4 major helical domains are postulated to exist. A 23 basepair deletion common to *Z. mays* and *E. gracilis* chloroplast rDNA may be considered as indication for their common divergence from the bacterial 16S rDNA.

Functional dissection of an H2A histone gene promoter

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Small deletions covering the complete 5′-flanking region of the H2A histone gene of the sea urchin *Psammechinus miliaris* were constructed in vitro in order to dissect the putative promoter region. These mutants were then tested functionally both in vivo by injection of the DNAs into centrifuged oocytes and in vitro using a HeLa cell extract. 3 promoter elements were identified in vivo which we have termed modulator, selector and initiator. The modulator element lying about 150–450 basepairs upstream of the H2A mRNA 5′-end is crucial for the expression of the H2A gene since manipulations in this region can alter the level

of H2A gene expression by a factor as much as 100. The mutants when tested in vitro gave similar results with the difference that deletion of the TATAAATA box does not give rise to a 5'-end heterogeneity of the H2A mRNA but abolishes initiation of transcription. The modulator element seems also to be important in vitro. We are narrowing down the DNA sequences causing the modulation of transcription.

Lactogenic hormone receptor from rabbit mammary gland: characterization of the binding site

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Binding of lactogenic hormones to membrane receptors is necessary for their biological activity, but the cellular events which allow them to exert their effect on gene expression are still unknow. In order to gain some insight in their mode of action we attempted to characterize the lactogenic hormone receptor. Plasma membranes from lactating mammary glands were solubilized with 0.5% TX-100. Scatchard analysis performed with eluates of soluble receptor from a sephadex G-200 column as well as the elution profile of a ^{125}I-hGH-receptor complex indicate heterogeneity of binding sites (Mr 100 to 400 KD). The heterobifunctional cross-linkers 2,4-dinitro-5-fluorophenyl-azide and ethyl-4-azidophenyl-1,3-dithiobutyrimidate. HCl were used to photolabel the binding site of the receptor with hGH. Upon SDS-PAGE this hormone-receptor complex was resolved as a 58-KD band. To confirm this data the soluble receptor was adsorbed to avidin-sepharose via biotinylated hGH, eluted, radiolabeled and analyzed by SDS-PAGE.

The effect of a heat shock on the differentiation of germ cells in *Drosophila*

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Early in embryogenesis the pole cells segregate from the somatic cells and become determined to form the germ line. Pole cells are characterized by intensive protein synthesis, while hardly any RNA synthesis can be detected. This protein synthesis occurs largely on maternal messengers which are being analyzed by cloning. In order to elucidate the role of the early protein synthesis in germ cell differentiation, a brief heat shock was applied to embryos before, during, and after pole cell formation. Heat shock is known to reversibly repress most of transcription and translation, and to activate only a small group of heat shock genes. Embryos heat treated at 35 °C for 30 min when pole cells have just formed, give rise to 35% of the adult flies lacking germ cells in their gonads. A heat shock of 37 °C for 10 min followed by transplantation of the pole cells to a nontreated embryo produces an 80% reduction in the percentage of transplants resulting in functional germ cells. These experiments suggest that the early intensive protein synthesis in pole cells is essential for their differentiation into germ cells.

Mouse B-globin and Adenovirus-2 major late transcripts are initiated at the cap site in vitro

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Initiation of transcription on DNA containing the cap site of the Adenovirus-2 major late region or of the mouse β-globin gene was studied using (β-^{32}P) ribonucleoside triphosphates as labeled precursor in vitro. The 5'-termini of discrete run-off transcripts of both templates were preferentially labeled. The β-phosphate of the cap structure was labeled by (β-^{32}P) ATP but not (β-^{32}P) GTP, indicating that the β-phosphate of the cap is contributed by the initiating nucleoside triphosphate (ATP in both cases). Therefore, capping occurs on the initial nucleotide of the transcripts.

Monoclonal antibodies to intestinal sucrase-isomaltase (SI) indicate synthesis of SI as a single-chain protein

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We use rat SI as a model for the study of membrane protein synthesis in the intestinal epithelial cell. Previous results have suggested that SI is synthesized as a precursor (P) which is cleaved into the sucrase and isomaltase subunit in the microvillus membrane (Hauri et al. PNAS *76*, 5183, 1979). Monoclonal antibodies to SI were produced by the hybridoma technique. One of these antibodies was specific for the sucrase subunit but also bound to P. This feature has enabled us to purify P by affinity chromatography from fetal intestinal transplants. In the transplants SI developed exclusively in the noncleaved P-form. Purified P was enzymatically active. Preliminary N-terminal analysis of P showed sequence homology with the N-terminus of isomaltase. Immunocytochemistry on intestinal cryosections provided evidence for an exclusive localization of SI in villus but not crypt cells. Conclusions: SI is synthesized as an enzymatically active single-chain protein that is not split into subunits in the absence of pancreatic proteases.

Characterization of a drug-induced membrane stabilization by ultrastructural and electrophysiological technique

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Electron microscopy has been used to help clarify the mechanism by which a test drug induces vesicles in rat lymphocytes. The vesicles observed at the light microscopic level were identified as myelin-like residual bodies at the ultrastructural level. Such structures are formed from drug-lipid complexes, and usually disappear upon drug withdrawal. Myelin-like residual bodies also formed in islets of Langerhans cultured in the presence of the drug, and most of them were still present 6 days after drug removal. In control cultures, the few residual bodies seen were found almost exclusively in the extracellular space. In contrast, cyproheptadine induced a completely reversible myelin-like residual body formation in the same system. Impaired lipid catabolism as a cause of residual body accumulation was ruled out by the presence of lysosomal acid phosphatase activity. The drug caused a reduction in the amplitude of action potentials in rabbit vagus nerve. This reduction was only partially reversible, suggesting that the drug has strong membrane stabilizing properties. It was concluded that the accumulation of myelin-like residual bodies is a result of reduced exocytosis.

DNA-binding proteins during in vivo differentiation of brain cortex and cerebellar neurons

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DNA-binding proteins of differentiating cortex and cerebellar neurons were investigated during normal development of the rat brain. Nuclei from various developmental stages were isolated, the proteins extracted with 0.6 M KCl, 0.1 M HEPES, pH 7.5, and ^{14}C-labeled by reductive methylation. After desalting, protein extracts were chromatographed sequentially on double- and single-stranded DNA immobilized on cellulose. After washing, the columns were uncoupled and the specifically bound proteins eluted with 2 M NaCl. Finally the ss-DNA and ds-DNA-binding proteins were analyzed on 2D-O'Farrell gels and visualized by fluorography. Several developmental changes were detected among the ss-DNA-binding proteins from cortex neurons affecting proteins of pI 6.5–8 and Mr 25,000–50,000. Similar fluctuations have been found earlier with the nonhistone chromosomal proteins (J. biol. Chem., in press). Both changes were temporally coincident with the arrest of cell division and the beginning of terminal differentiation.

Products of Balbiani ring genes of *Chironomus tentans* salivary glands

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Polytene nuclei of *Chironomus tentans* salivary gland cells contain some extraordinarily active chromosome regions, the Balbiani ring (BR) genes. The primary transcript of these genes is a large RNA with a mol.wt of $12–13 \times 10^6$. It was suggested that this RNA represents the mRNA for the secretion proteins (SP) the main product of the salivary gland. The proteins were extracted by an improved method and then characterized on acrylamid gradient gels. 2 of the 3 major protein species (SP I and SP II) have mol.wts in the range of 1×10^6 thus corresponding to the mol.wt calculated from the length of BR RNA. After experimentally induced depletion of the gland lumen by pilocarpine treatment of the larvae the synthesis of BR RNA as well as the synthesis of SP I and SP II was enhanced. This supports the assumption that the SP are coded for by BR RNA. Large amounts of BR RNA were obtained from pilocarpine stimulated glands and then used as a template in cell free in vitro translation experiments.

Influence of 5'-flanking sequences on tRNA transcription in vitro

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2 *Xenopus laevis* methionyl-tRNA genes are transcribed with very different efficiencies in a homologous cell-free system. The 2 genes differ at a single position intragenically and at several positions in their flanking regions. In vitro transcription of rearranged genes clearly demonstrates that the 5'-flanking regions are responsible for the different transcription efficiencies of the 2 genes. Deletion of most of the DNA 5' to the active gene has no effect on transcription efficiency whereas a comparable deletion of most of the DNA upstream of the relatively inactive gene results in a stimulation of transcription. We interpret this to mean that the sequences upstream of the active gene are nonessential, but are merely compatible with transcription, and that a sequence somehow inhibitory for transcription normally precedes the relatively inactive gene.

Stimulation of proliferation of hepatocytes cultured in chemically defined medium decreases induction of hemoprotein monooxygenases

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Hepatocyte cultures were prepared from 15-day-chick embryos (CE) and maintained in serum-free medium. Autoradiography of cultures exposed to ^{3}H-thymidine indicated continuation of DNA-synthesis initiated in vivo and subsequent cell division during the first 16 h. Later on only minimal entry into DNA-synthesis was observed (below 1% of the hepatocytes). Addition of insulin, hydrocortisone, inosine, triiodothyronine and glucagon (IHITG, Leffert et al., 1977) at 16–19 h of culture caused, after a lag period of 10 h, a rapid increase to 30% of the number of DNA-synthesizing hepatocytes within the next 8 h and subsequent cell division. Exposure of IHITG-stimulated hepatocytes to β-naphthoflavone resulted in decreased induction of cytochrome P450 (control: 280% of uninduced level; IHITG: 160%; $p < 0.05$) and of associated enzyme activities. The data suggest that cell proliferation is an important variable in the induction of hepatic hemoprotein monooxygenases.

Direct HPLC-determination of nucleosides in enzymatic digests of cell nuclei

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We looked for a method to rapidly estimate deoxynucleosides in order to quantify the DNA content and turnover as well as the base composition of DNA from rat cerebral cortex neurons and liver cells without prior isolation of DNA. Suspensions of nuclei were sonified and subjected to simultaneous enzymatic digestion by DNase I, phosphodiesterase, alk. phosphatase and adenosine deaminase (ADA). After centrifugation the nucleosides in the supernatant were analyzed by HPLC on a reverse phase column using a methanol gradient in potassium phosphate buffer. 2'-deoxy-5-fluorouridine was added as internal standard. Adenosine was determined as inosine through enzymatic peak shifting by ADA. Perfect separation of all main deoxynucleosides was achieved in less than 25 min. The method fulfills the requirements for rapid and precise quantification of nucleosides in the nmole range and has the potential for detecting minor changes such as DNA modification by carcinogens.

Characterization of cell-surface glycoproteins associated with the nonionic detergent insoluble cytoskeleton of murine lymphocytes plasma membranes

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Extraction of murine lymphoma cells with nonionic detergent yields 3 major insoluble fractions upon sucrose density fractionation. Nuclei and associated cytoplasmic structures sediment rapidly. An intermediate-density fraction contains free ribosomes. From light fractions on top of the gradient, detergent-insoluble material can be separated

from solubilized proteins and lipids. In this residue, actin and myosin are the 2 mayor proteins. 2-D gel analysis reveals the presence of minor components, some of which can be vectorially labeled on intact cells. 3 surface glycoproteins of 35, 52 and 160 kdaltons are enriched in the cytoskeletal residue. H-2 is only partially associated. The majority of surface labeled glycoproteins (e.g. Thy-1, T-200) are fully solubilized. Delipidation of lymphocyte plasma membrane thus leaves intact a transmembrane complex containing both cytoskeletal proteins and a distinct class of surface glycoproteins.

Initiation of transcription of influenza virus involves the synthesis of specific oligonucleotides in the absence of template

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The influenza virus has a segmented RNA genome which is transcribed by a virion-associated transcriptase upon infection of a cell. The transcriptase is composed of 3 large P-polypeptides (mol.wt 85,000-96,000) coded by the viral genome: in fowl plague virus the 2 largest polypeptides, P_1 and P_2, are basic, the smallest polypeptide P_3 is slightly acidic. - With detergent-disrupted virus, initiation of transcription requires specific primers. Experiments conducted with fowl plague virus using ApG or GpG as specific primers have shown that the transcriptase is capable of adding a C in the 3′ position of the primer and most probably an A next to it in the absence of a template. The polypeptide P_1 seems to carry the enzymatic activity for this reaction. The resulting oligonucleotide, 5′ A(or G)pGpCpA can base pair with the 3′-end of the viral template which reads 3′ UpCpGpU-. It is proposed that the influenza virus has an unique mode for initiation of transcription involving primarily an interaction between the primer and the transcriptase P_1 polypeptide.

Callosal neurons of monkey visual cortex: a study combining horseradish peroxidase and electron microscopy

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After injection of horseradish peroxidase along the area 17/18 border of the marmoset visual cortex, labeled neurons are found on each side of the contralateral 17/18 boundary. By light microscopy, most cells are identified as pyramids. The majority are in the supragranular layers but a few are infragranular. Computerized reconstructions of ultrathin serial sections through the centres of callosal cells show the spatial distribution of synapses on the soma, dendrites and spines. A comparison with callosal neurons of cat visual cortex defined by light and electron microscopy (Hornung and Garey, J. Neurocyt., 1981) shows that in monkey fewer are large spiny nonpyramidal neurons.

Synaptic maturation of monkey visual cortical neurons: a Golgi EM study

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Golgi impregnated neurons of primary visual cortex of monkeys aged from birth to several years were studied by electron microscopy after characterization by light microscopy. Computerized reconstructions show the spatial distribution of synapses on dendrites, spines and soma. The density of synapses per length of dendrite was calculated. By comparing results from animals at different ages, it is possible to obtain details of synaptic maturation on different cell types. Comparison is possible between morphological and physiological changes in developing visual cortex.

A new histone-like protein of *Escherichia coli* is immunologically related to the eukaryotic histone H2A

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A histone-like protein from *E.coli* has been purified to more than 98% homogeneity by using its capacity to inhibit DNA functions (U. Hübscher, H. Lutz and A. Kornberg, Proc. nat. Acad. Sci. USA *77*, 5097, 1980). This protein, called H-protein, resembles histones in its a) abundance, estimated at 30,000 dimers per cell, b) stability to heat and acid, c) binding to double- and single-stranded DNA and limiting of staphylococcal nuclease digestion and d) reannealing of complementary single-stranded DNA at low temperature. H-protein is related to the eukaryotic histone H2A, in particular in its amino acid composition and reactivity with an antiserum specific for histone H2A. The capacity of H-protein to bind DNA prevents its template or substrate functions in several in vitro DNA transactions (DNA and RNA polymerases, DNA-dependent ATPases, DNA topoisomerases). Together with other histone-like proteins, H-protein may organize the *E.coli* chromosome into nucleosomes such as in eukaryotic chromatin.

Genesis of IS mediated transposons

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Transposons are generally flanked by repeated DNA sequences and some of these repeats are known to be IS elements. In order to understand how IS-flanked transposons could have evolved, new Cm transposons flanked by IS1 were constructed in vivo from the Cm^r gene of pBR325 containing no IS1 sequence and the IS1 carried on the phage P1 genome. First, isolation of IS1-mediated pBR325::P1 cointegrates and subsequent segregation of the cointegrates resulted in pBR325::IS1 plasmids. From the cells carrying pBR325::IS1 and $\lambda plac5i^{21}$, nondefective λ-derivatives carrying the Cm^r gene were selected. In all cases studied, the Cm^r gene was flanked by 1 copy of IS1 on each side as direct (1 isolate) or as inverted repeats (5 isolates). All of these IS1-Cm-IS1 segments transposed as units from phage $\lambda plac5i^{21}$ to the genome of phage P1-15 hybrid2 carrying no IS1.

Microinjection of fluorescently labeled clathrin into living fibroblasts

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Clathrin of coated vesicles, a 175,000 d protein was isolated from brain and liver tissues and modified with rhodamine isothiocyanate. The labeled protein containing 5-7 covalently bound fluorochromes was microinjected into living human fibroblasts and its localization was monitored by videointensified fluorescence microscopy. Within less than 10 min the fluorochrome appeared in numerous small spots distributed throughout the cytoplasm. These spots were clearly different from lysosomes as examined by double labeling using the pH-dependent dye acridine orange. Whether the injected clathrin in fact traces the cellular coated pit-coated vesicle system is now investigated by electron microscopy.

Glucocorticoid domain of kidney

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Several mRNA species accumulate in rat kidney following a single injection of dexamethasone (dex). Such mRNAs were detected by translating total kidney poly(A)-rich RNA in a wheat germ protein synthesis system. Electrophoresis of the cell-free products according to O'Farrell revealed 9 polypeptides that were markedly (between 2- and 6-fold) and consistently induced 8 h after dex. The induction of these mRNAs occurred virtually without lag, as evidenced by their enhanced template activity 2 h after dex. The induction of the majority of the mRNAs was not dependent on concurrent protein synthesis, since it was not inhibited by cycloheximide. This drug, however, suppressed the hormone-dependent build-up of 1 mRNA, which was shown to code for the gluconeogenic enzyme P-enolpyruvate carboxykinase. The pattern of polypeptides encoded by the glucocorticoid-responsive mRNAs was kidney-specific, as demonstrated by analogous experiments performed with rat liver poly(A)-rich RNA. Furthermore, the response was specific to the glucocorticoids, as opposed to the mineralocorticoids.

Formation of intermediate filaments during mouse embryogenesis

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We describe the first appearance and formation of intermediate-sized filaments in eggs and preimplantation embryos of the mouse by a) electron microscopy; b) immunofluorescence microscopy using antibodies to the various cytoskeletal proteins; and c) 2-dimensional gel-electrophoresis of embryonic proteins and high salt-resistant cytoskeletal preparations. The first intermediate-sized filaments detected before implantation are bundles of filaments of the cytokeratin type which appear in the outer cells of morulae and in the trophectoderm of blastocysts. The formation of cytokeratin filaments, which apparently is exclusive to epithelial cells, also characterizes the trophectodermal cells of the mouse blastocyst as true differentiated epithelial-like cells, and may well represent the initial step in epithelial cell differentiation. We are presently investigating the various cell lineages during postimplantation development in order to gain further insight into the structural organization and biochemical composition of cytoskeletal elements during cellular diversification.

Influence of intraislet environment on B-cell function

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B-cells in the center of islets of Langerhans are surrounded by other B-cells while those situated at the islet periphery contact several non B-cells. To test whether this different environment affects B-cell function, the organelle content was evaluated in central and peripheral B-cells of islets isolated from either the splenic or duodenal portion of rat pancreas. After a 90-min incubation with 2.8 mM glucose, organelle content was similar in central and peripheral B-cells of both splenic and doudenal islets. After a 90-min stimulation with 16.7 mM glucose, central and peripheral B-cells differed in both islet types. Only central B-cells of splenic islets and peripheral B-cells of duodenal islets appeared degranulated, with increased endoplasmic reticulum and Golgi complexes. Lysosomes increased and microtubules decreased in all B-cells exposed to high glucose. Thus, B-cells form a homogeneous population, as judged by organelle content, under resting conditions, but respond differently to glucose stimulation depending upon their location within the islet and upon the location of the islet in the pancreas.

Importance of cell shape for the hormone responsiveness of rabbit mammary cells in primary cultures

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The development of the mammary gland during pregnancy is characterized by cell proliferation and expression of mild protein genes which are regulated by steroid and peptide hormones. Rabbit dispersed mammary cells were allowed to form spheroids which were then cultured for up to 20 days on attached or floating collagen gels, or embedded in collagen. Cell proliferation, as assessed by cell count, DNA content, and ^{3}H-thymidine incorporation, takes place on attached and embedding gels. Expression of milk proteins (caseins, α-lactalbumin, transferrin, secretory component) in response to lactogenic hormones as determined by radioimmunoassays, immunoprecipitation of biosynthetically labeled proteins and immunofluorescence occurs on floating and emedding gels. Differences in cell shape were observed under the various culture conditions suggesting that the geometry of the cell plays a role in the expression of biological activity.

Microinjection of affinity-purified antibodies against vinculin reduces the focal contacts of the recipient fibroblast

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Vinculin, a 130,000-dalton protein, was isolated and purified from chicken gizzards. An antibody against this protein was raised in rabbits and was affinity purified. The antibody specifically stained the focal contacts of spread fibroblasts (B. Geiger, Cell, 1979) and precipitated a single protein of 130,000 daltons from chicken embryo fibroblasts metabolically labeled with ^{35}S-methionine. This antibody (1 mg/ml) was microinjected into living MRC-5 human fibroblasts and the recipient cells were followed by reflection contrast microscopy. We found that the focal contacts of the cells were reduced in number and size 5-10 min after injection. As a control, microinjection of DEAE-purified rabbit IgG did not induce a significant change in cell attachment. The effect of antivinculin microinjection on the cytoskeleton organization was further investigated in cells with fluorescently traced stress fibres.

