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PHYSIOLOGIE – PHYSIOLOGY

Dynamics of Isovolumic Relaxation in the Canine Left Ventricle

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In the cat papillary muscle isometric relaxation is characterized by an exponential decline in tension. The half-time of the decline ($t_{\frac{1}{2}}$) correlates linearly with total load. In the present study the isovolumic relaxation period was analyzed in closed-chest dogs anesthetized with morphine and chloralose. Left ventricular (LV) pressure (tipmanometer) was measured after vagotomy and beta-blockade during volume (dextran) and pressure (methoxamine) loading and during Ca^{++}-infusion. After closure of the aortic valve an abrupt fall in LV pressure was found, followed by an exponential decline (25 to 50 msec) which deviated from a simple exponential only in the late phase; this latter part was excluded from analysis. A linear relationship was found between $t_{\frac{1}{2}}$ and the pressure existing at the onset of the exponential decay ($r = 0.74$). The enhancement of the inotropic state by Ca^{++} was not accompanied by a decrease in $t_{\frac{1}{2}}$. When the pressure at the end point of the phase of abrupt pressure drop exceeded 110 mmHg (± 2.5) the following decline in pressure was no longer exponential. Since velocity of lengthening of the contractile elements ($-V_{CE}$) is inversely related to $t_{\frac{1}{2}}$ one may state that 1. similarly to the findings in the papillary muscle there is a phase of constant $-V_{CE}$ during isovolumic relaxation except at very high pressures and 2. $-V_{CE}$ is dependent upon physical loading.

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Continuous Recording of $^{32}PO_4$-Uptake in Nerve at Different PO_4 Concentrations

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The influx of $^{32}PO_4$ into rabbit vagus nerve fibres was continuously measured in an apparatus in which the preparation, incubated at 37°C in radioactive Tyrode (pH 7.40), was slowly pulled along a counter, after passing through a tube, in which the extracellular space was washed free of adherent radioactivity. The output of the counter thus recorded the total $^{32}PO_4$-uptake from 0 to 60 min as a function of incubating time. Varying the extracellular PO_4 concentration between 0.08 and 8 m*M* at constant $^{32}PO_4$ showed that the total PO_4-uptake could be divided into a fraction that was saturated at about 0.5 m*M* PO_4 and a second fraction that increased linearly with extracellular PO_4. These two fractions were observed with incubation times exceeding 10 min. Lowering the temperature to 10°C almost abolished the first fraction while the second was little affected. Both kinds of experiments indicate that the PO_4 influx can be divided into an active and a passive part.

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Entrée présynaptique de calcium lors de la stimulation de l'organe électrique

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La présence de calcium ionisé dans le milieu extracellulaire est indispensable à la libération de l'acétylcholine. Nous avons mesuré le calcium intracellulaire (Ca_i) dans le tissu électrogène de la Torpille au repos et après différents temps de stimulation in vivo et in vitro. Dans les deux cas la stimulation fait augmenter le taux du Ca_i. Le gain net est de l'ordre de 80 pmoles/potentiel d'action/g de tissu frais. L'entrée de Ca se poursuit lorsqu'on continue à stimuler le tissu; mais sa vitesse diminue exponentiellement. L'effet a aussi été observé en utilisant du ^{45}Ca. La totalité du Ca est échangeable dans l'organe électrique pour autant qu'on respecte des temps d'équilibration suffisants. L'entrée de Ca mesurée ici concerne les terminaisons nerveuses présynaptiques, puisqu'elle se produit aussi quand la réponse des électroplaques est complètement abolie par le curare. Nous avons mis en relation l'entrée du Ca, la libération de l'acétylcholine et la répartition intracellulaire du médiateur.

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Calcium Absorption and Vitamin D Metabolism in Diphosphonate-Treated Rats

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Diphosphonates inhibit hydroxyapatite crystal formation in vitro. Ethane-1-hydroxy-1, 1-diphosphonate (EHDP), when given at large doses, inhibits bone calcification in vitamin D-repleted growing rats fed with a high Ca (1.4%) and a high P (1.3%) diet. This inhibition is accompanied by hypercalcaemia, hypercalciuria and a decrease in the intestinal calcium absorption. The intestinal response could represent an adaptation to the diminished deposition of calcium into bone, and might be due to an alteration in the vitamin D metabolism. Therefore, the organ distribution and the metabolism of double labelled vitamin D_3 (1, 2-^{3}H-4-^{14}C-cholecalciferol) has been studied in these animals. The most striking finding was a reduced accumulation of radioactive cholecalciferol and its metabolites in the kidney of EHDP-treated rats. Blood urea, glomerular filtration rate (C_{IN}), and renal plasma flow (C_{PAH}) were not modified by EHDP treatment. The change in the renal uptake of labelled vitamin D may be a consequence of the alteration induced by the diphosphonate on calcium metabolism; its relation, if any, with the decreased calcium absorption remains to be established.

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Postnatal Growth of the Rat Lung

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To investigate the formation of alveoli, which occurs after birth, 18 rats were sacrificed in groups of three animals at the age of 1, 4, 7, 10, 13 and 21 days. Their lungs were fixed and processed for light and electron microscopic morphometry. The postnatal lung growth can be divided into 3 phases. Phase I (days 1 to 4): The lung volume (Vl) increase is merely to due an expansion of the airspace volume (Va), the septal volume (Vs) showing practically no changes. The alveolar and capillary surface areas (Sa and Sc) remain stationary. Phase II (days 4 to 7): Sa and Sc become doubled; the increase in Vl is almost exclusively brought about by the doubling of Vs. Morphological data confirm a compartmentalisation of the primitive air sacs through the outgrowth of new septa. Phase III (7 to 21): This period is marked by a further doubling of Sa and Sc and by the fact that Va grows faster than Vs. This results in a thinning of the septa and of the airblood barrier.

Whilst the body weight shows a 5-fold increase from 7.2 to 38.5 g within 3 weeks and Vl a 4-fold one (0.57 to 2.25 cm^3), the morphometrically determined pulmonary diffusing capacity is augmented by a factor of 6. It increases steadily from 0.05 to 0.30 $ml \cdot min^{-1} \cdot mmHg^{-1}$. This indicates that lung function is not altered, despite the complex transformations of structure.

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Metabolic and Structural Lesions of a Sympathetic Ganglion by Black-Widow Venom

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In the presence of Latrodectus venom, synaptic transmission through the rat superior cervical ganglion rapidly failed in a manner which could be explained by depolarization of the presynaptic nerve endings. Creatine phosphokinase (CPK) activity, measured by an enzymatic fluorimetric method, was completely inhibited after one hour's incubation with the venom. The time course of CPK inhibition was parallel to the fall of synaptic transmission. The presence of cystein protected both, transmission and CPK, from the action of the venom. A 20 percent fall of the ATP content was observed in the presence of the venom; ATPase activity was not altered. When transmission was blocked, the ganglia did not show ultrastructural changes. Typical lesions of the presynaptic nerve terminals appeared later including cytoplasmic alterations and loss of synaptic vesicles. It is suggested that Latrodectus venom blocks synaptic transmission by interfering with metabolic processes in which CPK and other SH-containing enzymes are involved.

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High Potassium and Low Potassium Red Cells in Cattle

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K and Na content of red blood cells was measured by flame photometry in 283 animals of different breeds of cattle. Within one breed animals with high K cells (up to 70 m-moles/l cell) and low K cells (down to 7 m-moles/l cell) were found. Na content varies inversely with K content. The frequency distribution suggests the presence of a large group of animals with high K (HK) cells and a small group of animals with low K (LK) cells. In vitro HK cells show a higher activity of the Na-K pump than LK cells. In an assay medium containing 100 m*M* Na and 10 m*M* K isolated membranes from HK cells display a higher rate of ouabain-sensitive ATP splitting per mg of protein compared with those from LK cells. Much as in sheep and goats the higher rate of active Na-K transport seems to account for the elevated K concentration in HK cells. However, passive permeability of the membrane towards Na and K seems also to differ in the two types of cells.

Influence of Anoxia on the Membrane Potential of Retinula Cells in the Honeybee Drone

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In isolated heads of the honeybee drone, anoxia leads to a rapid decrease of the membrane potential of retinula cells stimulated regularly with strong flashes of light. Anoxia, however, does not affect the membrane potential of retinula cells kept in darkness, except in cells which have been depolarized with current applied through an intracellular microelectrode. This latter finding suggests that the decrease in membrane potential found in preparations stimulated with light is the consequence of the depolarizing receptor potential. During the receptor potential or the depolarization induced by current, potassium ions are driven out of the cell and accumulate in the narrow extracellular cleft which surrounds retinula cells. Readmittance of oxygen leads to a rapid repolarization in both cases. This might be the result of a potassium pumping mechanism which requires oxygen.

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Vagal and Extravagal Influences During Artificial Respiration in the Rabbit

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In artificial respiration with a positive-negative-pressure respirator one reflex contraction of the diaphragm is elicited by each forced deflation. Within certain limits the rabbit can be ventilated with a frequency different from its own respiratory rate. Lowering of the mean air pressure below atmospheric pressure increases both the strength of the reflex contractions and the maximal respirator frequency at which the reflex closely follows the respirator action. Elevation of the mean air pressure reduces the strength of the reflex contractions and lowers the maximal respirator frequency. At insufflation with about 15 cm H_2O the reflex contractions are abolished. After bilateral cervical vagotomy spontaneous respiration is slowed down. Artificial respiration with atmospheric or slightly negative pressure no longer influences the diaphragm contractions. Elevation of the mean air pressure to 15 cm H_2O increases both the strength and the frequency of the diaphragm contractions. This suggests that in artificial respiration with positive pressure the activity of the respiratory center is stimulated by extravagal influences and inhibited by vagal afferents from the pulmonary stretch receptors. The inhibition is dominant.

Slow Recovery of the Na-System from Inactivation in Ventricular Myocardial Fibres

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The kinetics of the inward Na current responsible for the rapid upstroke of action potentials (AP) in guinea pig papillary muscles were studied. We analyzed the maximum rate of rise, $[(dV/dt)_{max}]$ of the AP as a function of membrane potential (MP) and time. MP was varied between −90 and −60 mV by adding KCl to the bathing solution, and time was varied by progressively shortening the interval between two AP's. In the steady state there was a sigmoid relation between MP and $(dV/dt)_{max}$. The time constants for $(dV/dt)_{max}$ to reach the steady state value in the second AP, i.e. the recovery from inactivation, increased from less than 20 msec when MP ranged from −90 to −85 mV to approximately 100 msec when MP ranged from −65 to −60 mV. Increasing $[Ca]_0$ shifted both the steady state curve and the curve relating the time constants to MP in the depolarizing direction. Voltage clamp experiments indicated that the inactivation of the Na current at any given MP was a much faster process than the recovery from inactivation.

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Effects of Pyramidotomy, Motor Cortical Ablation, and Deafferentiation on a Conditioned Finger Movement in Monkeys

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Experiments were disigned to analyse the effects of pyramidal tract lesions and motor cortical lesions on force and speed of a conditioned hand movement requiring opposition of thumb and index finger. In a first type of experiment, the force threshold necessary for reward could be varied from 100 to 700 g. In a second type of experiment, speed of this movement was tested in a reaction time situation. After unilateral and bilateral pyramidotomy as well as after unilateral cortical ablations the monkeys could relearn the motor task even at high force thresholds. However, the building up of force was delayed as evidenced by an increase of the EMG summation time and of the rise time of the force curve. After unilateral pyramidotomy the monkeys trained for speed exhibited a significant increase of the response time without equivalent increase of the EMG latencies. Following total deafferentiaton of the arm, the conditioned movement could not be reestablished.

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Electrophysiological Properties of Spinal Neurones in Tissue Culture

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Microelectrode studies were made on neurones of rat spinal cord cultivated for 13–49 days in vitro. Most of the membrane potentials recorded were between −40 and −55 mV and did not exceed −70 mV. The membrane resistance varied between 2 and 14 MΩ. An increase of potassium in the bathing fluid caused a depolarization of the cell membrane which was proportional to the potassium concentration. Action potentials could be recorded during impalement by the microelectrode (injury discharge). On some occasions spontaneous discharges were also observed (extracellular recordings). On a small number of cells potentials which resembled synaptic potentials of spinal motoneurones in situ (J. C. Eccles, The Physiology of Synapses, Springer, Berlin 1964) could be evoked by passing depolarizing current pulses through a second microelectrode located 80–100 μm from the impaled neurone.

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Sensitivity to Central and Skin Thermal Stimulation in Control and Obese Subjects

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Using direct and indirect calorimetry, the thermoregulatory responses to moderate cold exposure (ambient temperature 20°C) for 90 min were studied in 2 groups of female subjects: controls ($n = 10$) and obese ($n = 30$). In the overweight subjects, the fall in internal temperature during the test was smaller than in the controls; in addition, the levels of mean skin temperature were significantly lower in the obese. Total heat loss and metabolic rate were both more elevated in the control group. However, the heat debt was found to be similar in both groups. The cutaneous thermal insulation of controls was lower than that of obese subjects. These results show that overweight subjects do not increase their heat production during exposure to moderate cold. This economy of calories results from increased cutaneous thermal insulation which induces lower heat losses. The final consequence is a more stable internal temperature. Since the mean skin temperature was lower in obese subjects than in controls, the cutaneous thermal drive for increasing their metabolic rate appears to be damped; this might be due to the lack of fall in internal temperature during the cold exposure.

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Action of Thyroid Hormones on TSH Secretion Rate and Catabolism in PTU-Treated Rats

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The action of 1.2 μg/day of thyroxine (T_4) and 0.4 μg/day Triiodothyronine added to 0.01% Propylthiouracil (PTU) in drinking water was studied in normal rats on goiter development, plasma and pituitary thyrotropin (TSH) concentrations and on catabolism and secretion rate of thyrotropin. In the PTU + T_3 group, addition of T_3 to PTU resulted in partial inhibition of the goitrogenic action of PTU. However, T_3 had no effect opposite to that of PTU on TSH secretion rate and plasma concentration during the first week of treatment when compared with the PTU group. At one month, the TSH secretion rate significantly increased, indicating that addition of a small quantity of T_3 to PTU may induce stimulation of the TSH synthesis. In the PTU + T_4 group after one month of treatment addition of T_4 to PTU affected the goitrogenic action of PTU, resulting in a larger goiter than that found in the PTU group. TSH secretion and plasma TSH con-

centration did not increase significantly, indicating a partial blockage of TSH secretion by PTU. TSH metabolism was prolonged in each group. Comparison between biological and radioimmunological TSH activities showed a good correlation for the pituitary TSH content, although some discrepancies were observed regarding plasma TSH content.

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Morphology of Axonal and Glial Membranes at Nodes of Ranvier

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Panoramas of freeze-etched material from rat and cat spinal cord and pigeon optic tectum reveal repetitive structures that implicate both axonal and glial membranes asymmetrically in the paranodal zone. In thin-sectioned e.m. these two cell types are only 30 Å apart in this region. Corresponding to each glial wrapping, there is a shallow indentation encircling the axon which is visible on both leaflets of axonal membrane. Within these indentations are evenly spaced bands which repeat at 250 to 300 Å intervals. These bands are seen on the outer leaflets of both axons and glia but they do not affect the corresponding inner leaflets. The bands appear as parallel rows of gross particles regularly spaced 65–70 Å apart on the glial side, and as shallow grooves on the axon which are punctuated by diagonal files recurring at the same interval. Four oval substructures can be seen along the diagonal files. The files shift their orientation with respect to the bands between approximately 45° to 60°. The width of the bands varies between 250–300 Å in correspondence with these angular differences. Thus, the structure connecting the two cells displays flexibility, like a stretch fabric. This elaborate interface may present a barrier to ionic flow along the intercellular cleft and, simultaneously, favor intercellular exchanges across the two membranes.

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Inhibition du transport intestinal et rénal par le *n*-butyl-biguanide

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Le *n*-butyl-biguanide à haute concentration a un effet inhibiteur sur le transport intestinal des acides aminés et des sucres mesuré in vitro sur des rondelles d'intestin de cobaye et de rat. Cet inhibiteur, tout comme l'ouabaïne, n'a pas d'influence sur la vitesse initiale du transport et son effet n'est mesurable que lors d'incubations prolongées. D'autre part si le tissu a été préincubé en présence d'ouabaïne ou de biguanide, on remarque une diminution de la vitesse initiale de résorption du substrat lors d'une incubation ultérieure. Ces observations permettent de supposer que le biguanide agit en modifiant le gradient de sodium. En outre dans des tranches de cortex rénal de chien, l'ouabaïne ou l'acide éthacrynique à concentration maximale n'inhibe que partiellement le transport des sucres et des acides aminés, tandis qu'un mélange d'ouabaïne et d'acide éthacrynique l'inhibe complètement. Ces deux produits agissent vraisemblablement sur deux pompes à sodium différentes. Le *n*-butyl-biguanide ne provoque également qu'une inhibition partielle, mais son effet n'est additif qu'à celui de l'ouabaïne et non pas à celui de l'acide éthacrynique. En plus, le biguanide n'a aucune action sur la Na^+–K^+-ATPase microsomale qui est très sensible à l'ouabaïne. Ces résultats pourraient indiquer que le biguanide agit sur la pompe à sodium sensible à l'acide éthacrynique.

