Union Schweizerischer Gesellschaften für experimentelle Biologie

Berichte der 2. Jahresversammlung

Union des Sociétés Suisses de Biologie expérimentale

Comptes rendus de la 2e Réunion annuelle

Union of Swiss Societies for Experimental Biology

Abstracts of the 2nd Annual Meeting

Fribourg, 23.–24. Mai 1970

Experientia 26, 677–696 (1970)

PHYSIOLOGIE · PHYSIOLOGY

Dynamic Properties of the Presynaptic Vesicular Grid

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Electronmicroscopy of conventionally prepared and freeze-etched synapses of the mammalian CNS established the presence of a highly specialized membrane complex in the presynaptic area. It consists of a triagonal array of densities forming the nodal points of a filamentous grid. In prenatal and neonatal animals it is not clearly differentiated; its susceptibility to anterograde degeneration is a consistent feature. The following relationships with synaptic vesicles are established: (1) Vesicles and grid appear almost simultaneously in ontogeny. (2) The grid constant (550–750 Å) is directly proportional to the size of vesicles and thus an important parameter of synaptic classification. (3) Vesicles lying within the grid loose their zinc-iodide-osmium tetroxide staining specificity during the initial stage of terminal degeneration. (4) Vesicles are protected within the grid from expanding on flattening under various experimental conditions. (5) Invagination of the presynaptic membrane within the grid holes may facilitate the contact with the vesicle membrane and thereby facilitate transport functions.

Supported by the Swiss National Foundation of Scientific Research (No. 4356 and 3.133.69).

Magnitude of the Bohr Effect in Whole Blood in vitro in Presence of CO_2

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We have reassessed the magnitude of the Bohr effect on human whole-blood in vitro in presence of CO_2 by measuring the quantity of base required to compensate the change of plasma pH associated with oxygenation at constant pCO_2. In the pCO_2 range studied (30–60 mm Hg), blood of normal acid-base status yields a $(-\Delta H^+/\Delta HbO_2)$ ratio at constant plasma pH of 0.35 mEq H^+/m*M* HbO_2, that is approximately 30% less than the accepted value obtained by other techniques. It is tentatively suggested that this discrepancy is due to the fact that the latter techniques disregard the part played by the carbamino system under physiological conditions. This interpretation is in qualitative agreement with observations of Rossi and Roughton[1] made on haemoglobin solutions.

[1] J. Physiol. (Lond.) *189*, 1–29 (1967).

Supported by Grants Nos. 2718 and 4846 of the Swiss National Fund for Scientific Research.

Renal Tubular Secretion of Uric Acid in Rats?

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In the unloaded rat, as in man, mongrel dog and monkey, uric acid is predominantly reabsorbed at the renal tubular level (Cur./GFR = 0.15). Using renal clearance methods in non-anaesthetized rats given additional uric acid, we investigated the possible occurrence of simultaneous tubular secretion. At all plasma levels, the clearance ratio remained below 1. It approached unity with increasing plasma levels of uric acid. Arguments supporting a hypothetical secretory mechanism are: (1) At plasma concentrations well below reabsorptive Tm, the renal excretion never fell to zero, but tended towards a constant low level. (2) Below reabsorptive Tm, the clearance ratio decreased with the increase of plasma level. This may be explained by a constant rate of secretion with an increasing rate of reabsorption. (3) Drugs known to inhibit uric acid secretion in other animals (PAH and pyrazinamide) depressed the clearance ratio at all plasma levels.

Supported by grants Nos. 49833 and 34468 of Fonds National Suisse de la Recherche Scientifique.

Effect of Oxygen Partial Pressure on Diffusion Capacity of Growing Lungs

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The ability of the lung to react with structural adaptation to environmental changes in oxygen partial pressure during postnatal growth was tested by exposing 3 groups of rats from the 23rd to the 44th day of their life to hypoxic, normoxic and hyperoxic environments. Group A was raised at a pO_2 of 100 mm Hg (Jungfraujoch, high altitude station, 3454 m). Group B stayed in Bern at pO_2 ~150 mm Hg. Group C was kept in an O_2-chamber with a controlled atmosphere of 40% O_2, pO_2 ~ 290 mm Hg. The lungs of these animals were fixed by standardized techniques and analyzed by morphometric methods.

The specific gas exchange surface area (i.e. surface area per 100 g body weight) showed statistically significant changes: it amounted to 0.33 m^2/100 g under hypoxia and to 0.24 m^2/100 g under hyperoxic conditions. The control value was 0.28 m^2/100 g.

The morphometrically calculated specific pulmonary diffusion capacity (D_L/W) was 0.6 ml/min·mm Hg·100 g in controls; it increased to 0.72 in high-altitude animals and decreased to 0.51 in O_2-chamber rats. These experiments brought evidence that environmental pO_2 variations can influence the quantitative development of the growing lung.

Supported by Nationalfonds grant No. 3.5.68.

Pulmonary Diffusing Capacity for Oxygen in Resting and Exercising Dogs

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The pulmonary diffusing capacity for O_2 (D_{O_2}) was measured in dogs, breathing 11% O_2 by means of a mask, at rest and during exercise of varied intensity. The exercise consisted in running on a treadmill with + 10% grade at variable speed (7–14 km/h). At steady state of exercise the O_2 uptake ($\dot{V}_{O_2}$) was determined by the open-circuit method, and arterial and mixed venous P_{CO_2} and P_{O_2} were measured by electrodes in blood samples withdrawn through chronically implanted catheters. D_{O_2} was calculated using the ideal-alveolar P_{O_2} obtained from inspired and expired gas composition and arterial P_{CO_2}.

On the average, D_{O_2} increased from about 30 ml/min·mm Hg before exercise to about 70 ml/min·mm Hg when $\dot{V}_{O_2}$ reached 70 ml/min·kg (corresponding to 60–70% of the maximum $\dot{V}_{O_2}$ of the dogs). The observed increase of D_{O_2} with increasing $\dot{V}_{O_2}$ was similar to that found by Piiper et al. (Respir. Physiol. *6*, 219, 1969) in anaesthetized dogs whose metabolism was increased by 2,4-dinitrophenol. On the basis of these results and of theoretical considerations D_{O_2} does not appear to be the factor limiting the maximum rate of O_2 consumption in the dog.

Alveolar Equilibration of a Blood-Borne Volatile Tracer during Rebreathing

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Constant-rate venous infusions of ether, used as a cardiac output tracer, were performed on narcotized dogs, during rebreathing. The time course of the tracer's partial pressure at the mouth was followed by mass spectrometry. Because of the high blood solubility of the tracer, its alveolar partial pressure (P_L) increases rapidly during an initial transitory phase. However, P_L does not reach the mixed venous tracer's pressure, due to the capacitive effect of lung tissue. Later on, P_L still increases, at a lower but constant rate. Assuming a complete alveolar-arterial equilibration of the tracer, a relation can be established between (a) cardiac output, (b) the tracer's partial pressure obtained by backextrapolation of the P_L tracing's rectilinear part to zero time of perfusion, (c) the ratio: volume of lung tissue to volume of the other tissues of the body.

Synthesis of ATP by Reversal of the Na-K-Pump in Rabbit Non-Myelinated Nerve Fibres

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Synthesis of ATP by reversal of the Na-pump has been found in erythrocytes ghosts with measurements of the rate of incorporation of radioactive P (Garrahan and Glynn, 1967). In the present experiments similar conditions were realized for non-myelinated nerve fibres. Desheathed rabbit vagus nerves were first incubated for 45 min in Na-free K-rich Locke containing iodoacetic acid. They were then divided into 2 groups: group A was transferred to K-free Locke containing iodoacetic acid, group B to a similar solution with, in addition, ouabain (0.1 mg/ml). After 15 min the Na and K contents were measured, and the ATP concentrations determined by enzymatic-fluorometric methods. The ATP content of group A was found to be $171 \pm 41\%$ ($n = 19$) of that of group B, indicating that net synthesis of ATP may occur in these conditions by reversal of the Na-K-pump.

Supported by FNRS grant No. 4540 and Boehringer GmbH.

Secretion of Renin in Anaesthetized Rats

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Rates of renin secretion were measured in pentobarbital anaesthetized rats receiving isotonic saline i.v. (0.02 ml/min). Renin activity was measured in renal venous and arterial plasma samples after adding excess amounts of partially-purified renin substrate. Total renal plasma flow was determined by the Fick Principle (H^3-PAH). Renin secretion was calculated as the product of renal plasma flow and arterio-venous difference of renin activity. Results: renal plasma flow averaged 3.12 ± 0.18 ml/min kidney ($n = 20$), with a PAH extraction of $85.0 \pm 1.2\%$ ($n = 22$); arterial plasma renin activity, expressed in angiotensin II equivalents, was 2.8 ± 0.26 ng/100 ml ($n = 23$) and renin secretion averaged 27 ± 14 ng/min kidney ($n = 20$).

Supported by grant No. 49072 from the National Science Foundation, Washington, D.C., and grant No. 5315 from the Fonds National Suisse de la Recherche Scientifique.

Properties of Nicotinic and Muscarinic Receptors of Isolated Rat Ganglion

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In isolated rat cervical sympathetic ganglia, discharges obtained by adding acetylcholine (ACh) or carbachol to the bathing solution can be blocked by low doses of atropine (Atr) but not d-Tubocurarine (dTC). This suggests, as reported for ganglia in situ, that this activity is due mainly to activation of muscarinic receptors. The discharge following preganglionic stimulation at rates of 1, 5, 10, 20, 50 and 100 shocks/sec in presence of 4 γ/ml eserine has also been recorded and a method developed for integrating and quantifying the response. The discharge increases after rates of 1 to 10, but falls markedly after 50 and 100 shocks/sec. This activity also appears to be due predominantly to activation of muscarinic receptors, based on the following evidence: Atr, 0.05–0.1 γ/ml, causes almost complete suppression of the discharge, whereas dTC, 75–100 γ/ml, results in enhancement of firing at frequencies of 10 shocks/sec and higher. The latter observation suggests that in absence of dTC the predominant effect of prolonged activation of nicotinic receptors by endogenous ACh is desensitization or complete depolarization of the ganglion cells, thereby reducing their sensitivity to activation via muscarinic receptors.

Grant No. 4994, Swiss National Funds.

Quantitative Adaptation of Lung Structure to Increased O_2 Consumption in Mice

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To study the problem, whether the pulmonary gas-exchange apparatus becomes enlarged when O_2 consumption of the organism V_{O_2} is chronically increased, we estimated the diffusion capacity D_L by morphometric methods in 5 normal white mice (NM) and 5 Japanese waltzing mice (JWM). Both belong to the species *Mus muris*, but JWM have a genetic defect in vestibular apparatus and brain, which causes them to be constantly in motion. The average O_2 consumption per gram body weight W was 0.082 ml/min·g in JWM, as compared to 0.046 ml/min·g in NM. This 80% increase in V_{O_2}/W was paralleled by a 70% increase in D_L/W in JWM. The improvement in D_L was achieved by a variety of structural changes: increase in lung volume (30%) and in alveolar and capillary surface density per unit volume (30%)

leading to a 60% increase in exchange surface, and reduction in mean barrier thickness. The lung has hence exploited all possibilities of structural adaptation to increase its gas-exchange efficiency as O_2 needs of the organism are augmented.

Supported by Nationalfonds grant No. 3.5.68.

Apparent Pulmonary O_2 and CO Diffusing Capacity in the Inhomogenous Lung

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In a theoretical lung model, where alveolar ventilation and diffusion capacity were unevenly distributed in various fashions with respect to pulmonary blood flow, a system of linear equations allowed calculation of the 'mean' and 'ideal' alveolar as well as of the 'mean' arterial and mixed venous P_{O_2} and P_{CO} for steady-state conditions. With these partial pressures the apparent D_{LO_2} and $D_{L\,CO}$ were determined and compared to the D_{LO_2} and D_{LCO} values attributed to the model (true D_L's). The equations were established for a given hypoxic inspiratory air with the following constants: Vo_2, Vco_2, total V_A, Q and D_L and the slopes of the O_2 and CO_2 dissociation curves of blood; the parameters were the V_A/Q and D/Q ratios of the different lung compartments. The results show that in an inhomogenous lung: (a) apparent D_L are smaller than true D_L both for O_2 and CO, (b) apparent $D_{L\,O_2}$ are smaller than apparent $D_{L\,CO}$ for most of the distributions considered, (c) the use of 'ideal' alveolar pressures reduces the errors in apparent $D_{L\,O_2}$ but may result in apparent $D_{L\,CO}$ larger than true $D_{L\,CO}$.

Supported by Swiss National Fund, grant No. 2718.