Split promoter of a eukaryotic tRNA gene

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To delimit the promoter of a eukaryotic tRNA gene, we constructed a series of insertion and substitution mutants in the $tRNA_I^{met}$ gene of *Xenopus laevis*. The 5'- and 3'-flanking region of the cloned gene have been resected enzymatically, the truncated genes were recloned and then tested for

transcriptional activity by oocyte injection and in a homologous in vitro transcription system. Both flanking regions can be removed without loss of the promoter function, hence the promoter lies within the structural gene itself. Deletion mutants lacking the 5'- or 3'-end of the structural gene are not transcribed. Deleting the middle region of the gene also caused it not to be transcribed, but its replacement, with Hind III linker DNA restored the transcriptional activity. In summary, we conclude that the promoter of the $tRNA_1^{met}$ gene is split and is composed of 2 short sequence elements which are located within the gene near the 5'- and 3'-end.

Protein kinases and growth control in human mammary carcinoma cells (MCF-7)

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The human mammary carcinoma cell line MCF-7 is used as a model for hormone-sensitive tumor proliferation. It was suggested that cyclic AMP, via protein kinases, is involved in growth control. However, we show that DBcAMP and theophylline have no effects on proliferation of MCF-7 cells. Analysis of protein kinase activity with DEAE cellulose chromatography showed 2 cyclic AMP-dependent and at least 2 cyclic nucleotide-independent protein kinases. The activities of these enzymes were studied under different growth conditions. Growth arrest caused by serum depletion correlates with a marked decrease of a cyclic nucleotide-independent protein kinase eluting at ~90 mM NaCl. The activities of the cyclic AMP-dependent protein kinases were not substantially affected. In MCF-7 cells, the role of cAMP in growth control needs therefore further critical evaluation.

Comparison of the morphology of rat liver chromatin at pH 7 and 9

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Different morphology of chromatin has been observed depending on whether the nuclei prepared for electron microscopy were lysed at pH 7 or pH 9. We have, therefore, examined the possible release or redistribution of chromatin components. Soluble chromatin was fractionated at pH 7 and 9 and at different ionic strengths to remove H1 and/or nonhistone components. The salt-dependent condensation of the fractionated chromatin was analyzed in the electron microscope. We show that the shift from pH 7-9 leads to less compact, higher order chromatin structures. At very low ionic strength nucleosomes are partially or totally unfolded, although there is no indication for a release or displacement of H1. The pH-effect is completely reversible. The data suggest a change in the mode of action of H1 in the formation of nucleosomes and higher order structures.

High affinity β_2-adrenergic receptors in mononuclear leucocytes: similar density in young and old normal subjects

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An age-related decrease of β-adrenergic receptors might explain the reduced responsiveness of β-adrenoceptor-mediated cardiovascular functions recently observed in older subjects. In order to study age-related receptor changes, β-adrenergic receptors on mononuclear leucocytes were characterized and their properties were compared in young and old healthy subjects. High affinity β_2-adrenergic binding sites were measured using a radioreceptor assay with (−) ^{3}H-dihydroalprenolol and were defined by rapid kinetics, structural specificity and saturability. Studies in normal subjects revealed no age difference between 14 young (18-40 years) and 8 old (53-65 years) subjects with respect to the binding capacity (53±4 fmoles/mg vs 67±8 fmoles/mg) or equilibrium dissociation constant K_D (0.6±0.5 nM for both). The discrepancy between β-adrenergic receptors or an age-related target cell defect distal to the adrenergic receptor.

Nucleosome phasing in genes of *Drosophila melanogaster*

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The arrangement of the nucleosomes with respect to the DNA sequence has been examined in a number of genes in *Drosophila*. The 146 basepair DNA fragments protected by the nucleosome core particles against micrococcal nuclease digestion were isolated and digested with various restriction enzymes. After separation of these double-digests in polyacrylamide gels and transfer to DBM papers, hybridization has been carried out with different probes. The results show that the nucleosomes are precisely phased in several frames.

Stimulation of Balbiani ring genes expression: refractory and responsive phases during development

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The salivary glands of 4th instar larvae of *Chironomus tentans* produce a silk-like secretion. There is good evidence that its corresponding mRNA is transcribed by the Balbiani rings (BRs) of their polytene chromosomes (see abstract by Hertner et al.). Stimulation of the secretory process, e.g. by pilocarpine or by depriving the animals of their housing tubes, enhances BR-expansion and BR-RNA synthesis up to 10-fold. This response of the BR-genes is greatest with animals which after termination of diapause reach within 3 days at least phase 5.5 (phases based on the imaginal disc status). Animals of phase 4.5/5 are refractory. Without stimulation these 2 phases do not differ in their BR-sizes and BR-RNA synthesis. However, they do so in their nuclear ion activities. - These findings are interpreted in terms of a 2-step model of gene activation in which an ion-dependent preparatory step is followed by the activation step of transcription.

Interrupted rRNA genes in *Drosophila melanogaster:* Nucleotide sequence of the transcription initiation site and of the insertion boundaries

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Many of the rRNA genes in *D. melanogaster* are interrupted in the sequence coding for 28S rRNA by insertions. Nuclear RNA transcribed from insertions represents less than one copy per embryo cell, more than 3 orders of magnitude below the concentration of nascent rRNA chains. A similar low level of insertion transcripts was found in ovaries, testes, in various developmental stages and in *bobbed* mutants with severe deletions of their rDNA.

We conclude that interrupted rRNA genes cannot be functional, unless a mechanism other than splicing is involved in their expression. The nucleotide sequence around the site where transcription initiates is identical in several genes with and without insertions. Therefore, the presence of an insertion per se seems to prevent transcription of interrupted genes. Both sides of an insertion are flanked by a direct repeat of 14 bases, present in the coding region of uninterrupted genes. We propose that ribosomal insertions arose from a true insertion event of a mobile genetic element into a preexisting uninterrupted gene.

Enzymatic and immunological properties of human lymphocytes in immunodeficiency states

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We have investigated the relationships between the activities of enzymes associated with the plasma membrane and the in vitro physiological properties of lymphocytes isolated from peripheral blood of normal donors and of patients with primary immunodeficiency. In contrast to normal lymphocytes, cells of patients were characterized by a marked imbalance of the enzymatic response, an absence of correlation between the various enzyme activities and a significant low level of 5′-nucleotidase. Low 5′-nucleotidase correlated with a deficient proliferative response to mitogens (ConA, PWM) and with the impaired capacity to induce the terminal differentiation of B-lymphocytes to cytoplasmic immunoglobulin containing cells. However, a negative correlation was found between the mitogenic response and the activity of other plasma membrane enzymes. In addition, coculture experiments showed that the suppressor effect mediated by patients' cells on the plasma blast differentiation of control B-lymphocytes was positively correlated with the activity level of the (Mg-Na-K) ATPase, determined in the lymphocytes of patients with primary immunodeficiency.

3′-terminal homology of mRNAs for surface antigens of different antigenic variants of *Trypanosoma brucei*

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Trypanosoma brucei, the protozoan parasite causing African sleeping sickness, protects itself from the host's immune response by frequent changes of its antigenic properties. This is achieved by successive synthesis of many different Variant Surface Glycoproteins (VSG). Cloning of DNA complementary to the mRNAs of VSGs from different variant strains was described previously (Hoeijmakers et al., Gene *8*, 391, 1980). All the cDNAs selected by differential hybridization were found to be lacking portions both at the 5′- and the 3′-ends. Using these incomplete copies as probes, several cDNA clones containing intact 3′-termini of 2 different VSG sequences were isolated. About 85 bp long regions at the 3′-ends were sequenced and found to be 80–85% homologous. This explains why 3′-end-containing clones were initially not selected by differential hybridization. The 3′-terminal sequences lack the otherwise ubiquitous poly-A-addition signal AATAAA and show heterogeneity of poly-A-addition sites.

A deletion mutation that phenotypically is reversible

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We have characterized a chloramphenicol and fusidic acid sensitive mutation of the resistance plasmid NR1 by restriction cleavage analysis and DNA sequencing. This mutation is an IS1-mediated deletion into the C-terminal end of the gene responsible for both antibiotic resistancies. Most unusually for a deletion, revertants can be found that have regained resistance to both antibiotics. The DNA sequence analysis of some of these revertants will be presented together with a molecular explanation of their phenotype.

Cell interactions in the preorganogenetic blastoderm of the cephalopod *Loligo vulgaris*

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On the basis of earlier studies on cephalopod embryogenesis, we argue that the phenomenon of cell and organ determination (the prerequisit of specific cell and organ differentiation) is basically explained by the existence of 2 primary systems of cell and tissue interactions (Marthy 1978-9; 1979). The first interaction system comprizes the contact of the blastoderm with the yolk syncytium (plus yolk); it conditions the nutrition of the cell material. The 2nd system is the combination of all cell relations *within* the blastoderm composed of ectoderm and mesendoderm; it is this interaction system which is essential for the specialization of the cell material in histologically differentiated structures. In this process of determination, the mesendoderm is thought to act as an organizer for the ectodermal cover. - According to this model, it is very likely, that the establishment and the maintenance of contacts between the cells throughout the cell generations of the preorganogenetic development are of primary morphogenetic importance (e.g. sites of passage for developmental information). The present study shows such cell and tissue contact sites; they are revealed on embryos of different preorganogenetic stages by SEM. The findings are considered as 'morphological arguments' in favor of the suggested determination model.

Comparing the length density of the capillary network in various muscles

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The development of structural models for the analysis of O_2 exchange from blood to the muscle cells requires a precise knowledge of some quantitative properties of the capillary network. A basic dimension characterizing the microvasculature is the length density of capillaries per unit volume of tissue, $J_V(c,t)$, which relates directly to the capillary volume and surface densities, and to the intercapillary mean distance. The parameter $J_V(c,t)$ is also related to the number of capillary profiles per unit area of tissue section by a coefficient which depends on the 3-dimensional concentration parameter K of the capillary segments orientation and on the sectioning angel. - We have developed a method to estimate the value of K in muscle tissue from 2 sets of sections taken perpendicularly and longitudinally to the muscle fibre axis. The length density of capillaries per unit volume of fibre, $J_V(c,f)$, in cat M. soleus is found to be 1061 $m \cdot cm^{-3}$. The degree of orientation of capillary segments in various muscles with different aerobic capacities is compared to that found in M. soleus, and the value of $J_V(c,f)$ in those muscles is estimated.

Transcription of 5S genes in vitro with extracts from polyoma-infected or mock-infected mouse kidney cultures

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Khandjian et al. (PNAS *77*, 1476, 1980) have shown that virus-induced cellular RNA (rRNA, hnRNA, tRNA and 5S RNA) and protein synthesis begin within 1-2 h after onset of T-antigen synthesis. Specific and complete synthesis in vitro of 5S RNA has been achieved by incubation of a cloned 5S gene from *Xenopus* in mammalian cell extracts containing RNA polymerase III and specific transcription factors (Engelke et al., Cell *19*, 717, 1980). To study the mechanism of polyoma-induced stimulation of 5S RNA synthesis in mouse kidney cells, we prepared cell extracts (S100) from mouse kidney cell cultures infected with polyoma virus for 25 h and from uninfected parallel cultures. As template we used a cloned 5S gene from *Xenopus borealis*. Cell extracts from polyoma-infected cultures transcribed the 5S genes at a 50% higher rate than extracts from mock-infected cultures. Stimulated transcription remained unchanged after removal of polyoma T-antigens by exhaustive immunoprecipitation. This and other results suggest that, in vitro, stimulation is linked to a increased concentration of certain host cell proteins.

HMW proteins are selectively associated with dendritic microtubules

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Antisera have been raised which react specifically with either tubulin or high molecular weight proteins (HMWs) from rat brain microtubules. When sections of rat brain were stained using an immunoperoxidase procedure, anti-tubulin reacted with microtubules in both glial cells and neurons and in both axons and dendrites. However, anti-HMW reacted only with dendritic microtubules leaving those in glia and axons unstained. This was most marked in large neurons such as cerebellar Purkinje cells and pyramidal cells of the hippocampus and cerebral cortex where anti-HMW stained dendritic microtubules while sparing the axonal microtubules of the same cells. Thus HMW proteins seem to be specifically expressed in neurons and selectively associated with their dendritic microtubules.

A cytoplasmic glucocorticoid receptor from *Xenopus laevis* liver: effect of alkaline phosphatase and molybdate on steroid binding

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The binding activity of the glucocorticoid receptor from *Xenopus* liver is rapidly lost at 10 and 20 °C. Sodium fluoride and protease inhibitors have no effect while molybdate and tungstate decrease and alkaline phosphatase increases the loss of steroid binding activity. This data is consistent with the loss of binding activity being due to dephosphorylation of the receptor. Binding of ^{3}H-dexamethasone to the receptor does not alter the rate at which steroid binding activity is lost but does increase the stabilizing effect of molybdate. Molybdate can interact with the receptor and we suggest that this interaction stabilizes the receptor in the steroid binding from. Long incubations at 0 °C, warming and exposure to ATP or salt which all 'transform' mammalian glucocorticoid receptors result in a loss of binding of the *Xenopus* receptor. This suggests that the *Xenopus* receptor cannot be 'transformed' and therefore we speculate that this process might not be important in the action of mammalian glucocorticoid receptors.

Sensitive staining of fully denatured nucleic acids with silver nitrate

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We describe the application to nucleic acids of a procedure for staining proteins with silver (Oakley et al., Analyt. Biochem, in press). RNA or DNA denatured with glyoxal is electrophoresed in agarose or polyacrylamide gels in the presence of 0.1% SDS (McMaster and Carmichael, PNAS *74*, 1977). The gels are rinsed in water (30′), immersed in a solution of ammoniacal silver (5-10′), rinsed again in water (2′) and transferred to a solution of citric acid (0.005%) and formaldehyde (0.019%) for 5-10′. Destaining is in rapid fixer (5-15′), followed by hypoclearing agent (5′). The gel is dried and photographed. The silver staining method is particularly suitable for use with glyoxalated nucleic acids. The presence of glyoxal increases the intensity of silver staining, whereas it decreases the intensity of ethidium staining. Silver staining is thus a sensitive method to detect fully denatured nucleic acids.

Comparison of canine parvovirus (CPV) with mink enteritis virus (MEV)

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In 1978 outbreaks of an apparently new contagious enteric disease in dogs were observed almost simultaneously throughout the world. The clinical and pathological picture of the disease closely resembled panleucopenia in cats and enteritis in mink, both caused by parvoviruses (FPV and MEV, respectively). Whether or not CPV can be distinguished from MEV and FPV by serological means is still in dispute. Therefore we compared the genomes of CPV and MEV by restriction enzyme analysis of their replicate form (RF) DNAs. Out of 79 mapped sites 68 or 86% were found to be common for both types of DNAs indicating that CPV and MEV are closely related viruses. Whether they evolved from a common precursor or whether CPV is derived from MEV, however, cannot be deduced from this data. Presently other isolates of these viruses are being compared. This approach might yield insight into the relation of these viruses among themselves and could help to trace the origin of CPV.

Demonstration of metabolic coupling between islet cells

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Exchange of molecules (metabolic coupling) between islet cells through gap junctions may participate in regulating secretion in islets of Langerhans. To demonstrate such a coupling, islet cells in monolayer culture were loaded with either exogenous carboxyfluorescein (by microinjection) or endogenous ^{3}H-uridine nucleotides (by exposure to ^{3}H-uridine) and cultured with unloaded islet cells. Exchange of the marker molecules from loaded to unloaded cells was assessed by fluorescence microscopy and autoradiography. Transfer of carboxyfluorescein and uridine nucleotides was observed between B-cells but also between B- and non B-cells. The impermeant properties of the 2 molecules, the relationships between loaded and unloaded cells and the time course of the transfer indicated that transfer did not occur via the medium but rather via gap junctions. Since gap junctions vary with the state of activity of B-cells (Meda et al., Science *209*, 1026, 1980), the coupling between islet cells may be modulated as well, a prerequisite for regulation of islet cells activities.

Stability and alteration of IS1-mediated Cm transposons

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IS1-mediated transposons are generally stable. However, a number of mechanisms can cause either their elimination from a genome or their alteration. This is documented in work with Cm transposons flanked by a pair of IS1 elements either in the same or in inverted orientation. In the former case loss of the Cm^r marker occurs most frequently by reciprocal recombination, a largely *recA*-dependent process, between the 2 flanking IS1 elements. The following other restructuration processes were observed to affect transposons flanked either by directly or by invertedly repeated IS1: a) IS1-mediated deletion formation; b) deletions formed by an unrelated IS element carried in the vicinity of the transposon, or by chance inside the transposon; c) precise or nearly precise excision of the transposon; d) gene amplification. Some of the structures studied are more complex than expected from a single restructuration event.

Reproducible isolation of somatic and oral cortices of *Paramecium tetraurelia*

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Cortical proteins of *P. tetraurelia* were studied by isolation of cortices and SDS-polyacrylamide gel-electrophoresis. Components of the extraction medium (EDTA, Tris, 2-Me, Triton X-100) following R.K. Peck and G. de Haller (J. Protozool. *23*, 16A, 1976) were tested and adapted for this species. - The existence of at least 50 proteins was shown. Most of the proteins appeared with 85-100% regularity in 13 different extractions. 8 bands (mol.wt < 17600) showed more variable results: 70% regularity - Several proteins were recognized: tubulins A and B and proteins from trichocysts. Heavy proteins had mol.wts similar to i-antigens or dynein subunits.

Large T-antigen associated with messenger ribonucleoproteins of CV-I cells infected by simian virus 40

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Large SV40 T-antigen has been proposed to serve as regulator of translation in the infected host cell (Michel und Schwyzer, Experientia *36*, 753, 1980). In support of this hypothesis, we find that cytoplasmic T-antigen (about 10% of total T-antigen) is associated with messenger ribonucleoproteins (mRNP's), as shown by the following observations: In discontinuous sucrose gradients, T-antigen cosediments with mRNP's to 1.2 g/cm^3. Upon zonal centrifugation of cytoplasm, T-antigen sediments predominantly with initiation complexes for translation (30-80S) and with free and membrane-bound polysomes. Nuclear T-antigen (7-15S) added before subcellular fractionation does not bind to these organelles. After disruption of polysomes with puromycin/KCl or EDTA, T-antigen remains associated with the released mRNP's.

A nonuniform sensitivity for radiation-induced chromatid breaks within the G_2-phase of Chinese hamster cells

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Parallel cultures of fibroblast-like cells of Chinese hamster (line 19/1) were consecutively irradiated at hourly intervals with 1.0 Gy X-rays after puls labeling with ^{3}HTdR. Cultures were treated with colchicine 0.5 h after irradiation and fixed after a further 0.5 h. Unlabeled metaphases represented cells irradiated during different sections of the G_2 phase. Aberration frequencies showed peaks at 1 and 4 h after irradiation (2.23 ± 0.06 and 2.42 ± 0.39 breaks per cell respectively). Results indicate that: a) small but highly sensitive subpopulations within a specific phase of the cell cycle could be missed by using a long sampling time; b) the position of the cells within a specific 'subphase' of the G_2 and not the time elapsing between irradiation and fixation plays a role for their radiation sensitivity.

Purification of an inhibitor of sponge aggregation

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An inhibitor of sponge cell aggregation has been extracted from membranes of *Microciona prolifera*. Purification on an aggregation-factor affinity column yields active material that can be iodinated. Further sedimentation on sucrose gradients resolves the activity into 2 peaks. Inhibitory activity from the main peak is sensitive to periodate and NEM, but not to EDTA or delipidation. Activity from the secondary peak, which is situated close to the top of the gradient, is sensitive to heat, and delipidation produces an 8-fold increase in its recovery. - The material from both peaks is currently being analyzed by isoelectric focusing.

Isolation, molecular cloning and sequence analysis of highly repetitive DNA sequences contained in the eliminated genome of *Ascaris lumbricoides*

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High mol.wt DNA from germ line and somatic cells of *A. lumbricoides* has been isolated and digested with different restriction enzymes. The resulting DNA fragments were separated by agarose gel-electrophoresis. Germ line DNA shows monomers about 120 bp long as well as multimers of this fragment, whereas somatic DNA contains no or only a very small number of these sequences. Southern blot analysis and double digestion experiments demonstrated that the eliminated DNA sequences are composed of a whole set of different but related gene families, all showing the same repeating unit length of about 120 bp. Several 120-bp fragments produced by digestions with different restriction enzymes have been cloned, using pBR 322/*Escherichia coli* HB 101 as a cloning vehicle. Sequence analysis of the cloned DNA fragments demonstrated their precise degree of heterogeneity.

Evidence for an albumin within the muscle cell

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Previously, albumin was thought to be synthesized only in liver cells (especially light hepatocytes), from where it is

released into the circulation to take up its many functions. However, in recent investigations albumin-like proteins have also been localized in tissues other than liver. Recently we found that serum albumin and the muscle protein β-actinin were indistinguishable by several physicochemical and immunological criteria (Proc. nat. Acad. Sci. USA, in press). Ultrastructural studies revealed the presence of this protein within the muscle cell bound to the myofibrils. In addition we have investigated whether it is synthesized in muscle. Chicken muscle was incubated in Krebs-Ringer buffer with ^{14}C-leucine, an amino acid known to promote protein synthesis and reduce degradation. Analysis of the muscle proteins by 2D-gel-electrophoresis showed an incorporation of radioactivity into a protein closely resembling serum albumin.

