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PHYSIOLOGIE – PHYSIOLOGY

Evaluation of Thermodilution Technique for Measuring Coronary Sinus Blood Flow

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In hydraulic non-occlusive models mixing effects were studied with slug-injection and constant-infusion technique. Measurements were accomplished by specially constructed catheters with thermistors. Constant-rate infusion of 5 ml/min of saline at room temperature with an infusion velocity of 2–5 m/sec at the catheter orifice showed homogeneity of mixing. The distance between catheter orifice, diameter 0.15–0.2 mm, and thermistor was 10–15 mm. The thermistor was carefully isolated to prevent direct influence of cold passing through the wall of the infusion catheter. Good correlation ($r = 0.939$) was found between measured flow and reciprocal change in temperature in the range from 50 to 450 ml/min. In anesthetized open chest dogs coronary sinus blood was drained through the right atrium into the jugular vein so that volume flow could be measured by graduated cylinder and stop-watch. An infusion catheter with a mounted thermistor was inserted through the drainage into the coronary sinus. In the range of 50–450 ml/min directly measured coronary sinus outflow and the reciprocal of the simultaneously registered changes in temperature showed a linear correlation ($r = 0.946$).

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Effect of Blocking Na-K-Pump or Metabolism on PO_4 Influx into Nerve Fibres

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The effect of ouabain and metabolic inhibitors on the influx of PO_4 into desheathed rabbit vagus nerves was investigated using $^{32}PO_4$. The $^{32}PO_4$ uptake by nerves incubated in Tyrode reached saturation after 15 min. The uptake was reduced by about 20% when ouabain was added to Tyrode, or when iodoacetic acid or 2-deoxyglucose were admixed to glucose-free Tyrode; a decrease of 50% was found with DNP or CN. The incorporation of PO_4 into ATP, ADP, AMP or GTP, measured after separation by thin layer chromatography, was also reduced by the metabolic inhibitors; ouabain had no effect. In other experiments the working of the Na-K-pump was reversed by transfering nerves, previously incubated in a K-rich, Na and glucose-free solution with IAA or 2DG, to a Na-rich, K- and glucose-free Tyrode with $^{32}PO_4$ and the same inhibitors. In these conditions ouabain also decreased the PO_4-uptake. The results suggest that PO_4 transport depends on the activity of the Na-K-pump and thereby indirectly on the level of intracellular nucleotides.

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Factors Complicating a Prediction of Whole-Blood Bohr Effect from Hb-Solution Work

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We have shown recently that in the physiological acid-base range, the $-\Delta H^+/\Delta O_2$ ratio of whole blood at constant PCO_2 and plasma pH is appreciably smaller than expected from the titration properties of Hb and HbO_2 solutions in absence of CO_2 (Arczynska et al., this Journal *26* 678, 1970). In turn, our values are significantly larger than those predicted by the oxylabile carbamino theory of Roughton and Rossi. Whereas the latter discrepancy may reflect in part the antagonizing effect of 2.3 DPG on carbamino reactions (Bauer, Resp. Physiol. *10*, 10, 1970), it must be stressed that the often-attempted prediction of whole-blood Bohr effect from Hb solution studies is made hazardous by the heterogeneous nature of whole blood. Thus, even if the behavior of Hb in its physiological environment is assumed to parallel that observed in dilute Hb solutions, it can be shown that the shifts of erythrocyte H_2O and above all the small changes of transmembrane H^+ distribution ratio known or suspected to occur on whole-blood oxygenation are bound to influence markedly the amplitude of the physiological Bohr effect as defined above.

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Die Wirkung von Colchicin auf den axoplasmatischen Fluss