Comparative Measurements of DLCO and DLO_2 in the Dog

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In anaesthetized dogs breathing hypoxic mixtures ($FI_{O_2} = 0.11$) containing carbon monoxide ($FI_{CO} = 0.001$) $D_{L\,O_2}$ and $D_{L\,CO}$ were determined from measurements of V_{O_2}, V_{CO_2}, V_{CO}, P_{AO_2}, P_{ACO_2}, P_{ACO}, Pa_{O_2}, Pa_{CO_2}, Pa_{CO} and P_{VO_2}. Using end expiratory alveolar partial pressures (PA) the average D_L was 14 ml/min·mm Hg for O_2 and 20 ml/min·mm Hg for CO; using the 'ideal' alveolar pressures (PAi) the values were respectively 28 and 70. The unexpected feature that D_{LCO}'s were larger than the $D_{L\,O_2}$'s could be explained by the functional inhomogeneities of the lung. This explanation implies that a non-negligible part of the $(PAi\text{-}Pa)O_2$ difference is due to uneven distribution of VA and D to Q. This is confirmed by the observation that in hypoxia the decrease of the slope of the O_2 dissociation curve caused by the presence of CO in the blood increases the $(PAi\text{-}PA)O_2$ difference.

Supported by Swiss National Fund, grant No. 2718.

Einfluss der cutanen Sensibilität auf zwei Typen von Pyramidenbahnneuronen der Katze

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An 30 mit Chloralose narkotisierten Katzen werden mit extrazellulärer Registrierung im motorischen Cortex 95 Pyramidenbahnneurone untersucht. Die Neurone werden durch antidrome Reizung identifiziert und die Leitungsgeschwindigkeit ihrer Axone bestimmt. Deren Reaktion auf elektrische Reizung von Hautnerven ergibt folgendes: Durch Hautafferenzen der contralateralen Vorderpfote werden die schnelleitenden Pyramidenbahnneurone mit kürzerer Latenz aktiviert als die langsamleitenden. Diese gekreuzten kurzlatenten Afferenzen gehen von einer Gruppe leichterregbarer Hautnervenfasern aus (Aβ-Fasern). Reversible und irreversible Ausschaltungsversuche zeigen, dass sie im Rückenmark ausschliesslich oder überwiegend über die Hinterstränge geleitet werden. Eine langsamere Aktivierung über die Vorder-Seitenstränge wird erst nach Ausschaltung der Hinterstränge sichtbar.

Unterstützt durch Schweiz. Nationalfonds für wissenschaftliche Forschung (Nr. 4447 und 4356).

Monoamines, LSD and Brain Stem Reticular Neurones

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A study was made of the actions of microelectrophoretically administered 5-hydroxytryptamine (5-HT), noradrenaline (NA), dopamine (DA) and lysergic acid diethylamide (LSD) on spontaneous or glutamate evoked firing of neurones in the bulbar reticular formation of unanaesthetized, decerebrate cats. 5-HT and NA had excitant, depressant or 'dual' excitant-depressant actions on the majority of neurones tested. The excitant effects were usually of a slow time course and sometimes showed desensitization on repeated applications, whereas the depressant actions were usually of a rapid time course and constant. DA frequently caused depression, although it excited a small proportion of cells and was without effect on approximately half the neurones tested. Neurones which were affected by all 3 monoamines showed various combinations of excitation and depression and seemed to be scattered diffusely throughout the bulbar reticular formation. LSD often reversibly blocked the excitant, but not the depressant, actions of 5-HT and NA.

Supported by the Swiss National Foundation of Scientific Research.

Membrane Potentials of Neurones and Glial Cells in Tissue Cultures

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Neurones and glial cells from cerebellum, brain stem and spinal cord of 2–4 day old rats were cultivated in vitro (up to 30 days). The various cell types were identified using phase-contrast microscopy in the living state, and specific staining techniques in both vital (methylene blue) and fixed (Nissl, Bodian) preparations. Cells were impaled with 3*M* KCl-containing microelectrodes (tip diameter less than 1 μ) under visual control using phase-contrast optics. Internally negative membrane potentials were recorded from neurones (−12 to −70 mV) and astrocytes (−10 to −40 mV). Small glial cells which were classified as oligodendrocytes from their morphological appearance, showed an internally positive potential ranging from +8 to +30 mV. The resistance of all the cell

membrane measured in a few large neurones of spinal tissue varied between 2 and 6 MΩ. These results suggest that neurones grown in cultures show similar membrane properties to those studied in vivo.

Reduced Hypothalamic Set Point Temperature During Exercise in Man

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Experiments were performed on 10 male subjects to determine the threshold hypothalamic temperature at which sweating and cutaneous vasodilatation are elicited during exercise. Cutaneous and respiratory heat losses were measured in a gradient calorimeter. Comparisons of evaporative heat loss during rest and exercise showed greater loss during exercise than at rest at the same tympanic temperature. Cutaneous thermal conductance also was higher during exercise than at rest at the same tympanic temperature. These results suggest that the set point of the temperature regulating centre is reduced during exercise. Under certain ambiant conditions, it was found that a very slight exercise (1 or 2 watts), with no increase in tympanic temperature, gave rise to sudomotor activity. This supports the hypothesis that reflexes arising from joints might contribute to shift the hypothalamic set point temperature during exercise.

Short-Circuiting Factor of Myelinated Nerve Fibres in the Sucrose-Gap

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Equations for the longitudinal potential distribution of a preparation in a non-homogeneous extracellular medium were derived and the formula was found that relates the true value of the membrane potential of the preparation in one of the regions to the potential difference measured between electrodes placed in two of the extracellular regions. A modified sucrose-gap apparatus, that allows the measurements of the extracellular potential distribution, was developed. The results of measurements with this apparatus were in good agreement with the theoretical values calculated from the derived equations.

Supported by FNRS grant No. 4540.

Fluxes and Location of Calcium in Rabbit Vagus Nerve

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Desheathed rabbit vagus nerves were loaded with ^{45}Ca by incubation; the Ca-influx showed a fast and a slow phase and after 2 h of soak-in the tracer content in the nerve ceased to increase.

The efflux followed a double exponential after an initial rapid phase, suggesting the presence of 3 different compartments; all components of the efflux curve had a low temperature dependence.

About $^1/_3$ of the initial tracer content remained in the nerve after 4 h of washing. Approximately $^9/_{10}$ of the calcium content could be extracted by homogenization; the amount not removable by this procedure increased only slightly after 120 min (as opposed to 15 min) of incubation. ATP was not required for the binding of calcium: inhibitors which strongly decrease the ATP level in this tissue did not stimulate the Ca-efflux.

The possibility that the slowly exchanging nerve Ca is bound in a similar way as in skeletal muscle seems to be ruled out by the ineffectiveness of several substances known to promote Ca-efflux from muscle.

Supported by FNRS grant No. 4540.

Electrophysiological Characteristics of Supraoptico-hypophysial Fibres

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Many similarities between the neurosecretory cells of the supraoptic nucleus and other neurons have been established. Exposure of the ventromedial surface of the diencephalon permitted a study of field potentials and of over 100 single cells recorded from the 'surface marked' supraoptic nucleus activated antidromically by electrodes placed on the pituitary stalk. NaCl-filled recording electrodes were inserted just lateral and posterior to the bifurcation of the middle and anterior cerebral arteries. The majority of fibres required a stimulus of 0.5 msec, 10–30 V. Estimated conduction velocities ranged from 0.5 to 0.2 m/sec (mean = 0.30 m/sec ± 0.04 S.E., $n = 69$). The fibres follow frequencies as high as 100/sec; however, the spike amplitude was greatly reduced at frequencies above 30/sec if the number of shocks exceeded a critical number, as few as 3–6 at 100/sec. These results suggest that the supraoptico-hypophysial tract functions as a C-fibre tract, e.g. the rabbit vagus nerve.

Supported by Swiss National Science Foundation and Hoffmann-La Roche Foundation.
* IBRO/UNESCO fellow.

Peak Measured Velocity (V_{pm}) in the Canine Left Ventricle

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The extrapolated maximal velocity of shortening of the contractile elements (V_{max}) has been shown to be a meaningful measure of myocardial contractility. In the ejecting heart the inverse portion of the stress-velocity curves is often very short and extrapolation to V_{max} becomes difficult. Therefore it was investigated if V_{pm} might be used instead of V_{max} as an index of contractility. When the left ventricular end-diastolic pressure was raised from 9 to 28 mm Hg by volume or pressure loading in closed-chest anaesthetized dogs with minimized cardiac reflex adjustments (propranolol, vagi cut) V_{pm} decreased from 2.2. to 1.4 circumferences/sec. Following calcium V_{pm} increased from 1.9 to 2.8 circumferences/sec. In contrast, the time from the onset of contraction up to V_{pm} (t-V_{pm}) remained unchanged under preload variations but shortened after calcium. It is concluded that (1) V_{pm} is not an absolute measure of myocardial contractility because it is dependent upon preload, and (2) t-V_{pm} may be a useful parameter for assessing the contractile state of the myocardium in the intact organism.

Supported by a grant from the Swiss National Fund.

Slow Outward Current in Ventricular Muscle

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Using a modified sucrose gap technique, Giebisch and Weidmann (Helv. Physiol. Acta *25* CR, 189–190, 1967) found no evidence for a time-dependent increase in outward current. However, they used clamp pulses of around 500 msec and if the clamp duration is increased to several seconds a slow increase in outward current becomes apparent. This slow increase in outward current resembles the x-currents found in Purkinje fibres (D. Noble and R. W. Tsien, J. Physiol., Lond. *200*, 205–231, 1969) in that: (a) the reversal potential is around −60 mV; (b) time dependence on repolarization is shown; (c) the outward current can be split into 2 exponentials, a fast and a slow component. These 2 components are clearly distinguished at positive membrane potentials (inside positive to outside) and at values more negative than −80 mV. Over the range −80 to 0 mV, inward current interferes with the analysis of the fast component. The rate constants of the x-currents are similar to those found in Purkinje fibres. Since the membrane resistance during the plateau phase of the ventricular action potential is relatively high (unpublished observations) it is suggested that this slow increase in outward current plays a part in repolarization.

A Visual Function of the Supraoptic Decussation in the Pigeon

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Anatomical and electrophysiological studies indicate that there is a complete crossing of the visual pathways in the pigeon. Relearning tests show that the homolateral hemisphere has access to monocularly learned information. The present investigation set out to determine which commissures are responsible for this interhemispheric transfer of visual information. Operant conditioning techniques and simultaneous colour and pattern discriminations were employed. After stereotactic section of tectal and posterior commissures the transfer of both types of discriminations was still possible. Lesions in the area of the supraoptic decussation led to a severe impairment in transfer. This decussation appears to connect mesodiencephalic structures with the telencephalon. Its contribution to the interhemispheric transfer of the visual information in the pigeon has been shown for the first time.

Supported by the Swiss National Foundation of Scientific Research (grants Nos. 4806 and 4356).

Effect of Glycerol Treatment on Heart Muscle

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Sheep heart and frog skeletal muscle were examined by electrophysiological and morphological methods before and after treatment with hypertonic glycerol-electrolyte-solutions. This treatment in frog muscle results in loss of contractility and disappearance of the afterpotentials, due to the disruption of the T-system. In these experiments the T-system could be labelled by horseradish peroxidase at any stage before the disruption of the T-tubes. Before this disruption the tracer could also be washed out. In heart muscle, the contractility never disappeared and the resting membrane and action potentials following electrical depolarization were not influenced by glycerol treatment. The time constant of the 'make' of a hyperpolarizing pulse did not change significantly. Morphologically it could be shown that T-system was intact before and after glycerol treatment, since the diffusion tracer could penetrate and be removed at all experimental stages. A slight hypertonic effect of glycerol solution (i.e. little swelling of T-system) was observed in heart muscle. Similar results in perfused sheep moderator band and perfused rat heart have been found. Morphological studies in rat diaphragm show results corresponding to frog skeletal muscle.

Action of Chlordiazepoxide on Contractile Mechanism in Single Fibres of Frog Muscle

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Chlordiazepoxide, 0.4 m*M*, increases twitch tension in a few seconds and this increase is maintained for as long as the drug is present. Tetanic tension (tet.) is reduced and cannot be sustained and local contractions are also seen. When tension has fallen to zero during tetanic stimulation, depolarisation by 190 m*M*-K elicits a contracture of normal height. Recovery from this contracture when tested by the method of Hodkin and Horowicz (J. Physiol. *153*, 1960) is diminished. Two explanations are considered: (1) under ClD fibres remain depolarized; (2) the drug inhibits the SR-Ca-pump. Potential measurements do not give any evidence for continuous depolarisation. The second explanation is in agreement with findings of Balzer et al. (Naunyn-Schmiedeberg *260*, 1968), who demonstrated an inhibition of SR-Ca-Pump by similar drugs. Ca-accumulation in the longitudinal part of SR, as found by Winegrad (J. gen. Physiol. *51*, 1968) in fibres after tet. stimulation, would also explain the potentiation of the single twitch.

Supported by Hoffmann-La Roche Ltd.