Reversible muscle fibre transformation

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The superior sternohyoid muscle of the rat displays only sporadically slow type IA-fibres (0-3 per muscle). After various surgical procedures (e.g. overloading of the muscle by excision of the contralateral one, or incision of the skin and the superficial fascia only) the number of type IA-fibres increases within a short time. There is a parallel increase of the number of intermediate fibres between the fast type IIA and the slow type IA (IIC, IB). A first maximum is reached after 9 days. During the following 3 days there is a drastic decrease of the number of type I fibres, increasing again within 6-9 days. Immunohistochemically the type I fibres display synthesis of slow myosin. - The results provide evidence for a relatively fast switch of the gen expression for the synthesis of a certain myosin. The muscle fibre transformation following skin incision only points to still unclear feedback mechanisms.

A study of in vivo coding rules in *Schizosaccharomyces pombe*

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In *S. pombe*, the loss of the wild-type function of the 2 genes *sup3* and *sup9* known to code for $tRNA^{Ser}_{UCA}$ (anticodon U*GA) is lethal when *both* genes carry mutant alleles (either single-mutant alleles coding for an active UGA suppressor tRNA with the mutant anticodon U*CA, or double-mutant alleles carrying an additional mutation and coding for a completely inactive tRNA). We favor the interpretation that in vivo no other isoacceptors than those mentioned above are able to read UCA efficiently. This contradicts the 'wobble hypothesis' of Crick which is supported by ribosomal triplet binding experiments and which predicts that serine tRNA containing inosine in the first anticodon position (anticodon IGA) should read UCU, UCC *and* UCA. A serine tRNA containing inosine exists in *S. pombe* (P. Agris and C. Gehrke, pers. commun.). The results are also at variance with the two out of three hypothesis' of Lagerkvist which is based on in vitro protein synthesis data and which predicts that any serine tRNA should be able to read the 4 serine codons UCU, UCC, UCA and UCG which differ in the 3rd position only.

Expression of human interferon α in *Escherichia coli*

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Human interferon (IFN) α cDNAs and chromosomal DNAs have been cloned and brought to expression in *E. coli*. 2 types of constructions have been examined: a) A bacterial promoter and ribosome binding site were joined to the DNA coding for the IFN signal sequence, so that a fused protein resulted which was found to be biologically active. b) The bacterial sequences were fused to the IFN gene in such a way that the initiator AUG abutted the first codon of the mature IFN. Part of the resulting IFN molecules carry an additional methionine, and part do not. Strains producing about 1 mg of interferon per liter culture medium have been obtained.

Expression of complement protein C3 in macrophage and liver cell lines

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We are interested in the regulation of C3 synthesis by glucocorticoid hormones and during inflammatory reactions. Liver hepatocytes and peritoneal macrophages are the principal producers of C3. Upon Starch induction of primary macrophages no significant increase of C3 synthesis was found. For these experiments cells were labeled with ^{35}S-methionine and C3 protein was assayed by immunoprecipitation and SDS-polyacrylamide gel-electrophoresis. 2 mouse macrophage cell lines, IC21 and J774.2 were found to synthesize and secrete C3, but not to secrete C4. Preliminary results with rat hepatoma cell lines indicate that the synthesis of C3 is stimulated by the synthetic glucocorticoid hormone dexamethasone. Levels of C3-mRNA in presence or in absence of the hormone are being quantitated by hybridization studies.

Light driven protein synthesis in isolated *Euglena* chloroplasts

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Isolated chloroplasts from photoheterotrophic *Euglena gracilis* use light as the only source of energy to incorporate labeled amino acids into protein (Ortiz and Stutz, FEBS Lett. *116*, 298, 1980). The process is chloramphenicol-sensitive and insensitive to cycloheximide or ribonuclease. Of the total incorporated counts, about 20% is associated with the stromal fraction and 75-80% is associated with the thylakoid membranes. We have now analyzed the stromal and thylakoid fractions on 12.5-15% gradient gels of polyacrylamide-SDS to study the products of the chloroplast translational apparatus. The principal translation product present in the stroma is a 55 kd polypeptide (the large subunit of ribulose bisphosphate carboxylase) in addition to 6-12 minor ones. In the thylakoid fraction, the major chloroplast translation products are comprised in the 10-45 kd range.

Selection of MMTV-transfected mink cells by cotransfection with SV-40 DNA

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Mink lung cells which do not contain endogenous MMTV sequences are able to integrate and express MMTV acquired by infection with exogenous virus. These cells were therefore chosen for cotransfection experiments with cloned MMTV DNA in which transformation by SV-40 DNA was used as a selective marker. SV-40 DNA transforms mink cells with a high efficiency (10^3 foci/μg DNA). Transfection was done by calcium-phosphate precipitation

of SV-40 DNA with a 10- to 20-fold excess of cloned MMTV DNA. Clones of transformed cells were isolated and so far all those examined have acquired new MMTV DNA. These clones are being tested for synthesis of viral RNA andproteins.

Chromatin structure of ribosomal RNA genes in *Physarum polycephalum*

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The rRNA genes of *Physarum* exist on palindromic, extrachromosomal deoxyribonucleoprotein molecules situated in the nucleolus. Limited digestion of either isolated nuclei or nucleoli with micrococcal nuclease produces an identical nucleosome repeat pattern, as visualized on agarose gels stained with ethidium bromide. After hybridization of these gels with radioactive rRNA or cloned fragments of rDNA, the same pattern is again produced. Thus the nucleosome structure of ribosomal chromatin appears to be similar to that of total chromatin. In order to characterize further the protein components of ribosomal chromatin, isolated nucleoli have been treated with Eco RI restriction endonuclease and then gently lysed. The resulting fragments of ribosomal chromatin have been separated on agarose gels, the proteins extracted and radioactively labeled in vitro. Analysis of these proteins by polyacrylamide gel-electrophoresis reveals that there are specific proteins associated with each fragment.

Chemiluminescence: an early event in the interaction of myxo- and paramyxoviruses with mouse spleen cells

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Influenza, Sendai, Newcastle, parainfluenza-3, measles and mumps viruses stimulate a burst of chemiluminescence (CL) starting within a few seconds on addition to a suspension of mouse spleen cells. CL is thought to reflect the generation of unstable oxygen species by the cells and depends on the presence of luminol (5-amino-2,3-dihydro-1,4 phthalazinedione). The extent and kinetics of light emission depend on the virus used to stimulate the cells and are correlated with the virus dose in a nonlinear fashion. In general, the peak of CL is reached at 2–8 min postinfection and thereafter declines to a level higher than before stimulation. CL measurement differs in several ways from conventional methods used in the study of virus receptors (e.g. cell agglutination). Most importantly, it reflects biochemical reaction(s) of the host cells triggered by the binding of the virus to the cell surface. Together with its exquisite sensitivity to low doses of virus, and the possibility to accurately quantitate light in a liquid scintillation spectrometer, this suggests that CL measurement represents a powerful tool in the study of virus receptors.

Protein synthesis in gynogenetic and androgenetic mouse embryos

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Gynogenetic and androgenetic mouse embryos were experimentally produced by microsurgically removing either the male or the female pronucleus from fertilized mouse eggs. The resulting haploid eggs were diploidized in a medium containing cytochalasin B. Protein synthesis was analyzed at different stages of preimplantation development using 2-dimensional polyacrylamide gel-electrophoresis. Both types of uniparental embryos synthesized a similar set of proteins independent of whether the maternal or the paternal genome was present. The homozygous-diploid embryos expressed a protein pattern that corresponded remarkably to normal embryos at the subsequent cleavage stage, i.e. 2-cell uniparentals resembled normal 4-cell embryos. These data suggest that the stage-specific protein synthesis during preimplantation development corresponds to nuclear replication rather than to cellular division.

Cross adhesion experiments in neoplastic and normal homogeneic cells

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Normal single cells (FG) and neoplastic cells (SGS-3A) released from monolayers with trypsin (0.25%) or EDTA (0.5 mM) have different adhesion kinetics. This observation induced us to perform a series of homologous and heterologous adhesion experiments in order to identify differences in the molecular adhesion mechanisms of the 2 cell lines. - In addition to a higher adhesive capacity of neoplastic cells we observed: a) Neoplastic cells carry on their cell coat at least 1 trypsin sensitive ligand which is involved in the homologous and in the heterologous adhesion. b) An analogous component is absent on fibroblasts. c) Ligands on the cell coat of fibroblasts are less trypsin-sensitive. d) There is a higher affinity between heterologous than between homologous ligands. e) The concentration of ligands on the cell coat of fibroblasts is lower than on neoplastic cells.

Ultrastructural studies on the in vitro replication of wild, no passage human cytomegalovirus

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Low passage HCMV is slow and of low yield. Cultures of human diploid cells infected with wild HCMV from an adult carrier's urine were maintained for 41 days. Lesions were not extensive. Thin sections after glutaraldehyde fixation, OsO_4 treatment and epon embedding were observed with a HU-12 Hitachi electron microscope. 2 distinct types of infected cells were seen: 1. After the start of virus maturation and release (mature virions in the nucleus, doubly enveloped virions in the cytoplasm). 2. At the start of virion maturation: The nuclei contained dense nucleoli, inclusion simulating electron translucent areas, granulated areas containing capsids and in the latter's vicinity, tubular structures 63–73 nm wide and up to 1.2 µm long. Such structures, seen by Nii (Biken's J. *18*, 145, 1975) in herpes virus cuniculi infected nuclei, were not seen in HCMV infected nuclei but in those infected with wild virus from the human carrier. They can either be precursors of capsids or, better, abnormal structures expressing the poor adaptation of the virus to the new host cells.

Induction of cytopathic effects (CPE) in Semliki forest virus (SFV) infected *Aedes* cells

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Usually no CPE is found in invertebrate cells infected with Alpha-Togaviruses. In contrast, cell clone C6/36 (Igarashi),

derived from *Aedes albopictus* (Singh) cells adapted to vertebrate culture media, reacts with spontaneous CPE to the infection with SFV, whereas the original *A. albopictus* (Singh) and *A. pseudoscutellaris* cells do not. In all cell lines of *A. albopictus* and even in the corresponding permanently infected cultures, however, a massive syncytium formation is inducible by lowering the pH, e.g. by addition of MES buffer, to a critical value, e.g. 6.1 in the case of C6/36. - In control experiments uninfected cells never showed any CPE after lowering the pH. By raising the pH to physiological conditions normal growth can be restored in such cultures, whether infected before or not.

Semliki forest (SFV) infection and secreted proteins (SP) of *Aedes* cells

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Intra- and extracellular proteins of host origin were analyzed before and after infection of *Aedes* cells (A: *albopictus* Singh, Flow; D: *albopictus* Singh, Yale; B: *albopictus* C6/36, Yale, adapted to vertebrate medium, high virus producer; *pseudoscutellaris*, Yale) with SFV. A, C, D were grown in invertebrate medium MM, B in MM or TCM 199. The biosynthesis of 2 protein was 'regulated' in B. 1. SP 54000 was inhibited from 24 to 48 h p.i. during the period of virus burst, irrespective of medium. SP 54 was again labeled, when cells went into the permanently infected state (72 h p.i.). 2. SP 62000 was only prominent, if C6/36 were in TCM 199. After infection SP 62 behaved as SP 54. Both were not affected in A, C, D (low virus producers). Fingerprints show SP 54/62 before infection = SP 54/62 72 h p.i. and SP/54/62 of A = SP/54/62 of B. SP 54 and SP 62 are structurally different monomeric proteins.

Biochemical changes during demyelination and remyelination in rabbit sciatic nerves

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In crushed sciatic nerves, proximal and distal stumps were dissected 2-120 days after the operation. Contralateral nerves served as controls. Lysosomal enzymes were measured: in distal stump, arylsulfatase A, NAc β-glucosaminidase and β-galactosidase activities were increased whereas cerebroside β-glucosidase and sphingomyelinase rose only slightly. The activities of 2 Schwann cell enzymes, cerebroside sulfotransferase (CST) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) decreased during the first 8 days of degeneration. Whereas CST activity increased between days 8 and 30, CNP increased only slowly. Myelin proteins were not detectable at 14 days but reappeared at 30 days. As in the case of CNP, the amounts of myelin proteins never reached control values. CST and CNP were lowest when demyelination was still incomplete and increased before myelin proteins became detectable. In conclusion, PNS myelin was degraded fast and as a unit and remyelination was not yet complete 120 days after the lesion.

Characterization of an interspersed repetitive DNA element consisting of a tandemly repeated subunit of 80 nucleotides from *Xenopus laevis*

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A genomic DNA library from *X. laevis* was screened with a repetitive DNA probe and a clone containing a 4.9 kb DNA segment (X-132) was isolated. The cloned fragment hybridizes to total RNA from oocytes and contains a repetitive element represented 100,000 times in the genome. Restricted genomic DNA probed with X-132 shows a large number of DNA fragments of different sizes hybridizing to the cloned fragment. The repetitive element is thus interspersed in the genome. The restriction map of X-132 confirms this result: the repetitive sequence is flanked by DNA which does not hybridize with repetitive DNA. Sequence analysis shows that the repetitive element itself consists of a basic 'unit' of 80 bp which is tandemly repeated at least 6 times. We have now isolated additional clones containing the same repetitive element and are investigating whether genes which are located close to these sequences are transcribed in a coordinate manner.

Isolation and expression in different tissues of genes from *Xenopus laevis* embryos

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Xenopus laevis embryonic DNA was digested with EcoR1 and cloned in Charon 9 λ vector to establish a complete genomic bank. This bank was screened for the presence of genes expressed during embryogenesis. For this purpose cDNA was synthesized on polysomal RNA extracts from stage 41 (prefeeding) embryos. - 110 clones were isolated by this technique and their expression in different tissues was tested. cDNA was made on cytoplasmic RNA from oocytes, tadpole liver, tadpole brain (stage 55, beginning of metamorphosis) and adult liver and hybridized with the clones. 50% of the recombinants contained genes expressed in all analyzed tissues while the remaining have shown different hybridization patterns. Differences were observed for oocytes, tadpole liver and tadpole brain, whereas the pattern was identical for tadpole and adult liver. The clones were classified in families according to their pattern of expression and are now analyzed for the presence of common sequences.

5-HT storage organelles in platelets and megakaryocytes of patients with Hermansky-Pudlak syndrome (HPS)

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HPS is a recessively inherited autosomal storage pool disease characterized by an increased bleeding time and a marked reduction of 5-HT adenine nucleotides (ATP, ADP) in platelets. In order to determine which platelet subcellular organelles are affected and whether these changes are already present in megakaryocytes (the stem cells), we examined, morphologically, platelets and sternal marrow megakaryocytes from 4 healthy volunteers and five HPS patients. Whereas in platelets of the former 5-HT organelles were positively stained with the uranaffin and chromaffin reactions (specific for ATP, ADP and monoamines respectively) and with mepacrine, those of HPS patients were either unstained or (with mepacrine) only weakly stained. Moreover, examination of megakaryocytes revealed numerous uranaffin-positive organelles in healthy volunteers but their total absence in HPS patients. These findings demonstrate that the storage defect is probably due to a deficiency of adenine nucleotides in 5-HT organelles of platelets which is already prevalent in their stem cells.

Mitosis in *Dictyostelium:* analysis of spindle elongation by time-lapse microcinematography

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Vegetative amebae from log-phase cultures of *D. discoideum* were enclosed in culture chambers specially constructed for light microscopy with oil immersion optics. We recorded mitosis in living cells at 23 °C on 16-mm film and analyzed spindle elongation in 6 amebae. On the average, elongation was completed within about 2 min. The mean velocity was 3.8 µm/min, about twice the rate observed in higher eukaryotes, but comparable to that reported for other lower eukaryotes (Heath, Int. Rev. Cytol. *64*, 1, 1980). The mean length of the spindle was 3.0 µm at metaphase and 10.8 µm at the end of telophase. In individual cells the spindle elongated by a factor of 3-4. This indicates that the spindle does not elongate simply by the sliding appart of 2 half-spindles, which could at the most produce a 2-fold elongation. Rather, the microtubules of the half-spindles either slide against free tubules, or they elongate concomitantly as they slide.

Effects of nocodazole on amebae of *Dictyostelium* in interphase and mitosis

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We treated log-phase cultures of *D. discoideum* with the microtubule inhibitor nocodazole and we are investigating, by light and electron microscopy, the cytological effects in relation to the concentration of the drug and the period of exposure. The microtubules (MT) of interphase cells treated for 190 min with 9.9 µg/ml nocodazole were not visibly affected. In cultures incubated for 12 min with 5 µg/ml nocodazole the frequency of mitotic cells was 2.3%, approximately as in the control cultures. The same concentration applied for 190 min produced 63% amebae that were arrested in mitosis (N-mitotic cells), 11.5% of which were binucleate. Recovery from this treatment by incubation for 45 min in conditioned medium without nocodazole was incomplete, for a substantial fraction of the cells still contained aberrant nuclei. Some N-mitotic amebae contained nuclei with short, presumably nonfunctional spindles; others contained spindle pole bodies and kinetochores, but few or no MT.

Surface proteins on SV40-transformed mouse cells

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Several authors suggested to existence of SV40 T-antigen (88,000 daltons) on the surface of SV40-transformed cells which would act as SV40-specific transplantation antigen. In an attempt to corroborate these reports, we have analyzed ^{125}I-labeled surface proteins by 2-dimensional electrophoresis (IEF and SDS-PAGE). A number of ^{125}I-labeled proteins occurring in SV40-transformed AL/N cells (SVAL/N) were absent in spontaneously transformed AL/N cells (TAL/N). One of these proteins, with a mol.wt of 96,000 daltons, equilibrating at pH 6.8 in an isoelectric focusing gradient, was immunoprecipitated by anti-SVAL/N sera. However, no ^{125}I-labeled protein corresponding to SV40 T-antigen could be detected, even after immunoprecipitation with antisera directed against native or denatured SV40 T-antigen. We therefore consider the possibility that either our techniques were insufficient to detect SV40 T-antigen on the cell membrane, or that the SV40-specific transplantation antigen corresponds to a virus-induced host protein(s).

Intracellular routes for markers of endocytosis

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Endocrine cell monolayer cultures from neonatal rat pancreas were exposed for various periods of time (from 5 min to 14 h) to the following markers: anionic (AF) and cationic (CF) ferritin, purified ferritin conjugates of concanavalin A (ConA-f) and of *Ricinus communis* agglutinin (RcA-f). The intracellular distribution of these markers was quantitatively assessed in thin sections. Following the initial surface and internalization steps, intracellular AF was localized exclusively to lysosomes; CF, ConA-f and RcA-f, on the contrary, were detected in lysosomes, in secretory granules and infrequently in Golgi cisternae. Secretory granules were labeled already in very early stages, suggesting that they acquired the marker directly by fusing with loaded endocytotic vesicles and not by budding from labeled Golgi. Markers binding to plasma membrane thus detect: a) alternative routes than lysosomal uptake for endocytosed material; b) a possible recycling of entire membrane segments; c) a fraction of secretory granules exposed to the exoplasmic space independently of exocytosis, exposure which may lead to crinophagy of the loaded granules.

In vitro culture and IgG binding of chick yolk sac endothelium

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The chick yolk sac sequesters yolk proteins which are degraded for use by the developing embryo. In contrast maternal yolk IgG crosses through the monolayer into the embryonic circulation. To culture this tissue, the connective tissue and blood vessels were dissected free from 4-day-old yolk sacs. Collagenase dissociated tissue was then pipetted, collected on a nylon screen, and then plated on collagen. Within 3 days the cells reorganized into a confluent monolayer. EM showed tightly applied cells with desmosomes and tight and gap junctions, which ruthenium red did not penetrate. With freeze etching characteristic tight junctions were noted. In scanning EM the cells appeared decorated with dispersed microvillae. From preliminary experiments, ^{125}I IgG bound specifically. The site of binding is being determined using biotinylated IgG-avidin gold. Electrical measurements and IgG transport studies are planned.

Establishment of a long-term in vitro persistent infection by a slow virus

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When tissue culture cells are infected with infectious particles of Sendai virus, they die within a few days. On the contrary, when defective interfering (DI) particles of the virus are mixed with the infectious virus in the inoculum (mixed virus infection), the infection leads to complete survival of the cells. These cells can be cultured for months after the initial infection. They all contain viral genomes and proteins and represent a culture persistently infected with the virus (Roux and Holland, Virology *93*, 91, 1979). Recently, we have analyzed the initial mixed viral infection leading to survival of the cells and persistency. Viral

replication, transcription and translation was measured by use of radioactive precursors incorporation. Surprisingly, no differences could be found between mixed virus infection and infection with the infectious virus alone, suggesting a main role of the infected cells in the decision of survival or death. The significance of these findings in understanding virus persistency will be discussed.