Origin of Proteins in Synaptic Organelles

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The contribution of fast (1) and slow (2) axoplasmic flows and local synthesis (3) to the origin of synaptic organelles was studied in the visual system of pigeon. Proteins in each flow were labelled with ^3H- and ^{14}C-proline or -leucine, respectively, by intraocular injection. Subfractions obtained after osmotic shock of crude mitochondria (V.P. Whittaker et al., Biochem. J. *90*, 293, 1964) are differently labelled by the three sources. Slowly migrating material predominates in all fractions, especially in those soluble (O) and mitochondrial (I). The fast flow carries material mostly for the small membrane (F) and plasma membrane (G, H) fractions. The specific activity of proteins labelled by local synthesis is too high to be due to microsomal contamination. Bands specific for the synaptic vesicle fraction (D) were investigated with respect to their origins by means of SDS polyacrylamide gel electrophoresis (P. Marko et al., FEBS Letters *17*, 261, 1971).

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Narrow Extracellular Clefts Between Cardiac Muscle Fibers as a Possible Site for Ion Accumulation

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Thin bundles of bullfrog auricular fibres were voltage-clamped using a double-sucrose-gap method. Current was measured during and following a depolarizing voltage step. Much of the outward current through a depolarized cell membrane is probably carried by K ions. These ions might accumulate within narrow intercellular spaces; the current from the clefts to the outside solution may be carried mainly by ions other than K. This hypothesis was tested by keeping the preparation depolarized for various durations, and determining the 'reversal potential' at the end of the depolarizing voltage step. This characteristic potential level, at which neither a 'tail' of outward- nor a 'tail' of inward-current is observed upon repolarization, was −60 mV with a depolarizing clamp of 3 sec, but −40 mV with a step of the same amplitude (80 mV) lasting for 15 sec. The reversal potential shifted approximately +35 mV with a 10-fold increase in extracellular K. It is thought that the change of the reversal potential as a result of prolonged outward current flow corresponds to a change of K equilibrium potential which in turn points to K accumulation within narrow extracellular clefts.

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The Dorsal Thalamus as a Relay in the Visual Pathways of the Pigeon

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1. Stereotactic intraocular injection of ^{3}H-leucine and proline was applied. Optic nerve terminals containing labelled proteins were observed in *N. lateralis anterior* (LA) and *N. dorsolateralis anterior* (DLA) with its subdivisions situated dorsally and partly rostrally of *N. rotundus*. No ipsilateral projections were found. 2. Following unilateral ablation of the Wulst, retrograde degeneration was seen in the medial and caudal part of DLA, leaving LA intact. 3. After electrical stimulation of one optic nerve papilla, a 2.5 msec latent response was recorded in the contralateral LA and DLA. Evoked responses were observed in the Wulst bilaterally after unilateral DLA stimulation. Latency of the ipsilateral response was 2.2 msec, while the contralateral was 4.5 msec. 4. Cryogenic blockade of the dorsal thalamic area led to a severe decrement of Wulst responses bilaterally following contralateral eye stimulation. Thus, visual information from one retina reaches the contralateral dorsal thalamus which in turn bilaterally projects to the Wulst.

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ATP Extracted from the Isolated Superior Cervical Ganglion of the Rat: Effect of EDTA

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The ganglion, excised from the rat and desheathed, was incubated in a standard Krebs-bicarbonate solution at pH 7.4. At the end of the incubation time the ganglion was frozen, dry-grounded and homogenized in perchloric acid at low temperature. The macromolecules were separated from the acidosoluble constituants by centrifugation at 11,000 *g* for 30 min. The amount of ATP was measured in the neutralized supernatant.

EDTA had been added at different steps of the extraction, its concentration varying from 0 to 5 m*M*. By adding this chelating agent it was possible to detect a surplus of ATP, which could not be found in the non-treated supernatant.

We have tried to explain the origin of this surplus on the basis of ultrastructural observations (F. Mir-Léchaire et al., J. Physiologie *63*, 256A, 1971) and biochemical findings (K. H. Berneis et al., Biochim-Biophys. Acta *215*, 547, 1970).

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Possible Role of ADH in the Regulation of Potassium Balance in the Rat

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It has been demonstrated recently that rats with genetic diabetes insipidus (D.I.) are hypokalemic. Injection of vasopressin to such animals causes potassium retention without affecting sodium balance (J. Möhring et al., Life Sciences *11*, 65, 1972). In rats heterozygous for D.I., no changes in either sodium or postasium balance were observed after vasopressin administration. In further experiments, the effects of 50, 100, 125 and 250 mU of vasopressin tannate on water, sodium and potassium balances were studied in male D.I. rats. After the injection of vasopressin, a dose-dependent potassium and water retention with a concomitant decrease in water intake and urine volume were observed. Sodium balance was affected only after the injection of 250 mU, which caused a slight sodium retention. These findings and the observation of kaliopenic nephropathy in D.I. rats support the hypothesis that ADH is of significance for the regulation of potassium balance in the rat.

Effect of Colchicine on Synaptic Transmission

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Colchicine, injected intraocularly in doses of 10 or 100 µg, reversibly prevented appearance in optic nerve terminals of material labelled in the ganglion cell bodies. Colchicine also induced reversible ultrastructural alterations in the terminals, delayed enlargement of synaptic vesicles and fibrillar proliferation. After intraocular injection of colchicine, electrical stimulation of the optic nerve yielded normal evoked potentials in the optic tract and on the surface of the optic tectum, whereas responses recorded in the depths of the tectum were reduced by 75%, suggesting a deficit in synaptic transmission at the optic nerve terminals. The colchicine-induced synaptic effect reached a maximum within a few days and was reversible. These results imply that material migrating with axoplasmic flow contributes to presynaptic structures and is important for synaptic transmission.

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Humoral Transmission of Sleep, Partial Isolation and Characterization of a 'Sleep Factor Delta'

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The previous technique for producing sleep dialysate from rabbit donors kept asleep during extracorporal dialysis was improved, as well as the assessment of the dialysate's hypnogenic activity. This was achieved by intraventricular infusion of dialysate to recipients submitted to electroencephalographic analysis (slow delta rhythms of sleep) and to behavioral kinesigraphic tests (actogram: in dyn). The 'crude' sleep dialysate was fractionated and the fractions tested with the same bioassays. Gel filtration on Sephadex G-10 desalted the material. The salt-free fraction (higher M.W.) contained the hypnogenic activity. A separation into 6 different Ninhydrin-positive fractions was achieved by TLC. The hypnogenic activity resided in one of them only. After rechromatography on Sephadex G-15, this material emerged as 3 different Ninhydrin-positive peaks. The hypnogenic activity was found in one of them with a Rf-value of 0.75 suggesting a M.W. around 800. High voltage paper electrophoresis revealed 4 different Ninhydrin-positive spots in this active peak. Two of them were identified by amino-acid analysis as serine and glycine.

The other two, after acid hydrolysis, yielded seven different amino-acids. The peptide nature of both compounds apparently associated with hypnogenic activity is suggested.

Respiratory Effects of Preoptic Diathermic Heating in Sleeping Cats

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In cats hypothalamic temperature was recorded by means of a thermistor placed 2–4 mm from the electrode pair (2 mm tip distance) used for unilateral diathermic heating (0.75 MHz, 90–180 mW) of the preoptic region. Environmental temperature was kept at 28 ± 3°C to maintain panting threshold low. Under such conditions hypothalamic temperature and respiratory frequency during synchronized sleep (SS) were 39.09 ± 0.06°C and 24 ± 5/min, respectively. Preoptic heating (90–140 mW, 1–3 min) increased hypothalamic temperature up to 39.12 ± 0.06°C and elicited panting (98 ± 31/min, $P < 0.001$) during SS, whereas during desynchronized sleep (DS) it raised hypothalamic temperature to 39.13 ± 0.05°C, but did not result in panting (41 ± 15/min). Stronger and longer heating (150–180 mW, 2–5 min), applied to the preoptic region during DS until hypothalamic temperature reached 39.19 ± 0.08°C, elicited panting (117 ± 25/min, $P < 0.001$). On this basis, the disappearance of spontaneous panting during DS at high environmental temperature (Brain Res., *6*, 789, 1967) may be related to an increase in preoptic panting threshold.

Calcium Uptake in Energy Depleted Human Red Cell Ghosts

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Three variants of K-rich ghosts were prepared from starved human red cells by reversal of osmotic hemolysis: 1. O-Ca-EGTA-ghosts (hemolysing medium [HM] containing no Ca but 2–4 mM EGTA), 2. Ca-ghosts (HM containing 0.1–4 mM Ca), 3. Ca-EGTA-ghosts (HM containing Ca and EGTA in various proportions to establish intracellular Ca concentrations between 10^{-8} and $10^{-5}M$). Ca uptake into O-Ca-EGTA-ghosts from media containing up to 14 mM Ca leveled off before equilibration was reached. The ratio of cellular to extracellular Ca was well below 0.25. Half of this uptake was bound to membrane sites and did not reach the intracellular space. Ca uptake into the membrane and into the intracellular space decreased with increasing concentrations of intracellular free Ca ($[Ca^{++}]_i$) as was shown using ghost preparations 2. and 3. $10^{-6}M[Ca^{++}]_i$ inhibited the uptake almost completely. In the presence of mersalyl, a sulfhydryl blocking agent, intracellular Ca uptake into O-Ca-EGTA-ghosts was greatly increased. These observations suggest the Ca permeability under these conditions to be controlled by Ca bound to specific membrane sites. The self-limitation of Ca uptake enables the cell to maintain a low $[Ca^{++}]_i$ even in the absence of ATP-dependent Ca outward transport.

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Amino-Acid Transport in Hypertrophic Rat Kidneys

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Renal compensatory hypertrophy was induced in rats by uninephrectomy or ligation of the ureter. The contralateral hypertrophic organs were studied three weeks after the operation and compared with those of sham-operated animals. An increase of more than 30% in cortical and total renal mass was observed. It was accompanied by a parallel increase in total cortical DNA, although the DNA per unit weight of cortex was unaltered. Transport of glycine by cortical strips was determined, and no change in absorptive capacity of the individual cells of hypertrophic kidneys was encountered. The transport, when expressed in terms of unit weight of tissue, was unchanged, and the distribution ratio between intra- and extracellular space was unaltered. On considering the increment in total tissue mass, however, there was a highly significant increase in the glycine transport capacity of the hypertrophic kidneys.

Factors Modulating the Activity of *A. sylvaticus* L. as Measured in an Actograph

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A $80 \times 80 \times 90$ cm wooden chest has been equipped for continuous actographic measurements. On the floor a box can be used as a nest; food and water are freely available; an exercise wheel is accessible at any time. Various control devices, mostly infrared beams allow to record the animal's activities, using a 20 channel Esterline Angus recorder and digital counters.

The observed animal, *Apodemus sylvaticus* L., is nocturnal and leaves the nest at nightfall. After a brief exploration of the environment he starts running in the wheel, and spends inside it about 70% of the time he is awake. He stops this activity rather frequently in order to explore again the enclosure, drink, eat or store food, gather sawdust in front of the entry of his nest or heave it on the top of his food. We have been able to show intrinsic and extrinsic factors which are likely to modify the wheel running during the life of an animal (between the 3rd and the 24th month). These factors include individual caracteristics, sex, age, weight, season and light.

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Intracellular Quantification of Alkaline Phosphatase: Studies with Ultramicromethods

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Investigators who are engaged in subcellular fractionation of renal tissue use alkaline phosphatase (a. P'tase) as a marker for the identification of the brush border membrane fraction. Cytochemical technique revealed conflicting results on the subcellular distribution of this enzyme. We reinvestigated this problem using an ultramicrotechnique for quantification of a. P'tase in the basal and luminal area of the epithelial cell of rat renal proximal tubule. Proximal convoluted (PTC) and straight (PTR) portions were dissected out from lyophilized rat renal sections. From each PTC and PTR (20 ng dry weight) the basal and luminal area (3 ng each) were separated and

a. P'tase was determined using oil-well technique and *p*-nitrophenylphosphate as substrate. The following table gives the moles of substrate used per kg of dry weight and per hour at 37°C, in the absence (ØCN) and in the presence of cyanide.

	⌀ CN	10 m*M* CN	CN sens. a. P'tase
Basal area			
from PTC	41.9	20.4	21.5
from PTR	11.7	3.8	7.9
Luminal area			
from PTC	137.8	3.6	134.2
from PTR	171.0	4.2	166.8

The values suggest that a. P'tase is localized in the whole surface membrane of the renal proximal tubular cell including basal infoldings.

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Effects of Diphenylhydantoin on Sodium Transport in Frog Skin

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Diphenylhydantoin (DPH), an anticonvulsive and antiarrhythmic agent, has been shown to affect sodium transport in several tissues, including brain and muscle. We have undertaken a study of DPH on amphibian epithelia. Pieces of frog abdominal skin were mounted in lucite double chambers with both sides exposed to identical aerated Ringer solutions. Potential difference (PD) and short circuit current (SCC) across the skin were monitored continuously by standard techniques. Addition to the external surface of the skin of DPH up to 33 μg/ml, elicited a rapid, sustained rise in both PD and SCC. The effect was reversible and could be induced repeatedly on the same preparation. The action of oxytocin (33 mU/ml) was not modified by the presence of DPH prior to, or after addition of, the hormone, the effects of both agents being additive. Addition of DPH to the internal surface of the skin was without effect in the range of concentrations used. Amiloride completely abolished the effect of DPH. The evidence obtained suggests that DPH increases sodium permeability at the outer surface of the skin.

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Peripheral Effects of the Hypothalamic Hypophysiotrophic Releasing Hormone TRH (Thyrotropin Releasing Hormone)

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The effect of TRH on the thyroid was studied in vivo in hypophysectomized Holtzman-rats which had been given a low iodine diet for 11 days and were treated by 5 times 100 ng TRH s.c. on the last 2 days. The in vivo uptake of ^{131}I in the thyroid was then measured. It was significantly decreased when compared to that in saline-treated controls (= 100%): 53.48–70.63% (mean 62.05± 8.59).

The standardized activity index NAI (W. Boguth and D. Frenzel, Histochemie *5*, 135, 1965) of the thyroid was determined by measuring the mean nuclear diameter of the thyrocytes and the volume percent of the colloid (interferometrically) in hypophysectomized TSH-substituted rats. The TRH-treated animals showed significant inhibition of the function of the thyroid in this system as shown by the decreased standardized activity index:

	NAI SD	n
Controls (TSH 6 I.U.)	0.42±0.018	9
Controls (saline)	0.21±0.041	8
TRH 0.3 mg	0.24±0.021	3
TRH + TSH	0.33±0.025	4

These data suggest that in hypophysectomized rats TRH exerts an inhibitory effect on the thyroid. In vitro studies have so far failed to reveal a peripheral effect on the thyroid, suggesting that the inhibitory effect is an indirect rather than a direct one.

Diffusing Capacity in Rat Lungs Fixed at Different Degrees of Inflation

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Rat lungs were fixed for electron microscopy by vascular perfusion under controlled physiological conditions at five points of the pressure-volume curve. This revealed the presence of collapsed alveoli in all lungs, but in an increasing proportion at lower inflation degrees. Such regions contained superimposed capillaries; the narrow space separating the air-blood barriers was filled by material of the alveolar lining layer. In addition, incomplete inflation causes folding of interalveolar septa. This results in a relative reduction of the real or functional alveolar surface area available for diffusion down to less than 50% of the tissue surface. The capillary volume depends on the alveolo-capillary pressure gradient. The harmonic mean thickness of the air-blood barrier shows the lowest value at highest inflation degrees, but it is generally lower than that estimated on fully expanded lungs because the folding of septa dislocates thick-barrier portions away from the surface. The resulting available diffusing capacity is a function of the degree of lung inflation, varying between 50% near FRC and over 90% near TLC.

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Cell-to-Cell Diffusion of TEA in Calf Hearts

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Right ventricular bundles (0.9–1.2 mm thick, 8–9 mm long) were exposed to ^{14}C-labelled tetraethylammonium-bromide (10 m*M*) over half of their length for 10 min. Subsequently, they were washed in nonradioactive Tyrode's solution for 2–6 hrs, frozen in liquid air and cut into slices. TEA was determined by a liquid scintillation method. Since the preparations at rest showed no measurable efflux of TEA in the later part of the washing period, the assumption was made that no TEA was lost through the surface membrane. On this assumption a plot of radioactivity against distance could be fitted by a theoretical line (eq. 2.14 by Crank, 1967). The apparent diffusion coefficient works out to 2×10^{-6} cm^2 sec^{-1} which is 4 times lower than that reported for ^{42}K in the same type of

bundles (Weidmann, J. Physiol. *187*, 323, 1966). The results suggest that TEA with a molecular weight of 130 can cross intercalated disks. TEA increased the duration of action potentials (Haldimann, Arch. int. Pharmacodyn. *146*, 1, 1963). This effect persisted in TEA-free solutions which would be expected if TEA stayed in the intracellular compartment and acted on the surface membrane from inside.