Activity of Hypothalamic Units During Sleep

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In free-moving cats hypothalamic units were studied during sleep. Hypothalamic temperature was recorded. Activity of anterior and dorsal area units decreases in late slow-wave sleep (SWS) and in fast-wave sleep (FWS). Lateral area, fields of Forel, zona incerta unit activity increases in FWS; some units in lateral area tuberal division are less active in FWS. Activity of fornix, anterior and medial mammillary nn, mammillothalamic tract units temporarily decreases in late SWS. These changes appear independent of hypothalamic temperature changes in FWS. Control experiments show that: olfactory tubercle units are unchanged; some internal capsule units, optic chiasma and tract units are less active in SWS and FWS; other internal capsule units, cerebral peduncle units are more active in FWS; reuniens and ventral posteromedial thalamic nn. unit activity respectively decreases and increases in FWS; posterior commissure n., red n., central grey unit activity increases in FWS. It is surmised that hypothalamic control of homeostasis is changed in FWS, and that this change begins in late SWS.

Electrophysiology of Ipsi- and Contralateral Visual Forebrain Connections in Pigeon

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Evoked potentials and single unit discharges were observed in the hyperstriatum accessorium (HA) in 39 equithesin-anaesthetized pigeons in response to stimulation of retina and supraoptic decussation (DS). Bilateral HA responses of similar wave forms appeared following monocular photic or electrical stimulation. The latency in the ipsilateral HA exceeded that in the contralateral by 3–18 msec. No responses could be evoked in one HA by stimulation of the opposite HA. Short latency (2 msec) potentials were seen in both HA following stimulation in the region of DS. Electrolytic lesions in this region decreases the amplitude of ipsilateral retinal responses; contralateral potentials were not significantly changed. HA units (110) activated by electrical stimulation of the optic nerve papilla had latencies of 13–70 msec. Units responding to ipsilateral, contralateral and bilateral retinal stimulation were observed. Units responding to stimulation of both eyes had longer latency (2–37 msec) to ipsilateral stimuli. Dorsally located units responded only to contralateral stimuli, while ventrally placed units were activated exclusively by ipsilateral stimuli. Units located in the intermediate zone responded to both ipsi- and contralateral stimuli.

Supported by Swiss National Foundation for Scientific Research (grants Nos. 4806 and 4356).

Auswirkungen von Hypoxie auf Atmung und Kreislauf beim Katzenhai

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An wachen Grossgefleckten Katzenhaien (*Scyliorhinus stellaris* (L.)) wurden die wichtigsten Kenngrössen der Atmung und des Kreislaufs gemessen, während der O_2-Partialdruck des Umgebungswassers von 142 bis auf 54 mm Hg erniedrigt wurde. Die folgenden Effekte wurden in Hypoxie beobachtet: (1) Die O_2-Aufnahme zeigte ein deutliches Absinken schon bei geringer Erniedrigung des O_2-Druckes (auf 120 mm Hg). (2) Die Atemfrequenz änderte sich nicht signifikant, das Atemzeitvolumen nahm jedoch zu und wies ein Maximum in milder Hypoxie auf. (3) Die Herzfrequenz war erniedrigt; das (nach dem Fickschen Prinzip gemessene) Herzzeitvolumen zeigte im Mittel eine leichte Abnahme, die jedoch wegen der erheblichen Streuung statistisch nicht zu sichern war. (4) Der mittlere Blutdruck in der ventralen Aorta und in peripheren Arterien sank ab.

Diese Beobachtungen stimmen weitgehend mit Literaturbefunden an anderen Elasmobranchiern überein, unterscheiden sich jedoch von Veränderungen, die bei einigen Teleostiern in Hypoxie gefunden worden sind.

Natriuretic Effect of Lissamine Green

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Lissamine green (Chroma Gesellschaft, Stuttgart) is used in renal micropuncture experiments as a selective dye for tubular fluid. As such it should be devoid of any effect on renal functions. It has, however, been reported to inhibit Na^+ transport through frog skin[1]. In the present experiments possible natriuretic effects were investigated in rats infused whit either 0.03 (non-diuretic) or with 0.2 ml/min of isotonic saline. After control periods, the dye was injected at the rate of one injection of 0.05 ml of a 10% solution every 5 min (10 injections).

In the non-diuretic rats the fractional Na^+ excretion was increased from $1.6 \pm 0.5\%$ by $0.8 \pm 0.2\%$ of filtered load ($p < 0.05$) (Filtered load = GFR; $[Na^+]$ plasma; GFR = C_{In}). In the diuretic rats fractional Na^+ excretion rose from $12.2 \pm 1.2\%$ by $5.1 \pm 1.2\%$ of filtered load ($p < 0.01$). Urine flow was not increased.

Lissamine green should, therefore, not be used in micropuncture studies of renal Na^+ excretion.

[1] A. Dörge and W. Nagel, Pflügers Archiv *313*, 11–18 (1969).

Supported by Fonds National Suisse de la Recherche Scientifique, grants Nos. 4983.3 and 3.44.68.

Analyse des Gasaustausches in der Vogellunge

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Die Vogellunge ist aufgebaut aus parallelen, luftdurchströmten Parabronchien, von denen Luftkapillaren entspringen, über die der Gasaustausch mit dem umgebenden Blutkapillarnetz erfolgt. Funktionell lassen sich diese Gegebenheiten mit einem «serial-multikapillären System» vergleichen, bei dem einzelne Blutkapillaren in verschiedenen Abschnitten längs eines Rohres Kontakt mit diesem aufnehmen. Da die Luft beim Durchströmen des Rohres wegen des Gasaustausches ihre Zusammensetzung ständig ändert, ist auch die Arterialisierung des Blutes der einzelnen Kapillaren, dessen Mischung das arterielle Blut ergibt, sehr unterschiedlich. Die von uns erhobenen Messwerte von Gasaustauschparametern an Hühnern sind mit diesem Modell vereinbar, besonders der bei Vorliegen einer Alveolarlunge nur schwer zu deutende Befund, dass in der endexspiratorischen Luft höhere CO_2- und niedrigere O_2-Partialdrucke gefunden werden als im arteriellen Blut. Die nach dem Modell berechnete Diffusionskapazität für O_2 steht in guter Übereinstimmung mit der Diffusionskapazität für CO, die mit einer Rückatmungsmethode gewonnen wurde.

Repeated Estimates of $D_{L\,O_2}$ in Man at Different Work Levels

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In 1955 Riley et al. found a plateau for the exercise $D_{L\,O_2}$ in the range of $\dot{V}_{O_2}$ higher than 1 l/min. This result was not confirmed in later determinations of $D_{L\,O_2}$ or $D_{L\,CO}$, the D_L was found to increase linearly with $\dot{V}_{O_2}$ up to 2 l/min (Turino et al. 1963, Johnson et al. 1965, Reuschlein et al. 1968). In a 25-year-old volunteer, the $D_{L\,O_2}$ was estimated in the course of 3 months, 5 times at 3 bicycle work-loads ($\dot{V}_{O_2}$ 1.4, 1.8 and 2.2 l/min). The subject breathed at all 3 work-levels during 15 min 20.9% O_2 in N_2, then during 15 min 16.0% O_2 in N_2. Ventilation, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured with a concentration stabilized metabograph of Fleisch. $P_{a\,O_2}$, $P_{a\,CO_2}$ and pH were determined at the end of each 15-min period with IL-electrodes, $S_{a\,O_2}$ with an IL-CO-Oximeter, $C_{a\,O_2}$ and $C_{a\,CO_2}$ with van Slyke's manometric method. The mean of the 5 $D_{L\,O_2}$ was found to be at slight, moderate and heavy work respectively 81.7 ± 24.3, 75.3 ± 6.7 and 71.2 ± 7.6 ml/min · mm Hg, the 3 exercise values do not differ statistically one from the other ($p > 0.05$). Thus, Riley's original result of an exercise $D_{L\,O_2}$-plateau can be confirmed.

Estimation of Pulmonary Diffusion Capacity by Morphometry in Dogs

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The pulmonary diffusion capacity D_L can be estimated from morphometric data on alveolar and capillary surface, capillary volume, and harmonic mean thickness of tissue and plasma, which can be determined on electron micrographs by stereologic methods. On 20 dogs D_L was found to be proportional to body weight W ($D_L = 7.2 \times W - 17$). Averaged: $\overline{W} = 15$ kg, $\overline{D_L} = 92$ ml/min·mm Hg. The largest group of dogs with W = 24 kg reached a D_L of 155 ml/min·mm Hg. This is about twice the highest physiological estimate obtained in exercise. For human lungs identical results are obtained: with W = 70 kg, $D_L = 127$ ml/min·mm Hg. The estimates for D_L obtained by the morphometric approach hence represent a 'true or maximal diffusion capacity', i.e. the optimum the lung could achieve under ideal conditions which are probably never met under physiological conditions.

Supported by Nationalfonds grant 3.5.68 and a grant from the Hoffmann-La Roche Stiftung.

'Aspiration Reflex', a Respiratory Reaction Extremely Resistant to Suppression

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Mechanical stimulation of the epipharyngeal mucosa provokes series of rapid and powerful sniff- and gasp-like inspiratory twitches associated with reflex hypertension and bronchodilation (J. Physiol. *200*, 25–49, 1969). In experiments on cats anaesthetized with pentobarbital (40 mg/kg i.p.) the reflex proved to be very resistant to chlorpromazine (5 mg/kg i.v.). At the same time cough and sneeze reflexes were absent or markedly suppressed. The aspiration reflex is very resistant also to antitussics and narcotics. It can be elicited in very deep anaesthesia and in agony, when other respiratory reflexes are absent and gasping develops.

Investigation of the aspiration reflex seems to be a suitable method of screening drugs for their inspiratory twitches suppressing activities with possible application in the therapy of sniffing, gasping and hiccup. Owing to its very great resistance to suppression, the aspiration reflex may be useful for resuscitation of cats during agony.

Change of Apparent Distribution Volume of $^{35}SO_4^=$ in Acute Respiratory Acidosis

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The effect of CO_2 breathing on the disappearance curve of arterial plasma activity after injection of $^{35}SO_4^=$ was studied in nephrectomized dogs. After a control period of air breathing, the animals were switched to 20% CO_2 at constant ventilation. This change shifted the disappearance curve downward: after 30 and 60 min, the amplitude of the shift with respect to the extrapolated control curve corresponded to a 7% rise of the apparent distribution volume of activity. Conversely, an upward step followed the return to air breathing. This phenomenon may conceivably be due to respiratory acidosis causing a real increase of the extracellular fluid volume, or to the existence of pH-dependent 'trapping' of $SO_4^=$ in the interstitial fluid. Both possibilities raise intriguing problems about the behavior of the extracellular phase in acute respiratory acidosis.

Supported by grant No. 3.181.69 of the Swiss National Fund for Scientific Research.

Biophysical Properties of Cultured Human Fetal Glial Cells

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Biophysical examination of cultured human fetal glia, identified by morphological, cytological and biochemical features as fibrous astrocytes, showed that the membrane time constant was extremely short averaging 311 μsec. This value is about $^1/_{25}$ that for cortical neurons but very similar to the glial time constant found in both in vivo and in vitro studies. The mean membrane specific capacitance was almost 1 $\mu F/cm^2$, a frequently observed biological value while the membrane specific resistance was significantly smaller than that observed for neurons, averaging 327 Ω cm^2. Glial membrane voltage responses to imposed current pulses were non-rectifying over the range examined. Regenerative responses were absent despite membrane potential alteration as great as ± 25 mV. An altered intracellular Na:K ratio, attributable to culture metabolic conditions and lowered temperature during examination resulted in a low membrane resting potential (X = −7 mV). Lowered resting potential does not prevent mitosis or growth and appears unrelated to membrane biophysical properties. The physiological significance of these data is considered.

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DNA Synthesis in the Mitotic Cycle of Physarum

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The myxomycete *Physarum polycephalum* can easily be grown as a syncytium containing about 10^8 nuclei in a single mass of cytoplasm. Since nearly all nuclei divide synchronously every 8–10 h, this organism is ideally suited for biochemical studies of the mitotic cycle.

The bulk of the chromosomal DNA is replicated during the S-phase, which lasts for 3 h immediately following mitosis. On the molecular level there is a predetermined time sequence of DNA replication. Two types of DNA can be distinguished from bulk chromosomal DNA on the basis of their physico-chemical properties: nuclear satellite DNA and mitochondrial DNA. These two types of DNA also differ in physiological properties from bulk chromosomal DNA: they are both synthesized throughout the mitotic cycle and not only during the S-period.

Effets du 3'5'-GMP cyclique et du 3'5'-AMP$_c$ sur le métabolisme glucolipidique in vivo

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De nombreuses expériences in vitro démontrent l'importance des nucléotides monophosphates cycliques dans le contrôle de la néoglucogénèse, de la glycogénolyse et de la lipolyse; toutefois les rôles respectifs de chacun d'eux sont encore mal définis.