Changes in human jaw muscle fibre type composition associated with temporal mandibular joint ankylosis

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Very little published work concerning the fibre type composition of human jaw muscles is available. This is partly due to the difficulty of obtaining normal tissue, and possibly also to some unusual features in these muscles compared to limb muscles. We are making an immunohistochemical survey of changes in the fibre tpye composition of jaw muscles of patients with altered jaw function due to temporal mandibular joint ankylosis, compared with normal controls. Results indicate that whereas the normal jaw muscle contains mainly fibre types I and IIB, the patient's muscle contains large numbers of transitional (IIC) fibres.

Transcription of structural genes in the *Xenopus* oocyte system, a comparison of viral and cell type specific genes

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Injected polyoma virus DNA is efficiently transcribed by RNA polymerase B. Half of the virus specific RNA formed has the size of known viral mRNAs. It is shown by S_1 analysis that initiation of transcription in early and late genes takes place on specific sites that are the same as in infected cells. - Cloned rabbit chromosomal β-globin genes are not specifically transcribed. Transcription extends over vector, leader and gene sequences. A cDNA clone is also transcribed. This means that transcription is due to spill-over of polymerase from the vector. The isolated and circularized globin insert with a leading sequence of 1.5 kb is only weekly transcribed. We compare the template activity of globin genes having different 5'-extensions, trying to delimit sequences that inhibit transcription in the absence of specific stimuli.

Transformation of *Xenopus laevis* embryos with a cloned rabbit β-globin gene

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In order to study the fate and possible expression of foreign DNA during embryogenesis of *Xenopus laevis*, a genomic rabbit hemoglobin β-chain gene (Maniatis et al., Cell *15*, 687, 1978) was injected into fertilized *Xenopus* eggs. Total embryonic DNA was extracted at various stages of development, fractionated by agarose gel-electrophoresis, transferred to nitrocellulose filters and hybridized to labeled β-globin recombinant plasmid DNA. It was found that the injected DNA (10^7 copies/egg) replicates extrachromosomally, reaching a maximum copy number at late gastrula, after which a large fraction of the foreign DNA is degraded. 6-week-old tadpoles still contain an average of about 10 copies of the globin gene per cell. Most of these genes are now present at tandem repeats and comigrate in agarose gel-electrophoresis with high molecular weight *Xenopus* DNA. Analysis of globin gene expression by S_1 mapping has shown that the rabbit β-globin promotor is recognized in the frog embryo and that the transcripts are correctly spliced.

Transcription of the single copy vitellogenin gene of *Xenopus* involves the expression of middle repetitive DNA

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6 of the 7 largest introns of the A1 vitellogenin gene of *Xenopus laevis* contain a middle repetitive DNA sequence. These repetitive sequences differ from one intron to the next and are not found in the closely related A2 vitellogenin gene. Hybridization of intron subclones to blots of genomic Eco RI digests demonstrates that the repetitive sequences are distributed over numerous Eco RI fragments. 3 repetitive sequences are not detected in the DNA of the related species *X. tropicalis* suggesting that these sequences are evolutionary unstable. Hybridization of intron subclones to nuclear poly(A)-containing RNA from the liver of an unstimulated male known to contain inactive vitellogenin genes revealed that all 6 repetitive DNA sequences are transcribed, suggesting that the repetitive sequences found in the A1 vitellogenin gene are also present in transcriptional units active in the absence of estrogen.

Comparison of polysomes from uninfected and SV40-infected cells

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Simian virus 40 was known to stimulate overal cellular protein synthesis (Khandjian et al., PNAS *77*, 1476, 1980). To analyze this stimulatory effect further, we examined different aspects of polysome formation and function. Polysome formation reacted to the viral stimulus more strongly (up to 2-fold increase) than protein synthesis. Polysomes from infected cells appeared to be more active than those from uninfected cells when added in equal amounts to a reticulocyte lysate and tested for translation in vitro. In a search for possible regulator proteins, a number of nonribosomal proteins (among others, actin and myosin) were found to be associated with polysomes. These proteins were much more prominent in infected cells. Large T-antigen was observed to be synthesized exclusively on 'heavy' polysomes and to be bound subsequently to 'light' polysomes.

Development of neurons in monkey lateral geniculate nucleus

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Maturation of lateral geniculate nucleus neurons in baby monkeys was studied in Golgi preparations. Of 5 main neuronal types in mature monkeys (Saini and Garey, Exp. Brain Res., 1981) 4 are identifiable in their immature forms. They are: multipolar neurons, bipolar neurons, neurons with 'axon-like' dendritic processes and capsular neurons. During the first postnatal month they pass through

stages characterized by dendritic growth cones and a profusion of spine-like protuberances on dendrites and somata. Capsular neurons mature first, followed by multipolar and bipolar neurons while cells with long cylindrical dendrites and axon-like processes are last. Magnocellular laminae mature earlier than parvocellular.

Neuronal types in monkey lateral geniculate nucleus

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In Golgi preparations of the lateral geniculate nucleus of old- and new-world monkeys 4 main types of neuron are described. Large, medium or small multipolar neurons are found in all laminae. Their dendrites are sparsely spiny. Some have a 'radiate' dendritic arbor and others have dendrites grouped in 'tufts'. The next commonest class is the bipolar neuron with 2 thick, sparsely spiny dendrites arising from opposite poles of the soma, and found mainly in magnocellular laminae. A few examples of medium-sized neurons with beaded dendrites were found in magnocellular laminae. There is a 4th class of small neurons with fine 'axon-like' dendritic processes. They are in all laminae and form 2 subgroups, one with very long, untapered dendrites and few axon-like processes, the other with shorter dendritic arbors and many axon-like processes. A class of large, capsular neurons is found in the circumgeniculate capsule between layer 6 and the pregeniculate nucleus. They have dendritic ramifications in layer 6. The interlaminar zones contain examples of all types except beaded and capsular neurons.

The effect of the proalbumin hexapeptide extension on albumin synthesis in isolated hepatocytes

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Albumin (alb) is synthesized via an intracellular precursor which differs from serum alb by the peptide extension Arg-Gly-Val-Phe-Arg-Arg-(HP) at the N-terminal end. To prove the hypothesis that HP is involved in the regulation of alb synthesis the effect of HP and one of its possible degradation products (TP) on alb synthesis were studied in liver cell suspensions. - HP was synthesized by stepwise N-terminal synthesis with the mixed anhydrides of the amino acids, purified by chromatography and purity confirmed by amino acid analysis. Enzymatically isolated hepatocytes were incubated for 8 h either with a single bolus of HP (1.13×10^{-4} M) or with sequential doses every 30 min (3.4×10^{-5} M). The control suspension synthesized alb at a rate of 2.44 ± 0.5 µg/10^6 cells/h. Alb synthesis could not be stimulated or inhibited by addition of HP or TP. - Physiologically HP is removed from proalbumin within the cell. It is not known whether the HP can cross the cell membrane. Additional experiments with a cell free system are required to exclude definitely that HP regulates alb synthesis.

Thymidine kinase activity of heat- and cold-sensitive mammalian cell cycle variants

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2 heat-sensitive (hs, arrested in G1 at 39.5 °C) and 2 cold-sensitive (cs, arrested in G1 at 33 °C) clonal variants were isolated from a clone of the murine P-815 cell line and tested for thymidine kinase (TK) activity. After shift of variant cells to the nonpermissive temperature, TK activity decreased and minimal levels (i.e. less than 3% of those observed for wild-type cells at the respective temperature) were attained within 16 h in hs and after 4 days in cs variants. After return of variant cells to the permissive temperature, TK of hs cells increased in parallel with DNA synthesis, whereas in cultures of cs cells, the labeling index began to increase 16 h earlier than TK activity. In wild-type cells, TK activity at 39.5 °C was approx. 25% of that at 33 °C. Upon shift from 39.5 to 33 °C, the increase of TK activity was inhibited by cycloheximide, but not by actinomycin D.

Transcription of the chloroplast DNA of *Chlamydomonas reinhardii*

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Several aspects of the transcription of the chloroplast DNA of *C. reinhardii* which consists of 190-kb circles have been examined. 1. Cloned chloroplast DNA restriction fragments, whose location on the map is known, have been radioactively labeled in vitro and hybridized with cellular RNA fractionated by electrophoresis on fully denaturing agarose gels. The results allow to draw a transcription map of the chloroplast genome and reveal regions on the map which are transcribed strongly and/or whose transcripts are stable; and regions which are transcribed weakly and/or whose transcripts are unstable. 2. The transcription of the chloroplast genome has been studied during a synchronous cell cycle. Cells were labeled with $^{32}PO_4$ at various periods of the cell cycle; the RNA was extracted and hybridized with chloroplast restriction of fragments. The results indicate that several chloroplast genes are not transcribed uniformally throughout the cell cycle.

Inhibition of progesterone-induced meiosis in *Xenopus laevis* oocytes by a methylase inhibitor of membrane phospholipids: a link with cAMP metabolism

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Progesterone interacts at the surface level when the steroid triggers the reinitiation of meiosis in *Xenopus laevis* oocytes, with modulation of membrane enzymes such as adenylate cyclase and concomitant protein synthesis. The evidence is now presented for a correlation between membrane phospholipid methylation and progesterone-mediated process by using a methylase inhibitor (SIBA = 5'-deoxy-5'-S-isobutyl-thioadenosine). When incubated with oocytes, this methylase inhibitor inhibts meiosis reinitiation induced by progesterone, the effect being dose-dependent. In addition, SIBA was found to enhance the cAMP content of oocytes exposed to this inhibitor. It appears thus that methylation of membrane phospholipid, cAMP metabolism and meiosis reinitiation mediated by progesterone are closely correlated.

Studies on *Escherichia coli* galactokinase (*gal*K) gene expression in mammalian cells

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The *gal*K gene of *E. coli* was put into a hybrid plasmid containing SV40 regulatory sequences for transcription initiation, RNA splicing and poly(A) addition. The construction should ensure the formation, in transfected cells,

of a mature *gal*K mRNA. By in vitro manipulations, the translation initiation codon of the *gal*K was positioned at various distances from the SV40 promoter either to become the first AUG on the mRNA or to be preceded by other AUGs. Transfection studies have been initiated and *gal*K expression is being monitored by starch gel-electrophoresis and by analysis of mRNAs. Such analyses will also be performed in cell clones that have been stably transformed with the *gal*K gene. Expression of a bacterial gene in a heterologous, mammalian background can potentially be used to complement, in cultured cells, a human genetic disorder such as *gal*K-deficiency galactosemia.

PWM-induced proliferation and differentiation of *Xenopus* splenocytes

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Splenocytes of *Xenopus laevis* undergo a strong proliferation upon pokeweed mitogen (PWM) stimulation in Click's medium containing 2-mercaptoethanol which is most evident after 6–10 days of culture. Light and electron microscopic examination reveals the presence of large number of lymphoblasts. A substantial proportion of them exhibit, from day 8 onwards, the presence of cytoplasmic 19S immunoglobulin (HMW-Ig) as shown by direct immunofluorescence. Few cells only synthesize 7S Ig (LMW-Ig) at day 8, but their number increases by day 11. Stimulated 8- and 11-day cell cultures were incubated with ^{35}S methionine, the labeled proteins were immunoprecipitated with class-specific antisera to HMW- and LMW-Igs and analyzed on SDS-polyacrylamide gels. The results show that *Xenopus* lymphoblasts synthesize HMW- and LMW-Ig subunits and assemble them into 2H2L monomeric units, but they secrete the HMW-Ig in their polymeric and the LMW-Ig in their monomeric form. Thus PWM is shown to be a potent agent for induction of in vitro synthesis of *Xenopus* Igs.

Role of acid phosphatase of the fission yeast *Schizosaccharomyces pombe* in cell surface growth and differentiation

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A. pase of *S. pompe* is an abundant cell surface glycoprotein. Over 70 mutants deficient in the enzyme activity have been selected and characterized. All map at the same locus designated *pho*1. Some of them show slow growth, aberrant cell morphology and altered agglutination. Based on genetic and biochemical criteria the *pho*1-locus codes probably for the structural gene of a. pase. Some of the misshaped mutants are defective in the processing of a. pase. A. pase may be directly involved in cell-cell contact, cell wall morphogenesis and cell-cycle regulation.

Genetic control of chromosome aberration formation in *Drosophila*

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In X-rayed (200 rad) neuroblasts of excision-repair (mei-9) and postreplication-repair (mei 41) deficient mutants a mutation specific significant increase of particular aberrations is observed depending strongly upon the time interval between irradiation and entrance of the cells into mitosis. Up to a 10-fold increase of chromatid breaks in cells entering mitosis within 6 h p.r. is caused by the mei-$9^{L}1$ allele. Chromatid exchanges and dicentrics are almost completely absent up to 14 h p.r. (average length of cell cycle). The mei-$41^{D}5$ allele causes a close to 10-fold increase of isolocus breaks in cells entering mitosis 4–10 h p.r. A failure in the repair attributed to these mutations alone cannot explain the aberration pattern and suggests either further metabolic degradation of nonrepaired sites in chromosomal DNA, the involvement of mei-9+ and mei-41+ gene products in further repair pathways or an increase of misrepair in the sense of Revells (1959) or Evans (1967) misrepair hypothesis of chromosome aberration formation.

A cloned mouse H4 histone gene: characterization and expression in L-cells

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I have cloned a genomic EcoRI restriction fragment from the mouse. The 5.2 kb long insert contains one H4 histone gene and probably no other. Structural analysis of the clone reveals that the H4 gene is not interrupted by introns and that regulatory elements flanking the coding region are similar to those found in the sea urchin. A sequence thought to be a hot spot of intergenic recombination in human globin genes is found downstream of the H4 gene. A set of 12 EcoRI fragments containing H4 genes can be detected in an inbred mouse strain, each of them being represented 1–3 times per haploid genome. Some of the H4 genes may be linked together. After hybridization of the cloned H4 gene to histone mRNA's of L-cells, the DNA becomes protected against S_1 nuclease attack and hence this particular gene appears to be expressed in the tissue culture cells. High mol.wt trancripts containing H4 sequences have not been detected at the level of 10 molecules per nucleus.

DNA from dairy cultures

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Genome sequence homology relationships and relative genome sizes were determined by free solution DNA-DNA hybridization for strains *Streptococcus lactis* ATCC 19435, ATCC 7962, C2, NCDO 763 ML3, C4, x 13, 606, *S. lactis* var. *diacetylactis* 216, 26-2, Pim 710, and *S. cremoris* Z-8, H-2, ML-1, Sc-87. With the exception of *S. lactis* C2 and ML3, strains could be grouped according to relative genome size, *S. lactis* var. *diacetylactis* 1.2–1.4, *S. lactis* 1–1.14 and *S. cremoris* 0.75–0.95. On plotting homology relationships all *S. lactis* except C2 and ML3 formed a cluster with *diacetylactis* varieties in the centre. This indicates that *S. lactis* are probably varieties of *S. diacetylactis*. *S. cremoris* strains with *S. lactis* C2 and ML3 plotted also as a separate cluster with only 30% homology to the lactis group. Although C2 and ML3 produce ammonia from arginine characteristic for *S. lactis*, they have over 80% sequence homology with *S. cremoris* strains and genome sizes 0.94 and 0.85 respectively. These results indicate that C2 and ML3 have been previously misidentified taxonomically.

A molecular view of the *Drosophila* chromosome

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We have collected overlapping cloned DNA fragments covering a 300-kb segment of the 3^{d} chromosome of

Drosophila. This region comprises 9 bands on the polytene chromosomes (87 D11-87 E6) and about 9 complementation groups (1G37, ry, pic, S8, B16-1, C9a, ace, G7, m32). The position and extent of the bands have been estimated and the genes have been located by deletion mapping. *Ace* (acetylcholinesterase) has been located and identified by hybrid arrested translation. Tissue specific DNAse-I sensitive sites are found in the vicinity of the *ace* locus. - We have also accumulated clones covering a 150-kb region containing the bithorax complex (a group of genes controlling segmentation). The localization of mutations on the DNA sequence is in excellent agreement with the genetic map. Most, if not all, the mutants analyzed are large rearrangements.

Intracellular collagen degradation is conformation-dependent

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Collagen (C), the most abundant extracellular protein, is important for the proper structure and function of most tissues. Levels of C result from synthesis and extracellular degradation by collagenase-mediated and/or phagocytic mechanisms but also from intracellular degradation (iD) of newly synthesized C (J. biol. Chem. *253,* 4356, 1978). We tested whether iD depends on the thermal stability of C and hence its triple helical conformation which is stabilized by the ascorbate-mediated hydroxylation (H) of proline to hydroxyproline (hyp). Human skin fibroblasts at different densities and temperatures were incubated for 8 h in medium containing ^{14}C-proline, 10% serum, ±50 µg/ml ascorbate. iD was estimated by the amount of dialyzable hyp per total hyp formed. The extent of H was determined after digestion with bacterial collagenase. At 37 °C iD ranged from 13 to 54% and was inversely correlated with H (from 45 to 16%). At 42 °C (i.e. above the melting temperature Tm of fully hydroxylated C) and at 23 °C (i.e. below Tm of underhydroxylated C) iD was high (49%) for fully hydroxylated C (H = 51%) and low (14%) for underhydroxylated C (H = 15%). We conclude that intracellular collagen degradation is considerable and indeed conformation-dependent and thus it may play an important role in the regulation of tissue levels and the quality control of this secretory protein.

Genetically displaced sensory neurons in *Drosophila* project into the same specific brain regions though by different pathways

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The homoeotic mutants *pb, Antp, ss*^{a} and *en* form ectopic populations of sensory neurons in the proboscis and antenna of *D. melanogaster.* Antennal neurons originating in the proboscis project into the normal proboscis center and into 1 antennal center of the brain (although in the latter they show an aberrant pattern). Leg axons from the proboscis may enter the brain at 4 different sites (in contrast to normal proboscis axons), and yet they terminate always in proboscis and antennal centers. Consequently the pathway between these 2 brains regions may be followed in opposite directions. Leg neurons from the antenna project into normal antennal and proboscis centers, too. - Why displaced leg and antennal neurons project exclusively into normal antennal and proboscis centers may be explained by affinities between the sensory neurons and the brain centers in question, the affinities being due to a) homologies between antennal, leg and proboscis neurons, and b) homologies between the 3 corresponding centers.

Characterization of rabbit milk protein mRNAs; construction of bacterial recombinants containing casein and α-lactalbumin cDNAs

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Milk protein synthesis in the rabbit mammary gland provides an attractive system to analyze prolactin-induced gene activation which occurs both at the transcriptional and posttranscriptional levels. The size of the most abundant mRNAs in lactating mammary gland was determined on denaturing agarose gels. Specific antisera against secretory component, transferrin, casein (α, β, $\varkappa$) and α-lactalbumin were used to immunoprecipitate the in-vitro translation products. Highly enriched casein and α-lactalbumin mRNAs were used as probes to isolate clones from a cDNA library. These cDNA clones are being used to study the expression of prolactin responsive genes.

The appearance of nucleosomes in the electron microscope

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We have observed 3 different conformational states of nucleosomes: 1. Nucleosomes where the DNA enter- and exit-points are on the same side. 2. Nucleosomes where the DNA enters and leaves on opposite sides. 3. Nucleosomes which are completely unravelled into DNA-like filaments. These conformational states are dependent on the chromatin composition, on the ionic strength and on the pH of the solvent used for the preparation. The folding/unfolding transitions are reversible supporting models of dynamic nucleosomal structures.

Serum stimulation of resting 3T3 cells induces synthesis of all major RNA species

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Resting 3T3 mouse fibroblast cultures kept in 0.5% bovine serum are stimulated to enter into a division cycle by addition of 10% fresh serum. DNA synthesis begins 10–11 h later and reaches a maximum around 24 h when 80% of the cells are in S-phase. An increase in total cellular RNA becomes detectable colorimetrically by 3–5 h after stimulation and RNA content has doubled around 20 h. Examination by gel-electrophoresis of nuclear and cytoplasmic RNA extracted from cultures labeled with [^{3}H]U suggests a sequential activation of transcription; it begins with stimulated synthesis of 5S RNA and tRNA which is rapidly followed by synthesis of precursor rRNA and hnRNA. Stimulation of all species of RNA, including hnRNA, occurs several hours before onset of DNA synthesis and also takes place if DNA synthesis is inhibited with araC. Our results thus show that the rate of hnRNA synthesis is not template limited as was previously suggested by Mauck and Green (PNAS, 1973) on the basis of their work with araC-inhibited 3T6 fibroblasts.