Digital Processing of Spike Train Data

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Input raw data are pre-amplified neuronal unit potentials, recorded by microelectrodes. The input potential is scanned at a rate of 40 kHz, and the digital samples displayed on-line. For each unit potential, the time of occurrence and the peak amplitude are recorded as 24-bit and 12-bit integers, respectively. Multi-unit records of a single electrode are then separated by use of a peak amplitude histogram. Associated event times are re-arranged in ascending order and stored as data files on a bulk storage device. Each analysis program refers to at least one of these train files. Single train processing is required for all first and second order property estimates of one unit discharge pattern. Functional dependence of simultaneous records are tested by lagged coincidence of forward and backward recurrence times. Histograms, function estimates, scatter diagrams and digital values are displayed on digital oscilloscopes for inspection. Furthermore, all these values are plotted.

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BIOCHEMIE – BIOCHIMIE – BIOCHEMISTRY

The Complement-Binding Sites on Rabbit IgG

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The modification of tryptophan in rabbit IgG and its fragment Fc with 2-hydroxy-5-nitrobenzyl bromide (NBB) substantially decreases their anticomplementary activity. In order to locate the position of the critical residues in the complement-binding site, modified IgG was digested with pepsin and subjected to gel filtration to yield a 5000 MW peptide fraction which contained NBB-modified tryptophan (PEP V, derived from Fc near the 'hinge region'). Since the modification of a few tryptophans may affect different parts of the molecule and therefore the binding sites for different components of complement, the fixation of ^{125}I-labelled Clq (a subunit of the first component of complement) by native and NBB-treated IgG and its fragments Fc and F $(ab')_2$ was also studied. Insoluble aggregates of IgG and its fragments were prepared by dioxan precipitation. One mg Fc fixed 1 μg ^{125}I-Clq, wehreas the NBB-treated Fc fixed 0.01 μg Clq, providing a control value to measure the inhibitory activity of peptides derived from Fc. Fragment $F(ab')_2$ did not fix Clq, confirming that the site of interaction is located on Fc. The finding that $F(ab')_2$ fixes complement when incubated with whole serum points to a mechanism involving the recently described 'bypass' (Jacot-Guillarmod).

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Positive and Negative Cooperativity in the Binding of Coenzyme to Yeast Alcohol Dehydrogenase

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YADH (yeast alcohol dehydrogenase) is a tetrameric enzyme which catalyses the interconversion of ethanol-acetaldehyde in the presence of nicotinamide adenine dinucleotide coenzyme (NAD-NADH). The four subunits are chemically equal (I. Harris, Nature *203*, 30, 1964). We found that the binding of NAD and NADH to YADH produces a quenching of the tryptophan fluorescence. This phenomenon bears direct evidence to the presence of tryptophan at the active site. The quantitative evaluation of the quenching process provides an accurate, fast routine method to determine enzyme-coenzyme binding constants and to detect cooperative subunit interactions accompanying the binding. The binding of NADH is characterized by positive cooperativity, with a Hill coefficient of 2.5 approx. in the first part of the titration range. The binding of the fourth subunit is the loosest one, and this explains some claims (F.M. Dickinson, Biochem. J. *120*, 821, 1970) that the enzyme has only three binding sites for NADH. The binding of NAD is characterized by a lower affinity in the last part of the titration range, and indicates weak negative cooperativity. Preliminary results show that the binding of coenzyme to lactate dehydrogenases from various sources proceeds with similar pattern, and positive and negative cooperativity upon coenzyme binding has been already found by other authors in yeast glyceraldehyde-3-phosphate dehydrogenase (R.A. Cook and D.E. Koshland, Biochemistry *9*, 3338, 1970). This seems to indicate that a common cooperative mechanism is operative in most tetrameric dehydrogenases in the binding of coenzyme.

Glutamate Dehydrogenase of Cortical Neurons and Brain Metabolism

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It has often been stated that the very high content of free glutamic acid in the central nervous system can operate as an 'energy booster' or a secondary energy source, under stress conditions and depletion of carbohydrate supply or after repeated firings of nerve signals. Considering the inability of (radioactive) Glu to penetrate into the neurons and the limited access of Glu, also taking into account the major functions of Glu as the precursor for the biosynthesis of a variety of nucleotides, amino acids and brain amines, as well as its postulated contribution as a neurotransmitter in certain cells or as a mediator in the attainment of subfiring level potentials, brain GDH occupies a key position in the metabolism of the central nervous system. Its regulation is likely to be dependent on numerous factors and to be strictly modulated. But could brain GDH really work in reverse within nerve cells? To answer

this and other intriguing questions, a study was initiated in our laboratory on the cellular location of the enzyme and its physical, chemical and kinetic parameters. A highly purified enzyme has been prepared in a crystalline state. Enzymatic properties were investigated under steady-state conditions and relative affinities towards substrates (and products) determined. Thermodynamic calculations based upon either theoretical heats of formation or the values of reduction potentials, when extended within the entire range of physiological variation in the availability of all reactants, preclude any reversal of the reaction, e.g. the formation of 2-oxo-glutaric acid and ammonia from Glu. The mechanism of the forward reaction appears to fit with a mixed sequential ping-pong pattern in a 'tri-tri' system (water being taken into account at the pH set at 7.0).

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Localization of Teichoic Acids in Cell Walls of Gram Positive Bacteria

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Some teichoic acids are known to be partially substituted by α-D-glucopyranosyl residues such as the teichoic acids of *Streptococcus faecalis* (strain 8191) and *Lactobacillus plantarum* (strain 17–5). They therefore will bind specifically the phytohemagglutinin Concanavalin A (Con A). Con A, labelled with mercury or colloidal gold coated with Con A have been used as markers for electron microscopy. Teichoic acids, localized by this technique showed areas of different density of labelling indicating an uneven distribution of the teichoic acids in the cell wall. The labelling pattern might correspond with distinct differentiations of the plasma membrane observed after freeze-etching. The densest label was observed over the newly forming septa.

Studies on the Structure of the Qβ-Genome

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5′ terminal segments of Qβ plus and minus strands were prepared by synchronized synthesis and their nucleotide sequences were determined as described earlier (Billeter et al., Nature *224*, 1083, 1969; Goodman et al., Proc. Nat. Acad. Sci. *67*, 921, 1970). The sequence of 330 nucleotides at the 5′ terminal region and 160 at the 3′ terminal region was deduced. A replicase binding site, comprising 100 nucleotides, was isolated and its structure elucidated. More recently resynchronisation of synthesis at the ribosome binding sites at the beginning of the coat or the replicase cistron was used to generate minus strand segments labelled in defined internal regions. It is estimated that the initiation sites of these two cistrons are approximately 800–1000 nucleotides apart, a distance greater by 400–600 nucleotides than that expected if this region comprised the coat cistron alone. We conclude that the read-through resulting in synthesis of protein A_1 (Moore et al., Nature New Biol. *234*, 204, 1971) extends into this region rather than into the replicase cistron.

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A Possible Role for Parvalbumins in the Regulation of Muscular Contraction

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Parvalbumins are low molecular weight proteins, found in the myoplasm of lower vertebrates which, up to now, have not been found to possess any biological activity. However, several chemical and physico-chemical parameters suggest a similarity with troponin A, a regulatory protein present in the muscles of higher vertebrates where parvalbumins are not found.

Binding of calcium by parvalbumins has now been studied quantitatively: using the ^{45}Ca-Chelex partition method of Briggs and Fleischman (2 m*M* Mg^{++}, 60 m*M* K^+, pH 6.7, Ca^{++} concentrations ranging from 0.5 to $100 \times 10^{-6} M$), a parvalbumin from fish (Hake) and two from frog were shown to have two high-affinity binding sites for Ca^{++} ($k_{dissoc.}$ between 0.1 and $0.4 \times 10^{-6} M$) and three to five sites with lower affinity for Ca^{++} ($k_{dissoc.}$ between 20 and $100 \times 10^{-6} M$).

These data, which are quite similar to those pertaining to troponin A, give further support to the hypothesis that parvalbumins could represent evolutionary precursors of troponin.

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Acid-Soluble Deoxyribonucleoside Triphosphates Pools and DNA Synthesis in Physarum

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Acid-soluble nucleoside triphosphates (NTPs) of *Physarum polycephalum*, an acellular Myxomycete with a synchronous mitotic cycle, were determined after ^{32}P label and separation by thin layer chromatography. Their levels, expressed in counts per minute, were related to protein content.

The distribution of individual triphosphates was calculated in percent of the sum of their activities. The deoxyribonucleoside triphosphates (dNTPs) vary from 3% to less than 1% of the total activity between the beginning and the end of S period and the ribonucleoside triphosphates between 97 and 99%. The pool sizes of dNTPs decreased in the following order: dTTP, dCTP, dGTP, dATP. During DNA replication, dTTP and dGTP decreased by à half, dCTP dropped tenfold and dATP became too small to be measurable. These low levels remained rather constant throughout G2 and increased again at the onset of the following S period.

This shows a parallelism between the supply of dNTPs and the rate of chromosomal DNA synthesis, but does not indicate whether there is a direct interdependence between the two. dCTP seems the most representative of DNA replication. The low remaining amounts of dNTPs in G2 could be related to mitochondrial and satellite DNA replication which takes place throughout the cycle.

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Reconstitution of Qβ Replicase from i Factor and Incomplete (α-less) Replicase

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Qβ replicase consists or 4 subunits, one of which (β) is coded for by the viral genome. Of the 3 host-specific sub-

units, α has been identified as the i factor of polypeptide initiation (Groner et al., pers. comm.) while δ and γ correspond to the polypeptide elongation factors Ts and probably Tu (K. Weber et al., pers. comm.). We have isolated an α-deficient enzyme ($R^{-\alpha}$) from a side fraction of replicase purification. $R^{-\alpha}$ synthesizes plus strands on a minus strand template, poly G on poly C and replicates '6 s RNA' (Banerjee et al., J. Mol. Biol. *45*, 181, 1969); however, replication of Qβ RNA was completely dependent on added α (and the host factor described by August and his colleagues). Subunit α was obtained by fractionation of Qβ replicase on low-salt gradients (Kamen, Nature *228*, 527, 1970) or from unwashed ribosomes, using the purification devised by Revel for i factor. These experiments suggest that α is required for specific recognition of Qβ RNA, presumably at an internal binding site (c.f. Schwyzer et al., Experientia, in press) while the $R^{-\alpha}$ complex comprises the polymerase activity and the recognition of the 3′ terminus of the template.

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Localization of the Dibutyryl Cyclic AMP Stimulated Lipase in Microsomes of Rat Adipose Tissue

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The localization of lipases in adipose tissue has been the subject of a number of studies. As the methods of potterization, as well as the centrifugation were very rarely described in detail (spleed, strokes, clearance) and as the experimental conditions were often quite different (Huttunen and Steinberg, BBA *239*, 411, 1971; Biale et al., BBA *152*, 28, 1968), it is difficult to compare the results of individual authors. Using the method of McKeel and Jarett (J. Cell Biol. *44*, 417, 1970), who have exactly described all operations, we found the following distribution of lipase activities (substrate 2-naphthol laurate, pH 7.4) in spec. units: nuclei 14 ± 1, mitochondria 35 ± 3, plasma membranes 286 ± 32, microsomes 664 ± 51, soluble fraction 163 ± 23. The total activities, however, showed the proportion of 58% in soluble fraction, 35% in microsomes, 5.5% in plasma membranes, 1 and 0.5% in nuclei and mitochondria, respectively. The role of DBcAMP in the stimulation of lipolysis is widely accepted. As the localization of triglyceride lipase is not certain, we applied DBcAMP i.p. in the dose of 50 μmoles/kg and at the peak of its effect (Chmelarova and Chmelar, Intern. Congr. Biochem., Luzern 1970) killed the animals, then separated the individual fractions of adipose tissue and estimated the lipase activity under the same experimental conditions as mentioned above for the control tissue. The only significant increase of specific activity was found in microsomes (from 664 ± 51 to 1228 ± 155).

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Influence of pH on Human Renin Activity with Different Substrates: Role of Substrate Denaturation

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The activity of human renin upon human substrate and synthetic N-terminal ileu^5-tetradecapeptide was compared as a function of pH. The angiotensin I generated during the enzymatic reaction was measured by radioimmunoassay. By incubating purified human renin with each of the following substrates, optimum pH was determined: pH 4.5 for ileu^5-tetradecapeptide, pH 5.5 for human substrate in plasma, pH 6 for partially purified human substrate and pH 7.5 for hog substrate. These different optima could be attributed partially to a direct effect of pH on the substrate itself. For verification of this hypothesis each substrate was progressively acidified to various pH prior to incubation at its optimum pH with renin. Human substrate was irreversibly inactivated below pH 5 whereas tetradecapeptide and renin were stable down to pH 3. The acid denaturation of human substrate seems to be an important factor that determines the acid side of the renin activity vs. pH curve and explains the observed optimum pH which is higher for the complete sequence than for the tetradecapeptide.

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Influence of Calcium (Ca^{++}) on Angiotensin II (AII)-Antibody Reaction

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Few reports have mentioned Ca^{++}-dependent antigen-antibody reactions (Maurer, J. Immun. *105*, 567, 1970), particularly concerning AII (Boyd, Protein and Polypeptide Hormones, Excerpta Medica, ICS *161*, 558, 1968). Among 19 rabbits immunized against AII, 7 showed antibodies which required Ca^{++} for maximum binding. This effect was determined by measuring the binding with radioimmunoassay of AII. The addition of an excess of EDTA totally inhibited the binding in 4 antisera and dissociated the complex formed at equilibrium. With the most Ca^{++}-dependent antiserum, this influence appeared simultaneously with detectable antibodies, was constant at all dilutions, increased the affinity constant and improved the sensitivity of standard curves of AII. Preincubations of the antigen or the antiserum alone with Ca^{++} were equally effective, the resulting binding being similar as in buffer with the same final Ca^{++} concentration. Ca^{++} was a much more potent activator than other divalent metals. The optimum concentration of Ca^{++} was 0.1 M, but the trace of Ca^{++} brought by the antiserum itself already partially improved the binding. Ca^{++} therefore appears to be an important factor in some polypeptide-antibody reactions.

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Subunit Molecular Weights of Acetylcholinesterases

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According to the model of Leuzinger et al. (J. Mol. Biol. *40*, 217, 1969) acetylcholinesterase (EC 3.1.1.7) from the electroplax has a tetrameric structure ($\alpha_2\beta_2$), whereas up to 6 equal subunits per molecular weight of 260,000 have been implied by others. To determine the molecular weights of the subunits by means other than ultracentifugation ^{3}H-DFP labeled acetylcholinesterase, purified by affinity chromatography to a specific activity of 13,500 IU/mg of protein, was subjected to SDS polyacrylamide gel electrophoresis (Lenard, J., Biochemistry *9*,

1129, 1970). The gels were calibrated according to Dunker and Rückert (J. Biol. Chem. *244*, 5074, 1969). Contrary to the findings of Dudai and Silman (FEBS Letters *16*, 324, 1971) only one radioactive species with a molecular weight of 66,000±1000 was obtained corresponding to the α-subunit of this enzyme. Protein staining revealed a second band with an apparent molecular weight of 77,000±1000, probably corresponding to the β-subunit described by Leuzinger. These results are consistent with the tetrameric structure $\alpha_1\beta_2$. For the enzymes from *Torpedo marmorata*, *Pleuronectes platessa* and human erythrocytes the corresponding values for the DFP labeled subunit were 74,000±2000, 76,000±1500 and 76,500±1500, respectively.

Isolation of the Two Active Subunits of the Sucrase-Isomaltase Complex

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The sucrase-isomaltase complex (SI), isolated from rabbit small intestine, has a mol. wt. of 220,000 and has two active sites, the one with sucrase, the other with isomaltase activity (J. Kolinska & G. Semenza, BBA *146*, 181, 1967). We have now shown SDS-polyacrylamide gel electrophoresis, by determination of the chemical composition and by fingerprint analysis of the tryptic digest, that SI is composed of two subunits of similar size and primary structure, having each a mol. wt. of 110–120,000. The two active subunits, one having sucrase, the other isomaltase activity, could be separated: treatment of SI at pH 9.6 and 37°C destroyed almost completely and irreversibly the sucrase. The active isomaltase and the residual active sucrase subunits could be isolated by two consecutive chromatographies on Biogel p-200 and Sephadex G-200. The latter retained isomaltase specifically (affinity chromatography). Preparative SDS-polyacrylamide gel electrophoresis in presence of Tris allowed a separation of the subunits with better yields, but in denatured form.

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Effect of Oestradiolpropionate and Progesterone on Monoamine Uptake in Rat Brain

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The influence of oestradiol propionate and progesterone pretreatment on the uptake of ^{3}H-dopamine (DA) and ^{3}H-serotonin (5-HT) in different brain regions was studied. Ovarectomised rats were killed 2 hours after s.c. injection of oestradiol propionate, progesterone (both 0,4 mg/rat), or control oil carrier. Brain slices from cortex, preoptic region, hypothalamus, thalamus, midbrain, and striatum were incubated (10′ at 37°C) with either ^{3}H-DA or ^{3}H-5-HT ($1\times10^{-7}M$). The difference in uptake (cpm/mg protein) between control and hormone treated slices was analysed by the Wilcoxon test. Pretreatment with oestradiol propionate resulted in lowered 5-HT uptake in the hypothalamus, and a tendency to lowered DA uptake in the thalamus. Pretreatment with progesterone resulted in an increased 5-HT uptake in the preoptic region, and no significant effect on DA uptake in any region.