C'est pourquoi nous avons comparé chez la souris vivante, les effets du GMP$_c$ et de l'AMP$_c$ sur la glycémie, la néoglucogénèse, la glycogénolyse et sur la synthèse des acides gras du foie, des graisses épididymaires et de la carcasse. Nos résultats montrent que:

- les glycémies sont élevées de manière significative par le GMP$_c$ et plus encore par l'AMP$_c$
- la concentration du glycogène hépatique s'abaisse des 2/3 sous l'influence de ces 2 nucléotides
- l'AMP$_c$ stimule la néoglucogénèse contrairement au GMP$_c$
- ces 2 dérivés sont sans action sur la synthèse des acides gras dans les graisses épididymaires (on y observe plutôt une légère stimulation) alors qu'ils provoquent une très forte inhibition de la lipogenèse hépatique. Dans cet organe, l'AMP$_c$ inhibe plus nettement l'incorporation du pyruvate ^{14}C que celle de l'acétate ^{3}H, le précurseur carboné étant vraisemblablement détourné vers la néoglucogénèse.
- Dans la carcasse, le GMP$_c$ provoque une légère stimulation de la lipogenèse, alors que l'AMP$_c$ ne produit pas de réponse significative.

Studies on the Antilipolytic Action of Nicotinic Acid

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Since the mode of action of nicotinic acid (NA), one of the most potent antilipolytic agents, has not been so far quite explained, its effects in vitro as well as in vivo on the rat tissues hormone-sensitive lipase (HSL) activities were studied. In adipose tissue in vitro NA significantly lowered the base-line activity of HSL as well as its stimulation by catecholamines. The reciprocal plots of dose-response curve of norepinephrine showed equal values for intrinsic activities E_{max} and different values for apparent affinities K_A in the presence and in the absence of NA. Therefore, kinetic competition of NA and norepinephrine was demonstrated. When NA (100 mg/kg) was administered in vivo and the activity of adipose tissue HSL was studied in tissue specimens obtained in different intervals following the administration, it was found a significant immediate decrease of lipase activity. In liver tissue, however, NA in vitro as well as in vivo significantly augmented the lipase activity. The participation of liver lipase in the rebound phenomenon is one of the possible explanations.

Isolation of Two Different Lipoproteins from Burned and Unburned Mouse Skin

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Germ-free inbred mice were used. The skins were freed of surface-lipids and cell content by organic extraction and milling. Heat was applied under standard conditions to the 'skin ghosts'. Not burned skin underwent an identical isolation procedure. All work was done at 4 °C. A lipoprotein was isolated from both starting materials. Gradient disc electrophoresis showed 2 protein bands for the burned in contrast to one band for the unburned compound. One of the 2 bands isolated from burned skin was identical for both products. The second band showed an identical electrical charge but a higher mol. wt. and toxic activity. Gradient ultracentrifugation separated the 2 burned moieties by density. Both the burned and unburned compounds contained 50% protein, 49% lipid and 1% amino sugar. The lipid parts showed 6 different fractions on thin-layer chromatography identical for both compounds. Thus, heat produced an increase in mol. wt. and density, while chemical composition and electrical charge were not influenced. The toxic product from burned skin was therefore concluded to be the polymeric form of a naturally occurring monomer.

Synthèse et migration de protéines neuronales néoformées. Etude biochimique

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L'apport en protéines fournies à la terminaison synaptique par le soma neuronal a été étudié dans la voie retino-tectale du pigeon. Après injection de leucine tritié dans l'humeur vitrée d'un œil, les lobes optiques ont été prélevés dans un délai variant de 3 h à 60 jours. Ils ont été fractionnés par centrifugation différentielle et la radioactivité spécifique des protéines a été déterminée et comparée entre les 2 lobes. Les protéines des fractions synaptosomale et axonale présentent un marquage important vers 4–6 h, 12–24 h et 2–4 semaines. Les fractions microsomale et soluble sont marquées uniquement entre 1 et 4 semaines. L'absence de marquage préférentiel de matériel soluble dans l'acide, à tous les temps étudiés, semble indiquer que la plupart de ces protéines ne sont pas synthétisées dans le toit. Ces résultats suggèrent l'existence de 3 vagues de migration au moins, ayant des vitesses respectivement de plus de 100 mm/jour d'environ 50 mm/jour et de 1–5 mm/jour. Les 3 semblent gagner la terminaison synaptique.

Travail soutenu par le Fond National de la Recherche Scientifique (No. 4806 et 4356).

Permissive Action of Glucocorticoids on Glycogenolysis in Heart and Liver

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Perfused hearts and livers from adrenalectomized rats exhibited impaired glycogenolytic responses to low doses of epinephrine in vitro. Cortisol treatment restored the responses to normal. With high doses of epinephrine, normal glycogenolysis was observed.

The reduced glycogenolytic responses were due to impairment of phosphorylase activation. This was not due to a defect in the activation of adenyl cyclase by epinephrine since normal increases in cyclic AMP were observed in tissues from adrenal-deficient animals. Consistent with the conclusion that the defect was situated beyond cyclic AMP formation was the observation that the glyco-

genolytic response to low concentrations of cyclic AMP was impaired in the steroid-deficient liver. Studies to further define the defect revealed that the activities of phosphorylase-b kinase and histone kinase were normal in hearts from adrenalectomized rats.

These observations indicate that lack of adrenal cortical hormones reduces the sensitivity of the phosphorylase activation system to cyclic AMP. In the heart, adrenal cortical secretion appears to be required for normal activation of phosphorylase-b by phosphorylase-b kinase.

Analyse des cellules et granules éosinophiles isolés à partir du sang de cheval

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L'analyse comparative des cellules et des granules lyophilisés a donné les résultats suivants: N 12,9 (12,1); lipides totaux 12 (9,5); cholestérol 1,06 (1,6); triglycérides 1,2 (0,8); P total 0,8 (0,3); P lipoidique 0,2 (0,12) et sucres 3,0 (1,3). Ces valeurs sont exprimées en mg/100 mg de cellules ou de granules (valeurs des granules entre parenthèses). La fraction soluble dans l'eau des cellules fait 61,5%, celle des granules 16,6%. 75 (27) % des sucres (glucose, galactose, mannose, xylose et ribose) se trouvent dans ces fractions solubles. L'électrophorèse des protéines solubles a révélé la présence d'au moins 8 fractions différentes dans les cellules et 5 dans les granules. Les 5 protéines solubles des granules ont montré une éosinophilie plus ou moins marquée. Les protéines des granules insolubles dans l'eau n'ont pu être dissoutes que dans l'acide acétique à 50%. Pour cette raison, l'homogénéité n'a pas encore pu être vérifiée. La teneur en métaux, exprimée en µg/100 mg de cellules ou granules lyophilisés était: Cu 7 (–); Co 4 (15,6); Fe 65 (36); Zn 25 (31); Mg 30 (25) et Ca 48 (47).

Action de l'insuline sur les enzymes de la synthèse des acides gras

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L'insuline, on le sait, rétablit la lipogenèse inhibée par le jeûne. Ce travail était destiné à voir si cette hormone exerçait cette action en augmentant la synthèse de l'acétyl CoA carboxylase et/ou de la synthétase des acides gras.

A cette fin, nous avons comparé l'incorporation d'acétyl CoA ^{14}C et de malonyl CoA ^{14}C par du surnageant (100000 g) et des homogénats de foie et de tissu adipeux de rats: témoins (à jeûn 24 h), traités à l'insuline avec ou sans actinomycine.

Dans le foie, l'insuline accroît fortement l'activité synthétasique de l'homogénat et du surnageant. L'activité de l'acétyl CoA carboxylase (pour autant que ce soit l'enzyme limitatif de la synthèse des acides gras) est légèrement stimulée par l'insuline dans l'homogénat, mais pas dans le surnageant. Aucun effet de l'insuline n'est observé sur l'activité de ces 2 enzymes dans le tissu adipeux. L'actinomycine, sans action sur les témoins, supprime les effets de l'insuline sur les enzymes du foie, suggérant que cette hormone stimule la synthèse de la synthétase des acides gras dans cet organe, mais est sans action sur les enzymes de la lipogenèse dans le tissu adipeux.

Subcellular Distribution of ^3H-labelled Transmitters in Rat Cerebral Cortex

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Cortex slices were labelled by incubation in Krebs-Henseleit buffer with ($\sim 10^{-7} M$) [^{3}H]-γ-aminobutyric acid (GABA), [^{3}H]-noradrenaline (NE), [^{3}H]-choline (Ch), [^{3}H]-glycine (Gly) respectively, and with [^{3}H]-leucine (Leu) for a control. After incubation (25 °C, 10 min) the slices were washed, homogenised and centrifuged (10 min, 200 *g*). Aliquots of the supernatent were separated by centrifugation (2 h, 60,000 *g*) on a (25 ml) discontinuous sucrose density gradient (0.25, 0.8, 1.0, 1.1, 1.2, 1.3, 1.7 m). Protein, LDH, GDH and radioactivity were determined in 1 ml fractions. The separation was checked by electron microscopy (EM). In addition to the supernatent and myelin fractions, 5 main peaks were obtained. According to EM, LDH and GDH, synaptosomes were present in different populations in the 5 fractions, but mainly in the second. Radioactivity except for Leu showed a distinct distribution. GABA and Gly showed differences in absolute activity but very similar patterns following roughly that of LDH. NE labelling was mainly in the middle and the 2 heavier fractions, while Ch was mainly in the middle and the lighter 2 fractions.

ATP Sulfurylase of Mast Cell Tumor

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ATP sulfurylase (ATP: sulfate adenylyltransferase, E.C. 2.7.7.4) of Furth mouse mastocytoma was purified by ammonium sulfate fractionation, gel filtration, hydroxyapatite and alumina chromatography, and Geon resin electrophoresis. The enzyme activity was determined by measuring ATP formation from adenylylsulfate (APS) and inorganic pyrophosphate (reverse reaction) using luciferase. The purified enzyme was found to be homogeneous by disc electrophoresis, and free of ATPase, inorganic pyrophosphatase, APS sulfohydrolase and APS kinase. The activity of the ATP sulfurylase was markedly enhanced by Mn^{++} and p-chloromercuribenzoate. Upon DEAE cellulose column chromatography of an enzyme fraction purified by ammonium sulfate fractionation and gel filtration, 2 distinct ATP sulfurylase peaks were obtained. Apparently, 2 molecular species of the enzyme carrying different net charges were separated under these conditions.

Supported, in part, by USPHS, NIH grant No. 5R01HE1596.

Vergleichende Analyse der in den eosinophilen Zellen und Granula enthaltenen Lipide

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Der mit KCl-Lösung gereinigte Lipidextrakt ergab, ausgedrückt in mg/100 mg lyophilisierte Zellen oder Granula (Werte in Klammern) folgende Analyse: N: 0,16 (0,03); P: 0,115 (0,06); Cholesterin: 1,0 (1,6); Glycerin: 0,13 (0,07); Zucker: 0,09 (0,02); Sialinsäure: 0,05 (–); Serin: 0,06 (0,05) und Äthanolamin: 0,07 (0,04). Eine 1. Kieselgel-G-Dünnschichtchromatographie erlaubte die nicht wandernden Phospholipide von den sich in Mono-, Di- und

Triglyceride, in Cholesterin und in Chol. ester auftrennenden unpolaren Lipide zu isolieren. Eine 2. DChrom. auf Kieselgel H ermöglichte die Auftrennung der Phospholipide in Lysolezithin, Sphingomyelin + Glycolipide, Lezithin, Inositphosphatide, in Serin- und Colamin-Kephaline, Cardiolipin und Phosphatidsäuren. Nach quantitativ durchgeführter DChrom. ergab die Analyse der oben erwähnten Lipidfraktionen folgende Werte: Cholesterin: 0,78 (1,1); Chol. ester: 0,31 (0,5); Monoglyceride: 0,08 (0,06); Diglyceride: 0,24 (0,2); Triglyceride: 0,58 (0,29); Sphingomyelin: 0,75 (0,2); Lezithin: 2,67 (1,0); Serinkephalin: 0,45 (0,35); Colaminkephalin: 0,84 (0,44) und Glycolipide: 0,34 (0,06).

Na and K Requirement of a Ca-Stimulated ATPase in Human Erythrocyte Membranes

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In the presence of 0.2 mM Ca^{2+} (in a medium of (mM) 100 Choline or Na or K or Li, 60 *Tris*, 0.7 Ca, 0.5 *Tris*-EGTA, 7 Mg, 175.4 Cl, pH 7.3), freeze-thawed 'white membranes' show 3 different ATPase activities: (1) A large basal activity not requiring Na or K. (2) An activity stimulated by Na or K alone. This part is negligible if Na or K are replaced by Li, it persists at [Ca] = 1 mM, is insensitive towards ouabain and vanishes at [Mg] = 0. Its affinity for K and Na is different (apparent $K_{mK} = 6 \times 10^{-3}$, $KmNa = 3.3 \times 10^{-2} M$), whereas similar maximal rates are reached at 100 mM Na or K. It is reminiscent of the membrane phosphatase activated by ATP, Ca and K, described by Pouchan et al. (BBA *173*, 151, 1969). (3) The well-known Na + K activated ATPase: the rate observed with 100 mM Na + 10 mM K exceeds the rate obtained with 100 mM Na or K alone. This increment is abolished by 10^{-4} g/ml ouabain or by 1 mM Ca.