Cell-survival as a dosimetry system of pion beams

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The Piotron at the Swiss Institute for Nuclear Research (SIN) is built for the use of negative pi-mesons in radiotherapy. The pions are extracted by 60 channels to enter the treatment volume radially and each beam can be switched off individually. In the initial experimental phase cells were irradiated by 15 beams and our main concern was the relative biological effectiveness (RBE) of pions as opposed to X-rays. Both proliferation- and colony-forming ability of CHO-cells in vitro were investigated. According to the method, modified by Tremp et al. (1979), cells were embedded in tissue-equivalent gelatine, which turns fluid at 37 °C and solid at the irradiation temperature of 19–21 °C. After irradiations small slices of gel were cut off and the cells tested to their colony-forming ability. Cell-survival curves and RBE-values were presented for Peak pions as well as for X-rays. These studies were carried out in combination with thermoluminescent dosimeters (TLD) in order to measure the exact position of the cell-gel mixture and to estimate the absorbed physical dose.

The effect of hyaluronidase (hyase) and hyaluronic acid (HA) on the Ca-dependent aggregation of myogenic cells

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Cultured cells detached from plates according to Takeichi (J. Cell Biol. *75*, 464) show a Ca-dependent adhesion while cells detached by EDTA also have a Ca-independent mechanism. To investigate the Ca-dependent aggregation its rate was measured with detached primary chicken breast muscle cells. We counted particles which remained in the aggregation system in the presence of testicular hyaluronidase. Cells were preincubated with hyase and trypsin inhibitor in order to inactivate eventual trypsin-like activity in the hyase preparation. Hyase completely blocked aggregation with and without trypsin inhibitor present. An increased aggregation, however, was found with cells preincubated with HA and Ca whereas HA treatment alone had no such effect. Whereas others (Exp. Cell Res. *117*, 155) have reported a Ca-independent aggregation, our results indicate that HA can also be involved in a Ca-independent aggregation where HA may function as a link in conjunction with Ca.

4 clustered *Drosophila melanogaster* heat shock genes

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4 small heat shock protein genes have been isolated from chromosomal site 67B. 1 major type of arrangement of these genes has been defined by whole genome Southerns using cloned cDNAs as probes. The same organization was deduced from the analysis of 17 independently isolated genomic clones carrying either 2 or all 4 genes. The 4 heat shock genes are clustered in an 11-kb DNA segment and are separated by spacers of 1–4.7 kb. 3 of these genes exhibit alternating polarities suggesting that they are transcribed individually. As judged by the absence of cross-hybridization, the 4 genes are not closely related. – A 2nd type of arrangement was observed in one of the genomic clones. In this clone the heat shock gene cluster is flanked by sequences not found in any of the other isolates. This unusual type of organization is probably due to an insertion of a 5.3 kb dispersed repetitive sequence element into the DNA region immediately adjacent to the heat shock genes. – In order to identify possible common transcriptional signals the 5′-flanking regions of various heat shock genes are being analyzed by cross-hybridization and direct sequencing.

The gene for the precursor of the bacteriophage T4 internal peptide: structure and function

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We have defined the genomic location of a T4 late gene, which codes for the precursor of the internal peptide II of the phage head. This could be managed by a gene expression assay from cloned T4 DNA using specific antiserum against its gene product. Because classical mutagenesis failed to give mutations in this gene, we constructed frameshift mutations at specific restriction sites, using the DNA sequence of that gene as a basis. We will present the DNA sequence of that gene and the results of the mutagenesis approach. The unusual AS sequence of its gene product will be discussed.

Methylation of specific genes inhibits their expression after microinjection into viable mammalian cells

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Many organisms contain methylated purines and pyrimidines in their DNA. The biological function of these modified bases is not known, but they are believed to play a role in gene regulation. We therefore methylated in vitro specific genes which had been cloned into plasmids and studied their expression after microinjection into viable mammalian cells. First, we methylated the HSV thymidine kinase (TK) gene with EcoR1 methylase and microinjected it into ts13TK$^-$ hamster cells. To check for cells expressing TK we labeled them for 24 h with (^{3}H)thymidine and counted the number of labeled cells after autoradiography. We found a 2-3-fold decreased expression of the TK function after microinjection of the methylated HSV TK gene compared to the unmethylated form of this gene. Likewise, we studied the expression of a cloned gene coding for the SV40 T antigen before and after methylation.

Do rat spermatids function as a flagellated cell layer?

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Recently, motility of both human and rat spermatid flagellae was demonstrated on single cells (Walt, Eur. J. Cell Biol. *22*, 559, 1980). Video analysis of intact, viable pieces of germinal epithelium of the rat revealed motility of spermatid flagellae as well. Furthermore, scanning electron microscopical investigation of seminiferous tubules, freed of sperm, displayed an inner surface endowed with spermatid flagellae, which point towards the tubular lumen. This morphological aspect resembles the inner surface of a choanocyte channel in sponge, which is covered by a flagellated epithelium. The results demonstrate that flagellae of spermatids, integrated in the epithelial complex, behave as do flagellae of single spermatids in vitro. There is strong support for the suggestion that spermatid flagellae are involved in the convection of tubular fluid and in the

introduction of just matured sperm into the spermatic flux. The mechanical force, originating in this flagellated cell layer, may be regarded as an additional factor in sperm transport.

Human chromosomal α-interferon genes: structure and expression in mouse cells

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The structure of several of the not less than ten different human chromosomal α-interferon (IFN) genes have been determined. The gene of IFN α1 was inserted into the late region of a polyoma pBR322 vector. Mouse cells transfected with these hybrids produced IFN α1 after 20 h; synthesis declined after 40 h. S1 mapping showed that the 5'-terminus of the IFN mRNA from mouse cells mapped close to but not at the same position as that of IFN mRNA from human leucocytes.

Specific antibodies against the proalbumin hexapeptide

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Proalbumin, the intracellular precursor of albumin (alb), differs from serum alb by the peptide extension Arg-Gly-Val-Phe-Arg-Arg- at the N-terminal end. This hexapeptide (HP) is removed shortly before alb is released from the hepatocyte. To follow the hitherto unknown fate of this HP, antibodies against synthetic HP were produced. - The HP was synthesized by stepwise N-terminal synthesis with the mixed anhydrides of the amino acids, purified by chromatography, and purity confirmed by amino acid analysis. The pure HP was coupled to rabbit alb by carbodiimide, and 2 rabbits were immunized by intradermal injection. - After 5 weeks precipitating antibodies against the coupled HP could be detected by immunodiffusion in both animals. With an enzyme linked immunosorbent assay (ELISA) a strong binding to coupled and uncoupled HP of both antisera was found. A minor and much weaker binding was found for rabbit alb treated with carbodiimide. - The results demonstrate that rabbits produce a specific antibody against the chemically synthesized HP extension of proalbumin, which will allow to determine the biological fate of the proalbumin hexapeptide extension.

2 related albumin genes are expressed in *Xenopus laevis* liver

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Double-stranded cDNA was synthesized from 18S poly(A)+ RNA from male *Xenopus* liver, inserted into the Pst 1 site of the plasmid pBR 322 and cloned in *Escherichia coli.* Several liver specific clones were isolated which cross-hybridized to each other and were shown to contain albumin sequences by translation of RNA positively selected with plasmid DNA immobilized on DBM filters. Restriction mapping, hybrid melting experiments and R-loop and heteroduplex analysis showed that the cloned cDNA covered 1700 of the 2300 nucleotide long albumin mRNA and that there are 2 closely related sequences which are mismatched by about 5%. These cDNA clones have been used to isolate fragments of genomic DNA from a *Xenopus* DNA library (provided by W. Wahli) cloned in Charon 4a and the structure of these cloned fragments has been determined.

Stage-specific expression of *Xenopus* globin genes

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cDNA clones containing larval and adult *Xenopus* globin sequences were characterized by cross hybridization and restriction analysis. In both, adults and larvae, 2 unrelated main groups of clones were identified each of which comprising 2 related subgroups. The unrelated main groups were shown to be α- and β-globins by sequence analysis. Melting curves of hybrids formed between the subgroups suggest that the larval subgroups are less related than the adult ones. These results suggest that 2 α- and 2 β-globin genes are expressed in both, larvae and adults as a result of a genome duplication but that these genes have subsequently diverged to different degrees.

Characterization of cloned cDNA for mouse complement C3 and of the mouse and human C3-genes

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From a collection of cDNA-clones carrying sequences homologous to mouse liver mRNA on pBR 322 we have identified 8 different C3-cDNA plasmids. Identification was achieved by hybrid selection of mRNA followed by its cell-free translation, immunoprecipitation and SDS polyacrylamide gel-electrophoresis of the products. The inserts of those plasmids cover in total 4.6 kilobases of the C3-mRNA which was estimated to be 7.5 kilobases long. - Mouse C3-cDNA sequences were found to cross-hybridize with restriction fragments of human DNA. By means of this cross-hybridization we have isolated 1 lambda phage carrying part of the C3-gene from a human gene library. At present we are constructing mouse gene libraries to isolate and characterize the mouse C3-genes.

Drosophila cells respond specifically to insulin

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The *Drosophila* Kc cell line has a high cloning efficiency (c.e.) in a synthetic medium supplemented with fractionated yeast extract and 1% horse serum (Wyss, Somat. Cell Genet. *5*, 23, 1979). Bovine insulin reduces c.e. in a dose-dependent manner starting at 10 ng/ml. This effect is not seen if insulin is pretreated with insulin antibodies. Pure insulin-like growth factor I (IGF I, Rinderknecht and Humbel, Proc. nat. Acad. Sci. *73*, 2365, 1976) has no effect up to 1 µg/ml. Cells from 6 h *Drosophila* embryos die within 12 h in the unsupplemented synthetic medium. Bovine insulin (1 ng/ml to 10 µg/ml), however permits these cells to survive and differentiate into muscle, nerve and fat cells. The effect of insulin is abolished by antiinsulin. IGF I up to 1 µg/ml has no effect on these cells. Due to inhibitory material in partially purified *Drosophila* extracts insulin-like activity could only be detected immunologically but not in culture.

Transformation of a mutant *Drosophila* cell line in vitro

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Line MDR3 is a mutant of the Kc line, deficient in adenine phosphoribosyltransferase activity and therefore unable to grow in TAM (thymidine, adenine, methotrexate) supplemented media; its reversion to TAM resistance is very rare (Wyss, Somat. Cell Genet. *5*, 29, 1979). MDR3 cells ($2-5\times 10^6$) were exposed to DNA (20-50 µg) for 2 h at 25 °C in buffered saline. Phenol extracted DNA was either sheared or digested with EcoR1. To enhance uptake DNA was added as Ca-precipitate or complexed to DEAE-dextran or poly-L-ornithine. After an expression time of 24 h in nonselective medium cells were plated in TAM supplemented soft agar medium. Colonies were scored after 3-4 weeks. With DNA from MDR3 cells no transformation was found. Wild type Kc DNA, however, produced up to 10^{-4} transformation if DNA-polyornithine was used. Little or no effect was seen, when the DNA was added differently. Sheared and EcoR1 digested DNA was equally effective.

Genetic and functional analysis of heat- and cold-sensitive mammalian cell cycle variants

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A heat-sensitive (hs, reversibly arrested in G1 at 39.5 °C) and a cold-sensitive (cs, reversibly arrested in G1 at 33 °C) clonal variant isolated from an undifferentiated murine mastocytoma line (P-815) were analyzed by PEG-induced fusion of arrested cells with 'wild-type' (WT) cells arrested in G1 by serum deprivation. For identification of heterokaryons, cells used for fusion were labeled by ingestion of latex particles of different size. After fusion of hs or cs with WT cells and reincubation at the nonpermissive temperature with 10% serum and ^{3}H-dThd for up to 48 h, hs-WT, but not cs-WT binuclear cells entered the S-phase, indicating recessive expression of the hs and dominant expression of the cs phenotype. After shift to the nonpermissive temperature, cs, but not hs variant cells underwent cellular differentiation with a marked increase in numbers of mast cell granules, as determined by electron microscopy and toluidine blue staining. The cs variant cells thus appear to be arrested at 33 °C in a 'G0'-like state.

PHARMAKOLOGIE - PHARMACOLOGIE - PHARMACOLOGY

Selective antagonism by caffeine of diazepam-induced muscle relaxation in mice

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Muscle tone can be assessed in mice by palpation of the abdominal wall (S. Irwin, Psychopharmacologia *13*, 222, 1968). In a randomized treatment situation with a blind evaluation schedule, a dose-dependent reduction of abdominal muscle tone was found after diazepam (ED_{50} = 1.2 mg · kg^{-1} p.o., 0.2 mg · kg^{-1} i.p.) and after phenobarbitone (ED_{50} = 19 mg · kg^{-1} i.p.). Caffeine, administered p.o. after diazepam i.p. (1 mg · kg^{-1} = ED_{90}), antagonized the diazepam-induced muscle relaxation with an ED_{50} of 0.53 mg · kg^{-1} (95% confidence interval: 0.17-1.68 mg · kg^{-1} p.o.). Against phenobarbitone (100 mg · kg^{-1} = ED_{90}), an antagonism by caffeine could not be found up to 30 mg · kg^{-1} p.o.

Distribution and metabolism of theophylline in the pregnant rat: presence of a blood brain barrier

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Theophylline (T) is a major drug in the treatment of asthma and the control of neonate apnea. In agreement with observations on preterm children, we showed (Nut. Rev. *38*, 196, 1980) that [8-^{14}C]T given orally to pregnant rats was methylated into caffeine in the fetus. Only trace of caffeine was produced, in utero, because T did not accumulate in the fetus and was excreted in the urine of the pregnant rat. The analysis of urinary metabolites at the 18th day of pregnancy, showed that unchanged T corresponds to 70± 10% of urine radioactivity in contrast to 35± 3% in non-pregnant rats. This result demonstrates a reduced capacity of T metabolism during pregnancy and can explain the prolonged half-life of methylxanthine already described. T distribution in the organs of the fetus and the mother was studied and showed the presence of a blood brain barrier in the adult animal. The ratio of brain/blood T concentration was 0.41±0.03 in the adult animal and 0.88±0.09 in the fetus. As the ratio of blood T concentration in the pregnant rat/fetus is 0.89±0.06, these results demonstrate the total absence of a blood brain barrier in the fetus for T. In the premature infants, a ratio of 0.88 for cerebrospinal fluid/serum (Somani et al. J. of Pediatrics 96, 1091, 1980) has been reported and no data is available in the adult human.

Placental transfer of the major caffeine metabolite in the rat using the 6-amino-5[N-formylmethylamino]1,3[Me^{14}C]dimethyluracil

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6 - Amino - 5 [N - formylmethylamino] 1, 3 - dimethyluracil (DAU) called previously the 1,3,7-trimethyldihydrouric acid is quantitatively the most important caffeine metabolite in several animal species (20% of rat's urine metabolites). After oral administration of caffeine (8 mg/kg), only 1% of the dose was excreted as DAU in the urine of human volunteers. This important species difference initiated research on the effects of daily oral administration of DAU on rats during pregnancy. The absence of effect reported (Muther, Food Chem. News, Nov., 47, 1980) has to be validated by the demonstration of the placental transfer which has never been studied. In order to demonstrate the presence of this molecule in the fetus, [1-Me^{14}C] caffeine was administered to pregnant rats and the metabolites present in the fetus were identified. DAU was identified in the fetus and its blood concentration was the same in the fetus and the pregnant rat showing its rapid transfer through the placenta. To confirm this transfer, labeled

DAU was synthesized. One hour after oral administration to pregnant rats, labeled DAU was identified in the fetus both by whole animal body autoradiography and by radiochemical extraction and chromatography. Urine analysis confirmed the absence of metabolism of DAU.

Metabolism of [^{14}C] emodin in rats

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Emodin represents the active component in most laxative drugs from plant origin. These drugs usually contain beside the emodin glycosides 3-5% impurities as free emodin and other anthraquinones. [^{14}C] emodin was biosynthesized by cultures of *Penicillium islandicum* from [^{14}C] acetate as a precursor. Metabolism studies were performed in female rats and absorption, excretion and tissue distribution were studied after a single oral dose of 50 mg/kg (equiv. to approx. 8×10^5 dpm). Urinary excretion amounted to 18±5% after 24 h and 22±6% after 72 h respectively. Metabolites found in urine were unchanged emodin and small amounts of emodic acid. In feces the metabolites amounted to 48±11% of dose within 48 h and to 63±8% within 72 h respectively. Biliary excretion was studied in bile duct cannulated rats and reached a maximum at 6 h after administration. 49% of dose were excreted within 15 h, mostly as conjugated emodin. Radioactivity levels in most organs decreased between days 3 and 5, but in kidneys [^{14}C] activity was still equivalent to 4.33 ppm emodin 5 days after administration. Mesenterial and fat tissue showed increasing [^{14}C] activity from 72 to 120 h.

Antidepressives in saliva: comparison with free and total plasma levels

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In clinical practice, the determination of plasma levels of tricyclics has been introduced in order to improve the pharmacotherapy of depressives. However, the relationship between the plasma level and the clinical outcome is not clear. As tricyclics are bound to some extent to plasma proteins, it may be necessary to take into account their free level. - For some drugs, saliva levels reflect the free plasma levels. This explains why some authors recommend the determination of certain drugs in saliva. In the present study, the relationship between the free and total plasma, on one hand and saliva levels on the other hand have been investigated for amitriptyline and nortriptyline. Depressive hospitalized patients were treated with amitriptyline, either p.o. or i.v. Plasma and saliva were regularly collected during the treatment. - The results indicate that the concentrations of these drugs in saliva exceed by far the free plasma levels, approaching the total plasma levels. The findings are discussed with regard to the basic and lipophilic properties of antidepressive drugs.

Etude du Sulpiride et de ses métabolites sur la sécrétion de la prolactine in vivo chez le rat

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L'action du Sulpiride (SU) sur la sécrétion hypophysaire de la prolactine (PR) est bien connue et décrite dans plusieurs de nos publications. Nous avons aussi étudié le métabolisme de ce médicament et déterminé la structure de plusieurs métabolites. - Cette communication donne les résultats des taux plasmatique et adénohypophysaire de la PR déterminés par une technique radioimmunologique chez le rat après administration du SU et de 3 de ses métabolites: N-[(éthyl-1 pyrrolidinyl-2)méthyl] hydroxy-2 sulfamoyl-benzamide (A); N-[(oxo-5, pyrrolidinyl-2)méthyl] méthoxy-2 sulfamoyl-5 benzamide (B); N-[(éthyl-1, oxo-5, pyrrolidinyl-2)méthyl] méthoxy-2 sulfamoyl-5 benzamide (C). Le SU est très actif vis-à-vis de l'excrétion de la PR 2 h après administration et présente des effets stimulants sur la libération de la PR à 8 h alors que les métabolites A et B sont inactifs. Le métabolite C présente un effet inhibiteur à 2 h et stimulant à 8 h. - La libération massive de la PR sous l'effet du SU est accompagnée d'une diminution significative du taux adénohypophysaire de la PR ce qui n'apparaît pas lors de l'administration des métabolites, démontrant ainsi leur inactivité vis-à-vis de ce processus

Drug effects on lisuride-induced mounting in female rats

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Lisuride (LIS)-induced mounting (M) behavior of rats was demonstrated to depend on simultaneous dopamine (DA) stimulation and reduced 5-hydroxytryptamine (5-HT) receptor activation (Da Prada et al., Neurosci. Lett. *6*, 349, 1977). Accordingly, LIS-induced M was suppressed either by the DA receptor blocker pimozide (0.25 mg/kg i.p.) or by compounds enhancing 5-HT receptor activation by different mechanisms. These include the precursor amino acid 5-hydroxytryptophan (100 mg/kg i.p.), 5-HT receptor agonists (quipazine, 5 mg/kg i.p., m-chlorophenylpiperazine, 10 mg/kg i.p.), and antidepressant drugs inhibiting the uptake of 5-HT (chlorimipramine, 10 mg/kg i.p., citalopram, 10 mg/kg i.p., (-)paroxetine, 3 mg/kg i.p., Ro 11-2465, 3 mg/kg i.p.). Moreover, a possible involvement of opioid receptor mediated mechanisms in this behavior is proposed, since morphine (1 mg/kg s.c.) and the synthetic enkephaline analogue FK 33-824 (0.1 mg/kg s.c.) both inhibited LIS-(0.5 mg/kg i.p.)-induced M in female rats and, as to be expected, this inhibition was reversed by naloxone (3 mg/kg s.c.).

A selective benzodiazepine antagonist: Ro 15-1788

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The imidazobenzodiazepinone Ro 15-1788 is a benzodiazepine (BD) antagonist active in BD binding but completely devoid of BD-like activities (Hunkeler et al., 1981). The depressant effect of diazepam (D) in the horizontal wire test was antagonized by 15-1788 with a p.o. ED_{50} of 0.2 mg/kg in mice and 0.06 mg/kg in rats. Phenobarbitone (Ph), meprobamate (M) and ethanol were not antagonized. Given p.o. to dogs, 15-1788 abolished BD-induced ataxia. The loss of righting reflex and sleep-like posture induced in squirrel monkeys by i.v. flunitrazepam was completely reversed after p.o. 15-1788. 15-1788 given p.o. after a dose of D fully protecting mice from pentetrazole tonic seizures reversed the effect of D with an ED_{50} of 2.8 mg/kg but was inactive against Ph or M. 15-1788 itself did not interact with pentetrazole. The LD_{50} (mg/kg p.o.) for 15-1788 was 4300 in mice and 6000 in rats. As a potent and selective BD antagonist, 15-1788 should be useful in various clinical situations.