It has already been established that the monoamines in specific brain regions control ovulation and oestrus behaviour. These preliminary findings suggest that, vice versa, the ovarian hormones may perhaps play a role in the uptake mechanism of the monoamines themselves.

Mechanistic Probes for Enzymatic Reactions: Oxidation-Reduction Indicators as Probes for Intermediary Carbanions

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Intermediates of enzymatic reactions may be inferred from the occurrence of specific reactivities acquired transiently by certain groups of the enzyme-substrate complex in the course of catalysis. Thus, the oxidation-reduction indicators phenazine methosulfate, 2,6-dichlorophenolindophenol, hexacyanoferrate(II), porphyrindin, and porphyrexide are reduced to their leuco form when added to a solution of aldolase and its substrate dihydroxyacetone phosphate, but not when added to either enzyme or substrate alone. The initial rate of reduction of the indicator depends on the substrate concentration and follows apparent Michaelis-Menten kinetics. By analogy to the reaction of tetranitromethane reported previously (Biochemistry *7*, 1531, 1968) the carbanion-enamine intermediate of dihydroxyacetone phosphate with aldolase ($^{\ominus}\ddot{C}HOH.C = NH^{\oplus}R.CH_2OPO_3^{2-}$, H_2NR = lys residue of aldolase) is thought to be the reactive species. Both the indicators and tetranitromethane result in the oxidation of this intermediate to hydroxypyruvaldehyde phosphate ($CHO.CO.CH_2OPO_3^{2-}$). The indicators and tetranitromethane are also reduced by enzyme substrate intermediates of pyruvate decarboxylase, aspartate aminotransferase, and 6-phosphogluconate dehydrogenase, indicating the general applicability of these reagents to mechanistic studies of enzyme reactions thought to involve carbanions.

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Preparation and Properties of ^{125}Iodide Labelled Clq Containing Full Biological Activity (Clq: Subunit of the First Component of Complement)

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Understanding the molecular mechanism of the initial stage of complement action is facilitated by the use of radioactive Clq. However, ^{125}I incorporation by the 'Chloramine-T' method results in a drastic reduction of Clq activity. The use of lactoperoxidase has now been found to provide an efficient and rapid labelling of Clq. When 1 atom of iodine was introduced per 2 molecules of Clq between 90 and 100% hemolytic activity was retained and over 95% of the radioactivity precipitated with either trichloracetic acid or monospecific rabbit anti-Clq. The same percentage was found to bind to ovalbumin-anti-ovalbumin immune precipitates. The labelled material migrated as a single component in SDS gel electrophoresis; in the ultracentrifuge it sedimented as a homogenous preparation with a coincident relationship between hemolysis, radioactivity and protein concentration.

This ^{125}I Clq used to study the possible involvement of carbohydrate residues in two of its biological parameters: oxidation by $5\times10^{-3}M$ periodate at 0°C produced a rapid decrease in hemolytic activity, whereas the ability to bind to immune precipitates was only slightly reduced.

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In Vitro Uptake of ^{3}H-Glycine and ^{3}H-l-Glutamate into Cat Dorsal and Ventral Spinal Cord

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Transport of ^{3}H-glycine and ^{3}H-l-glutamate into ($1{,}0 \times 0{,}5$ mm) slices of cat dorsal and ventral spinal cord (L_{6+7}) has been studied. Conditions for incubation ($5 \times 10^{-7} M$ isotope), centrifugation (90 min), and fractionation (30 fractions) were essentially as described (Honegger et al. Experientia *27*, 728, 1971). In cat spinal cord about 3–4 times more ^{3}H-glycine than ^{3}H-glutamate is taken up. ^{3}H-glycine is about 60% and ^{3}H-glutamate 35% higher in the dorsal than in the ventral half. After centrifugation, synaptosomes separated into 4 peaks (characterization by EM, LDH, GDH, protein). Approximate uptake into these peaks was 20% dorsal, 13% ventral for ^{3}H-glycine and 9% dorsal, 6% ventral for ^{3}H-glutamate. The labelling pattern for ^{3}H-glycine showed differences between dorsal and ventral, the uptake in the lightest fraction being considerably higher in the dorsal synaptosomes. These data for glycine are at variance to present electrophysiological and biochemical concepts. Amino acid analyses of synaptosomal fractions are in progress to clarify these questions.

Specific Glucose Transport in Isolated Brush Border Membranes from Rat Small Intestine

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Brush border membranes of epithelial cells have been preparated. Uptake of labeled D- and L-glucose has been measured with a Millipore filtration technique. An intact glucose carrier system in the isolated membranes can be demonstrated by the following: 1. D-glucose is taken up and released faster than L-glucose. 2. Sodium stimulates D-glucose uptake 3–5-fold; no other cation shows this effect. 3. D-glucose uptake and release is inhibited by phlorhizin. 4. Countertransport of D-glucose can be demonstrated. 5. D- and L-glucose reach the same level after prolonged incubation. 6. The uptake of D-glucose is inhibited by higher concentrations of D-galactose and vice versa.

Glucose uptake represents transport into an intravesicular space rather than binding. Exposure of the membrane to increasing cellobiose concentrations leads to osmotic shrinkage of the intravesicular space and decreased glucose uptake. Sodium in the medium, but not intravesicular sodium stimulates D-glucose absorption. We conclude that the glucose carrier remains intact in isolated brush border membranes. Our results are consistent with Crane's model for glucose transport (Fed. Proc. *24*, 1000, 1965).

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Mono- and Oligomeric Structures of Murine IgA Myeloma Proteins

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It is known that immunoglobulins of the IgA class exist not only as monomeric 7S units, but also as oligomeric aggregates of various sizes. Incorporation of labelled amino acids by cells from murine plasmacellular tumors indicated that each tumor has a characteristic ratio of mono- to oligomeric molecules which it secretes. In order to study in a more direct way the mono- and oligomeric units of IgA that are present in serum, a modified technique of crossed antigen-antibody electrophoresis (first described by Laurell) was used. Initial electrophoresis of the paraproteinemic mouse serum was performed on a polyacrylamide gel having a retarding effect according to molecular size. The second electrophoresis was carried out at a 90° angle to the first, in agarose containing the antibody. This resulted in the formation of a precipitin profile. For a number of IgA myeloma sera a typical profile was obtained for each. This permits evaluation of the ratio of mono- to oligomeric assembly of IgA in these sera and will facilitate the investigation of the mechanism and significance of this phenomenon.

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Primary Structures of Acomys and Mouse Insulins

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In *acomys*, a single insulin was found. The amino acid compositions of two different *acomys* strains (*A. cahirinus minous* and *A. cahirinus dimidiatus*, resp.) were found to be identical. Amino acid compositions and studies on the isolated chains using digestion with aminopeptidase O and with trypsin are in accord with the assumption that the A chain sequence is identical to the one of rat A chain, and that the B chain sequence is identical to the one of rabbit B chain.

Two different mouse insulins could be separated by gel electrophoresis. RAE A and B chains of mouse insulins I and II were digested with trypsin, chymotrypsin and/or thermolysin. The resulting peptides were separated and their amino acid sequences determined by stepwise chemical and enzymatic degradation. The complete amino acid sequences of the A and B chains of mouse insulins I and II are described; they are identical to the ones of rat insulins I and II.

Biological activities of mouse and *acomys* insulins are within the normal range. No indication for an 'abnormal' insulin in *acomys* has been found.

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Reciprocal Induction and Repression of Serine-dehydratase and Phosphoglyceratedehydrogenase by Proteins and Dietary Essential Amino Acids

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The impact of protein nutrition upon serine biosynthesis and catabolism in rat liver was investigated by determining the activity of two key enzymes in the pathway of serine metabolism: Phosphoglyceratedehydrogenase on the pathway leading from pyruvate to serine, and Serine-dehydratase on the catabolic way transforming serine to pyruvate. It was found that increasing the protein content of the diet induced Serinedehydratase and repressed reciprocally Phosphoglyceratedehydrogenase. By feeding amino acid mixtures lacking one amino acid at a time it was established that only four essential amino acids, i.e. methionine, tryptophan, threonine and valine, are active

in regulating the two enzymes. The regulating action of dietary proteins on the two enzymes can thus be expressed in terms of their content in the four 'active amino acids'. Finally a coherent theory for the reciprocal Serinedehydratase repression and Phosphoglyceratehydrogenase induction in rat liver by lack of an 'active' amino acid is proposed. It is based on the presence of a posttranscriptional repressor, which both inhibits messenger translation and promotes messenger degradation, and on the fact that the translation of small proteins is less impeded by amino acid deficiency than that of bigger polypeptide chains.

Pyruvate Carboxylase Activity in Rat Liver Mitochondria Preloaded with Calcium

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Purified pyruvate carboxylase (PC), the first enzyme in the gluconeogenic pathway, is known to be inhibited by Ca^{2+}. On the basis of experiments with perfused rat livers and with mitochondria, Rasmussen et al. postulated a regulatory role for Ca^{2+} on PC and gluconeogenesis (Biochim. Biophys. Acta *222*, 41, 1970). In order to further study this hypothesis, the influence of large intramitochondrial Ca^{2+} pools on PC activity was studied by incubation of mitochondria preloaded with different amounts of Ca^{2+}. During preloading mitochondria accumulated up to 150 nmoles of Ca^{2+} per mg of protein with complete retention of respiratory control. ATP, ADP and Mg^{2+} were accumulated in parallel with Ca^{2+}. During the incubation, intramitochondrial ADP was further varied by addition of octanoate. The results showed a correlation between PC activity and the intramitochondrial concentration of total ADP, MgADP, ATP_{total}/ADP_{total}, MgATP/MgADP and CaATP/CaADP ($p < 0.01$). If only the values in absence of octanoate were considered, PC activity decreased with increasing intramitochondrial Ca^{2+}. It is suggested that the inhibition of PC activity by Ca^{2+} in intact mitochondria is an indirect effect and is due to the accumulation of ADP caused by Ca^{2+}.

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Isolierung und Enzymanalyse eosinophiler Pferdeleukocyten und ihrer Granula

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Eosinophile Zellen wurden in vitalem Zustand isoliert. Mit Na-Dihexylsulfosuccinat (Aerosol MA) erhielten wir daraus die eosinophilen Granula in Gramm-Mengen und in hoher Reinheit, aber in leicht denaturiertem Zustand. Mit mechanischem Zellaufbruch konnten native Granula in guter Präparatreinheit, aber nur in kleinen Mengen erhalten werden. In ihnen fanden sich Peroxydase und Kathepsin in 2,5fach höherer Konzentration als in den ganzen Zellen. Ausserdem enthielten die Granula saure und alkalische Phosphatase, Glucosamidase, aber kein Trypsin und Chymotrypsin. Im Homogenat der mechanisch isolierten Granula sedimentierte nur die alkalische Phosphatase. Dabei wurden die nicht strukturgebundenen Enzyme der Granula mit stark erhöhter spezifischer Aktivität im Überstand gefunden. Aus unseren Ergebnissen können wir schliessen, dass die eosinophilen Granula Enzyme enthalten, die bisher in Peroxysomen und in Lysosomen gefunden wurden. Unsere Ergebnisse stimmen überein mit den von D.F. Bainton und M.G. Farquhar (J. Cell. Biol. *45*, 54, 1970) elektronenmikroskopisch-histochemisch aufgestellten Befunden.

Coupled Oxygenase-Hydrase in Microsomes from Rodent Liver

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Hepatic monooxygenases catalyze formation of reactive arene oxides from aromatic hydrocarbons. There is increasing evidence that such arene oxides are responsible for toxicity and carcinogenicity of many aromatic compounds. Hepatic expoxide hydrase (EH) converts arene oxides to less reactive dihydrodiols. Besides 'free' EH there now appears to be a coupled monooxygenase-epoxide hydrase system. After removal of glutathione in guinea pig liver homogenates 0.5 m*M* trichloropropene oxide effectively inhibited formation of naphthalene dihydrodiol (ND) from naphthalene oxide (inhibition of 'free EH') but not from naphthalene (no inhibition of 'coupled EH'). ND formation from naphthalene oxide was much more enhanced after pretreatment of rats with phenobarbital than with 3-methylcholanthrene. For the formation of ND from naphthalene the converse was true. Purified P-450 showed high EH activity but other microsomal enzymes were absent. This apparent coupled oxygenase-hydrase system may be of greater importance for inactivation of in situ formed arene oxides than the 'free' epoxide hydrase.

Proteolysis of Bovine Immunoglobulins G

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Bovine IgG_1 prepared from serum and colostrum, and IgG_2 from serum have been exposed to trypsin (pH 8.0) and pepsin (pH 4.5) at an enzyme-substrate ratio of 1:20 and 1:60, respectively. The kinetics of appearance of free amino groups have been measured by trinitrophenylation according to Habeeb. IgG_1 derived from colostrum and serum have identical kinetics, with trypsin as well as with pepsin. On the other hand, IgG_2 derived from serum is much more susceptible to trypsin than IgG_1: 3 times after 3 h, 2 times after 25 h, and 1.5 times after 72 h digestion (average values from 5 experiments). Even more striking is the finding that pepsin degrades IgG_2 to a smaller extent than IgG_1 (0.6 times after 3 h, 0.75 times after 12 h and 0.9 times after 32 h). The fragments were analyzed by electrophoresis in polyacrylamide gel in the presence of 0.1% sodium dodecylsulfate. The results are consistent with those measuring the free amino groups: after 32 h tryptic digestion, 37% of the IgG_1 and 22% of the IgG_2 remained intact. On the other hand, 12 h peptic digestion left 5% of the IgG_1 and 24% of the IgG_2 unaltered. The size and nature of the fragments with respect to their biological activity is discussed.

Heterogeneity of Pyridine Nucleotide-Dependent Aldehyde Reductase in Human and Rat Brain

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In brain aldehydes derived from biogenic amines are mainly reduced to the corresponding alcohols by an aldehyde reductase. The accumulating evidence for biol-

ogical activities of biogenic aldehydes and alcohols indicates that this enzyme may play a specific role in the central nervous system. In human brain aldehyde reductase occurs in four multiple forms. Two major fractions contain between 80 and 90 % of the total activity, one of them corresponding to the enzyme in bovine brain described by Tabakoff and Erwin (JBC *245*, 3263, 1970). The single forms were isolated by chromatography on DEAE-, CM-cellulose and Sephadex G-100. The purified enzymes differed with respect to coenzyme and substrate specificity with aliphatic and aromatic aldehydes as well as inhibition by barbiturates, butyraldoxime and *p*-chloro-mercuribenzoate. K_M values measured at saturating NADPH concentration ranged from $9 \cdot 10^{-6}$ to $2 \cdot 10^{-4} M$ for *p*-nitrobenzaldehyde. Identical pH-rate profiles were found for all enzymes. No activity was detected with NAD or NADP and alcohols. Disc electrophoresis showed the four enzymes to migrate differently, while their molecular weights are all about 70,000. In contrast to human brain only the two major multiple forms are found in rat brain.

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Glycogenolytic Action of Conversion Products of ^{3}H ATP and Cyclic AMP Injected into Living Mice

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Swiss albino mice were injected i.p. with 25 µc of 8-^{3}H 3′5′ cyclic AMP (0.003 or 3.5 mg) or adenosine ^{3}H-G-ATP (0.003 or 3.5 mg) and were sacrificed 10 or 30 min later. The 3′5′ cAMP, ATP and 5′AMP of livers and adipose tissues were isolated according to the method of Krishna. Their radioactivity and the glycogen concentrations were measured.

1. *^{3}H cyclic AMP injection:* Considerable radioactivity was found in the ATP + ADP fraction of both liver and adipose tissue in mice sacrificed 10 min after the injection. Only 22.6% of the radioactivity recovered in the three fractions were found in the cyclic AMP of liver (10% after 30 min). In adipose tissue, the corresponding values are 58% and 37%.

2. *^{3}H ATP injection:* In liver, the ratios of $\frac{\text{ATP+ADP}}{\text{cyclic AMP}}$ radioactivities were 15.8 after 10 min, and 7.3 after 30 min. A proportionally greater fraction of ^{3}H ATP was recovered into cyclic AMP of adipose tissue than into that of liver. The observed difference of behaviour of ^{3}H ATP in liver and adipose tissue excludes that the metabolization of ATP occurs only in the blood. The glycogenolytic effect observed in vivo after administration of cyclic AMP cannot be attributable to its degradation products. These observations are difficult to reconcile with an absolute impermeability of liver cells to cyclic AMP.