Heterogeneity and Polymorphism of Human Liver Alcohol Dehydrogenase

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Multiple molecular forms exist for human liver alcohol dehydrogenase (ADH; E.C.1.1.1.1). At least 7 fractions can be distinguished by electrophoresis and chromatography. This heterogeneity seems to be due to 3 isoenzymes and conformers. By monomerization and subsequent reactivation it is shown that 2 fractions behave like isoenzymes with subunit composition BB and AB respectively. The catalytic properties of these isoenzymes reveal differences between the subunit A and B. Human isoenzyme BB was hybridized with horse isoenzyme AA, and the interspecies enzyme was isolated and characterized. Analogous to the conformers of horse liver ADH, the conformers of human liver ADH seem to represent random combinations of 2 conformations of subunit B (B and B') to dimers. A polymorphism of human liver ADH is known to exist. In a population an atypical variant can be distinguished from the normal by its kinetic parameters. Multiple molecular forms are also found in atypical livers. Normal and atypical isoenzym BB reveal a difference in the pH optimum (normal pH 10.8; atypical pH 8.5), previously observed as characteristic for normal and atypical liver homogenates.

Supported by Schweiz. Nationalfonds grant No. 5021.3 and U.S. PHS grant No. MH16202.

DNS-Synthese in synchronen Zellkulturen

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Die Zentrifugation in vitro gezüchteter Zellen (Mäuse-Mastocytom P-815-X2) in isotonischen Saccharose-Gradienten erlaubt eine Abtrennung postmitotischer Zellen von der Gesamtpopulation. Bei Weiterzüchtung der postmitotischen Zellen liess sich eine Synchronie der Zellvermehrung mit rhythmischer Variation der Zahl DNS-synthetisierender Zellen sowie der Mitosetätigkeit beobachten. Die Geschwindigkeit des Einbaus mehrerer Nucleoside (Thymidin-^{3}H, Uridin-6-^{3}H, Cytidin-5-^{3}H und Deoxyadenosin-^{3}H) in die DNS (bezogen auf die in DNS-Synthese befindlichen Zellen) zeigte einen gleichartigen zeitlichen Verlauf, der auf ein Maximum des Einbaus ungefähr in der Mitte der S-Phase schliessen lässt.

Mit Unterstützung des Schweizerischen Nationalfonds.

Further Chemical and Physical Characterization of Lipoproteins Derived from Mouse Skin

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Two different lipoproteins were isolated from mous skin before and after burning. Both compounds were shown to contain 50% protein w/w. The products appeared as one single band in the gradient disc electrophoresis with different migration distances. The change to an acid pH with addition of urea and mercaptoethanol showed a microheterogenicity of 6 different oligomeric peptide chains in both compounds. An identical electrophoresis at neutral pH and 0.1% SDS made it possible to calculate the mol. wt. for each chain and compute a total weight of 540,000 for the protein moieties. After protecting the –S–S– bonds from disrupting and reshuffling with iodoacetamide only 2 bands appeared representing a total mol. wt. of 270,000. The amino-acid analysis showed 17 different acids and a total of 970 residues per 100,000 g of protein in both compounds with no difference in the amino-acid composition. The ratio hydrophilic/apolar amino acids was 2.1. These results showed a surprising similarity to structural proteins in biological membranes for the components present, the microheterogenicity of the protein and the amino-acid content.

Synthèse et migration des protéines neuronales néoformées. Etude autoradiographique

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Le destin des protéines néoformées des neurones rétino-tectaux du pigeon, a été étudié par autoradiographie en microscopie optique, après injection intra-oculaire de l-Leucine H3.

Deux heures après injection un marquage sélectif des 1ère (axones rétino-tectaux) et 5^e (riche en terminaisons rétino-tectales) couches tectales contro-latérales était déjà observé. La concentration des grains au niveau de la 5^e couche culminait à 8 h, 24 h et 15–21 jours après injection. Elle était toujours précédée par une accentuation du marquage au niveau du chiasma et de la 1er couche. Ceci renforce l'hypothèse d'un flot axonal de protéines qui pourrait se décomposer en 3 phases, dont les vitesses de migration serait respectivement de 250 mm/jour, 50 mm/jour et 1 à 5 mm/jour.

La destination synaptique des protéines de la phase rapide a été démonstrée par autoradiographie en microscopie électronique. 12 h après injection de précurseur, 67% des grains de la 5e couche était retrouvés sur les terminaisons nerveuses. Au niveau de la 1re couche, 70% des grains étaient intra-axonaux.

Le marquage semblait prédominer, sur les membranes plasmatiques et le reticulum agranulaire axonaux et sur les mitochondries et vésicules synaptiques.

Travail soutenu par le fond National de la Recherche Scientifique (No. 4806 et 4356).

Indications for an Impairment of Insulin Secretion in Spiny Mice (Acomys cahirinus)

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Plasma glucose, immunoreactive insulin (IRI) concentrations and pancreatic IRI content were measured in fasted, fed and glucose injected spiny mice. Two populations were found, one with low and one with high plasma IRI concentrations irrespective of the degree of pancreatic stimulation. Pancreatic IRI content was higher than in any other known normal or hyperglycemic laboratory rodent, increased progressively with age, and was unrelated to body weight or plasma IRI concentrations. Both absolutely, and relative to pancreatic IRI content, plasma IRI concentrations even of hyperinsulinemic spiny mice were lower than those of normal Swiss mice. These data and results of electron microscopy of the B-cells prevalence of pale, confluent β-granules, lysosomal destruction of β-granules, absence of autonomous nerve endings and of signs indicative of insulin release after stimulation) suggest that even normoglycemic spiny mice may suffer from an impairment of insulin release. The possible importance of such a defect, and of the finding that spiny mice synthesize 2 structurally different insulins, for the pathogenesis of the diabetic syndrome occurring in some spiny mice is discussed.

Supported in part by grants Nos. 4848.3 and 3.154.69 Fonds National Suisse de la Recherche Scientifique.

Regulation of Pyruvate Metabolism in Rat Liver Mitochondria

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In intact rat liver mitochondria pyruvate metabolism yields acetyl-CoA and oxalacetate. Only the latter can serve as a substrate for gluconeogenesis. The channelling of pyruvate into one or the other reaction is subject to metabolic control. Octoanoate and acyl-L-carnitines inhibit the pyruvate oxidation; oleoyl-L-carnitine being the most potent inhibitor tested so far. The pyruvate carboxylation can be regulated by the adeninenucleotide levels in the matrix. Addition of an ADP generating system to the mitochondrial incubations elevated the matrix ADP and inhibited the pyruvate carboxylase (PC) in this compartment. Addition of octanoate to this system lowered the matrix ADP and released the inhibition of PC. Oleate also released this inhibition but the results indicate that the 2 fatty acids act in a different way. By these mechanisms, fatty acids direct pyruvate towards carboxylation. Physiological concentrations of ADP and deate were used in the incubations and, therefore, the observed effects offer an explanation for the stimulation of gluconeogenesis by fatty acids.

Transport de l'acide α-aminoisobutyrique (AIB) dans la cellule adipeuse isolée de souris

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Une technique permettant d'estimer simultanément la vitesse d'entrée et la vitesse de sortie de l'AIB a été mise au point. Les résultats obtenus sont les suivants: l'entrée et la sortie d'AIB se font toutes 2 principalement via un processus saturable auquel se surajoute un processus de diffusion quantitativement beaucoup moins important.

L'absence de potassium, ou de sodium, ou de calcium + magnésium a pour effet de diminuer l'entrée de l'AIB, la sortie n'étant pas modifiée. La détermination des paramètres de vitesse a été faite au «steady state» en utilisant une méthode cinétique dont la précision s'est révélée satisfaisante. Il a été ainsi possible de montrer que l'absence de potassium, ou de sodium, ou de calcium + magnésium diminuait la V_{max} d'entrée mais pas le K_m ni la constante de diffusion. D'autre part, le contenu en ATP intracellulaire était diminué par l'absence de sodium ou de potassium, étant au contraire augmenté par l'absence de calcium et de magnésium et, à un moindre degré, par la présence d'ouabaïne. La signification de ces résultats sera discutée.

Particle-Bound Aminopeptidase from Pig Kidney, a Zinc Metalloenzyme

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Particle-bound aminopeptidase contains 2 gram-atoms of firmly bound Zn per mole of enzyme of molecular weight 280,000. The protein contains a carbohydrate moeity accounting for about 20% of the molecule and consisting of glucosamine, galactose, mannose, fucose and sialic acid. During the course of purification the Zn content increases while the concentration of all other metals decreases. Metal-complexing agents reversibly inhibit enzymatic activity. Electrodialysis or treatment of the enzyme with chelating resins or metal-binding agents results in an inactive Zn-free apoenzyme. The metal-depleted enzyme can be fully reactivated by the addition of Zn. Certain other divalent metal ions can also reactivate the apoenzyme; the extent of reactivation varies as follows: Cu > Co > Zn > Ni. Though the enzyme displays more activity with Cu or Co than with Zn, it seems to bind more firmly the latter metal. Indeed, when Zn is used to reactivate the apoenzyme, amounts as small as 2 gram-atoms per mole can restore full activity.

On the Intracellular Location of Pyruvate Carboxylase in Rat Liver

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Pyruvate carboxylase (PC) catalyzes the first step of gluconeogenesis from pyruvate to glucose. The work of

Pette's group and of other authors has shown that the PC is localized inside the mitochondria. However, the results of Seubert's group indicate that also a considerable extramitochondrial PC activity exists. The observed preferential extraction of PC as compared to glutamate dehydrogenase (GDH, a mitochondrial marker enzyme) with certain media have lead to this conclusion. Our results show that under certain conditions the GDH is unstable and that therefore it is difficult to conclude as to what extent the mitochondria are ruptured by this procedure. The hypoglycemic agent 5-methoxyindol-2-carboxylic acid, which inhibits mitochondrial PC but not soluble PC, inhibited gluconeogenesis from pyruvate in the perfused liver completely, indicating that there is no extramitochondrial soluble PC activity in the liver cell. These results support the concept of an exclusive mitochondrial localization of PC.

Hämoglobin-Oxydationsintermediäre; Erklärung von Kooperativität und Bohreffekt?

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Oxydationsintermediäre vom Typ $\alpha^{h}{}_2{}^{+++}\,\beta^{h}{}_2{}^{++}$ und $\alpha^{h}{}_2{}^{++}\,\beta^{h}{}_2{}^{+++}$ werden durch Mischen der beiden Ketten im jeweiligen Oxydationszustand synthetisiert und gereinigt. Bei pH 7 ist die O_2-Affinität der beiden Moleküle viel höher als für Hämoglobin. n als Wert der Kooperativität ist 1 für $\alpha_2{}^{++}\,\beta_2{}^{+++}$ und ca. 1,2 für $\alpha_2{}^{+++}\,\beta_2{}^{++}$. Der alkalische Bohreffekt ist gleich für beide Moleküle aber der saure Bohreffekt fehlt bei $\alpha_2{}^{++}\,\beta_2{}^{+++}$. Konformationsänderungen der zweiwertigen Ketten bei Oxygenierung beziehungsweise Deoxygenierung, wurden mit ORD, Differenzspektroskopie und Reaktivitätsmessungen der SH-Gruppen in β_{93} nachgewiesen.

Das induced fit Modell von Koshland et al. beschreibt also die allosterischen Phänomene beim Hämoglobin besser als das klassische von Monod et al. Die Änderung der Quartärstruktur wird durch die Oxygenation der β-Ketten herbeigeführt.

RNA Synthesis in the Mitotic Cycle of Physarum

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The rate of RNA synthesis, measured by Ur-H^3 incorporation, shows a biphasic pattern in the synchronous mitotic cycle of *Physarum polycephalum*. In mitosis itself, and also $4^1/_2$ h after mitosis, the rate of Ur-H^3 incorporation is low. Since slight differences were detected in sucrose gradient patterns of RNA labelled at different times of the mitotic cycle, the question was asked whether different populations of RNA were synthesized at different times of the mitotic cycle. These RNAs were compared by competition-hybridization experiments. We did not find any difference among the RNA populations compared, and we conclude that there is no switch from 'early' to 'late' RNA in the mitotic cycle. Thus protein synthesis must be controlled at the translational level, at least for the most abundant genes, the products of which are the only RNA species detectable by hybridization experiments in an eukaryotic cell. This does not exclude a transcriptional control for RNA molecules which are present at low concentration and thus not detectable by the available methods.