Sleep deprivation: effect on specific binding of neurotransmitters in rat brain

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Rats subjected to 24-h sleep deprivation (SD) by forced locomotion exhibit a massive compensatory increase of slow wave sleep and REM-sleep in the recovery period (Borbély and Neuhaus, J. comp. Physiol. *133*, 71, 1979). To test for effects on cerebral neurotransmitter receptors, rats were killed at 4-h intervals during the last 13 h of a 24-h SD period, and during the first 11 h of the recovery period. In nondeprived controls a 24-h rhythm was evident in the number of binding sites for alpha- and beta-adrenergic, muscarinic, benzodiazepine and opiate ligands in forebrain, and for dopaminergic ligands in striatum. The minor differences between the SD-group and the control group consisted mainly in a reduced amplitude of the 24-h binding rhythm in the SD-schedule for the first 4 neurotransmitters mentioned above. The values of the 2 groups correspond particularly in the recovery period. The results indicate that neither 24-h forced locomotion nor the subsequent prominent sleep rebound are reflected by marked changes in neurotransmitter receptor binding.

Pyrrolizidine alkaloids in *Symphytum officinale* L. and their dermal absorption in rats

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Several species of *Symphytum* (comfrey) are used (mainly externally) as a medicinal herb in many European countries. Hirono et al. (1978) found that leaves and roots of this plant containing the pyrrolizidine alkaloids symphytine and echimidine can induce liver tumors in rats. A GC-MS analysis of a commercial sample originating from Poland with a total alkaloid content of 0.07% revealed the presence of 5 pyrrolizidine alkaloids (present as N-oxides): 2 closely related isomers with a M^+ of 341 were tentatively identified as 7-acetylintermedine and 7-acetyllycopsamine (0.056%); 2 further compounds had mol. wts of 295 and 297 (0.014%) but only traces of symphytine ($M^+ = 381$) were found. The dermal absorption of these alkaloids was investigated in rats, using a crude alcoholic extract of the plant corresponding to a dose of 194 mg alkaloid N-oxides/kg b.wt. The excretion of free alkaloids and N-oxides in the urine during 2 days were in the range of 0.08-0.41% of the dose. The oral application led to a considerably higher excretion in the urine with 3.4-9.4% of the applied dose.

Specificity of action of a benzodiazepine antagonist on drug induced changes of cerebellar cyclic GMP in rats in vivo

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Cerebellar cGMP levels in rat were decreased in a dose-dependent manner 30 min after oral administration of diazepam ($ED_{50} = 2$ mg/kg). Ro 15-1788 (5 mg/kg), an imididazobenzodiazepinon had no effect on cGMP levels per se, but caused an increase of the ED_{50} valued for diazepam ($ED_{50} = 9$ mg/kg). The decrease of cerebellar cGMP due to diazepam 5 mg/kg could be antagonized with increasing doses of Ro 15-1788, yielding a very steep dose-response curve between 2 and 5 mg/kg ($ED_{50} =$ 3.6 mg/kg). Ro 15-1788 antagonized the effect of diazepam on cGMP if given before (prophylactic) and after diazepam (curative). Ro 15-1788 did not influence the effects of other drugs (ethanol, phenobarbitone, methaqualone, meprobamate, haloperidol, ethazolate, muscimol or apomorphine) on cerebellar cGMP. The antagonistic action was maximal 30 min after oral administration and was almost completely terminated after 90 min. It is concluded that Ro 15-1788 is a specific benzodiazepine antagonist in vivo, equipotent to diazepam and of short duration of action in rats.

Quick freezing, a method to catch the rapid biochemical and morphological changes underlaying synaptic transmission

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The mechanism of synaptic transmission of nerve impulses will only be elucidated after biochemical and morphological studies at the msec time level. We are studying transmission in the electrogenic tissue of *Torpedo*, using a stimulator coupled to a rapid tissue freezer. 24 isolated prisms are stimulated at intervals of 10 msec or greater and frozen simultaneously at the last stimulation. Significant changes in acetylcholine and ATP levels were found within 100 msec. Freeze fracture showed good preservation of synaptic membranes. The quenching rate has been estimated theoretically and the time resolution predicted. The method should be of general value to catch rapid processes in excitable or other tissues.

Computerized observation technique for the assessment of drug-induced behavioral changes in dogs

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A computerized observation technique allowing the recording and analysis of 64 individual and 64 social activities of 8 dogs has been developed. The method is based on simultaneous recording of the frequency of occurrence (event) and duration (timed event) of behavioral responses by means of an Observation Registration Equipment. This consisted of an acquisation keyboard, a 6-Digit LED-display and 8 3-Digit numbers to monitor the timed behavior. The logic of the system is an interactive standalone program on DEC LSI-11 microcomputer. The behavioral effects of 4 standard drugs (imipramine, amphetamine, haloperidol and diazepam) were compared by means of this method. The results showed that even discrete behavioral changes produced by drugs can be recognized and the activity profiles of drugs readily differentiated. The advantage of the method over classical rating scale procedures is that it provides a means for quantification of behavior in terms of frequency and duration and assessement of drug effects on social components of behavior.

Heterogeneity of transport of an organic anion along the proximal rat renal tubule

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Heterogeneity of transport of organic ions has been previously shown to occur along the renal proximal tubule (PT), but its characteristics differ between mammalian species. We investigated the segmental transport of an organic anion (PAH) in the rat PT, using 2 different methods: micropunctures in vivo or substrate accumulation

in vitro. Micropunctures: single cortical nephrons were punctured at 3 sites successively (distal, late proximal, early proximal); fluid samples were analyzed for PAH to calculate net PAH secretion (at saturating plasma levels) in the convoluted or straight segments of PT. Results indicate that, per unit length, the straight portion secretes 1.9 times more PAH than the convoluted. Uptake in vitro: straight or convoluted segments of PT were incubated (30 min) with ^{3}H-PAH, and steady-state accumulation in tubular cell was measured. Straight segments accumulated 1.6 times more PAH than the convoluted. This uptake was inhibited by probenecid. These differences are qualitatively similar, but quantitatively less as compared to those reported for the rabbit PT.

Effects of cyclosporin A (CY-A) on the adrenal gland in rodents

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CY-A is a cyclic endecapeptide with new immunosuppressive activity. In toxicological studies performed in mice and rats with CY-A, changes in adrenals associated with a slight eosinopenia were observed. We investigated whether CY-A has an effect on the hypothalamic-pituitary-adrenal axis, either by stimulating ACTH release or by a direct action on the adrenals. Female rats (IFFA CREDO) and male mice (OF-1, Sandoz) received single and multiple doses of up to 250 mg/kg CY-A p.o. in olive oil. Controls received olive oil only. - CY-A increased the adrenal weights and the amount of corticosterone (CS) in adrenals of animals dosed 50 mg/kg and higher. $ACTH_{1-24}$-induced CS release and CS plasma levels were increased in treated animals. Histological examination revealed a slight hyperplasia in cells of the zona fasciculata. In vitro steroidogenesis in adrenals from treated animals was increased compared to controls. Based on these results, CY-A seems to stimulate CS production in adrenals of rodents when given in high doses. The immunosuppressive activity, demonstrated at lower dose of CY-A, has been shown not to correlate with CS plasma levels.

Synthesis and antimetabolic activity of DL-threo-3-fluoroaspartate (FAsp) and DL-threo-3-fluoroasparagine (FAsn)

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FAsp and FAsn were synthesized by a new stereoselective chemical procedure which mimics in part the biological degradation of orotate. All steps were performed at ≤ 37 °C. 5-Fluoro-6-dimethoxymethyluracil + H_2 (Rh) then
KOH → $(MeO)_2CH-CH(NHCONH_2)-CHF-COOH$
+ HNO_2 at pH 1 → $(MeO)_2CH-CH(NH_2)-CHF-COOH$
+ Ac_2O → $(MeO)_2CH-CH(NHAc)-CHF-COOH$
+ O_3 → $MeOOC-CH(NHAc)-CHF-COOH$
+ HCl → FAsp + $SOCl_2$ in MeOH then NH_3 → FAsn.
Growth of *Escherichia coli* 1345 in a minimal medium was 100% inhibited by 0.6 µg/ml FAsp. The action of graded FAsp doses was reversed exclusively by equimolar L-Asp doses. FAsn was 100 times less inhibitory. In cultures of L1210 and L5778Y cells containing 5 µg/ml Asn, FAsn caused dose-dependent growth inhibition (ID_{50} = 5 µg/ml). 25 µg/ml caused lysis. In presence of 55 µg/ml Asn complete protection against the toxicity exerted by 10 µg/ml FAsn was achieved whereas 100 µg/ml FAsn caused lysis. Asp offered no protection and FAsp had only marginal antileukemia activity. Additional data characterize FAsp and FAsn as very selective antimetabolites of their unfluorinated counterparts.

Irreversible stimulation of MSH receptors

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3 new derivatives of α-MSH containing photolabile groups at different positions were synthesized and tested in a new melanophore assay system: a) [N^{α}-(4-azidophenylacetyl)-serine^1]-α-MSH, b) [2′-(2-nitro-4-azido-phenylsulphenyl)-tryptophan^9]-α-MSH, and c) [4′-azidophenylalanine^{13}]-α-MSH. Upon UV-irradiation, all 3 derivatives produced rather stable covalent complexes and an irreversible pigment dispersion in *Xenopus* melanophores which lasted for at least 5 h. Control experiments (no UV, excess of α-MSH, scavangers) proved that the effect is specific and due to photolytic incorporation of the α-MSH derivatives. Calcium is indispensable for the labeling of MSH receptors and for the irreversible stimulation of the cells by covalent MSH-receptor complexes. This indicates a double role of calcium in the binding of the hormone to the receptor and in the transduction of the hormonal message through the cell membrane.

Time of onset of dopaminergic (DA) action of bromocriptine (BR) in biochemical and behavioral tests

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The behavioral and biochemical effects of BR s.c. or i.p. occur with a delay, either due to a) slow absorption or b) to a prior conversion of BR to an active metabolite. Proadifen (PR), a microsomal enzyme inhibitor, reduced BR-induced circling in Ungerstedt rats, favoring explanation b. PR did not affect BR-induced reduction of DA metabolism in striatum and cortex. BR given i.v. induced behavioral effects without delay. The in vivo reversal by BR of the enhanced activity of striatal tyrosine-hydroxylase after γ-hydroxybutyrate showed a delay after i.p. but not after i.v. administration. Our results suggest that BR itself is the causative agent in inducing circling behavior and regulating the activity of DA neurons. The reduction by PR of only the behavioral effects of BR might be due to the sedative action of PR.

'Renin dependent' 2 kidney, 1 clip hypertension (2KlC): role of renal renin

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Early (1 month) 2KlC hypertension is said to be maintained by increased renin release from the clipped kidney, the contralateral renin-depleted kidney contributing little or not at all. We tested this hypothesis by removing one or both kidneys, and, 24 h later, blocking of the renin-angiotensin system with captopril (3 $mg \cdot kg^{-1} \cdot min^{-1}$, 10 min, i.v.). In intact rats captopril produced a −39% fall in blood pressure (BP) and a +45% increase in plasma renin level (PRL). After contralateral nephrectomy, captopril induced a similar fall in BP, but no rise in PRL. After removal of the clipped kidney, although PRL fell to 4% that of intact rats, captopril still induced a 25% fall in BP and

doubled PRL. After binephrectomy, PRL fell to the limit of detection but captopril again induced a fall in BP. Therefore 2KlC hypertension may be partly maintained by the contralateral kidney and by an extrarenal pressor system.

Towards quantitative structure activity relationships (QSAR) between enkephalin analogues and their μ-receptors

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The interaction of enkephalin analogues of general formula H-Tyr-ala-Gly-X-Y-NH_2 (X, Y amino-acid residues) with their μ-receptors has been studied in terms of binding and analgesic potencies. Results in all tests performed have shown the preponderant importance of electronic factors in X, of lipophilic and steric (including resistance towards proteolysis) factors in Y. Artifical amino acids have been devised in which one or the other factor is stressed and the corresponding side chain parameter $p_{i,j}$ takes an extreme value. Analysis of the potency 1/c according to a QSAR equation of the type $1/c = \sum_{i,j} k_{i,j} p_{i,j} + k_0$ shows the empirical constants $k_{i,j}$ and k_0 to vary only within narrow limits. All the analogues designed according to this scheme display higher activities than the natural enkephalins or the reference, H-Tyr-ala-Gly-Phe-Leu-NH_2.

Measurement of dopa and 5-hydroxytryptophan accumulation after central decarboxylase inhibition by HPLC with electrochemical detection

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Brain regions from rats pretreated with the decarboxylase inhibitor, Ro-4-4602 (800 mg/kg i.p.), were homogenized in 0.4 M perchloric acid, titrated to pH 2.5-2.8 with KOH and DOPA and 5-hydroxytryptophan (5-HTP) isolated on 3×10 mm Amberlite CG 120 II columns. They were quantitated by liquid chromatography with electrochemical detection, using a C_{18}-μBondapak column operated at 35 °C. The mobile phase was citric acid/Na_2HPO_4 (0.1 M each, 3:2 v) containing 5% methanol and 0.3 mM sodium octylsulphate, pumped at 1 ml/min. The electrochemical detector (Bioanalytical Systems, West Lafayette, In., USA, cp_w type) was set at +0.85 V. Retention times varied somewhat during lifetime of the columns and from one column to another. Typical values were 5 min for DOPA and 14 min for 5-HTP. Representative control accumulations were (in ng/g/h):

	Striatum	Cortex	
DOPA	949±67	69±1	
5-HTP	218±13	116±6	
	Hippocampus	Brain stem	
DOPA	< 50	141±8	
5-HTP	170±15	214±6	(n = 7)

Prostaglandins and the rebound contraction which follows nonadrenergic relaxation in the guinea-pig taenia coli

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The finding that indomethacin (Indo) at 50 μM abolished the rebound contraction following stimulation of intramural nonadrenergic inhibitory nerves in the guinea-pig taenia was taken as evidence by Burnstock et al. (Eur. J. Pharmac. *31*, 360, 1975) that the rebound was due to prostaglandins (PG). In view of the high concentration used, we have tested the specificity of this effect of Indo. When Indo 50 μM inhibited the rebound, the inhibition could be reversed by an increase in the frequency of stimulation or by the addition of 3-6 mM K^+ to the medium. Added K^+ also abolished the inhibition by Indo of carbachol contractions. On the other hand, the arachidonic acid (1.6 μM) contraction was completely blocked by Indo at 5 μM; added K^+ had no effect on this inhibition. Finally, adrenaline 10 nM mimicked the inhibition of the rebound, an effect which was also reversed by 3-6 mM K^+. These results indicate that Indo depresses smooth muscle contractility and that PG play no specific role in the rebound mechanism.

Prolactin secretion stimulation by an ergopeptine

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Certain ergot compounds inhibit prolactin secretion, which is due to their dopaminomimetic property. An ergopeptine has now been found, which stimulates prolactin release: 1,11'β-dimethyl-9,10-dihydroergocristine (I). I increased serum prolactin levels in rats dose-dependently. In male rats it was about 10 times less potent than perphenazine. In female rats treated at metoestrus with I prolonged luteal function was induced. In inseminated rats treated with a nidation inhibitory dose of bromocriptine pregnancy could be maintained by concomitant treatment with I. In prepubertal female rats precocious opening of the vagina could be induced. I had only marginal hebavioral effects and did not affect biogenic amine metabolism in the brain, but it antagonized the emetic action of apomorphine in the dog. In vitro it blocked the DA sensitive adenylate cyclase of the rat striatum ($pA_2 = 7.7$) and showed a high affinity to spiroperidol binding sites of rat caudatum.

Structure activity studies with N_6-homologs of bromocriptine

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N_6-homologs of bromocriptine, N_6-H, -Me (= bromocriptine), -Et, -nPr, -iPr, -nBu, -nHept were evaluated for the following properties: inhibition of prolactin secretion (nidation inhibition and lowering of serum prolactin levels, both in rats); central dopamine like actions (induction of circling in Ungerstedt rat and induction of stereotyped hebavior in rats, induction of emesis in dogs); uterotonic property (rabbit); interactions in vitro with catecholamine and serotonin receptors on blood vessels and adenylate cyclase systems; interactions with dopaminophilic radioligands on rat brain membranes. It will be shown that the homologous series of N_6-substituents affected these properties differentially.

Characterization of CNS-active drugs through multidimensional scaling of receptor binding data

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IC_{50}-values from displacement curves of 7 radioligands at their respective binding sites (^{3}H-dopamine and ^{3}H-spiroperidol in calf caudate; ^{3}H-serotonine and ^{3}H-WB 4101 in

whole rat brain; ^{3}H-clonidine, ^{3}H-mepyramine and ^{3}H-quinuclidinylbenzylate in rat brain minus cerebellum; ^{3}H-spiroperidol in rat frontal cortex) were used for the characterization of clinically tested and newly synthesized drugs with proven or assumed activity in the mammalian central nervous system. A multidimensional spatial scaling system was applied which arranges the drugs according to their in vitro potencies. Each binding site and each drug are thus represented by a point in a space where the distance between binding site and drug is given by the respective inverse pIC_{50}-values. The resulting clusters represent clinically meaningful sets of drugs for indications like schizophrenia, depression and parkinsonism.

Tolerance phenomena in hippocampal pyramidal cells after acute application of opioid peptides

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Bath application of 10^{-6} M FK 33-824, a stable enkephalin analogue, to cultured hippocampal pyramidal cells slightly increased excitatory and markedly decreased inhibitory postsynaptic potentials and led to bursting discharges and depolarization shifts. Despite continuous bath application of the opioid peptide, the response of a proportion of pyramidal cells returned toward control values after about 6–8 min. Following a 30-min washout period, 10^{-6} M FK 33-824 was, however, equally or less effective again. In contrast, bursting indiced by the GABA-antagonist bicuculline-methochloride (BMC) persisted during prolonged application (up to 40 min). BMC also elicited bursts in pyramidal cells which had been rendered tolerant to the action of FK 33-824, a finding which supports the view that the 2 substances produced their very similar effects by activation of different receptors. Cross-tolerance occurred, however, between FK 33-824 and $(D\text{-}Ala)^2=(D\text{-}Leu)^5$enkephalin.

Immunoprecipitation of the catalytic subunit of Na,K-ATPase in various cell fractions of toad kidney

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Kidney slices from the toad *Bufo marinus* were incubated with ^{3}H-leucine (40 µCi/ml) for 20 h at 25 °C. After a 4-h chase cytoplasmic, microsomal and purified Na,K-ATPase fractions were prepared from the tissue. Specific polyclonal antibodies raised against the catalytic subunit of Na,K-ATPase immunoprecipitated (M.-L. Maccechini et al., Proc. nat. Acad. Sci. USA *76*, 343, 1979) ~1%, 2.7% and 21% of the total counts in the 3 fractions respectively. These data correlate well with the 3-fold and the additional 8.5-fold increase of the enzyme activity in the microsomal and the purified fractions respectively. A single band of immunoprecipitated material from either of the 3 enzyme preparations can be identified by autoradiography on SDS-PAGE. Its apparent mol.wt of 98 KD is identical to that of the catalytic subunit. According to the present results, antibodies will be useful probes for studying the biosynthesis of Na,K-ATPase.

Induction of cytochrome P450 (P450) in hepatocyte culture by metyrapone (MP) and nicotinamide (NA)

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MP, a widely used inhibitor of monooxygenase reactions, and NA, another substituted pyridine, prevent the rapid decrease of P450 concentration in cultured rat hepatocytes. The mechanism of this protective effect is unclear. We studied the effect of MP and NA in cultured chick embryo hepatocytes, a system characterized by less degradation and preserved inducibility of P450. MP (0.5 mM) or NA (25 mM) caused a 2- to 3-fold increase in P450 concentration. This increase was additive to induction of P450 by phenobarbital and β-naphthoflavone, accompanied by increased ethoxycoumarin deethylase activity and abolished by cycloheximide. LiDS-PAGE of microsomes after MP and NA treatment showed an increase of a protein band of mol.wt 52,000. The increase in P450 was associated with increased δ-aminolevulinic acid synthetase, the rate-limiting enzyme of heme synthesis. In cultured chick embryo hepatocytes MP and NA induce a form of P450 resembling the phenobarbital induced hemoprotein. Induction and inhibition properties of MP and NA may both be related to their binding affinity to P450.