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Recherche sur le transport des précurseurs de la Gluconéogénèse et de la Synthèse des Acides Gras

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La gluconéogénèse, de même que la synthèse des acides gras dans les différentes fractions cellulaires de foies ou dans du tissu adipeux épididymaire ont été étudiées 5 ou 15 min après l'administration i.v. de divers précurseurs radioactifs, précédée ou non d'une injection i.p. de 10 mg d'amino-oxy-acétate (AOA), inhibiteur des transaminases. On constate que sous l'influence de l'AOA, la gluconéogénèse à partir de pyruvate 2-^{14}C ou de lactate 2-^{14}C s'abaisse d'environ 75%. L'AOA n'exerce aucun effet inhibiteur sur les incorporations d'acétate 1-, 2-^{14}C ou ^{3}H dans le glucose sanguin. L'incorporation du pyruvate 1-^{14}C est nettement moins diminuée que celle du pyruvate 2-^{14}C. La synthèse des acides gras augmente nettement dans le foie sous l'influence de l'AOA après administration de pyruvate 2-^{14}C, de lactate 2-^{14}C ou d'acétate 1- ou 2-^{14}C, mais l'AOA exerce un effet différent dans le tissu adipeux.

Ces résultats suggèrent que le malate est un meilleur transporteur hors des mitochondries des éléments à 4 C nécessaires à la gluconéogénèse que l'aspartate et que le citrate n'est pas l'unique transporteur d'acétyl-CoA pour la synthèse des acides gras. L'acétyl-aspartate est aussi proposé.

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Sérum de lapin anti-cellules thymo-dépendantes de souris

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L'immunisation de lapins avec des membranes de thymocytes de souris a permis d'obtenir un antisérum (AMTS) présentant une activité cytotoxique sur 100% des cellules thymiques et 40–50% des cellules spléniques de souris.

Afin d'étudier la spécificité de cet antisérum pour les lymphocytes T ou B, les cellules spléniques de souris C57BL/6 immunisées avec des cellules tumorales de souris DBA/2 ont été traitées par cet antisérum en présence de complément de lapin, puis testées in vitro selon la technique décrite par J.C. Cerottini et al. (J. exp. Med. *134*, 553, 1971). Les résultats obtenus ont indiqués que ce traitement supprimait l'activité des cellules T immunes sans diminuer la formation de plaques de lyse due aux cellules B immunes. L'analogie des propriétés de cet antisérum hétérologue avec celles d'un sérum isologue anti-θ, spécifique des cellules thymo-dérivées (A.E. Reiff et J.M.V. Allen, J. exp. Med. *120*, 413, 1964) a été confirmée par les observations suivantes: l'effet cytotoxique de l'AMTS sur les cellules lymphoïdes de la souris a) est inhibé par l'absorption avec du cerveau de souris; b) n'est pas affecté par l'absorption avec des cellules spléniques débarassées de cellules θ-positives.

Ces résultats indiquent un effet spécifique de l'antisérum hétérologue sur les cellules thymo-dépendantes de la souris.

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A New Acid Phosphatase (Exoplasmodial) from *Physarum polycephalum*

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Investigations on nucleolytic enzymes appearing in the broth *of Physarum polycephalum* cultures revealed a second ribonuclease, besides RNAse I discovered by Braun, and a new phosphomonoesterase. A purification procedure was worked out in order to supply all three enzymes in high yields and purity. The exoplasmodial acid phosphatase has a MW of 49,000 daltons, as established by equilibrium sedimentation, amino acid composition

and gel exclusion. Its isoelectric point is 4, as ascertained by gel electrofocusing. It is highly soluble in water, but extensive dilution impairs activity, suggesting a possible dissolution into subunits. Freezing inactivates, but glycerol or BSA afford some protection, more efficiently provided by non-ionic detergents. EDTA, other chelating agents or thiols have no effect on activity, but NaF strongly inhibits. Main features: Highly active on *p*-nitrophenyl phosphate, with a K_M of $2.5 \times 10^{-4} M$ established by a double regression best fit program on the hyperbolic Michaelis function, and by Eadie plots, this phosphatase is nonetheless just as active against 3'CMP, and somewhat less against other 3'-nucleotides, with a distinctive base specificity. 5'-nucleotides are dephosphorylated at half the rate of the 3' (or 2')-N, however, these substrates cannot qualify as 'activated esters' as compared with PNP. Compounds having a vic. hydroxyl on the alkyl moiety of the ester, when sterically favorable, are preferred substrates, perhaps due to cyclization by H-bonding. Citrate is a strong activator and significantly shifts the optimum of the pH profile curves from 4.5 for other buffers to below pH 3.0.

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Glutamic Acid Dehydrogenase of the Brain (Bovine): Preparation and Some Properties

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The preparation of a highly purified GDH from brain tissue has never been described. Methods have been worked out in our laboratory, using fresh bovine brain cortex, through steps involving either a prior isolation of the mitochondria of the nerve cells, or the preparation of an acetone powder of the cortex. The complete purification ends with a crystallization, following a combined application of ion exchange chromatography, gel sieving and salt fractionation, to yield thin, flexible crystals. Among the physical parameters established for this enzyme, an isoelectric point was measured at pH 4.9, which allows at pH 8.6 a higher mobility in agarose gels than the liver enzyme. The specificity of brain GDH toward substrates and co-enzymes, as established by in vitro reactions proceeding in the direction of deamination, is very narrow and restricted to L-Glu and L-Nval, among the 16 compounds investigated. However, both NADH and NADPH can act as co-enzymes, a property that specifies brain GDH as an E.C. 1.4.3 dehydrogenase. Studies of enzymatic activity versus pH for both substrates (reverse reaction) or 2-oxo-glutaric acid and similar compounds (forward reaction) provided interesting results proving that specificity is highly dependent of pH. For instance, the ionization of the amino-group of Glu is essential in the formation of an enzyme-substrate complex, a condition which strictly defines the pH range and pH optimum of the overall reaction.

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Reconstitution of Sucrase-Mediated Sugar Transport in Lipid Membranes

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The effect of the sucrase-isomaltase complex (SI) from rabbit small intestine on bilayer-lipid-membranes (BLM) was investigated. A crude lipid extract from hamster brush-border membranes or purified egg lecithin were used to draw BLM's as described by Läuger et al. (BBA *135*, 20, 1967). BLM's made in the presence of 100 µg/ml and 5 mM NaCl had a much larger *specific* permeability for glucose and fructose (^{14}C sucrose was used as a substrate). Using both ^{14}C and ^{3}H labelled compounds in 5 m*M* NaCl or KCl the following permeability coefficients P (cm²/sec) were measured: $P_{\text{Sucrose}} < 10^{-8}$ in the absence, 5×10^{-5} in the presence of SI, respectively. The permeating substrates were identified as glucose and fructose by paper chromatography and autoradiography. The presence of the SI did not significantly affect the P of other compounds, such as mannitol, free glucose or free fructose. Finally, SI produced a two-orders of magnitude decrease in the ohmic resistance of BLM's in 5 m*M* NaCl. The characteristics of the SI-mediated sugar transport in BLM's correspond to those of the disaccharidase-associated sugar transport system(s) reported by Crane et al. (Fed. Proc. *29*, 595, 1970) in the intact small intestine.

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On the Chemical Composition of Particle-Bound Aminopeptidase from Pig Kidney

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Particulate aminopeptidase contains a carbohydrate moiety accounting for about 22% of the molecule and consisting of glucosamine, galactose, mannose, fructose and sialic acid. The enzyme is free of phospholipids.

Upon prolonged incubation with glycosidic enzymes (sialidase, emulsine, α-mannosidase and β-N-acetylglucosaminidase), virtually all of the sialic acid, approximately 65% of the neutral sugars and 45% of the N-acetylglucosamine could be split off. The degraded aminopeptidase differs from the native enzyme by its electrophoretic mobility on disc gel and by its solubility properties. However, no differences were found with respect to catalytic activity and substrate specificity. Susceptibility towards inhibition by EDTA or *o*-phenanthroline was similar to that of the undegraded enzyme.

The high amount of carbohydrates present in the native enzyme had precluded an accurate determination of its tryptophan content. Corrected for carbohydrate interference by extrapolation, the amount of tryptophan could now be estimated at 60 ± 2 residues per mole as compared to a previously published value of 83.

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Oligomeric Forms of Acetylcholinesterase from *Electrophorus Electricus*

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According to Massoulié et al. (European J. Biochem. *11*, 441, 1969) acetylcholinesterase (EC 3.1.1.7) extracted from the electric eel contains 3 species A, C and D, differing in their sedimentation coefficients. A fourth species B is obtained by tryptic digestion or by autolysis in toluene. We found all 4 species A, B, C and D upon sucrose density gradient centrifugation of crude extracts of toluene-treated electroplax. The forms B, C and D could be purified by affinity chromatography, while form A was lost. Subsequent gel filtration on Sepharose 4B yielded 51% B, 26% C and 23% D with specific activities of

15,200, 14,300 and 13,300 IU/mg protein, respectively. The molecular weights calculated according to Martin and Ames (J. Biol. Chem. *236*, 1372, 1961) gave a ratio of B:C:D = 1:1.5:2 based on the sedimentation coefficients of 11.9, 15.6 and 18.6 S. Species C and D converted to the apparently more stable form B upon standing in 1 M NaCl for several weeks. These results complement a model of Leuzinger et al. (J. Mol. Biol. *40*, 217, 1969): Acetylcholinesterase has a dimeric protomer ($\alpha\beta$), which can aggregate to oligomers containing 2, 3, 4 and probably more protomers.

Impairment of Glucose Metabolism in Epididymal Adipose Tissue of Fat-Fed Rats

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By using specifically labelled glucose (20 m*M*) as a substrate in the presence of insulin (1 mU/ml), fluxes of glucose carbon through the major metabolic pathways in adipose tissue of rats fed either a fat-rich or a carbohydrate-rich diet was calculated. The impairment induced by fat diet resides mainly at the level of glycolytic pyruvate utilization. While fatty acid synthesis was reduced to 5% of the control, up to half of pyruvate carbon was derived into lactate. This can be related to the abnormally high value (20–25) of the ratio lactate: pyruvate in the medium. Lipogenesis in adipose tissue of fat-fed rats is not limited either by the maximal activities of lipogenic enzymes or by the supply of NADPH, as indicated by the considerable increase (150–200%) of total fatty acid synthesis induced by addition of acetate (20 m*M*) to glucose and insulin. Under these conditions, the ratio actate: pyruvate was still high. Then the cytoplasmic origin of reduced coenzymes used in oxidative phosphorylation to meet the ATP requirements for fatty acid synthesis is questioned.

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The Digestion of Colostral Bovine Immunoglobulins in Infants

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To study the eventual effectiveness of orally administrated heterologous immunoglobulins against enteric infectious, the intestinal passage of bovine colostral IgG_1 was studied in infants.

Ten healthy babies aged less than 3 weeks were fed with 2g/kg/day of lyophylized bovine colostral lactoserum containing 70% of IgG_1. Up to 20% of the administrated IgG_1 were found in the stool in undigested form. The average value in 4 infants was of the order of 13%. One gram wet stool contained between 5 and 70 mg of undigested IgG_1. By gel filtration on Sephadex G-200 and subsequent examination with the Mancini method undigested IgG_1 and fragments of the molecular weight of about 55,000 were found. These fragments were similar to Fab and Fc with respect to their electrophoretic mobility and immunological properies. When specific colostral anti-E. coli antibodies were given, the agglutinating titer in the stools rose parallel to the amount of undigested IgG_1 present.

PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

Interaction of Histamine, Imidazole Acetic Acid and Convulsants on Brain Stem Neurones

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Microelectrophoretic application of histamine and imidazole acetic acid (IAA) to spontaneously firing brain stem neurones of the cat markedly depressed their discharge rate (Hösli and Haas, Experientia *27*, 1311, 1971). The action of the convulsants bicuculline and strychnine on the depression by histamine and IAA was also investigated. Electrophoretically administered strychnine with currents sufficient to markedly reduce glycine inhibition was without influence on the actions of IAA or histamine. However, the ejection of bicuculline onto single neurones shifted the dose response curves of IAA and γ-aminobutyric acid (GABA) to the right without altering the responsiveness to glycine and histamine. These results suggest that IAA is acting on GABA-like receptors, and that histamine is acting on receptors distinct from those affected by GABA or glycine. Comparisons of the effects of histamine and cyclic 3′–5′ adenosine monophosphate revealed that both compounds have depressant actions on brain stem neurones.

E.G.A. on leave from Dept. Pharmacology, Univ. of Illinois, Chicago, Ill.

Conditions of Cyclohexylamine Formation in Rats Chronically Ingesting Cyclamate

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Male Wistar rats were given a long-term treatment of 500 mg/kg of sodium cyclamate (CYC) in the drinking water. For several months a mean of only 0.1% of the daily dose was excreted as the metabolite cyclohexylamine (CHA). After about 6 months CHA excretion began to increase and after 2 more months it reached a plateau with a mean of 40% in all of the tested rats. Removal of CYC was followed by a decline of excreted CHA to zero within 1 week. Subsequent single test doses of 100 mg CYC were again converted to CHA as before. However, test doses of CYC given 2 or more weeks after interruption of long-term treatment were no longer converted to CHA. The ability to form CHA was then only regained by another chronic treatment with CYC for 1–2 weeks. Under uninterrupted CYC treatment the CHA excretion decreased to near-zero values within a few days after adding neomycin to the drinking water. The ability to convert CYC was not regained within 16 weeks after removal of neomycin. ^{14}C-CYC injected parenterally (i.p.) into CYC converting rats led to a negligible excretion of ^{14}C-CHA. The results suggest that an interaction between CYC and

the intestinal flora is responsible for these unusual long-term effects on a drug metabolism reaction.

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Multi-Channel Telemetry of EEG and Other Parameters in the Rat

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A miniature 4-channel EEG-transmitter was used for recording spontaneous and evoked potentials from cortical and subcortical areas in the rat. The transmitter was plugged into a socket which was fixed to the animals' skull, and connected to 5 chronically implanted electrodes. Thus the transmitter could be easily removed for the exchange of batteries, or replaced with a dummy while experimentation was not in progress. Motor activity, water consumption and food intake were recorded continuously along with the EEG. Data reduction and analysis was a major problem in long-term experiments. The records illustrate changes of the electrophysiological and behavioral variables in entirely unrestrained rats before and after administration of neuropharmacological agents.

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Isolation of Antibacterial Basic Proteins from Granules of Chicken Polymorphonuclear Leukocytes

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Homogenates of chicken polymorphonuclear leukocytes (PMN) obtained from peritoneal exudates were separated on sucrose density gradients. By this means we isolated three different classes of membrane-bound particles differing in their morphological, biochemical and biophysical properties.

The largest class (1–3 μm in diameter), banding at a specific density of 1.23, comprised rod-shaped, electron dense granules which were associated with lysozyme and at least three other basic proteins. They formed the main cytoplasmic granule component of chicken PMN. The second class of granules, intermediate in size (0.3–0.8 μm) and specific density (1.19), consisted of two types of granules which showed only slight differences in their morphology but differed in their biochemical composition. The smallest class of granules (0.1–0.2 μm), banding at a specific density of approximately 1.17, comprised possibly two or more types of small, electron transparent granules and various membrane fragments.

Extracts of the largest class of granules inhibited the growth of *E. coli*, *Serratia marcescens* and *Staphylococcus albus*. They contained lysozyme and basic proteins.

Treatment of Hyperacute Graft-Versus-Host Disease in Mice with Cytosine Arabinoside

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A severe and rapidly fatal graft-versus-host disease was induced by the intravenous injection of CBA spleen cells in BALB/c mice primed with cyclophosphamide. The mortality could be markedly reduced by cytosine arabinoside. Methotrexate, procarbazine, cyclophosphamide and antilymphocyte serum were ineffective. The protection by cytosine arabinoside was seen at dosages which were ordinarily lethal (if tested for toxicity in conjunction with the priming dose of only cyclophosphamide, without the application of spleen cells). Thus, cytosine arabinoside prevents the injurious consequences of the allogeneic lymphoid cells whereas the cellular inoculum protects against the lethal effects of the therapy. A less severe from of graftversus-host disease was also partially precluded by cyclophosphamide. No mortality occured if the cell donors had been trated with procarbazine or antilymphocyte serum.

Disposition of Acetylcholine and Analogues in the Rat Uterus: Study by the Oil-Bath Technique

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Kalsner and Nickerson (Can. J. Physiol. Pharmacol. *46*, 719, 1968) have shown that aortic strips contracted by exposure to a pharmacological agent and then, immersed in oil, relaxed at a rate governed by the rate of disposition of the agent in the tissue. Using this technique we have exposed potassium-depolarised rat uterine strips to acetylcholine (ACh), acetyl-β-methylcholine (MCh), carbachol (C), and diazoacetylcholine (DCh) (Frank and Schwyzer, Experientia *26*, 1207, 1970) and recorded relaxation after replacement of the aqueous medium with oil. After submaximal contraction by ACh, relaxation was complete in 1–2 min; after equipotent MCh, it was 2–3 times slower; and after C or DCh no relaxation occurred for 1 h or more. When 1.5 μM physostigmine (P) was added 1 min before ACh, relaxation was 3–4 times slower than after ACh alone; 10 μM P prevented relaxation for 30 min or more.