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Effects of Salicylic and Acetylsalicylic Acid on Nervous Conduction

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Experiments in desheathed rabbit cervical vagus nerve showed, during incubation in salicylic acid in concentrations above 2 m*M*, a progressive loss of excitability, a concomitant increase in the content of inorganic P and a decrease in intracellular K; acetylsalicylic acid was ineffective in concentrations up to 5 m*M* and blocked nervous conduction at 10 m*M*. The loss of excitability during incubation in acetylsalicylic acid was not entirely due to the salicylic acid formed by hydrolysis, which amounted, after 90 min incubation, to at most 0.6 m*M*. In this preparation acetylsalicylic acid thus appears to be about 10 times less effective than salicylic acid.

Supported by FNRS grant No. 4540.

Tissue Level of Cyclic AMP in Rats Treated with β-Pyridylcarbinol or Nicotinic Acid

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In tissues of starved rats cyclic adenosine 3′,5′-monophosphate (cyclic AMP) was determined by the modified enzymatic isotope dilution method of G. Brooker et al. (Biochemistry 7, 4177, 1968). A single p.o. application of 1 mmol/kg β-pyridylcarbinol (or nicotinic acid) caused a bi-phasic change of cyclic AMP in epididymal fat, liver and brain: initially a transient decrease of about 30% occurred for 30 min followed by an increase of up to 150% of controls after 1 h. The levels returned to normal after 3–5 h. The increase of cyclic AMP was only partially related to an increased activity of adenylcyclase in liver homogenate. Cyclic AMP decreased simultaneously with

the free fatty acids (FFA) in plasma, but the subsequent increase of cyclic AMP did not parallel any further change in blood plasma, i.e. increase of FFA (rebound) and of urea or decrease of cholesterol and triglycerides. In conclusion, it is unlikely that the known changes of plasma metabolites due to β-pyridylcarbinol or nicotinic acid are in general mediated by variations of total cyclic AMP in the tissues studied.

Premières données sur le métabolisme de la vitamine K_1 tritiée chez le rat

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Nous avons utilisé pour cette étude une vitamine K_1 tritiée avec une activité spécifique de 8 μCi/mg. Nous l'avons administrée chez le rat mâle par voie intrapéritonéale et orale à raison de 20 mg/kg. Le taux sanguin est maximal à la 3e heure après administration; il est de 1,4% après injection i.p.

Le taux de liaisons protéiniques varie selon le mode d'administration: il est de 90% après injection i.p. et de 50% après administration orale. Par voie urinaire, l'élimination est de 5% en 3 jours, par voie fécale elle est de 75 à 80% en 2 jours; au niveau biliaire elle est très faible. Nous retrouvons le 60 à 70% de la radioactivité injectée. A la 3e heure, la rétention corporelle est de 10%, la substance radioactive se localise dans le foie et l'estomac.

L'autoradiographie chez la souris montre une localisation de la radioactivité au niveau du foie, du système digestif, et de la vessie, parfois faible au niveau du poumon et du testicule. Nous observons donc une rétention corporelle réduite et une élimination rapide. En outre, nous détectons la formation d'eau tritiée éliminée par voie urinaire.

Cette étude préliminaire a pour but de déterminer la dose de radioactivité à injecter chez l'homme.

Slow Synaptic Potentials in the Cat Sup. Cervical Ganglion (SCG) in situ

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Slow synaptic potentials evoked by the action of presynaptically released ACh on postsynaptic sites resistant to hexamethonium (C_6) and d-tubocurarine (d-TC) are recorded extra- and intracellularly from various sympathetic ganglia in vitro (Eccles and Libet 1961; Dunant and Dolivo 1967; Koketsu 1969).

In the cat SCG in situ, P- and LN-waves (positive and late negative) can be recorded from the ganglion surface in response to orthodromic stimulation after blockade of nicotinic receptors with C_6, d-TC, TEA, and nicotine. In most preparations, P- and LN-waves are too small to be detected in response to a single volley but they appear regularly after trains of repetitive stimuli (summation). During the LN-wave, low amplitude asynchronous discharges are conducted in the postganglionic nerve and represent impulses transmitted through the 'muscarinic' pathway. During the P-wave, discharges elicited for example by KCl and pilocarpine are depressed. Slow waves and late discharges are altered by various substances. The muscarinic pathway is relatively unimportant in the cat SCG as compared with other sympathetic ganglia.

Effect of Isoproterenol (IS) on the Membrane Potential (MP) of Vascular Smooth Muscle (VSM)

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The direction of MP changes produced by IS in avian slow muscle varies with external potassium (K_o) and hyperpolarization is blocked by ouabain (Somlyo and Somlyo, Fed. Proc. *28*, 1634, 1969). Measuring MP with intracellular microelectrodes in a mammalian VSM (rabbit main pulmonary artery) a similar effect of IS was found. IS (0.2 μg/ml) hyperpolarized this VSM in 1.0 m*M* K_o (59.1–67.0 mV, $p < 0.01$, $n = 10$) and depolarized it in 10.0 m*M* K_o (50.5–44.8 mV, $p < 0.01$, $n = 7$). IS caused no change when K_o was 5.9 m*M*. Pronethalol (10 μg/ml) and oligomycin (5 and 10 μg/ml) blocked the hyperpolarizing effect of IS. A lower concentration of IS (0.12 μg/ml), which by itself did not produce hyperpolarization increased the MP (59.6–67.6 mV, $p < 0.001$, $n = 8$) when the arterial strips had previously been exposed to theophylline (4.0 m*M*) for 30 min. Theophylline alone did not change the MP.

The β-adrenergic effect of IS on the MP of vascular smooth muscle cells of the rabbit main pulmonary artery depends on K_o; IS-induced hyperpolarization is potentiated by the phosphodiesterase inhibitor theophylline and blocked by the Na+K-ATPase inhibitor oligomycin.

Supported by NIH K3-17, 833, HE08226, NSF GB7188 and the Harrison Fund for Gynecological Research.

Morphine-Induced Changes in Catecholamine Content of Nerve Cells in Mouse Brain

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In mouse brain prepared according to the histochemical fluorescence method of Falck and Hillarp, the intensity of the catecholamine fluorescence was measured in nerve cell bodies of substantia nigra, ventromedial tegmental area and reticular formation of the midbrain. Groups of 5 male mice were given 40 mg/kg of morphine s.c. and sacrificed 20 or 60 min later. As compared to controls, which had received the same volume of saline, morphine-treated animals showed an increase in the mean intensity of the catecholamine fluorescence in the nerve cell bodies, which was significant in the substantia nigra and the ventromedial tegmental area after 20 min and in the reticular formation after 60 min ($p < 0.01$). Thus, changes in amine concentration are found both in dopamine containing cell groups and in probably mainly noradrenaline containing ones (midbrain reticular formation) at a comparatively early time after morphine administration. They may indicate changes in the activity of the respective neurons.

Supported by grant No. 4563, Swiss National Foundation for Scientific Research.

Effects of Nephrectomy and Ureteral Ligation on Development of Methylacetamide Diabetes

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Single lethal doses of N-monomethylacetamide (NMMAA) induce severe diabetes in normal rats: the blood-sugar level increases steadily until death[1].

In animals nephrectomized before drug administration, an initial moderate rise in blood sugar was followed by a fall toward normal values. However, the glucose tolerance of the animals remained poor.

When nephrectomy was performed 2 h after NMMAA administration, the same decrease in blood-glucose level after a transient rise was observed. On the other hand, ligation of both ureters 2 h after NMMAA did not prevent a high drug-induced hyperglycemia.

These findings suggest a role of the kidney, independent of its excretory functions, in the development of NMMAA diabetes.

[1] R. Guidoux, Diabetologia *5*, 11–21 (1969).

Supported by Fonds National Suisse de la Recherche Scientifique, grant No. 5316.

Pharmacological Modifications of Benzoquinolizine-Induced Geniculate Spikes

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Monophasic spikes have been observed in the lateral geniculate nucleus after reserpine injection in the cat: they are suppressed by 5-hydroxytryptophan (5-HTP) and slightly increased by 3, 4-dihydroxyphenylalanine (DOPA) (Delorme, Jeannerod and Jouvet, 1965). We have studied the effect of i.v. cumulative doses (mg/kg) of precursors of biogenic monoamines and of some psychotropic drugs on the geniculate spikes (GS) induced by the reserpine-like compound Ro 4-1284 (20 mg/kg i.p.).

L-Tryptophan (1–100), DL-5-HTP (0.1–10), LSD (0.01–0.3), imipramine HCl (0.1–3), amitriptyline HCl (0.1–3), cocaine HCl (0.1–3) and (+ –) amphetamine sulphate (0.1–10) decrease the frequency of GS. L-DOPA (0.1–10) and chlordiazepoxide HCl (0.1–10) increase the frequency of GS.

The effects of all the compounds increase with increasing doses. The frequency of GS is not affected significantly by atropine sulphate (0.01–0.3), chlorpromazine HCl (0.1–10), hexobarbital Na (0.1–10), pheniprazine (0.1–10).

[1] F. Delorme, M. Jeannerod and M. Jouvet, C. r. Soc. Biol. *159*, 900–903 (1965).

Ovalicin and Other Drugs in Experimental Allergic Encephalomyelitis (EAE)

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Rats, rabbits and dogs received bovine spinal cord emulsified in complete Freund's adjuvant, i.c. Daily oral treatment was started on the day of immunization: in rats 5 days a week for 2 weeks and 3 days in the 3rd week, in rabbits 5 days a week for 4 weeks, in dogs 6 days a week for 5 weeks. Paralysis was seen in 65–100% of the controls. DE-50-dose preventing permanently signs of EAE in 50% of test animals compared to controls. DL-50/DE-50 (therapeutic index) in rats treated p.o. were as follows: ovalicin 120/6.0 (20), cyclophosphamide 11/1.2 (9.2), azathioprine 30/3.0 (10), 6-mercaptopurine 28/20 (1.4), procarbazine > 30/30 (DL-50 determined with treatment schedule indicated above). 30–100 mg/kg ovalicin inhibited completely the manifestation of EAE. In rabbits, doses of 1–5 mg/kg had only a temporary effect, animals became paralyzed after stopping treatment. In dogs a dose of 2 mg/kg produced a strong delay in the manifestation of the EAE, and no animals became paralytic after stopping treatment.

Concentrative Transfer into Bile of a Lipophilic Drug and its Uptake into Bile Micelles

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Rats were given intraperitoneal injections of imipramine. During the following hours a large portion of imipramine-like material can be detected in the small intestinal contents of intact or sham-operated animals or in the bile of fistula rats. Comparable amounts are obtained in the bile of isolated perfused rat livers. Besides large amounts of glucuronides the bile also contains unchanged imipramine. Its bile/plasma concentration ratio is in the order of magnitude of 50. In an attempt to elucidate this concentrative transfer both bile from imipramine-treated animals and micellar model systems containing bile salts, phospholipid and the drug were subjected to equilibrium dialysis and to ultracentrifuge sedimentation. Both techniques revealed that the lipophilic molecules, imipramine and desmethylimipramine, but not the conjugated and other polar metabolites are incorporated into the bile micelles. A possible participation of biliary proteins or non-micelle-forming small molecules could be excluded by experiments with predialyzed bile and by its dilution beyond the critical micellar concentration.

Unterschiede in der Reaktion von Widerstands- und Kapazitätsgefässen

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An Katzen mit offenem Thorax wurde aus dem li. Vorhof der venöse Rückfluss in ein Reservoir (R) abgeleitet und daraus ein konstantes Volumen in die Aorta desc. gepumpt. Der Druck in der Bauchaorta und das Blutvolumen im R wurden fortlaufend registriert. Unter diesen Bedingungen entsprechen Veränderungen des arteriellen Blutdrucks (BD) direkt Veränderungen des peripheren Gesamtwiderstands; Änderungen des Volumens (V) im R beruhen auf Blutverschiebungen aus dem Niederdrucksystem in das R oder aus dem R in das Tier. Die Wirkungen von Acetylcholin (Ach, 0,1–1,5 γ/Tier), Isoprenalin (Iso, 0,5–5,0 γ), Adrenalin (Adr, 1–12 γ) und Noradrenalin (Nor, 1–9 γ) i.v. auf BD und V wurden untersucht. 0,8 γ Ach senkten BD um 34 mm Hg und veränderten V nicht ($n = 8$). 3 γ Iso senkten BD gleich stark, steigerten jedoch V um $7{,}8 \pm 0{,}7$ ml ($n = 7$). Auf BD aequieffektive Dosen (+ 40 mm Hg) von Adr und Nor (12 bzw. 3 γ) steigerten V um $13{,}4 \pm 1{,}1$ bzw. nur um $4{,}6 \pm$

0,5 ml (*n* je 4). Diese Differenzen werden durch eine unterschiedliche Beeinflussung von Widerstandsgefässen und Niederdrucksystem erklärt.