The influence of temperature on the effect of piperidine-4-sulfonic acid, THIP, muscimol and GABA on the isolated rat sympathetic ganglia

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At the isolated superior cervical ganglion of the rat the DC-potential was recorded using a suction electrode with an Ag/AgCl-element and another Ag/AgCl-cell as reference. The ganglia were superfused with a modified oxygenated Krebs solution at 22 °C and 36 °C. The effect of PSA (piperidine-4-sulfonic acid), THIP (4,5,6,7-tetrahydroisoxazolo(4,5-c)-pyridin-3-ol) and muscimol was compared with the effect of GABA. Generally the depolarizations by GABA were weaker at 36 °C than at 22 °C. The sequence of the depolarizing action (in concentrations up to 100 µM) was muscimol > GABA > PSA. These depolarizing actions could be blocked by bicuculline-methochloride. THIP in concentrations up to 10 µM had only a weak depolarizing action but at higher concentrations (1 mM) a distinct depolarizing action was evident. This effect of THIP could be observed at both temperatures and was antagonized by bicuculline-methochloride (50 µM).

Substance P (SP) and the baroreceptor reflex (BR)

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Recent studies from our laboratory suggested a participation of SP in transmitting baroreceptor signals in the nucleus tractus solitarii (NTS). Cats responded to bilateral electrical stimulation of the carotid sinus nerves with decreases in blood pressure and heart rate. Microinjection of SP antibody into the NTS reversibly inhibited the effects of sinus nerve stimulation. Vehicle of SP or SP antibody saturated with SP prior to microinjection were ineffective. On the other hand, in adult rats, injected at birth with capsaicin in order to destroy SP containing primary afferent neurons, BR function was normal. While the SP content of a dorsal medullary region including the NTS was reduced by 50–60%, SP immunohistochemistry showed a marked loss of medullary V, IX and X axons and V terminals, but less marked terminal losses in the NTS containing IX and X terminals. Thus, the NTS might contain, in addition to IX an X afferents, other (intrinsic?) SP terminals involved in the BR.

Effects of exogenous and endogenous arginine-vasopressin (AVP) on renal and mesenteric vascular resistance in rats

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AVP was injected i.v. in anesthetized Sprague-Dawley rats, in which renal or mesenteric blood flow was measured with an electromagnetic flow probe. A dose of 50 ng/kg, which elevated blood pressure (BP) by 24±3 (SEM) mm Hg, increased mesenteric vascular resistance (MVR) by 13±3 mm Hg · min/ml ($p < 0.01$), but renal vascular resistance (RVR) by only 4±1 mm Hg · min/ml ($p < 0.01$). The effects of endogenous AVP were studied in rats with glycerol-induced acute renal failure, in which plasma AVP is markedly increased (Hofbauer et al., Circulation Res. *41*, 424, 1977). A competitive antagonist of the vascular effects of AVP, $d(CH_2)_5VDAVP$ (100 µg/kg, i.v.), injected 2 h after glycerol, induced a fall in BP (−29±6 mm Hg, $p < 0.01$). The increase in MVR which occurred after glycerol (6±1 mm Hg · min/ml) was abolished (−5±1 mm Hg · min/ml, $p < 0.01$), whereas the rise in RVR (21±4 mm Hg · min/ml) was only partially reversed (−5±3 mm Hg · min/ml, n.s.). These results suggest that AVP increases BP by selective effects on certain vascular beds, such as the mesenteric, while RVR is only slightly affected.

Recent advances in cholinergic receptor preparation

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Cholinergic receptors have been isolated and characterized by numerous research groups. The preparation of active receptor in a reasonable time and in sufficient amount however is still a problem. The most serious problem remaining is: there is still no good assay available for the detection of activity and purity of the receptor. - Our starting material for receptor preparation was the electric organ of *Torpedo marmorata*, kindly supplied by Institut Universitaire de Biologie Marine, Arcachon, France. After homogenization and preliminary filtration of the crude proteins on sephadex, the resulting solution was subjected to affinity chromatography. The column contained a gallamine affinity ligand of pachy-curare type, bound to agarose by a spacer of about 50 Å. The column could be washed with buffer of considerable ionic strength (0.5 M NaCl), without losing the specifically bound receptor protein. Elution was performed with free gallamine and throughout preparation all steps were performed under protection of CO_2, as the receptor is relatively sensitive to ambient oxygen. The overall yield of cholinergic receptor protein was 0.02% (calculated on wet weight of electric organ).

Another approach for the treatment of organophosphate poisoning

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Almost all organophosphates blocking acetylcholinesterase (ACHE) may be lethal. The lethal effect is not caused by direct action, but by acetylcholine (ACH) accumulated as a result of ACHE-blockade. The only therapeutic methods suggested in literature are application of atropine or analogues and some oximes. Atropine however counteracts only some symptomes and with oximes alone no beneficial effects could ever be shown. Our aim was primarily not to reactivate ACHE but to suppress ACH-production by inhibiting choline-O-acetyltransferase (CAT). We are fully aware that our approach, in this early stage, is not suited for clinical treatment, because all relevant blockers of CAT act lethally. In rats after sufficient doses of methyl-methanethiol-sulfonate (MMTS), death occurs after about 1 h. The lethal effects of organophosphates however are established after a few minutes. The almost instantaneous lethal effects of fluoromethyl-isopropylphosphate, with severe convulsions, salivation and reduction of heart rate, could be significantly reduced and survival-time significantly increased. An important observation of our experiments was, that ACH is not stored for longer time in the synapses, but is synthesized in the range of a few minutes prior to use.

Phosphate influx, extracellular ATP and ecto-ATPase in nerve fibres

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Influx of radiophosphate into rabbit vagus nerves was continuously monitored during incubation in Locke containing either $^{32}PO_4$ or γ^{32}P-ATP, and carrier PO_4 or ATP at 0.02–1 mM. The influx rates, as well as the Na^+-dependence of the influx, were about the same in both cases. When the calcium of the incubation solution was omitted, influx from γ^{32}P-ATP was inhibited, whereas that from $^{32}PO_4$ was increased by about 80%. Uptake from $^{32}PO_4$ was inhibited on addition of nonradioactive ATP, and this inhibition suppressed in Ca^{2+}-free media. Inhibition of uptake from γ^{32}P-ATP by PO_4 was less pronounced. The results indicate competition between ATP and inorganic phosphate for the phosphate transport mechanism, with a higher affinity for the γ-PO_4 from the hydrolyzed ATP. Addition of ADP or AMP does not significantly affect this process. The observations suggest the presence of a Ca^{2+}-sensitive ecto-ATPase localized near, and involved with, the phosphate transport system.

Cathinone, an alkaloid from khat leaves with amphetamine-like releasing properties

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In certain countries of East Africa and the Arab Peninsula, khat leaves are widely used as a stimulant. A new alkaloid, (−)cathinone, has recently been isolated from khat and this compound has been shown to produce amphetamine-like behavioral effects. To test whether on the cellular level as well, the effects of (−)cathinone would be analogous to those of amphetamine, its effect on the efflux of radioactivity from rabbit striatal slices prelabeled with ^{3}H-dopamine was examined. It was found that low concentrations of (−)cathinone enhance the release of label in a dose-dependent manner, and that (−)cathinone was capable of sustaining the enhanced release induced by (+)amphetamine. Pretreatment of the tissue with cocaine, which is known to prevent the induction of release by (+)amphetamine, inhibited the efflux increase caused by (−)cathinone. The results of these experiments suggest that the stimulating properties of khat leaves are due to the presence of an alkaloid that acts also at the cellular level like amphetamine.

Antagonism by Ro 15-1788 of the effects of diazepam on the dopamine turnover in rat brain

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Ro 15-1788, an imidazobenzodiazepinone which has been shown to antagonize the pharmacological effects of diazepam (DIAZ) in various animal species (Hunkeler et al., submitted) was investigated in rat brain. The α-methyl-p-tyrosine-induced disappearance of dopamine (DA) from brain was decelerated by DIAZ, an effect which was prevented by Ro 15-1788 while the drug per se did not alter the DA level. Ro 15-1788 was also able to counteract dose-dependently (3-30 mg · kg^{-1} p.o.) another typical action of benzodiazepines, i.e. the DIAZ-(10 mg · kg^{-1} i.p.)-induced reduction of the increase of HVA provoked by chlorpromazine (5 mg · kg^{-1} i.p.). Electrical footshock stress-induced elevation of HVA in the cerebral cortex, limbic forebrain and striatum was significantly attenuated by DIAZ. This protecting effect of DIAZ was antagonized by Ro 15-1788 in all 3 brain regions. Thus, in conditions of enhanced dopaminergic activity, the DIAZ-induced attenuation of cerebral DA turnover is antagonized efficiently by Ro 15-1788.

Easier binding studies: no more washing!

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In brain receptor binding studies, it has been conventional practice to wash the centrifuged homogenate in order to remove endogenous ligands from putative receptor sites. However the concentration of endogenous neurotransmitters in the first homogenate are too low to modify antagonistic ligand binding characteristics. Thus for physiological (although not for drug) studies, the centrifuge wash steps are theoretically unnecessary. To test this idea we measured ^{3}H-WB4101, ^{3}H-DHA and ^{3}H-QNB binding in homogenates from frozen rat brain: parallel assays were carried out with fresh and twice-centrifuged-and-washed homogenate. Displacement curves (using phenoxybenzamine, l-propranolol and atropine respectively) were identical in both preparations. However there were clear differences in the number of receptors remaining after centrifugation and washing (56% for alpha$_1$-, 80% for beta-adrenergic and 107% for cholinergic receptors). The amount of protein washed out was of the order of 50% in all cases. These studies show that binding assays can be carried out directly in homogenized tissue.

Ro 15-1788 reverses the effects of midazolam on multiunit activity recorded in 'encéphale isolé' rats

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Multiunit activity was simultaneously recorded from 4 nuclei in 'encéphale isolé' rats. Impulses were selected from firing of small populations of neurons by an adjustable trigger and the mean impulse frequency determined during successive 3-min epochs. On statistical stationarity (z-test) midazolam was injected i.v. in cumulative doses at 6-min intervals (0.1, 0.3, 1,10 µmoles · kg^{-1}). Firing was significantly decreased (t-test) in the CA$_1$ region of hippocampus (38±11% of control activity remained after the final injection; $n=6$, $p<0.001$), the substantia nigra pars compacta (42±8%, $n=4$, $p<0.001$), the locus coeruleus (37±13%, $n=3$, $p<0.001$) and the raphé dorsalis (34±10%, $n=3$, $p<0.05$). Subsequent injection of the imidazobenzodiazepinone Ro 15-1788 (10 µmoles · kg^{-1} i.v.), 6 min after the final injection of midazolam resulted in the following recoveries: hippocampus (77±10%, $n=6$, $p<0.05$), substantia nigra pars compacta (92±2%, $n=4$, $p<0.05$), locus coeruleus (114±6%, $n=3$, $p<0.05$) and raphé dorsalis (92±12%, $n=3$, n.s.). Furthermore, Ro 15-1788 did not reverse the pentobarbitone-induced reduction in firing of these nuclei. It is therefore concluded that Ro 15-1788 specifically reversed the effect of midazolam on a molar basis at some benzodiazepine receptors.

Effect of capsaicin (CA) on substance P (SP) neurons in spinal cord and medulla oblongata of newborn rats

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In adult rats, treated at birth with CA (50 mg/kg s.c.), RIA and immunohistochemistry of SP showed a loss of SP cells in spinal ganglia by 32% and of SP fibres in the substantia gelatinosa by 50%. Other spinal SP systems appeared unaffected. In the medulla oblongata SP fibres were selectively markedly reduced in pathways and terminal areas of the V, IX and X cranial nerves e.g. (nucleus) tractus spinalis n.V. However in the nucl. tract. solitarii (NTS) containing IX and X afferents (e.g. baroreceptor), SP fibres were, unexpectedly, only slightly reduced while SP cells appeared unchanged in number. Since SP (perhaps from baroreceptor afferents) was suggested to participate in baroreceptor reflex function, the latter was tested in CA rats and was found normal. Thus functionally baroreceptor afferents in the NTS did not appear heavily damaged by CA. Immunohistochemistry suggested the persistence of an intrinsic SP system in the NTS after CA treatment.

Effect of metabolic inhibition on uptake and release of adenosine in nonmyelinated nerve fibres, at rest and during activity

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Previous experiments showed that ^{3}H-adenosine is rapidly taken up by nonmyelinated nerve fibres and incorporated into the main nucleotides within a few minutes. Washing these preparations with nonlabeled solution shows a release of radioactivity mostly in the form of inosine and hypoxanthine; electrical activity increasing the liberation of these compounds. We have now observed that these phenomena strongly depend on the metabolic conditions: application of cyanide or 2,4-dinitrophenol (DNP) slows the uptake of ^{3}H-adenosine by about 50%. A 7-fold increase in efflux of radioactivity is found after application of glucose-free solution, a 70-fold increase after addition of 2-deoxyglucose or cyanide and a 30-fold increase with DNP. There is also a change in the composition of the effluent; metabolic inhibition causing a larger release of adenosine, in resting and active nerve. This larger appearance of adenosine may be due to the slowing of ATP synthesis.

Rat renin antibodies: characterization and uses

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Renin specific antibody formation was induced in rabbits by intradermal injections of purified rat renin (single band on electrophoresis, 260 Goldblatt units/mg protein, 6000-fold purification). The specificity of the antibodies for renin was demonstrated by double immunodiffusion and

electrophoresis. The same purified rat renin was radioiodinated by the lactoperoxidase method using the immobilized enzymes technique. Homologous reactivity of the antibodies to this radioactive renin was confirmed by radioimmunodiffusion, electrophoresis and competitive protein-binding assays. Cross-reactivity of these rat renin specific antibodies for various heterologous renins was studied by immunodiffusion and radioimmunoassay. A direct radioimmunoassay for rat renin, using these renin specific antibodies and the radioiodinated renin, will be described and compared to the commonly used indirect method based on incubation of renin with its substrate and subsequent angiotensin I radioimmunoassay.

Bromocriptine and CM 29-712 stimulate different types of dopamine receptors

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Bromocriptine and CM 29-712 are 2 ergot derivatives which exert dopamine-like effects in various in vivo models. CM 29-712 stimulates and bromocriptine inhibits dopamine-sensitive adenylate cyclase in homogenates of bovine retina. Furthermore bromocriptine, like apomorphine, inhibits and CM 29-712 facilitates stimulation evoked acetylcholine release from rat brain striatal tissue slices. The effect of bromocriptine is antagonized by spiroperidol and that of CM 29-712 by apomorphine. This suggests that bromocriptine stimulates and CM 29-712 inhibits dopamine receptors involved in modulating acetylcholine release. In conclusion these results indicate that the dopamine-like effects of these 2 ergot derivatives are mediated by different types of dopamine receptors.

Pharmacological characterization of substance P receptors in the isolated rat portal vein

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Substance P (SP; $ED_{50} = 1.5 \times 10^{-6}$ M) dose-dependently provokes a tonic contraction and increases the time integrated spontaneous contractions of the everted, perfused rat portal vein. Since the contractile responses to SP are similar to those of NA and ACh, the possibility was considered that these transmitters mediate the actions of SP, by either a presynaptic action or by an action on adrenergic or cholinergic receptor sites on the muscle cells. However, a concentration (3.5×10^{-6} M) of phentolamine or atropine, which completely blocked the actions of NA and ACh, respectively, did not modify the SP response. An AII antagonist (Sar^1, Ile^8 AII, 10^{-7} M), and 2 histamine antagonists (mepyramine 10^{-4} M; cimetidine, 10^{-3} M), which blocked the actions of their respective agonist on the rat portal vein, did not modify that of SP. SP seems to express its inotropic actions by the stimulation of smooth muscle cell receptors.

Sulfation of tauro-3 β-OH-5-cholenoat (TCH) does not abolish its cholestatic effects

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Taurolithocholate (TL) is a cholestatic model compound in animals. Since sulfo-TL lacks cholestatic properties, it has been assumed that sulfation of monohydroxy bile acids (MBA) prevents their adverse effects on bile flow presumably by increasing solubility. To test this hypothesis, we prepared TCH - a bile acid implicated in infantile cholestasis - and sulfo-TCH labeled with ^{14}C in the taurine, and compared their effects on bile formation in anesthetized (pentobarbital) male Sprague-Dawley rats (210±25 g b.wt). Whereas bile formation following sulfo-TL infusion (100 nmoles min^{-1} 100 g^{-1} b.wt, n=6) was comparable to controls, bile flow 2 h after start of equimolar sulfo-TCH (n=6) was reduced from 9.0±SD 1.0 to 1.3±0.7 µl min^{-1} 100 g^{-1} b.wt, due to a fall of both bile salt-dependent and independent fractions. The finding that biliary concentration of sulfo-TCH achieved in the development of cholestasis. It is concluded that sulfation prevents the cholestatic effects of some, but not all, MBA.

The cholinergic regulation of neuronal activity in the rat locus coeruleus

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Noradrenergic cell bodies of the locus coeruleus (LC) stain for acetylcholinesterase and are excited by microiontophoretically administered acetylcholine. These findings suggest that there might be an activating acholinergic input to this nucleus. Biochemical studies have revealed that oxotremorine, physostigmine (PS) as well as the muscarinic blockers atropine (AP) and scopolamine accelerate brain noradrenaline turnover. In the present study, low doses of AP (0.1, 1.0 mg/kg) had no effect and higher doses (3, 10, 30 mg/kg) increased cell firing in LC. Similarly, while PS was inactive at a dose of 0.1 mg/kg, most neurons were excited and a few depressed by PS at a dose of 0.3 mg/kg. AP (10 mg/kg) antagonized the PS elicited effects if given after PS administration or prevented them if given prior to PS. In conclusion, the results cast doubts on the hypothesis that LC neurons are tonically activated by a cholinergic input.

Free and conjugated normetanephrine in platelets from patients with storage pool disease

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Human platelets, which possess high phenol sulfotransferase activity (PST), contain amines (e.g. 5HT, catecholamines, CA and normetanephrine, NMN) both in free and conjugated form and are able to take up and conjugate amines in vitro to different extents. In particular, CA-methoxy derivatives are excellent substrates for platelet PST. The concentrations of endogenous free and conjugated NMN (radioenzymatic method) as well as the in vitro uptake and conjugation of ^{14}C-NMN were examined in platelets from 6 patients with inherited defects of amine storing organelles (SPD) and compared with those of 4 healthy controls. Free but not conjugated NMN was significantly lower in SPD than in control platelets (free: 0.90±0.14 vs 1.59±0.21 pmoles/mg prot, $p < 0.05$; conjugated: 4.47±0.13 vs 5.08±0.27, n.s.). Accordingly, SPD platelets accumulated in vitro (30 min incubation with 1 µM ^{14}C-NMN) less free (5±0 vs 37±3 pmoles/mg prot, $p < 0.001$) but similar amounts of conjugated ^{14}C-NMN (415±30 vs 394±24) in respect to controls. From these and other results it is concluded that SPD platelets, which exhibit a reduced granular storage of free amines, have a normal capacity to conjugate amines and to retain them extragranularly in conjugated form.

Effects of the selective benzodiazepine antagonist Ro 15-1788 on the cat spinal cord

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In unanesthetized spinal cats, the imidazobenzodiazepinone Ro 15-1788 (1-10 mg/kg i.v.) did not affect segmental dorsal root potentials (DRPs), spontaneous activity of γ-motoneurons and the Renshaw cell (RC) discharge evoked by ventral root volleys. The enhancement of DRPs as well as the depression of spontaneous γ-motoneurons and RC discharge induced by the bezodiazepines (BD) diazepam, midazolam and 3-methylclonazepam (0.1-1 mg/kg i.v.) were antagonized by 15-1788. 15-1788 also blocked the BD-life effects of zopiclone (1 mg/kg i.v.) and the triazolopyridazine CL 218 872 (3-10 mg/kg i.v.), drugs which bind to the brain BD receptor, without affecting phenobarbitone (10-20 mg/kg i.v.) induced spinal cord effects. Since 15-1788 is active in BD binding but devoid of BD-like actions (Hunkeler et al., 1981), this drug might be of crucial importance in analyzing the mechanisms of action of drugs acting on the spinal cord.

Digital plethysmography (DPG), a noninvasive method to assess the bioavailability of glyceryl trinitrate (GTN)

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GTN, a very potent vasodilatator, may be given by several routes of administration (sublingual, p.o., i.v., percutaneous). The purpose of the study is to establish a noninvasive method to assess the bioavailability of GTN. - In 12 fasting subjects 4 graded i.v. infusion of GTN ranging from 7.5 to 27 µg/min were administered until a steady-state was achieved for each infusion rate. Measuring the pharmacological effect by DPG it was possible to construct linear dose-response curves with correlation coefficients > 0.98. In order to establish the 100% availability of i.v. doses each subject received an increasing followed by a decreasing i.v. infusion of GTN (non-steady-state conditions). The effective dose computed with the use of the dose-response curve and an integration procedure corresponded to 96±SD 19% of the infused dose. It was possible to measure pharmacological effects after sublingual doses of only 100 µg which demonstrate the high sensitivity of the method. By contrast no effect was recorded after 800 µg p.o. in 9 healthy volunteers, due to first pass elimination in the liver. Thus, DPG is a highly sensitive and noninvasive method to assess the bioavailability of GTN given by various routes.