It is concluded that the depolarised rat uterus is suitable for use with the oil-bath technique, and that the disposition of ACh in this tissue is due at least in part to the action of acetylcholine esterase.

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Membrane Potential and Contraction in Vascular Smooth Muscle

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Change of membrane potential as recorded by intracellular microelectrodes (MP) and contractile responses to KCl or noradrenaline (NA) were measured in vascular smooth muscle of rabbit pulmonary artery (VSM). Decrease in MP was linearly related to log concentration of external potassium in the range of 10–100 m*M* KCl with a slope of 43 mV for a tenfold change of $[K]_o$. 71% of the maximum contraction occurred in the MP range of 38–21 mV. In concentrations between 10^{-7} and $3 \times 10^{-7} M$, NA gradually reduced the MP from a mean of 59 mV to 45 mV without any further major depolarization when higher concentrations (up to $10^{-4} M$) were applied. Most of the NA-induced contraction occurred in the absence of MP changes. Maximum contraction was equal for KCl and NA. While in calcium-free solution contraction to KCl (160 m*M*) was small, that to NA in depolarized VSM reached 50% of the maximum. Omission of calcium from normal Tyrode solution caused a similar depolarization as

NA (10^{-6}–$10^{-4}M$). The results can be explained on the assumption that NA releases calcium from the cell membrane and that part of this membrane-bound calcium controls sodium permeability in VSM.

Investigations on the Mode of Action of Ergotamine in the Isolated Femoral Vein of the Dog

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The effects of Ergotamine (E) were investigated in isolated femoral vein strips of the dog by the cumulative dose-response technique (van Rossum, Arch. int. Pharmacodyn. *143*, 299, 1963) according to the following parameters: intrinsic stimulating activity, affinity to receptor sites as compared with noradrenaline (NA), competitive and noncompetitive components of NA-antagonism.

The affinity of E to the smooth muscle stimulating receptors was 355 times higher than that of NA (calculated from the pD_2-values). The intrinsic stimulating activity was found to be about 35% of the maximal NA-effect. The competitive NA-antagonism was characterized by a pA_2-value of 9.02 and the non-competitive NA-antagonism by a pD'_2-value of 7.57. Since the stimulating activity of E was antagonized by pretreatment with α-blockers, E could be characterized as an α-blocking drug with considerable intrinsic α-stimulating activity.

Muscarinic and α-Adrenergic Blockade of Cyclic AMP Increase in Stimulated Ganglia

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Previous experiments showed that the cyclic AMP content of isolated rabbit superior cervical ganglia increased severalfold after stimulation of the preganglionic nerve trunk. The cyclic AMP level did not change in ganglia that were stimulated antidromically or in stimulated cervical vagus or nodose ganglia. Thus, these observations indicated that this increased level of cyclic AMP was associated with synaptic activity. In subsequent experiments, it was found that the cyclic AMP response was largely inhibited by low doses of atropine but not of hexamethonium, suggesting that a muscarinic receptor is involved. Furthermore, a marked inhibition was found with the α-blocker, phentolamine, at low concentration, while high doses of the β-blocker MJ 1999 were ineffective. These results indicate that ACh released during activity stimulates the muscarinic sites of interneurons which secrete a catecholamine, probably dopamine; dopamine may then cause the cyclic AMP increase in the ganglion cells with concomitant hyperpolarization (S-IPSP).

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Effect of Vincristine Sulfate (VCR) on Blood Platelets

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Clinical experience showed that VCR affected platelets less than other oncolytic agents. Moreover, VCR sometimes caused marked thrombocytosis. The mechanism of this unusual side-effect is not known. Rabbit platelets incubated with VCR showed swelling at 0.83 mcg/ml and released platelet factor (PF) 4-like activity at 8.3 mcg/ml. Platelet swelling also occurred 10 min after 0.25–0.5 mg/kg i.v. in rabbits. After 30–60 min the plasma contained many small particles (platelet fragments?). Platelet functions (ADP-induced aggregation, clot retraction) were impaired. The small particles were removed 24 h after VCR. In guinea pigs PF3- and PF4-like activities were demonstrated in plasma 30–90 min after 0.5 mg/ kg i.v. The experiments showed that VCR caused platelet damage followed by platelet loss. Since VCR has little effect on megakaryocyte maturation (Morse and Stohlman, J. Clin. Invest. *45*, 1241, 1966), the system was able to respond swiftly to thrombopoietin stimulation. Thrombocytosis might be explained as overreaction to this stimulus.

The Effects of Ergotamine and Dihydroergotamine on Skin and Skeletal Muscle Vasculature

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Dihydroergotamine (DHE) was shown to cause dose-dependent constriction of skeletal muscle capacitance vessels with no significant simultaneous constriction of resistance vessels (Mellander and Nordenfelt, Clin. Sci *39*, 183, 1970; Owen and Stürmer, Br. J. Pharmac. *42*, 655P, 1971). As an extention of these observations, the effects of Ergotamine (E) have been compared with those of DHE on denervated skin (Arturson and Mellander, Acta physiol. scand. *62*, 457, 1964) and skeletal muscle (Mellander, Angiologica. *3*, 77, 1966) vascular bed preparations.

On the skeletal muscle vasculature E was as inactive as DHE on the resistance vessels, but about 10 times more potent than DHE in constricting the capacitance vessels. On the skin vasculature, however, E constricted both resistance and capacitance vessels, whereas DHE constricted only the capacitance vessels.

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

Controlled, Stepwise Synthesis of RNA by Qβ Replicase

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A method has been developed permitting stepwise, substrate-limited synthesis of viral RNA in vitro. Its use is illustrated by the synthesis of a Qβ minus strand segment, 71 nucleotides long, in 4 discrete elongation steps, and elucidation of the hitherto unknown nucleotide sequence between positions 52 and 71. Qβ replicase, Qβ RNA, host factor, GTP, ATP and CTP were incubated under conditons leading to initiation and elongation up to position 23 where U was required for further elongation (cf. Goodman et al., Proc. Nat. Acad. Sci. *67*, 921, 1970). The replication complex was filtered through Sephadex to remove the substrates. Polyethylenesulfonate was then

added to prevent initiation by free enzyme and incubation was carried out with UTP, CTP and ATP, to allow elongation to position 37, where G is required. In a third (GTP, ATP and UTP) and fourth (CTP, ATP and UTP) elongation step synthesis proceded up to positions 62 and 71, respectively. The segments of unknown sequence were synthesized using one α-^{32}P-labelled and two nonradioactive substrates at a time. Analysis was carried out as in Billeter et al. (Nature *224*, 1083, 1969), yielding the

52 62 71
sequence...(G) AUAAUUUAUUCACAAUUA (G).

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Ultrastructural Localization of Intracellular Antigen by the IgG Bridge Method

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The immunoglobulin-enzyme bridge method (T.E. Mason et al., J. Histochem. Cytochem. *17*, 563, 1969) was used on a model previously described (J.P. Kraehenbuhl et al.: J. Cell Biol. *50*, 432, 1971). A recognizable antigen, ferritin, was absorbed into the jejunal epithelium of piglets, or deposited in the glomerulus of rats after i.v. injection. The ultrastructural localization of ferritin was studied on nonfrozen sections of tissues fixed in 0.125% glutaraldehyde, treated with thiosemicarbazide (J.P. Kraehenbuhl, personal communication) and incubated with the following reagents: 1. papain-digested rabbit anti-ferritin serum; 2. sheep IgG anti-rabbit Fab; 3. rabbit IgG or Fab anti-peroxidase; 4. peroxidase, revealed histochemically. As controls, papain-digested nonspecific rabbit serum was used for steps 1. and 3. Specific labeling of ferritin by peroxidase reaction product was observed in most cases if ferritin was present in large amounts; isolated molecules were frequently nonlabeled. Nonspecific labeling was unimportant if rabbit Fab anti-peroxidase was used for step 3.

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Regulation of λdv Plasmids

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Plasmids are stable genetic elements of bacteria which replicate in approximate synchrony with the bacterial chromosome, although physically autonomous of it. Bacteriophage λ mutants, that form plasmids in *E. coli*, have been selected. The class designated λdv is a deletion of phage λ in which more than $^4/_5$ of the phage genome has been lost, and only the immunity region and DNA replication genes remain. λdv plasmids are small covalently closed circular molecules, and are present in multiple (25 to 100) copies per cell.

Cells carrying λdv inhibit the growth of superinfecting *λvir* (*λvir* is a mutant phage which overcomes the immunity of an integrated λ^+ prophage.). The inhibited *λvir* genomes are diverted to a plasmid state in cells carrying λdv. Direct construction of λdv plasmids from phage defective in gene *cI*, and radioimmune measurements of *cI* repressor levels demonstrate that λdv inhibition of λ phage growth is not dependent on the λ repressor. The regulatory properties of λdv suggest that a bacterial component (such as a replication-transcription site) absolutely required by λ for growth may be titrated by the multiple copies of λdv.

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Nucleotide Sequence at the Binding Site on R-17 RNA for Coat Protein

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The RNA and the coat protein isolated from the bacteriophage R-17 can interact to form two types of complexes: complex I in which from one (Spahr, Farber and Gesteland, Nature *222*, 455, 1969) to six (Sugiyama, Herbert and Hartman, J. Mol. Biol. *25*, 455, 1967) molecules of coat protein are bound to one molecule of RNA, and complex II which contains about 180 molecules of coat protein per molecule of RNA. Complex I has been implicated in the control of translation since it directs the in vitro synthesis of the cistron B product (coat protein) but not that of the cistron C product (RNA synthetase) indicating that coat protein acts as a repressor of the translation of cistron C. Using highly labelled native ^{32}P RNA and unlabelled coat protein to form complex I and digesting with T_1 RNase, a fragment retained on millipore filters has been isolated. Data on the nucleotide sequence of this fragment reveal that it contains the punctuation signal between cistrons B and C, suggesting that this is the site on the RNA where the coat protein acts as a repressor.

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Demonstration of a Contractile Actomyosin-Like Protein in the Pillar Cells of Fish Gills

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The secondary lamellae of fish gills consist of epithelial sheets. The blood flows between the two sheets which are held together by characteristic pillar cells. EM studies revealed the presence in these cells of filaments arranged in compact bundles. The possibility that such filaments might represent a contractile mechanism of physiological importance has been suggested (G.M. Hughes and E.R. Weibel, J. Ultrastruct. Res,. in press 1972).

In order to test this hypothesis, tips of rainbow trout gills were extracted in a glycerine-containing buffer, then incubated under conditions favoring the superprecipitation by ATP of actomyosin-like proteins. Fixed and sectioned samples displayed the characteristic structures of actomyosin-like superprecipitates in the pillar cells. This was clearly recognizable by the presence of spindle-shaped needles as was described for thrombosthenin from blood platelets (M. Bettex-Galland, E.F. Lüscher and E.R. Weibel, Thromb. Diath. haemorrh. *22*, 431, 1969).

It is concluded that pillar cells contain a contractile mechanism which will be discussed in relation to control of blood flow through the fish gills.

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Effects of Cytochalasin B on Chemotaxis and Immune Reactions

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Cytochalasin B (CyB), known to inhibit a number of cell functions which are connected to surface motility, has variable effects on microfilament structures. The present study shows that the chemotactic migration of rabbit neutrophils is reversibly inhibited after addition of CyB (1 μg/ml) to the cell suspension; P-815 mastocytoma cells assume spherical shape and lose their pseudopodia. The phagocytosis of latex beads by peritoneal rat neutrophils is also negatively affected by CyB. Furthermore, CyB exerts some influence on the immune reaction. The cytotoxicity of sensitized mouse spleen lymphocytes (as measured in vitro by the target cell destruction test) is reversibly suppressed in the presence of CyB. The i.p. application of CyB to mice shortly before immunization with erythrocytes produces reduction of the number of antibody forming cells (PFC). CyB also inhibits cell multiplication in vitro and, in some models, in vivo.

CyB is also known as Phomin (W. Rothweiler and Ch. Tamm, Experientia *22*, 750, 1966).

Localisation ultrastructurale des sites de synthèse de DNA dans des cellules de Mammifères

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La localisation des sites de synthèse du DNA est étudiée dans des cellules de souris en culture (mastocytome Px-815). Les cellules en phase de croissance exponentielle sont marquées pendant 30 secondes par la thymidine tritiée. Elles sont soit immédiatement fixées, soit fixées après une heure d'incubation en présence de thymidine non-marquée en excédent. L'examen autoradiographique à haute résolution montre que la radioactivité incorporée intéresse tout le noyau. Toutefois, le rapport du nombre de grains par unité de surface est plus élevé dans la région périphérique du noyau que dans la région intérieure de celui-ci, avec cependant, plus de 50% du nombre total des grains au niveau de cette dernière.

Après une heure d'incubation en présence de thymidine non-marquée, ce rapport n'est pas sensiblement mofidié.

La densité plus élevée des grains dans la région périphérique parait indiquer un plus grand nombre de sites de réplication dans cette région, qui peut être interprétée comme étant liée à une concentration plus élevée de DNA.

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Isolation of Specific Polysomes by Affinity Chromatography and their Recovery with Puromycin

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A general method is described to isolate a specific type of polysome from a heterologous mixture, based on affinity chromatography. The isolation uses antibody (Ab) covalently coupled to agarose and directed against the polysomal-synthesized specific proteins, thus occurring via the nascent polypeptide chain still attached to the peptidyl-tRNA. Only immunochemically-competent peptides react with the Ab. After calibration and standardization of the immunochemical reactivity with radiolabeled kappa chain immunoglobulin, the column is tested with a ^{32}P-labeled polysome fraction from murine kappa chain producing myelomas, MOPC-70E and 41.

The specifically absorbed polysomes may be recovered upon treatment with moderate salt and puromycin. Some of the ribonucleoprotein released has polysomal character according to sucrose gradient sedimentation, indicating the presence of mRNA. The column may then be regenerated for further use with a brief treatment of high salt.

The evidence that these polysomes are kappa chain specific comes indirectly through the use of controls to quantitate specific and nonspecific interactions. These data indicate approximately a ten-fold enrichment of specific polysomes, when compared with these controls.

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Cooperation of Antibodies in the Selective Lysis of B-Lymphoid Cells

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B-lymphoid cells, in contrast to the T-cells, cannot selectively be killed by specific antibodies (Ab) and complement. Anti-IgG Ab, which are considered specific for B-cells, were not cytotoxic. The same observation was made with heterophile anti-Forssman (Fo) Ab, despite the presence of Fo hapten on mouse lymphoid cells. However, a mixture of rabbit anti-Fo and anti-IgG Ab was able to kill 32% spleen and 18% mouse lymph node cells, but no bone marrow or thymus cells. The cytotoxic titration curve of anti-IgG Ab in the presence of anti-Fo Ab showed differences between the surface IgG of spleen and lymph node B-cells. Saturation and a titer of 1:1260 were measured with spleen cells. A titer of 1:40 and no saturation were observed with lymph node cells. The titration of anti-Fo in excess anti-IgG Ab showed that anti-Fo Ab were limiting for the cytotoxicity of spleen, but not of lymph node cells. This also suggested surface membrane differences with respect to Fo hapten density on both cell types.

Assay of cAMP Using a cAMP Binding Protein Coupled to Sepharose

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Partially purified cAMP binding protein from adrenals was covalently attached to Sepharose 2B with the cyanogen bromide method. The resulting product retains its binding properties unchanged for several months. Equilibrium with cold and tritiated cAMP is established within one hour without shaking and the insolubilized binding-protein-cAMP complex can be isolated by filtration over a 35 μm polyester net (filtration and washing takes about 20 sec). Both bound and free ligand can be determined; neither Sepharose nor polyester net interfere with scintillation counting. The compound has been used for assay of picomole and subpicomole amounts of cAMP in biological samples. The results compare well with those

of parallel immunological assays (Steiner, PNAS *64*, 367, 1969). pH and ionic strength influences of K_{ass}, maximal binding capacity and the Hill coefficient are discussed.

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Plaquettes et muscle lisse: analogies de structure et de fonction

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Il est bien établi que les plaquettes contiennent un système contractile (thrombosthénine) biochimiquement semblable à celui du muscle et responsable de la contraction du caillot. Par conséquent, des caillots en forme de ruban, placés dans un bain et suspendus au levier d'un kymographe, devraient pouvoir répondre à quelques-uns des agents pharmacologiques actifs sur la musculature lisse. Nous avons testé cette hypothèse; il s'avère en effet que pendant la contraction spontanée le caillot se relâche sous l'effet de la papavérine, la théophylline et la cytochalasine B. Lorsque ces substances sont enlevées par lavage, la contraction reprend. Cela suggère qu'il devrait être possible de mettre en évidence les plaquettes par immunonfluorescence, avec un sérum anti-muscle lisse (selon une technique que nous avons utilisée récemment pour démontrer la ressemblance entre myofibroblastes et musculature lisse: J.exp. Med., avril 1972). En employant du sérum humain contenant des anticorps anti-muscle lisse (sérum obtenu chez un patient souffrant d'hépatite chronique) nous avons trouvé qu'il est possible, chez le rat, de marquer les plaquettes sanguines, soit isolées par centrifugation, soit incorporées à un thrombus formé in vivo.