Subcellular Storage of Nucleotides

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Subcellular organelles storing biogenic monoamines, e.g. 5-hydroxytryptamine in blood platelets and catecholamines in adrenal medulla show considerable concentrations of adenosine- and guanosine-5′-triphosphate (ATP, GTP). On incubation of isolated blood platelets of rabbits with ^{14}C-adenine or ^{14}C-adenosine, but not with ^{14}C-ATP, a marked accumulation of ^{14}C-ATP occurs in the 5-hydroxytryptamine storage organelles. Similarly, platelets incubated with ^{14}C-guanosine or ^{14}C-guanine accumulate ^{14}C-GTP in the organelles.

Analytical ultracentrifugation of aqueous solutions of ATP or GTP shows that bivalent but not monovalent cations induce a marked temperature and concentration dependent increase of the apparent molecular weights (up to several hundred thousands). From homogeneous aqueous solutions of ATP or GTP plus bivalent cations, a second, highly viscous and transparent phase may spontaneously separate, in which ATP and GTP respectively are accumulated.

It is concluded that in blood platelets ATP and GTP are synthesized outside, but stored within the organelles, where, due to the presence of bivalent cations, the nucleotides form aggregates of high molecular weight.

Distribution, élimination et métabolisme du chlorhydrate de Clobenzorex-7-^{14}C

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Le chlorhydrate de (+)-N-(*o*-chlorobenzyl) α-méthylphénéthylamine (chlorhydrate de Clobenzorex) est un nouvel anorexigène de synthèse. Les expériences sont faites chez le rat à l'aide du produit marqué au ^{14}C (17,2 mCi/m*M*). *Distribution au niveau des organes*: La radioactivité retrouvée dans le cœur, la rate, les poumons, le cerveau, les reins, est toujours très faible. Par contre, elle est importante au niveau des intestins après administration p.os ou i.p. La radioactivité au niveau du foie reste constante et égale à 4%. Trois heures après administration orale, 12% de la dose injectée sont présents dans l'estomac, et seulement 0,8% après injection i.p. *Elimination*: 70% de la radioactivité administrée est éliminée en 24 h: 6/7 dans les urines et 1/7 dans les selles. 0,03% est expirée pendant 8 h sous forme de $^{14}CO_2$. *Métabolisme*: Le taux sanguin du médicament est maximum 5 mn après l'injection et décroît rapidement. Les principaux métabolites des urines de 24 h correspondent aux conjugués de dérivés du Clobenzorex et de la *p*-hydroxyamphétamine, à l'amphétamine, à la *p*-hydroxyamphétamine, à l'acide hippurique. Le médicament excrété par la bile, sous forme de produits conjugués, subit un cycle entérohépatique au moins partiel. La radioactivité des selles se répartit en produit inchangé et dérivés conjugués.

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Studies of T4D Phage Internal Proteins

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Three basic structurally unrelated internal proteins of relatively low molecular weight have been isolated from the T4D phage particle. Many molecules of each protein are located within the phage head, probably in association with the DNA, and together with three proteins which form the capsid shell, comprise most of the head structural proteins. The purified proteins were characterized by physicochemical and immunological techniques; a radio immuno assay allowed measurement of their synthesis in phage infected bacteria. Each appears to be synthesized at both early and late times after infection. Their structural genes, shown to be present in the phage genome, are not among the known amber mutant-containing genes of T4D, but their genetic origins are apparently unrelated to those of the internal peptides. Puls-chase experiments with two of these proteins show that they are incorporated into certain defective T4D heads. Whether or not they are incorporated appears to depend on the degree of completion of these heads, perhaps with respect to DNA packaging.

Specimen Preparation Method for Dark-Field Electron Microscopy of Biomacromolecules

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High resolution and excellent contrast are obtained with dark-field electron microscopy provided very clean and thin (< 200 Å) specimen are used. We have rendered carbonfilms positively charged by submitting it to a glow discharge in amylamine. Nucleic acids and acid proteins are selectively adsorbed. They are randomly disposed and escape flow artefacts currently observed on normal films. On slightly uranylacetate stained DNA, we have made length measurements and found the standard error to be about half that obtained with Kleinschmidt's method. DNA appears in its real width. Therefore, the method is particularly adapted to study protein-nucleic acid interactions as exemplified on the DNA dependant RNA-polymerase. Contrast as limiting factor is replaced by beam-destruction and fine structure of the supporting carbonfilm.

Autoradiographie der enterochromaffinen Zellen der Ratte

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Die häufigste unter den endokrinen Zellen des Magendarmtraktes ist die enterochromaffine Zelle (EC-Zelle). Ihre Funktion soll die Bildung von Serotonin sein. Wir versuchten mittels ultrastruktureller Histoautoradiographie die Frage zu klären, ob die EC-Zelle zur Serotoninsynthese spezialisiert ist. 15 Minuten bis 27 Stunden nach Injektion von tritiummarkiertem Tryptophan (Try), 5-Hydroxytryptophan (5-HTP) oder Serotonin (5-HT) wurden die Ratten perfusionsfixiert und für die ultrastrukturelle Histoautoradiographie aufbereitet. Die radioaktive Substanz ist nach Injektion von Try und 5-HTP eingebaut, während 5-HT nicht in die EC-Zellen aufgenommen wird. Durch Try sind andere Zellen schwach markiert, was auf einen Einbau in Proteine bezogen wird. In den EC-Zellen dürfte ein Eingang des Try in die Serotoninsynthese erfolgen. Durch 5-HTP werden neben den EC-Zellen die polypeptidhormon-bildenden Zellen markiert, die anscheinend zur Decarboxylierung befähigt sind. 5-HT selbst wird von den EC-Zellen nicht aufgenommen. Aus den Versuchen ergibt sich, dass die EC-Zelle für die Serotoninsynthese spezifisch ist, denn sie kann die Vorläufer des Serotonins einbauen aber nicht das Stoffwechselendprodukt selbst.

Mit Unterstützung der Foundation Sandoz.

Réactions «de type Feulgen» en microscopie électronique

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En microscopie photonique, une hydrolyse acide ménagée permet de colorer les structures contenant du DNA à l'aide du réactif de Schiff ou d'autres colorants basiques, dits «de type Schiff», qui comprennent tous un aminogroupe primaire (F. H. Kasten, Int. Rev. Cytol. *10*, 1, 1960; G. Prenna, Mikroskopie *23*, 150, 1968). L'intérêt que présenterait une technique analogue *en microscopie électronique* est évident. Nous avons cherché dans ce but des colorants «de type Schiff» livrant une augmentation de contraste suffisante pour être aisément décelable, ce qui n'est pas le cas du réactif de Schiff. *L'Acriflavine* décolorée par SO_2 peut être ainsi employée, après hydrolyse, dans une réaction «de type Feulgen» sur des coupes de tissus fixés par un aldéhyde et inclus en plastique. Il semble en être de même pour certains produits de dégradation du *Rouge de Ruthénium* décoloré par SO_2, utilisés en conditions analogues. Des hydrolyses chlorhydriques et perchloriques à concentrations et températures variables permettent l'étude critique des résultats obtenus.

Modifikation des Zellteilungszyklus in metabolisch limitierten Zellkulturen

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Durch Kombination von mikrofluorometrischer DNS-Bestimmung und Autoradiographie nach Inkubation mit Thymidin-^{3}H wurde in «steady state»-Kulturen des Mäuse-Mastocytoms P-815-X2 die relative Häufigkeit von G_1-, S-, G_2-Zellen und Mitosen bestimmt. Zellkulturen unter optimalen Wachstumsbedingungen wurden mit solchen verglichen, die infolge Limitierung der Konzentration einer Komponente des Mediums (Glucose, Leucin, Serum) oder durch Zugabe eines Hemmstoffs (Actinomycin D, Amethopterin) eine verlängerte Generationszeit und Zellzahlverdoppelungszeit aufwiesen. Unter optimalen Bedingungen fand sich folgende Verteilung der Zellen: G_1-Phase 31%, S-Phase 55%, G_2-Phase 12%, Mitose 2%. Zellzahlverdoppelungszeit und Generationszeit betrugen ca. 10,5 Std. Bei Zusatz von Actinomycin D oder Limitierung von Glucose oder Leucin war die relative Häufigkeit der G_1-Zellen auf 43% bis 74% erhöht, diejenige der S-Zellen, G_2-Zellen und Mitosen erniedrigt. Die Zellzahlverdoppelungszeit betrug 15 bis 25 Std.

Mit Unterstützung des Schweizerischen Nationalfonds.

Nucleotide Sequence Analysis of Qβ RNA: The Sequence at the 3′ End

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We have previously described a method for determining the nucleotide sequence of segments of viral RNA prepared in vitro by synchronized enzymatic synthesis. The nucleotide sequence of the 5′ terminal region of Qβ RNA comprising 175 nucleotides, has been reported (Nature 224, 1083, 1969). We have now prepared labeled segments of the 5′ terminal region of the Qβ RNA *minus strand* by an analogous procedure. The nucleotide sequence of a segment of about 150 nucleotides is being determined. Since the 5′ end of the minus strand is complementary to the 3′ end of the plus strand, it will be possible to deduce the sequence of the latter.

Supported by the Schweizerische Nationalfonds and the Jane Coffin Childs Fund.

Fragilité des structures phagiques

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Les têtes produites par les mutants amber du bactériophage T4 se divisent en 2 groupes:

- Les têtes fonctionnelles par exemple 10$^-$ qui complémentent in vitro avec des queues. Cependant, même dans ce cas favorable, seules 1 à 5% des têtes produites sont actives dans ce test; une modification de la technique est présentée qui porte ce chiffre autour de 30 à 50%. La vulnérabilité de ces têtes à divers agents physiques et chimiques a pu être montrée. Morphologiquement ces têtes apparaissent pleines après une fixation appropriée.
- D'autres têtes, celles produites par les mutants du groupe X apparaissent peu ou pas compétentes. Les phages produits par ceux de ces mutants qui sont «leaky» ne semblent pas plus fragiles que les sauvages. Au microscope, même après les préparations les plus soigneuses certaines de ces têtes sont vides.

La fragilité variable de ces particules pourrait expliquer leur compétence différente.

Quantitative Bestimmung humoraler Anti-Peroxydase-Antikörper

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Im Hinblick auf die weitverbreitete Verwendung von Meerrettich-Peroxydase als Antigen in immunohistochemischen Untersuchungen kommt der Möglichkeit einer quantitativen Antikörperbestimmung grosse Bedeutung zu. Die Enzymaktivität der Peroxydase bleibt nach Kombination mit spezifischem Antikörper erhalten. Die Peroxydase-Aktivität in der Globulinfraktion eines mit einer bekannten Enzymdosis versetzten Antiserums entspricht deshalb der Menge pro Volumeneinheit des Serums gebundenen Antigens. Primäre und sekundäre Antikörperproduktion in der Maus nach Stimulation mit verschiedenen Peroxydase-Präparaten werden verglichen.

Mit Unterstützung des Schweizerischen Nationalfonds.

Localisation of the Qβ Coat Protein Cistron on Qβ RNA

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Ribosomes bound to $Q\beta$ RNA under conditions of initiation of protein synthesis attach predominantly to the beginning of the coat protein cistron and render this segment of RNA resistant to degradation by pancreatic RNase (Hindley and Staples, Nature *224*, 964, 1969). In order to determine the location of this segment, a collection of full-length $Q\beta$ RNA strands, radioactively labeled for various lengths from the 5′ end was prepared enzymatically. Ribosomes were bound to the partially labeled molecules and the RNA segment corresponding to the binding site was isolated. Only those molecules which had been labeled for about $^1/_3$ of their length yielded a labeled RNA segment. It is therefore very probable that the coat protein cistron is the second cistron on the genome.

Supported by the Schweizerische Nationalfonds, the Jane Coffin Childs Fund and the British Science Research Council.

Uptake and Retention of Particles from the Intestine by Peyer's Patches in Mice

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The uptake of particulate material from the lumen of the intestine was studied in young adult mice given India ink via a stomach tube. At various time intervals following the administration of India ink, Peyer's patches (P.P.) and sections of ileum between P.P. were fixed, embedded in Epon, sectioned at 1 μ, and examined for the presence of carbon particles.

Within 6 h after administration, carbon particles were detected in the epithelium covering P.P. and, to a lesser extent, in the epithelium of intestinal villi. At later time intervals, accumulations of particles were observed in phagocytic cells of the reticular zone beneath the P.P. epithelium. Macrophages containing carbon remained visible in P.P. for at least 1 week after the last dose of India ink, a time when no particles could be found in intestinal villous structures. Similar studies were performed in newborn mice.

Mit Unterstützung durch den Schweizerischen Nationalfonds.