Properties of β-adrenergic binding sites in intact cells

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We have measured equilibrium dissociation constants (Kd values) for β-adrenergic ligands and the density of their specific binding sites in beating monolayers of heart cell cultures. In the same preparation we determined the activation constant (Ka) of isoprenaline (I) in stimulating cAMP formation. A hydrophobic ((±) carazolol) and a hydrophilic ((±) CGP 12177) antagonist were bound with high affinities (50 and 400 pM respectively). Binding was saturable and displaceable by β-adrenergic ligands. The density of specific binding sites was 5000/cell. Kd values for (−) I were 27 or 1200 nM if calculated from specific displacement of CGP or carazolol respectively. The Ka value of I was 100 nM. The Kd/Ka ratio of I seemed to be determined by receptor-antagonist interactions rather than by the 'true' affinity of the agonist. On the basis of Ka-Kd discrepancies ($Ka \ll Kd$) in cardiac preparations a large surplus of spare receptors over functional receptors has been postulated. Our results offer a possible alternative explanation for this discrepancy.

Autoradiographic localization of [^{3}H] Ro 15-1788, a selective benzodiazepine antagonist, in rat brain in vitro

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The benzodiazepine (BD), Ro 15-1788, has been shown to selectively antagonize all known CNS effects of BD (anxiolytic, sedative, muscle relaxant and anticonvulsant) although per se it lacks major pharmacological activity. In order to determine its site of action, cryostat sections of rat brain were incubated for 40 min at 0 °C in 2 nM [^{3}H]15-1788 (s.a. 26.8 Ci/mM) in Tris-HCl. Darkfield microscopy revealed intense radiolabeling in amygdala, molecular layers of cerebellar cortex, hippocampus and dentate gyrus, layer IV of cerebral frontal cortex and dorsal horn of spinal cord but only moderate labeling in granular layers of cerebellar cortex and dentate gyrus, pyramidal cell layer of hippocampus, layer V of cerebral cortex, median eminence and ventral horn of spinal cord. Only background levels without regional variations were seen in sections incubated with the radioligand + 1 µM flunitrazepam, a potent BD agonist. Thus, the BD antagonist has a high affinity for the same brain structures as BD agonists previously visualized autoradiographically. This is in line with the selective antagonism of central BD effects by 15-1788.

The antimineralocorticoid action of triiodothyronine (T_3) is not mediated by the regulation of mineralocorticoid receptors

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In the urinary bladder of the toad *(Bufo marinus)*, T_3 inhibits the aldosterone-dependent Na^+ transport. We have studied whether T_3 might mediate its antimineralocorticoid action by controlling the level of mineralocorticoid receptors. After 18 h of exposure to T_3 (60 nM), there was no change in the affinity or the number of ^{3}H-aldosterone binding sites (type I and type II) as measured in cytosolic extracts. Moreover, there was no difference in the time course of ^{3}H-aldosterone uptake (10 nM, up to 90 min) into cytoplasmic and nuclear fractions. However, under the same conditions, T_3 inhibited significantly the aldosterone-dependent Na^+ transport ($p < 0.01$, $n = 10$) already 90 min after addition of aldosterone (80 nM). We conclude that T_3 does not down regulate the mineralocorticoid receptor. The antimineralocorticoid action of T_3 seems to occur at a step beyond the aldosterone receptor.

Phosphate transport in rabbit nerve and intestine after application of diphosphonate

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Previous experiments showed that injection of ethane-1-hydroxy-1,1 diphosphonate (EHDP) at doses inhibiting the production of 1,25 $(OH)_2D_3$ to rabbits abolished the Na-depend phosphate uptake across duodenum brush border, and that this transport process recovered after administration of 1,25$(OH)_2D_3$ (Danisi, Bonjour and Straub, Pflügers Arch., in press). We have now studied whether EHDP treatment also affects the Na-dependent phosphate transport in nerve (Anner, Ferrero, Jirounek, Jones, Salamin and Straub, J. Physiol., Lond. *260,* 667 (1976). Rabbits were treated for 3, 5 or 7 days with EHDP and the phosphate transport measured in desheathed vagus and duodenum. The experiments showed that phosphate influx and efflux in vagus appeared to be unaffected as compared to controls from untreated rabbits, while the Na-dependent phosphate uptake in the duodenum of the EHDP-treated animals was nearly abolished.

Inhibition of lymphocyte proliferation by vetradidine

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The steroidal alkaloid veratridine is a valuable tool in neurobiology to study the gated uptake of Na^+ in nerves and neuroblastoma cells (for review: W.A. Catterall, A. Rev. Pharmac. Toxic. *20,* 15, 1980). - Regarding lymphocyte activation and proliferation little is known about changes in Na^+-fluxes into the cell. It was therefore of interest to see whether a Na^+ ionophore could influence the stimulation of lymphocytes. We used mouse mesenteric lymphnode cells and as mitogenic stimuli, concanavalin A and lipopolysaccharide from *Escherichia coli.* The ^{3}H-thymidine incorporation as a measure of the proliferative response after 48 h was reduced to 50% after 30 µM veratridine and completely blocked after 60 µM veratridine. Basal ^{3}H-thymidine incorporation in unstimulated cultures was not changed by veratridine. In addition, veratridine was not toxic for these cells, and electronmicroscopic examination of cultured cells showed no structural changes. Tetrodotoxin, a well-known antagonist of the Na^+ ionophore, could not reverse the inhibiting effect of veratridine. - The possibility of veratridine induced ionic changes in lymphocytes, which could lead to the inhibition of the mitogenic response, will be discussed and evaluated further.

Angiotensinogen in cerebrospinal fluid (CSF) and plasma of rats

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Angiotensinogen was measured in CSF of male Sprague-Dawley rats under different states known to alter the plasma substrate concentration, namely nephrectomy (NX) for 24 h; adrenalectomy (ADX) for 4 days; hydrocortisone treatment (HC) for 2 days and the treatment with the converting enzyme inhibitor Captopril® (CI) for 4 weeks. - High angiotensinogen values were measured after NX in plasma (370% of controls) and in CSF (150%). There was no correlation between the substrate content in plasma and CSF. The HC treatment influenced plasma (142%) and CSF (137%) angiotensinogen levels in parallel and a significant relationship existed between each other. The ADX and the CI treatment diminished the plasma content of angiotensinogen by 36% (ADX) and by 68% (CI) while no significant change was observed in CSF of either instance. - It is concluded that CSF angiotensinogen does not necessarily reflect changes in plasma substrate levels.

The postimplantation rodent embryo culture system: a potential prescreen in teratology

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Mice and rat embryos were removed from the uterus horns on days 9.5 and 10.5 and dissected free from maternal tissue and Reichert's membrane, the yolk sac and the ectoplacental cone being left intact. Subsequently, the conceptuses were cultured in roller bottles in pure male rat serum at 37 °C for 48 h. At the end of the culture period, conceptuses were examined for their morphological appearance and their degree of differentiation. Growth was assessed by measurement of DNA and protein contents. - Cadmium chloride (Cd), valproate (Val), cyclophosphamide (Cpa) or acrolein (Ac) were added to the medium at the start of the culture. Specific morphologic lesions were observed after Cd and Val treatment, whereas conceptuses exposed to Cpa exhibited dysmorphogenesis only after addition of liver microsomes and NADPH. Ac had no dysmorphogenic effects, although DNA and protein contents were dose-dependently reduced. These findings are in good accordance with those observed in in vivo teratology studies. It is, therefore, suggested that this system represents a valuable additional screening procedure for new drugs and environmental chemicals.

VIP- and glucagon-induced accumulation of cAMP in intact mammalian and avian retinae

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Isolated retinae of rabbits were exposed to various neuropeptides in order to study their effects upon the cAMP content. VIP was found to be very potent to stimulate cAMP synthesis, with a 2-fold increase over control tissue at 0.1 nM and a 67-fold increase at the highest concentration tested (10 µM). In contrast, glucagon (up to 10 µM) was ineffective. However, the latter peptide (1-10 µM) caused a 4-fold increase in cAMP in pigeon retina. Up to now, only VIP in rabbit retina and glucagon in pigeon retina were able to stimulate the synthesis of cAMP. Attempts to block the cAMP generated by VIP by either somatostatin or dopamine receptor antagonists (+ butaclamol) were not successful, the latter finding implying that VIP interacts with cyclase coupled-mechanisms or -receptors different from those of dopamine. The intact retina may therefore provide a useful model to study some biochemical related effects of peptides in CNS.

Fluorescent labeling of MSH receptors with tobacco mosaic virus derivatives

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Tobacco mosaic virus (TMV) was chemically modified to contain about 250 rhodamine and 200 α-melanotropin (α-MSH) molecules per virion. The biologically active compound was found to stain cultured Cloudman S 91 mouse melanoma cells in a typical manner, indicating a strong and practically irreversible reaction with α-MSH

receptors followed by (probable) internalization. This notion is supported by a number of control experiments. The method may perhaps be generalized for mapping polypeptide and other agonist receptors and following their fate.

The effects of 1-dopa on retinal cAMP in light or dark: modulation by GABA?

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Rabbit retinae were isolated in light, then incubated in dark for 40 min in Krebs-Ringer under oxygenation. Each retina was then cut in 4 pieces under red light. The final incubation was performed in presence of 10 mM theophyline with or without l-dopa (1 μM up to 1000 μM). The maximal increase (3-fold) of cAMP induced by newly formed dopamine was obtained after 20 min exposure to 100 μM l-dopa. Similar experiments were performed in light or dark with 10 μM l-dopa for 30 min. The increase of cAMP over control tissues was of 183% in light and of 130% in dark. Attempts to modify this difference by GABAergic mechanisms were performed with a) 10 mM mercaptopropionic acid, a GAD inhibitor, b) 100 μM picrotoxin, c) 100 μM bicuculline, d) 100 μM muscimol, e) 10 μM amino-oxyacetic acid, a reversible inhibitor of GABA-T, + 10 μM GABA, f) 100 μM gabaculine, an irreversible GABA-T inhibitor, + 10 μM GABA, g) 100 μM(−)-baclofen h) 100 μM γ-butyrolactone. Up to now, these treatments were unable to modify the difference of cAMP increase observed between light and dark conditions.

Differences in stability and localization of rabbit liver epoxide hydrolase activities towards different substrates

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Epoxide hydrolase activity towards 6 substrates was measured in rabbit liver microsomes and cytosol after various periods of storage at 4 °C. Microsomal HEOM (a dieldrin analogue) hydrolase was very unstable; activity towards the other 5 substrates was markedly more stable. Activity in the cytosol for all substrates was very stable and exceptionally high relative to other species. β-trans-Ethyl styrene oxide hydrolase activity was high in cytosol and low in microsomes; the opposite distribution pattern was observed for the other 5 substrates. These results provide evicence for multiple EH forms within microsomes and between microsomes and cytosol in rabbit liver.

In vivo assessment of acetanilide hydroxylation with a tritium tracer method

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A new method to measure acetainilide (AA) hydroxylation in vivo was investigated. It consisted in the i.v. administration of ring-G-^{3}H-AA as test compound (18 μCi) followed by assessment of ^{3}HOH in exhaled water which was collected in a could trap. Half lives of ^{3}H-AA in blood, determined with an isotope dilution method, were well correlated with the time (T_{50}) needed for the formation of 50% of total ^{3}HOH production ($r = 0.90$, $n = 12$). T_{50} was 31±SD 10 min in 16 normal control rats, 20±6 ($n = 13$) after enzyme induction with 3-methylcholanthrene ($p < 0.01$), 21±6 ($n = 9$) after phenobarbital ($p < 0.01$), 36+6 ($n = 13$) 3 weeks after portacaval shunt and 31±4 ($n = 6$) 2 days after bile duct ligation. The results in these animal are compatible with the idea that following administration of a suitable test compound the rate of ^{3}HOH formation reflects the rate of hydroxylation in vivo.

Central actions of THIP

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THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol), a rigid analog of muscimol, was found to have a GABA-like action when applied iontophoretically to neurons recorded extracellularly in the cerebellum, hippocampus and cortex of urethane-anesthetized rats. THIP and GABA reversibly depressed the firing rate of all 27 neurons on which both were tested and these effects could be antagonized by bicuculline methochloride. In the cerebellum THIP appeared to be approximately twice as potent as GABA, but equipotent in the hippocampal pyramidal cell layer. Following systemic (i.p. and i.v.) application of THIP (10-60 mg/kg) or muscimol (7.5 mg/kg), recurrent inhibition in the hippocampus was enhanced, with little or no change in excitability as judged by the amplitude of CAl field potentials evoked by fimbrial stimulation. These results show that THIP acts iontophoretically as a potent GABA agonist in the cerebellum and hippocampus and suggest that in the hippocampus it may prolong GABAergic synaptic inhibition as well.

Characterization of microsomal epoxide hydrolase in hyperplastic nodules

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Microsomal epoxide hydrolase (EH) is considered a marker of early preneoplastic lesions in the liver. Whilst treating rats with certain hepatocarcinogens led to a 2-3-fold increase in microsomal EH activity in hyperplastic nodules (HN), no increase in cytosolic EH was seen. Several characteristics of microsomal EH from HN were strikingly similar to those from control microsomes (CM). 1. Microsomal EH activity towards 4 endogenous and exogenous substrates was in the same ratio in both CM and HN. 2. Diagnostic inhibitors produced the same degree of microsomal EH inhibition in both CM and HN. 3. Double diffusion and immunoprecipitation analysis showed no immunological difference between microsomal EH from CM and HN. Higher absolute amounts of antibody were required to precipitate the higher amount of microsomal EH in HN. Therefore EH induction in HN appears to be an increase in protein, not activation. Thus microsomal EH from HN is identical or very similar to that of CM with respect to all criteria tested.

Lack of genotoxic activity of the hypolipidemic drugs clofibrate and fenofibrate

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Covalent binding to DNA of the target organ is known to be a common feature of the great majority of genotoxic

chemical carcinogens. This type of activity can be studied in vivo by analyzing the DNA of rodents which had been administered radiolabeled test compound. [^{14}C]-labeled clofibric acid and fenofibric acid were administered p.o. to 200-g male and female rats. After 10 h, liver nuclear DNA and protein were isolated and the radioactivity was determined. Binding to protein was clearly measurable whereas no binding to DNA could be detected from any drug. A comparison of the limit of detection of such DNA binding with well-known chemical carcinogens revealed that the known hepatocarcinogenicity of clofibrate cannot be based upon an initiating, DNA damaging mode of action but must be due to other, nongenotoxic, possibly promoting mechanisms. The respective role of the marked peroxisome proliferation, hepatomegaly, or cytotoxicity of these drugs due to protein binding remains to be elucidated. This type of activity has to be taken into account for an interpretation of the carcinogenicity data in rodents and for a risk assessment in man.

Interaction of α-agonists and -antagonists with dopaminergic transmission

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The preferential α_2-antagonists yohimbine (Y), esproquin (E), piperoxane and rauwolscine and the preferential α_1-antagonists WB 4101 (WB) and corynanthine increased homovanillic (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in rat striatum, mesolimbic area and cortex. Prazosine (P) slightly reduced HVA and DOPAC and antagonized their increase after haloperidol (H) moderately. Clonidine (C) decreased HVA and DOPAC slightly and reduced their increase by H strongly, that by Y significantly, though less efficiently. The effects of Y and H were not additive at any H dose. Y, E and WB inhibited ^{3}H-spiperone binding in vivo in rat striatum, frontal cortex and hippocampus. The effects were stronger in the latter 2 regions. In frontal cortex, only a fraction of the ^{3}H-spiper binding sites were inhibited. Little or no effects in all 3 areas were seen with P and C. Thus, drugs with α_2-blocking properties seem to enhance dopamine metabolism by blockade of receptors labeled by ^{3}H-spiperone. This could mean that either these α_2-antagonists also possess antidopaminergic properties, or that spiperone also labels α_2-receptors.

Reduction of the baclofen-induced increase in rat striatal serotonin metabolism: a model for agonistic effects on serotonin autoreceptors

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Baclofen (B) increased striatal serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels. 5-HT agonists like MK-212 (3/10 mg/kg i.p.), m-chlorophenylpiperazine (1 mg/kg) and quipazine (2.5/5 mg/kg) reduced the effect of B (30 mg/kg i.p.) on 5-HIAA. The 5-HT antagonists pipamperone (10/30 mg/kg), GP 50302 (2-methyl-2,3-dihydro-1 H-dibenzo[2, 3 : 6, 7]thiepino[4, 5-c]pyrrole methanesulphonate; 1/3 mg/kg), methiothepin (1 mg/kg), cyproheptadine (30 mg/kg) and methysergide (5/10/20 mg/kg), but not mianserin (5-20 mg/kg), cinanserin (1-30 mg/kg) and pizotifen (1-10 mg/kg), did the same. Methergoline (0.25/0.5 mg/kg) increased the effect of B, but this was reversed at 1 mg/kg. Haloperidol and spiroperidol (0.1-0.3 mg/kg) were inactive. The effects on the 5-HT increase after B were similar, but much less clearcut. Dopamine (DA) agonists reduce the increase of DA levels or synthesis after B or gammahydroxybutyrate. This is commonly interpreted as an action on DA autoreceptors. Since B reduces the firing of both 5-HT and DA cells, our results might be explained in terms of agonist effects on 5-HT autoreceptors.

Pharmacological properties of the tranquilizing factor in cephalopod eggs

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The perivitelline fluid (PVF) of the eggs of the Cephalopod *Loligo vulgaris* contains a tranquilizing factor first observed and described by Marthy, Hauser and Scholl (1976). This tranquilizing factor is not only effective in the *Loligo* embryos themselves but also in other species such as other cephalopods, crustaceans and even small fish. - Further investigation of the pharmacological and chemical properties of the PVF led to the following results: When injected i.p. the PVF is also effective in mammals (mice) in whom it causes a decrease in curiosity and locomoter activity as well as a slight prolongation of hexobarbital sleeping time. - In *Loligo* embryos the tranquilizing effect of the PVF can be antagonized by centrally acting drugs e.g. bicuculline and pentylenetetrazol. A comparison with other tranquilizing drugs shows that those drugs are also effective in *Loligo* embryos but their effects differ in some way from that of the PVF. - By thin-layer and column chromatography a ninhydrine positive fraction was isolated which has a tranquilizing effect in mice. The effect of this fraction in *Loligo* embryos was identical with that of the PVF. - A comparison with proteins of known molecular weights showed that the mol.wt of this active fraction was about 60,000 daltons.

Psychoactive drugs modify circadian rhythms in rat brain neurotransmitter receptors

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We have recently demonstrated the existence of endogenous circadian rhythms in the number of many rat brain neurotransmitter receptors: forebrain alpha- and beta-adrenergic, muscarinic cholinergic, opiate, benzodiazepine and striatal dopaminergic (Experientia *36*, 703, 1980). Chronic treatment with the antidepressant drugs imipramine, clorgyline and lithium, and with the neuroleptic drug, fluphenazine, profoundly modified some or all rhythm characteristics of wave-form, amplitude, phase and 24-h mean of all receptors investigated. The drug effects were complex in that they were not restricted to any one receptor nor to one rhythm parameter. The issue of specificity and selectivity remains to be studied. However one conclusion is warranted, that psychoactive drugs have powerful effects on the circadian system.

Hepatic metabolic capacity is the primary determinant of Zomepirac clearance

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The effects of liver disease on the fate of commonly used analgesics eliminated in man primarily by glucuronidation are as yet ill defined. We therefore studied the pharmacokinetics of a model compound Zomepirac (Z), a substance almost exclusively glucuronidated in the liver, in 6 subjects with Gilbert's syndrome (GS) and in 10 patients with chronic liver disease (CLD), defined in severity by the initial disappearance of bromsulphthalein (BSP-k_i) and by galactose elimination capacity (GEC). Following a single oral dose of 200 mg Z, plasma and urinary concentrations of Z were measured by HPLC · Z did not share the glucuronidation defect of GS, since approximately 95% of Z in urine was liberated by alcaline hydrolysis consistent with its occurrence as a glucuronide. $AUC_{0\text{-}5h}$ of Z in CLD patients was significantly ($p<0.01$) higher ($17\pm$ SD 5 μg h ml^{-1}) than in controls (10 ± 5). GEC (but not BSP-k_i) correlated closely with total clearance ($r=0.8$, $n=10$) indicating that the metabolic capacity of the liver is the primary determinant of the systemic availability of Z. Presumably, therefore, dosage adjustment of Z will only be necessary in severe parenchymal liver disease.

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