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Reversible Dissociation of Avian Globin Synthesizing Polyribosomes

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Duck globin synthesizing polyribosomes can be completely dissociated into subunits by treatment with 0.02–0.05 μmoles EDTA/A_{260} unit of polyribosomes as shown by glycerol gradient analysis. Under these conditions the dissociation is reversible after restoration of the original Mg^{++} concentration when a polyribosomal pattern can again be observed. These reformed polyribosomes have retained their ability to translate the endogenous mRNA in a cell-free system and the analysis of the labelled product on a CM cellulose column revealed the presence of the three duck globin chains. Furthermore, it was shown by dansylation of the NH_2-terminal valine that the reconstituted polyribosomes were more active in initiation than untreated control polyribosomes.

By incubating cells with ^{3}H-uridine for 40 min it was also possible to study the behaviour of the rapidly labelled polyribosomal mRNA following EDTA treatment and during subsequent reconstitution.

Although after EDTA treatment a heterodisperse radioactivity profile is found on glycerol gradients, CsCl-density gradient analysis indicated that the mRNA is released as mRNP particle which bands at a density of 1.40–1.47 g/cm^3 and is not bound to any subunit.

Translation Re-Initiation in the i-Gene of the Lac Operon of *E. coli*

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The properties of mutants containing nonsense mutations in the i-gene of the Lac Operon in *E. coli* were studied. Several amber mutations mapping in the early part of the gene were shown to generate fragments of the i-gene product due to reinitiation of polypeptide synthesis after chain termination at the amber site. One of these fragments was isolated and purified. The sequence of the amino terminal end of the reinitiation fragment was ala-glu-leu-asn. The sequence of the wild-type i-gene product around the first internal methionine was shown to be ala-ala-met-ala-glu-leu-asn. This work demonstrates that the first internal AUG in the i-gene message is an efficient initiation signal in vivo if chain termination occurs nearby. Experiments to locate the precise position of the amber mutation in this mutant are in progress.

Sites of Chromosomal DNA Synthesis in a Eukaryotic Cell; Biochemical Studies

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We have studied the location of newly-replicated DNA within nuclei of exponentially-growing mouse cells (line P815), using a labelling time of 30 seconds to minimise displacement of newly-made DNA from its replication site. We find that: 1. The DNA which remains associated with nuclear membranes purified by density-equilibrium centrifugation is not enriched in newly-replicated DNA. 2. Newly-replicated DNA is present in a fraction of the chromatin which, from its density, probably contains some extra proteins, possibly those concerned with DNA replication. This material does not contain detectable phospholipids. 3. 'Replication complexes' of newly-replicated DNA (e.g. Friedman & Mueller, Biochem. Biophys. Acta *174*, 253, 1969) in cells treated with detergents are often artefacts due to non-specific binding of newly-replicated, partially single-stranded DNA to detergent-denatured proteins. From these results, together with studies by high-resolution autoradiography (Fakan & Hancock, these Abstracts), we conclude that DNA replication in eukaryotic cells takes place at sites throughout the nucleus and is not associated with the nuclear membrane.

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DNS-Synthese in Zellkulturen aus regenerierender Rattenleber

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Mittels einer Modifikation der Methode von Berry und Friend (Perfusion der Leber mit einer Collagenase-Hyaluronidase-Lösung sowie einer Versen-Lösung, J. Cell Biol *43*, 506, 1969) wurden 20 h nach partieller Hepatektomie erwachsener Ratten Leberzellsuspensionen hergestellt. Die Zellen wurden in einer halbsynthetischen Nährlösung suspendiert und bei 37° inkubiert. In 12 Versuchen fanden sich zu Beginn der Inkubation im Mittel $56 \pm 3\%$ lebende (d.h. trypanblau-negative) Zellen.

Die mittlere Zahl überlebender Zellen betrug nach 1 h 94±10%, nach 5 h 78±4% und nach 21 h (7 Versuche) 55±14% des Inokulums. Zu verschiedenen Zeiten nach Ansetzen der Kulturen wurde ^{3}H-Thymidin zugesetzt, und 1 bzw. 5 h später wurden die Zellen fixiert und zur Herstellung von Autoradiogrammen verwendet. Im Mittel von 12 Versuchen ergab eine Inkubation mit ^{3}H-Thymidin während der 1. Stunde nach Ansetzen der Kulturen einen Markierungsindex der Hepatocyten von 17± 4%, in der 5. Stunde 22±3%, während der ersten 5 Stunden 30±2%, in der 10. Stunde (3 Versuche) 25±5% und in der 21. Stunde (7 Versuche) 3±1%.

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Evidence for the Generation of Free Minus Strands on Multi-Stranded RNA with R17

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In vitro studies provided evidence for a mechanism of RNA replication in which viral plus strands are generated on minus strands. Such complexes can be obtained, apparently in collapsed form, as multi-stranded (MS) RNA from infected cells. Are minus strands generated similarly? If so, MS-RNA should be detectable which contains nascent minus-stranded tails. The latter should be annealable to viral plus strands.

In vivo evidence for the occurence of such a structure was obtained: 1. Viral RNA annealed to preparations of MS-RNA isolated from infected cells. This reaction was strain-specific: Homologous RNA competed with the reaction, heterologous phage RNA did not. 2. Up to 4% of the MS-RNA annealed viral plus strands. The annealing efficiency was highest with MS-RNA thought to be less rich in single strands. 3. Pre-digestion with RNase removed the capacity of MS-RNA to anneal plus strands.

The hospitality of the PHRI (NYC) is acknowledged as is support by H. Noll (grants from USPHS and ACS).

Stimulierung der Lipolyse in isolierten Fettzellen unabhängig von cAMP?

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Werden isolierte Fettzellen mit ACTH stimuliert, so beobachtet man in 3–5 Minuten einen Anstieg des cAMP-Spiegels auf etwa das Doppelte und hernach ein rasches Absinken bis fast zur Norm der unstimulierten Zellen. Im Gegensatz dazu steigt die Lipolysegeschwindigkeit schnell an und bleibt während etwa 1 Stunde erhöht. Um festzustellen, ob der Anstieg an cAMP eine de novo Synthese von lipolytischen Enzymen induziert, hat man die Hemmung der Proteinsynthese mit Actinomycin versucht. Überraschenderweise wird die Wirkung des ACTH auf die Lipolyse nicht blockiert, sondern gesteigert und verlängert, während der kurzfristige Anstieg des cAMP vollkommen unterdrückt wird. Bei der Stimulation mit Adrenalin ist die Wirkung dieses Antibiotikums viel geringer, und auf die Glukagon-stimulierten Zellen hat es praktisch keinen Effekt. Ebenfalls unbeeinflusst bleibt die Lipolyse der dcAMP-stimulierten Zellen (dcAMP = dibutyryl-cyclisch-Adenosin-Mono-Phosphat). Es scheint deshalb, als ob das Actinomycin einen Mechanismus beeinflusst, der im Reaktionsgeschehen zwischen der Erkennung des Hormons und der Umwandlung von ATP zu cAMP liegt, oder aber eine andere (direkte?) Wirkung des ACTH auf die Lipolyse vermittelt.

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Ontogeny of Fructose Diphosphate Aldolase B in the Chick

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Three homologous fructose-1,6-diphosphate aldolases (A, B, and C) are found in vertebrate systems. In addition, hybrid molecules formed by the combination of parental subunits into tetramers can be produced in vitro and are observed in tissues in which two of the parental subunits are synthezised. Although five membered A-B, A-C, and B-C hybrid sets are readily produced in vitro, only A-B and A-C sets have previously been detected in vertebrate tissues. The present studies demonstrate the presence of B-C hybrid molecules in vivo. B-C hybrids were found in early embryonic chick liver (5± to 8± days) and in yolk sac membranes. The electrophoretic profiles of embryonic chick liver suggest that a transition in synthesis from aldolase C to predominantly aldolase B subunits takes place in liver cells, presumably parenchymal, during early development. The timing of the C to B transition is consistent with the proposed role of aldolase B in gluconeogenesis. The present studies are discussed in relation to other aldolase transitions associated with vertebrate tissue embryogenesis and with certain states of disease.

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An Ultrastructural Study of Pancreatic Exocrine Cells in Monolayer Culture

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Pancreatic cells derived from neonatal rats were grown in monolayer culture for up to 19 days. Endocrine B (insulin producing) and A (glucagon producing) cells maintained their differentiated ultrastructure and physiological competence at all times during their growth in culture. In contrast, differentiated exocrine cells were absent after 2½ days of culture. Reduced in number by cell necrosis in the initial hours of culture, the surviving exocrine cells underwent progressive dedifferentiation giving rise to a population of almost uniformly degranulated epithelioid cells. These modified exocrine cells were rich in free ribosomes and poor in rough endoplasmic reticulum, therefore devoid of the major organelle involved in production of export protein. In addition, these cells possess large numbers of 50–80 Å microfilaments, numerous microtubules, vesicles and bundles of coarse filaments resembling tonofilaments. Clearly distinguishable from the endocrine cells and fibroblasts, this population of dedifferentiated exocrine cells is proposed as a potentially valuable model for studying mechanisms modulating pancreatic exocrine cell differentiation and function and the possible neo-formation of endocrine cells.

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Formation asymétrique des fragments d'Okazaki chez *E. coli* K 12

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Lorsque des bactéries sont marquées par de la thymidine tritiée pendant un temps bref, une partie du marquage est incorporée dans du DNA de faible poids moléculaire, généralement appelé «fragments d'Okazaki».

Nous avons pu montrer que ces fragments, pour un marqueur génétique donné, ne sont complémentaires que de l'un seulement des deux brins du chromosome. Dans une souche lysogène pour le phage lambda, les fragments d'Okazaki spécifiques du prophage sont complémentaires du brin l du DNA phagique et non du brin *r*: ils seraient formés sur le brin se terminant en 5'P, compte tenu de l'orientation moléculaire du génome phagique à l'état intégré et du mode de replication du chromosome d'*E. coli*.

Les fragments d'Okazaki étant vraisemblablement des intermédiaires dans la replication du DNA, ce résultat est compatible avec un mode de replication continu-discontinu.

Protection of Portions of the 16S Ribosomal RNA of *Escherichia coli* by two Ribosomal Proteins

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We have investigated the structure of regions of the 16S rRNA molecule protected against ribonuclease A digestion in the presence of specific ribosomal proteins. The complex between 16S RNA and the protein in question was treated with ribonuclease A, and the products separated by electrophoresis on polyacrylamide gels in presence of Mg^{++}. Under these conditions, only two of the five ribosomal proteins which bind directly to 16S RNA protect a portion of the RNA molecule. Protein S4 binds to a fragment having a sedimentation coefficient of 7S; protein S15, to one of 5S. Using ^{32}P-labeled 16S RNA and two dimensional electrophoretic techniques, we have studied the oligonucleotide composition of these fragments and have located their position within the molecule. The results imply that the binding sites of ribosomal proteins are complex, and depend on a highly ordered secondary structure within the RNA molecule.

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Studies on Mouse Lymphocytes and their Cell Membrane Antigens

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Antisera can be raised in rabbits which, after proper absorption, contain antibodies binding selectively to certain lymphocytes depending whether they are thymus derived or not. These lymphocytes are characterized by different membrane antigens which can be called B (or 'MSBA') and T (or 'MSLA') antigen. Using anti-B and anti-T antibodies bound to tracers (viral particles or peroxidase), it is possible to follow the ultrastructural pattern of differentiation of B and T cells into various cell types. In addition some mouse lymphocytes bear surface IgG (sIgG). Using double labelling methods it can be shown that sIgG are restricted to B cells but that not all B cells bear sIgG. Incubation of lymphocytes with anti-IgG or anti-B antibodies leads to surface modification, rapid pinocytosis of antibodies and partial or complete disappearance of sIgG and B antigen; this process is partially sensitive to cytochalasin B. Although the surface changes affect both sIgG and B antigen, B antigen reappears much faster than sIgG indicating that the two membrane antigens have a different turnover and/or structural relationship to the membranes.

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Les cellules corticotropes de l'hypophyse du Crapaud *Bufo bufo* L.

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L'interrénalectomie bilatérale par thermocautérisation a été pratiquée sur des crapauds adultes afin d'identifier les cellules corticotropes (ACTH) de l'hypophyse.

Les résultats observés au microscope électronique suggèrent que l'ACTH est produite par des cellules glycoprotidiques à grains de sécrétion fins, de forme assez hétérogène. Ces cellules semblent différentes des cellules glycoprotidiques de type II caractérisées par des granules plus fins, de forme plus homogène et auquelles il a été attribué, suivant les auteurs, soit la fonction thyréotrope (TSH) soit la fonction corticotrope (ACTH). Ces deux types cellulaires sont souvent côte à côte le long des capillaires de la zone antérieure de la pars distalis et jusqu'à maintenant ils n'avaient pas été différenciés l'un de l'autre. La réponse hypophysaire à la suppression des corticoïdes est très rapide. Déjà 24 à 48 heures après l'opération on note des modifications nettes au niveau des cellules à grains hétérogènes: dégranulation massive, extension de l'ergastoplasme et de l'appareil de Golgi. En 4 jours une partie d'entre elles sont transformées en volumineuses cellules chromophobes.

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High Resolution Autoradiographic Localization of DNA in Fixed Tissue Sections Incubated with Terminal Deoxynucleotidyl Transferase

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Calf thymus terminal deoxynucleotidyl transferase treatment of fixed tissue sections results in the incorporation of ^{3}H-dAMP by end-addition synthesis of poly dA using free 3'-OH and of DNA in situ (Modak & Bollum, Exper. Cell Res. *62*, 421, 1970).

Ultrathin sections of fixed tissues were incubated with this enzyme and ^{3}H-dATP and the incorporation was detected and localized by electron microscope autoradiography using the gold latensification Elon ascorbic acid procedure. Incorporated precursor was found to be TCA-insoluble and DNase-sensitive, and its localization indicated sites containing DNA or polydeoxynucleotides.

With this method, we detected DNA in the nucleus, nucleolus, mitotic chromosomes, mitochondria, the endoplasmic reticulum and the basal bodies of cilia.

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Model for a Transcriptional Control in Terminal Lens Cell Differentiation

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The process of cell differentiation involves a progressive acquisition of specialized structures and/or functions. These events are seen particularly clearly in terminally differentiating lens fiber cells. In this system, the sequence of molecular events has been studied in detail and it includes: 1. cessation of DNA synthesis and cell division, 2. activation of RNA synthesis, 3. synthesis of lens-specific proteins, 4. appearance and accumulation of strand-breaks in the DNA, 5. decrease and stoppage of RNA synthesis and 6. the nuclear degeneration, DNA loss and disappearance. An inverse relationship is found between the RNA synthetic activity and the physical integrity of DNA. A model is proposed which attributes a controlling role to the DNA-repair function in maintenance of the physical integrity of the genome. The loss of repair function would result in an accumulation of damage to DNA and thus effect the in vivo transcriptional events. The model is also valid for the aging of differentiated tissues.

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Further Studies on Duck Globin Messenger Ribonucleoprotein Complex (mRNP)

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Globin mRNP can be isolated in large amounts from duck immature red blood cells (Morel, Kayibanda and Scherrer, FEBS Letters *18*, 84, 1971). It contains 2 main proteins of MW 73,000 and 49,000 daltons and its RNA has biological activity assayed either by *Xenopus* oocytes microinjection (Lane, Curdon, Morel and Scherrer, in prep.) or on a protein-synthesizing cell-free system from rabbit reticulocytes (Stewart, Gander and Scherrer, in prep.). We have further characterized this mRNP in two ways: 1. Behaviour under increasing salt concentrations: this was done by centrifuging labelled 9S mRNA together with unlabelled mRNA (15S) on sucrose gradients at different ionic strengths. Although in control gradients containing SDS a complete dissociation in RNA and protein was observed, this could not be obtained with salt concentrations up to 0.75 *M* LiCl. In contrast, artificial RNP complexes are 80% dissociated at 0.1 *M* NaCl, and completely dissociated at 0.5 *M* NaCl (Baltimore and Huang, J. Mol. Biol. *47*, 263, 1970). 2. Particle gel electrophoresis on exponential polyacrylamide gels: when purified mRNP was subjected to electrophoresis on 2.5–20% gels (buffer: TEA 25 m*M* pH 8.0 – EDTA 1 m*M*), 3 components were resolved (named alpha, beta and gamma in order of increasing mobility). Components alpha (the major one) and gamma are RNP particles, and component beta is free protein, as judged by staining characteristics. No free RNA component has been observed. These individual components are now under investigation.

Binding of 30S Ribosomal Proteins to Fragments of the 16S Ribosomal RNA

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Interaction of 30S ribosomal subunit proteins of *Escherichia coli* with RNA fragments arising from limited ribonuclease hydrolysis of 16S ribosomal RNA has been studied. Among six proteins known to interact directly with 16S RNA, proteins S4, S8, S12, S20 and S13 bind with a 12S fragment (900 nucleotides) which covers the entire 5′-terminal half of the 16S molecule. An 8S fragment (5–600 nucleotides), derived from 3′-terminal half of the molecule seems to contain the binding site for protein S7. A 9S fragment (500 nucleotides) which lies at the 5′-terminus of 16S RNA exclusively binds protein S4. Protein S15 interacts specifically with a fragment of about 135 nucleotides (4S) obtained by further ribonuclease hydrolysis of the 12S fragment, The distribution of ribosomal protein binding sites on the 16S RNA will be discussed.