Mol. Wt. Differences of Geneproduct P23 in Headrelated Variants of Phage T4

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Genetics, Urea-gelelectrophoresis and serology show that the major component of all the morphological head variants of phage T4 is P23 (Kellenberger, Virology *34*, 549; Laemmli et al., J.M.B., in press; Laemmli, thesis 1498, Geneva; Hosoda et al., Virology *34*, 709). By SDS-gelelectrophoresis (Weber et al., J.B.C. *244*, 4406) the subunit derived from the tubular variant gives a M.W. of 61,000, while the one derived from capsids is of 49,000, in agreement with 46,000 from equilibrium centrifugation in Guandine-HCl (Larcom, thesis, Pittsburgh).

Without P31, geometrically all-defined 'lumps' of P23 are produced (Laemmli et al., J.M.B. *47*, 69). Its major protein is also 61,000.

Most plausible hypothesis: the polypeptide P23 is reduced in its length prior or simultaneously to the assembly into capsids.

In vitro Synthesis of Mutagenized Phage RNA Containing Diaminopurine

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A novel method has been developed for the preparation of RNA phage mutants. This method involves the synthesis in vitro of RNA using $Q\beta$ replicase with $Q\beta$ RNA as template, in the presence of the base analogue, 2, 6-diaminopurine triphosphate, which is incorporated into the RNA in place of ATP. Full-length viral RNA was isolated, introduced into spheroplasts and the resulting phages were examined for temperature sensitive mutants. Under conditions where 6.8% of the A was replaced by diaminopurine (DAP), 11 mutants were found out of 1053 phages examined; no mutants were found among 1199 progeny prepared in a similar fashion, however, without use of diaminopurine triphosphate. Among 1100 particles originating from normally propagated phage, one mutant was found.

Supported by the Schweizerische Nationalfonds, the Jane Coffin Childs Fund and EMBO.

Structure and Function of Different Kinds of Ribosomal Subunits from Mouse Plasmocytoma

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An understanding of the mechanisms of protein synthesis in animal cells requires a better knowledge of the structure and function of the subunits (s.u.) of animal ribosomes. A systematic study of the conditions of dissociation of mouse plasmocytoma ribosomes into s.u. was undertaken, either when Mg^{++} was gradually lowered or when KCl was increased. This has permitted the preparation of new kinds of s.u., under conditions milder than those previously reported. Physico-chemical studies of derived and native s.u. showed differences in sedimentation coefficients and the absence of 2 distinct ribosomal proteins in the high KCl s.u.

Whereas low Mg^{++} and native s.u. could not reassociate spontaneously into monosomes, complete reassociation

was obtained with KCl s.u. The biological activity of the different s.u. preparations was tested in a polyU-directed protein synthesis system: Low Mg^{++} s.u. are all inactive, 0.8 *M* KCl and native s.u. are moderately active and 0.3 *M* KCl s.u. are considerably more active. These s.u. could also be prepared on a large scale by zonal centrifugation, starting with 0.5 g of ribosomes.

Supported by the Fonds National Suisse.

Les cellules glycoprotidiques de type I de l'adénohypophyse du crapaud Bufo bufo L.

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Les cellules les plus typiques de la *pars distalis* de l'hypophyse du Crapaud sont les cellules glycoprotidiques de type I (Doerr-Schott 1965, Mira-Moser 1970). Elles sont caractérisées par leur grande taille et par la présence de volumineux granules à côté de grains de sécrétion plus fins. On pense que ces cellules sont responsables de la sécrétion de l'hormone gonadotrope FSH.

Les granules de grande taille ressemblent à des lysosomes secondaires et contiennent des structures myéliniques, des microvésicules ou des granules denses. Certains ressemblent à ceux décrits par Smith et Farquhar (1966) dans les cellules LTH de l'hypophyse de Ratte allaitante et par Orci et coll. (1968) dans les cellules A du pancréas endocrine de l'Acomys diabétique. Ces organites sont également le siège d'une activité phosphatasique acide.

La fonction de ces lysosomes reste, dans le cas particulier, encore à déterminer. Peut-être pourraient-ils intervenir, comme dans d'autres types de cellules endocrines (Smith et Farquhar, Orci et coll.), dans la régulation de la sécrétion hormonale.

Travail réalisé grâce au Fonds National Suisse.

Glycogen-Loaded Lysosomes in Renal Tubular Cells in Experimental Diabetes

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Renal tubular glycogen infiltration is regularly seen in human and experimental diabetes. In a systematic study of an experimental model of diabetes mellitus with genetic and environmental components (Acomys Cahirinus), the ultrastructural aspects of 'glycogen nephrosis' were studied. This has led to the discovery of lysosomes filled with glycogen particles. Such structures have thus far not been observed in diabetic organisms but are regularly seen in liver in type II glycogenosis and in newborn rats or mice. In these instances, either a permanent deficiency in acid α-glucosidase (glycogenosis) or a physiological, transient deficiency in glycogenolytic enzymes (newborn rats and mice) may be the cause of lysosomal glycogen accumulation. The mechanisms responsible for its occurrence in diabetes are unknown. They may involve relative enzyme deficiency in a situation where hyperglycemia, glucosuria and tubular gluconeogenesis contribute to the excessive availability of glucose, or to impaired glycogen disposal secondary to insulin deficiency, although the glucose transport system of tubular cells is not insulin sensitive.

Supported by grant-in-aid from the Fonds National Suisse de la Recherche Scientifique.

Komplex zwischen Glucose-6-phosphat-dehydrogenase und einem ACTH-Derivat

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N^{ε}-Dansyllysin^{21}-ACTH-(1-24)-tetrakosipeptid wurde synthetisch hergestellt. Die Verbindung besitzt dieselbe maximale Wirkung auf isolierte Fettzellen der Rattenepididymis (Lipolyse) wie Synacthen® (ACTH-(1-24)-tetrakosipeptid) und zeigt dieselbe Calcium-Abhängigkeit dieser Wirkung; die Form der log-Dosis/Wirkungs-Kurve ist fast identisch, nur sind etwa 20mal höhere Dosierungen nötig (pharmakologische Versuche von Dr. Peter Bally, Bern). Dagegen besitzt die Verbindung eine nur sehr kleine steroidogene Wirkung in Nebennieren der Ratte (Mitteilung von Dr. Werner Rittel, Basel).

Eine $1{,}1 \cdot 10^{-7} M$ Lösung von N^{ε}-Dansyllysin^{21}-ACTH-(1-24)-tetrakosipeptid in Acetat-Puffer (0,3 *M*, pH = 5,5) weist eine sehr kleine experimentelle Fluoreszenz-Polarisation, p_{min}, von nur 0,075 auf (schnelle Brown'sche Rotation). Bei der Titration mit Lösungen von kristallinen Glucose-6-phosphat-dehydrogenase (G6PD) aus Rinder-Nebennieren (Geschenk von Prof. P. G. Squire, For Collins, Colorado) nimmt p zu, bis p_{max} von 0,23 bei etwa 30fachem Enzymüberschuss erreicht wird. Die Scatchard-Auftragung zeigt nur geringe experimentelle Streuung; ein grosser, signifikanter Bereich von $\bar{s}$ (dem durchschnittlichen Sättigungsgrad) ist gedeckt. Die Ergebnisse zeigen, dass G6PDH für dieses ACTH-Derivat entweder eine Bindungsstelle mit einer Assoziationskonstante oder mehrere mit derselben Assoziationskonstante ($1{,}5 \cdot 10^{6}$) besitzt.

Evolution of Phosphagen Kinases in Chordates

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Phosphagen kinases appear to be homologous enzymes. Creatine kinase, a phosphagen kinase typical for vertebrates may have evolved from a phylogenetically older precursor enzyme, probably from an arginine kinase-type enzyme as found in most invertebrates. Creatine Kinase exists in mammals as isoenzymes; probably the results of gene duplication.

Animals of various vertebrate classes differ considerably in the DNA-contents of their genomes. One therefore might expect that a greater divergence of multiple molecular forms of enzymes would arise if the difference in DNA-contents is a result of genome duplications. However investigations on creatine kinase isoenzymes in chordates show the mammalian pattern already existing in reptiles, amphibians, teleostei and sharks although most of them have lower DNA-contents than mammals.

Notable exceptions were observed among birds and some species of teleostei which show very complex patterns of creatine kinase isoenzymes inspite of relatively low DNA-contents. In Amphioxus on the other hand only one creatine kinase was found in supernatant fractions of several tissue homogenates. So far no phosphagen kinases other than creatine kinase were observed in some 300 species of vertebrates.

Grants Nos. 113F, Hoffmann-La Roche Stiftung, and 3.165.69 Schweiz. Nationalfonds.

Étude au microscope électronique de mutants du coliphage T4

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Lors de la réplication du bactériophage T4, une cinquantaine de gènes contribuent à la synthèse et l'assemblage des divers constituants protéiques de l'enveloppe du phage, et l'assemblage final en un phage complet et actif. Un défaut dans l'un quelconque des 8 gènes groupés sous le nom de gènes X cause l'apparition dans les lysats non plus de phages complets mais de queues et de têtes séparées, les têtes étant incapables d'être complétées *in vitro* pour donner des phages actifs.

Des études détaillées sont menées par ultramicrotomie, par lyses sur place et par étude globale des lysats pour subdiviser ce groupe X en gènes qui agissent très tôt dans la formation de la tête du phage, et en gènes plus tardifs qui assurent la stabilité d'une structure déjà assemblée. On observe que dans certains cas les têtes qui apparaissent vides dans les lysats sont pleines à l'intérieur des bactéries et que dans d'autres cas les têtes et les queues ne sont pas complètement dissociées.

Réponse immunologique primaire accelerée par des complexes antigène-anticorps

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Les complexes spécifiques peroxydase du raifort (HRP)-anticorps anti-HRP du mouton ont été précipités dans la zone d'équivalence. Après une injection dans le coussinet plantaire de la souris, la distribution topographique du complexe hétérologue puis de l'anticorps anti-HRP néoformé a été suivie dans le temps en microscopies conventionelle et électronique au niveau du ganglion poplité. Dans ce système expérimental, comparé aux résultats obtenus après une injection d'antigène seul, *l'apparition plus précoce* et des centres germinatifs néoformés et des cellules plasmocytoides spécifiques a pu être mise en évidence. Ces résultats, observés après une injection du complexes pré-formés, suggèrent le developpement dans le temps de réactions cellulaires semblables à une réponse de type secondaire.

Avec l'appui du Fonds National Suisse.

Multiplicity of tRNAs in Mammalian Tissues

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By partition and reverse phase chromatography three serine tRNAs from rat liver could be separated. Their coding properties corresponded to UCU, UCC and UCA for serine tRNA I, UCG for serine tRNA II, AGU and AGC for serine tRNA III, respectively.

Sequence studies on the primary structures of serine tRNA II and III indicated that they differ from serine tRNA I (Staehelin et al., Nature *219*, 1363, 1968) only by modifications in the anticodon region and the double helical parts of the molecule. Sequence studies of serine tRNA II showed that it consists of two species with different base sequences in the stem region. One of them contained the anticodon CUG and the other one the same trinucleotide sequence in a modified form. Similarly serine tRNA III was found to consist of a mixture of species differing only on the stem region. Multiple tRNAs coding for the same trinucleotide, but differing in their primary sequence seem to exist, therefore, in mammalian cells.

Gene Product Position in T4 Phage

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Electron microscopic examination of T4D phage particles after reaction with an anti-T4D serum showed a visible layer of anti-body molecules coating the surface of the phage particles. A procedure was developed for determining the positions of individual gene products in the phage structure: an antiserum specific for a given gene-product was prepared by depleting the anti-T4 serum with a lysate of non permissive bacteria infected with the corresponding amber mutant. The association of the remaining antibodies with the homologous antigen in the particle was detected by electron microscopy. This procedure identified the positions of seven gene products in different parts of the T4D particle: head proteins (gene 23), tail sheath proteins (gene 18), distal-half fibers (gene 37), joining parts of the two half fibers (gene 36), proximal half fibers (gene 34), structures located at the bottom of the base plate (gene 12) and structures in the region of the head-tail junction. This latter structure seems to be under the control of gene 49.

CONGRESSUS

The Netherlands Symposium of the International Atomic Energy Agency IAEA

in Rotterdam 31 August–4 September 1970

The Symposium will be concerned with dynamic studies with Radioisotopes in Clinical Medicine and Research. Scientific Secretaries: Dr. T. Nagai and Dr. E. H. Belcher, Internat. Atomic Energy Agency, Kärntnerring 11–13, P.O. Box 590, A-1011 Wien (Austria).

France
La 21ème réunion annuelle de la Société de Chimie physique

à Paris du 22 au 25 Septembre 1970.

Elle sera consacrée à une discussion sur le sujet de l'Etat de Transition réactionels: modèles généraux, systèmes hydrocarbonés.

Pour tous renseignements, s'adresser au Secrétariat Général de la Société de Chimie physique, 10, rue Vauquelin, F-75 Paris 5e (France).