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Fenestration of the Rough Endoplasmic Reticulum as Seen with Freeze-Etching

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Early electron microscopic studies have shown that the rough endoplasmic reticulum (RER) is a network of cisternae interconnected by canalicular bridges and probably is fenestrated (Palade, G. E. J. Biophys. Biochem. Cytol., suppl. *2*, 85, 1956). Since evidence for fenestration is not always convincing in thin-sectioned material, the RER membranes of exocrine pancreatic cells were studied with the freeze-etching technique. Large areas of intracellular membranes are exposed in the exocrine cell which correspond in arrangement and location with the RER cisternae seen in thin sections. The fenestrae of exposed RER membranes are revealed by the presence of numerous circular depressions, about 700 Å in a diameter. These closely resemble the nuclear pores and suitably thin sections indeed demonstrate the presence of diaphragms closing the RER fenestrae. The extension and the functional implication of the fenestration is as yet unknown; we suggest that it might contribute to the compartmentalization of the intracisternal spaces.

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AMV-RNA-Directed DNA Polymerase: Chemical Evidence for the Covalent Linkage of Product to Primer RNA

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AMV specific DNA formed by both crude and purified RNA-directed DNA polymerase isolated from Avian myeloblastosis virus appears linked to an RNA primer by the following evidence. The buoyant density of the product as well as its behaviour on hydroxyapatite chromatography are those of an RNA-DNA hybrid, even after heat denaturation. After alkaline hydrolysis of an α-^{32}P-TMP-labelled product formed by crude polymerase 0.21% of

the radioactivity is recovered as 2′ (3′) AMP. Moreover, mild heat-treatment of 70 S AMV-RNA or exposure to dimethylsulfoxide abolishes its capacity to serve as template for the viral DNA polymerase. This capacity is regained by the addition of oligo dT. Erikson and Erikson (J. Virol. *8*, 254, 1971) have shown that on heat-treatment of 70 S small, tRNA-like molecules are released from the larger RNA. These findings are compatible with our earlier proposal (Experientia *27*, 740, 1971) that native 70 S AMV RNA is hybridized to short RNA pieces which serve as primers for DNA synthesis.

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Regulation of cAMP Action on the Cellular Level: a Compartment Model

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Several biological systems in which cAMP mediates hormone action show similarities in kinetic and steady-state features: 1. Hormonal stimulation causes a rapid increase in total cAMP in the cell followed by a fall to the initial or a slightly increased steady-state level. 2. Phosphodiesterase inhibitors increase the steady-state as well as the peak level of cAMP; at high inhibitor concentration the peak may disappear. 3. The biological response persists over the whole period of stimulation. 4. The total cAMP concentration even in unstimulated tissue is much higher than necessary for full activation of protein kinases; only a small pool of cAMP can thus have access to kinases, the larger part being 'biologically nonactive'.

To rationalize these phenomena we propose a model with two cAMP compartments and a nonconstant feedback from a late stage in the chain of events to the cAMP distribution between the two pools. Its properties have been studied by an analog computer in relation to two biological systems: isolated fat-cells stimulated by ACTH or epinephrin (U. Lang and R. Schwyzer, FEBS-Letters, *21* 91, (1972)) and frog skin epithelium stimulated by oxytocin (M. Bernard and S. Jard, in preparation). The essential features of the time course of cAMP concentration and response (lipolysis and Na-transport, respectively) could be modelled.

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Thyroxin-Induced Changes in the Pattern of RNA Synthesis in Tail Muscle of *Xenopus* Larvae

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RNA labelled 'in vivo' for 24 h was extracted from tail muscle of tadpoles either maintained in prometamorphic condition by thiourea or after induction of metamorphosis by $5.2 \times 10^{-8} M$ L-thyroxin (4 days); RNA was separated by polyacrylamide gel electrophoresis. In prometamorphic larvae radioactivity was found in the 40 S r-precursor, 28 S, 18 S, 5 S r-RNA and 4 S t-RNA; a heavily labelled fraction was also detected between 28 S and 18 S r-RNA, and tentatively identified as myosin m-RNA. The ratio of labelling between 28 S and 18 S r-RNA was 5:1 which is in contrast to the expected 2:1 ratio. With the aid of a homogeneously ^{14}C-labelled RNA it was possible to demonstrate that the presumed 28 S r-RNA had a mobility characteristic of its direct 32 S precursor. The discrepancy in labelling of the 28 S and 18 S r-RNA, therefore, may be attributed to a faster degradation of the 18 S r-RNA. In response to thyroxin a 2:1 ratio of labelling of the 28 S and 18 S r-RNA was observed, together with a shift in radioactivity from the 32 S precursor to the 28 S r-RNA; the presumed myosin m-RNA was absent. It is concluded that thyroxin has a marked effect on the processing of r-RNA.

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X-Ray Sensitivity and Repair of Synchronous Cells in Culture

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The X-ray response of partially synchronous mastocytoma cells, which were obtained by sucrose density gradient centrifugation, presented a sensitivity high in early S and in G_2 + mitosis, and low in G_1 and in late S, although these cells have a short G_1. Survival curves of cells in different cell cycle phases indicated that the differences in sensitivity were due primarily to changes in extrapolation number. The degree of repair of sublethal damage, as measured by the split dose technique with a 1 h interval, was assessed as a function of time of irradiation in the division cycle. Pronounced rhythmic variations with time of the survival ratio were observed in partially synchronous cultures, indicating that the cells can repair radiation damage in any phase of the division cycle, but most effectively during the second part of S and in G_1. X-ray sensitivity or resistance of cells in different cell cycle phases appears, therefore, to be correlated with a lesser or greater ability to repair sublethal damage.

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Studies on the Template Requirements of Qβ Replicase

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Qβ replicase has specific requirements as to the templates it will replicate (Haruna & Spiegelman, Proc. Nat. Acad. Sci. *54*, 579, 1965). We have reinvestigated the claim (Haruna & Spiegelman, Proc. Nat. Acad. Sci *54*, 1189, 1965) that only intact Qβ RNA serves as template. Qβ RNA was fragmented by mild alkali treatment, indroducing less than one nick per strand and the fragments were resolved by sucrose gradient centrifugation. Each size class contained fragments of equal length comprising either the 3′ or the 5′ end. Fragments with a length substantially less than one half that of intact Qβ RNA elicited synthesis of a complementary RNA as long as the template. Only fragments comprising the 3′ terminus were competent as templates. Qβ RNA fragments shorter than 16 s were inefficient templates. These experiments support the view that template recognition involves an internal site on the Qβ RNA strand, as well as the 3′ terminus (cf. Weber et al., Nature, in press).

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DNA-Replication in Polyoma Infected Mouse Kidney Cells

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Nuclear lysates of polyoma-infected mouse kidney cells can be separated by high salt density-gradient centrifugation into two fractions: a bulk fraction banding at a buoyant density of 1.702 in CsCl and a minor fraction banding on top of the gradient. The latter contains about 5% of the total nuclear DNA, which represents the replicating DNA as shown by its labelling characteristics (^{3}H-TdR). This DNA is bound to some nuclear component, presumably the membrane, and can be released by pronase treatment. Density gradient analysis of this replicating DNA indicates a) that all ^{3}H-TdR incorporated is recovered in DNA, b) that all species of nuclear and viral DNA are replicating at these sites, and c) that during pulse-labelling sites replicating viral DNA are 'saturated' with ^{3}H-TdR faster than the ones replicating cellular DNA. This suggests that the cellular replication units are longer than the viral ones. The structure and localization of these replication sites are currently under investigation.

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Molecular Mechanisms of Specific Lymphocyte Stimulation by Antigens in vitro

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The capacity of various antigens carrying the penicilloyl (BPO) antigenic determinant of specifically stimulating DNA synthesis in lymphocytes from penicillin-sensitive patients has been investigated. A comparison between BPO conjugates by which the carrier molecule is a protein, a polypeptide, an oligopeptide or a single amino acid shows that a simple 'bridging' by antigen of specific receptors on the membrane of antigen-sensitive lymphocytes is not the activating mechanism. Our results show that an active covalent conjugation of BPO groups is required. Effective conjugation does not occur with soluble proteins from the medium but with the cell membrane itself. Isolated populations of autologous cells (lymphocytes, erythrocytes etc.) were incubated briefly with penicillin and added to penicillin-sensitive cells. Penicilloylated erythrocytes, soluble BPO conjugates and possibly specific antibody have an inhibitory effect, whereas BPO-leucocytes are highly stimulating. The possible models of cell cooperation required for lymphocyte stimulation in vitro will be briefly discussed.

Messenger RNA Synthesis During Avian Erythrocytes Maturation

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The pattern of mRNA species synthesized in immature duck erythrocytes is dependent on the degree of maturation of the cell population investigated. In a highly immature population one observes several mRNA species synthesized, two of them being defined: the 9S globin mRNA and a 12S mRNA of unknown function. In a more mature cell population the synthesis of mRNA species other than 9S is considerably reduced. A qualitatively similar pattern is observed for the mlRNA of the non-polyribosomal cytoplasmic ribonucleoprotein fraction; however, compared with the polyribosomal mRNA's, its heterogeneity is wider. A free cytoplasmic 9S RNA of the same electrophoretic mobility as polyribosomal 9S globin mRNA is found. A kinetic relationship between free cytoplasmic 9S RNA and functional 9S globin mRNA can be demonstrated, consistent with (but not a proof of) a precursor product relationship. The more heterogeneous pattern of free cytoplasmic mRNA compared to polyribosomal mRNA suggests a translational control of mRNA expression. The selective cessation of synthesis of mRNA species different from 9S in later stages of erythrocyte maturation suggests a transcriptional control of mRNA synthesis.

Preparation of a 9S RNA from Duck Immature Red Blood Cells and its Translation in a Rabbit Reticulocyte Cell-Free System

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A globin messenger RNA of approximately 9S has been prepared from the polyribosomes of immature red blood cells of the duck. The polyribosomes were either dissociated directly with SDS, or treated with EDTA to liberate a 15S ribonucleoprotein particle (Morel, Kayibanda & Scherrer, FEBS Letters *18*, 84, 1971), which was then dissociated with SDS. An RNA of about 9S was subsequently isolated on SDS-sucrose gradients. On exponential gradient polyacrylamide gels the RNA appeared heterogeneous, with a molecular weight in the range 1.8–2.2 $\times$ 10^5 Daltons.

In a rabbit reticulocyte lysate cell-free system (Adamson et al., Arch. Biochem. Biophys. *125*, 671, 1968), the RNA strongly inhibited the translation of the endogenous message. However, the translation of polypeptides already being synthesised by the endogenous message was not affected. The radioactive product of the cell-free system was analysed on 10% polyacrylamide gels to detect the presence of duck haemoglobins and on CM cellulose columns for duck globins. It was thereby shown that in spite of the inhibition, some of the duck 9S RNA had been translated into duck globins in the rabbit cell-free system. The inhibition and the low efficiency of translation of this RNA is presently under investigation.

Fluorescence and Electron Microscopy as Complementary Methods for the Study of the Nucleic Acids

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Thick sections of aldehyde-fixed and resine-embedded specimens of human epidermis may be treated with some fluorochromes and observed by fluorescence microscopy (A. Gautier et al., Proc. Internat. Congr. Electron Micr. 1970; J. Musy et al., Acta anat. 1970). DNA-Feulgen reagent or Acriflavine, as Feulgen-like stain, revealed stronger nuclear fluorescence of basal layer cells, whereas the nuclei of the upper layers were rather blurred (M. Stoian, 1971, accepted for publication: Dermatologica). With other fluorochromes such as Acridine-orange staining both DNA and RNA, we observed light-yellow nuclear as well as orange cytoplasmic fluorescence prima-

rily in the basal cell layer and in the lower strata of the epidermis. Methyl green-Pyronin conferred to the same cells a distinct scarlet-red nuclear and bright-yellow cytoplasmic fluorescence. Similar results were obtained with thick sections of skin tumors, expecially basal cell epitheliomas where such cells displayed a more intense nuclear fluorescence than others in the same cell-nest. Ultrathin sections treated with Schiff-like reagents, such as Acriflavine or Quinacrine, and studied under the electron microscope showed that the above mentioned bright-fluorescent nuclei are more electron dense than those of the upper strata of the epidermis.

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Gel Bound Cyclic 3',5'-Adenosine Monophosphate; Synthesis and Chemical Properties

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Starting with the sodium salt of 8-bromo cyclic AMP, 8-(N^6-aminocapropylaminoethylthio)-cyclic AMP was prepared by reaction with cysteamine followed by acylation with p. nitrophenyl tert. butyloxycarbonyl-N^3-aminocaproate and subsequent removal of the protecting group with 90% trifluoroacetic acid. The product was completely stable in water and in 1*M* ammonia. Bromocyanogen activated Agarose 2B and 4B reacted with this derivative in borate buffer (0,025*M*, pH = 10.2) to yield conjugates, that contained 1.9 and 3.3 μmoles of the nucleotide per ml of settled gel, respectively. Uncoupled nucleotide was easily washed out of the gels with some 50 ml of borate buffer, but some traces remained, as determined by the functional test of Fisch et al. (in press, 1972). Washing with tris buffer (0.02*M*, pH = 7.4) was useless, but washing with large volumina of sterile water eventually reduced the concentration in the eluates to less than 20 picomoles per ml. Quite unexpectedly, concentrations rose again to values over 500–1000 pmoles per ml after standing over night in the room. The same difficulties were encountered with matrices of other types (Sephadex and cellulose).

Association of Polyoma DNA with Host Cell DNA in a Lytic Infection

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Cells transformed by polyoma virus or SV 40 may contain the viral DNA in an 'integrated' state. To test a possible integration in the lytic infection, leading to the production of progeny virus, the following approach was used: Mouse embryo cells were grown in the presence of BUdR and FUdR. Up to 80% of the DNA of these cultures exhibited an increased buoyant density due to the substitution of thymine by bromouracil in one strand. After infection with polyoma these cultures underwent a lytic infection, proceeding essentially as the infection in control cultures with respect to time course and virus yield. The mouse DNA was separated from the DNA of the infecting virus by a series of CsCl density gradient equilibrium centrifugations and hybridized on filters to radioactive polyoma RNA made in vitro with *E. coli* polymerase. Within 6 hours after infection, viral DNA associated with mouse DNA could be detected. At 6 and 12 h p.i. viral DNA corresponded to 10–15 viral genome equivalents per cell. This value remained unchanged after an additional pronase digestion and a subsequent centrifugation. The nature of this tight association is under investigation.

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Intermolecular Interactions of Proteins at an Interface

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It is known that proteins in solution lower the surface tension of water (as do detergents). In our investigation of the nature of the forces generated by the contractile proteins myosin and actin, we found that these and other proteins develop a high surface viscosity (SV). The viscous drag generated between the cone and plate of the viscometer was found to increase with time to a stable level. KCl or NaCl up to 2 molar accelerated the development of the SV. The addition of triton X-100 or Na-dodecyl sulfate in a mole to mole ratio with the protein abolished the SV and reverted the system to Newtonian behaviour. In a solution of actomyosin the lowering of the viscosity by the addition of Mg-ATP, as found in outflow viscometry, did not occur. Our findings suggest that the proteins migrate to the surface where they build a two dimensional structure. This structure is produced even at protein concentrations as low as 10 μg/ml. Its rate of formation increases with increasing rate of shear, indicating an ordering of intermolecular interactions. Interactions of this type are thought to be involved in the ordering of the contractile proteins in muscle as well as of membrane proteins. The effect of small molecular compounds may be studied by this technique.

Studies on the Initial Binding of Ribosomes to mRNA and on the Structure of a Complex of one Ribosome with one mRNA in HeLa Cells

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We isolated from HeLa cells a mRNA-ribosome complex containing one ribosome bound to the mRNA at or near the initiation site. The structure is useful to study mRNA structure and function. We produced this complex in the cell as follows: cells were incubated in hypertonic medium, which leads to a reversible disaggregation of polyribosomes by a block of initiation of protein synthesis. Cycloheximide was added and some minutes later the tonicity of the medium was restored to normal values. Under these conditions reinitiation of protein synthesis takes place but translocation is blocked. The cells which are labelled with ^{14}C-uridine in rRNA and ^{3}H-uridine in mRNA are lysed and the mRNA containing structures analysed. The results are as follows: all mRNA bound to polyribosomes before treatment is now found in the form of complexes of one, two or three ribosomes with one mRNA molecule. Experiments show that the reason for the binding of more than one ribosome to mRNA is not a lack of efficiency of cycloheximide. The most straightforward interpretation is that part of the mRNA molecules contain more than one ribosome binding site. Electronmicroscopic and biochemical studies of the complexes containing one ribosome show that the mRNA therein is a RNA-protein complex of characteristic size and shape. Studies on the mRNA segment bound to the ribosome in such a complex are in progress.