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Der axoplasmatische Fluss in den retino-tectalen Neuronen der Taube und seine Beeinflussung durch Colchicin wurden untersucht. Colchicin wurde 24 h vor oder 1 h nach ^{3}H-Leucin in den Glaskörper des rechten Auges injiziert. 5, 12, 24 h oder 14 Tage nach der ^{3}H-Leucin-Injektion wurde das Tier getötet, die Tecta wurden einzeln durch Differentialzentrifugation in Fraktionen getrennt. Die Einbaurate von ^{3}H-Leucin in die Retina-Proteine wurde durch 24 h früher injiziertes Colchicin nicht beeinflusst; die in den tectalen Proteinen gefundene spezifische Aktivität nach 5, 12 und 24 h betrug 21% der Kontrollwerte; nach 14 Tagen betrug sie 40%. 1 h nach ^{3}H-Leucin injiziertes Colchicin verursachte eine leichte Verminderung der spezifischen Aktivität der tectalen Proteine innerhalb 24 h; nach 14 Tagen fand sich eine signifikante Verminderung in der Fraktion der löslichen Proteine. Diese Resultate weisen darauf hin, dass die Proteinsynthese durch Colchicin nicht wesentlich beeinflusst wird. Dagegen scheint es, dass die axoplasmatischen Transportsysteme gehemmt werden, das schnelle stärker als das langsame.

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Insulin Secretory Dynamics: Studies in Rodents With Spontaneous or Induced Obesity

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Dynamic aspects of insulin release, in vivo, have been examined in laboratory rodents with hereditary or acquired obese hyperglycemic syndromes. Both *obob* mice and mice rendered obese by the administration of gold thioglucose (GTG) had elevated basal plasma immunoreactive insulin (IRI) levels. After intraperitoneal or intravenous glucose administration, a rapid, though excessive and prolonged, elevation of plasma IRI was observed in both types of mice – absolute values being higher in *obob* than GTG animals of the same weight. Weight reduction of *obob* mice to body weights equal to those of control mice, while diminishing plasma IRI somewhat did not cause a reversion to normal. Abnormalities of insulin secretion were not seen in ad libitum fed GTG mice before the onset of obesity. Pancreatic IRI content was markedly elevated in *obob* mice but that of GTG mice was equal to controls. In Spiny Mice (Acomys cahirinus), a species with pancreatic IRI content equal to or greater than that of *obob* mice, insulin release following glucose was generally not as great as in *obob* mice. It is concluded that these syndromes in rodents are characterized by distinct abnormalities of insulin synthesis and secretion. They are considered useful models for the study of pancreatic function in obesity and diabetes.

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A Metabolic Response Synchronous to Neuronal Activation

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Most metabolic responses recorded in nervous tissue are slow and late as compared to the electrophysiological response. In order to obtain a better temporal resolution the surface fluorescence of the reduced pyridine nucleotides (PNH) has been correlated with well controlled electrical or chemical stimulations. The neuronal responses can be recorded on the postganglionic nerve.

The results show that this metabolic response appears immediately at the onset of the stimulation and lasts over for some time after the end of it. It is proportional to the number of activated neurones and to the frequency of the electrical stimulation. Two compartments, the cytosolic and the mitochondrial, are involved in this metabolic response. Their contribution to it are partly dependent upon the experimental conditions, namely the composition of the perfusing medium.

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Membrane Potential of Resting Brown Fat Cells: Metabolic Requirements

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The maintenance of a membrane potential of about 60 mV in brown adipose tissue in vitro has several requirements: external K^+ close to physiological concentration, presence of bicarbonate and traces of Ca^{++}. The potential is reversibly reduced to 50% in the absence of external Na and is sensitive to ouabain. These requirements suggest that the membrane potential is more directly dependent on chemical free energy sources than the membrane potential of excitable cells. This prompted us to analyze the metabolic dependence of the membrane polarization. One of the most striking effects was its remarkable sensitivity to a low pO_2. To our surprise 2.4 DNP and KCN were without effect. One possible explanation of this oxygen paradox can be offered for we have found that propranolol, a β-blocking agent, prevents the depolarization observed in hypoxia. Since propranolol also blocks catecholamine induced depolarization, this pO_2 effect is probably mediated by short sympathetic neurones.

Oligomycine produced a slow depolarization that could be reversed by addition of glucose. 2-deoxyglucose, a well-known glycolytic blocking agent, depolarized the cells. Thus, the high energy bonds needed for the maintenance of the potential depend on the glycolytic pathway.

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Induction of RNA Synthesis by Stimulation of a Sympathetic Ganglion

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The influence of neuronal stimulation on the RNA synthesis in the cervical sympathetic ganglion excised from the rat was studied by labelling with tritiated uridine. Preganglionic stimulation as well as externally applied acetylcholine (ACh) caused a transient fall in the RNA specific radioactivity followed by an increase. These changes were prevented when synaptic transmission had been blocked during stimulation by *d*-tubocurarine or mecamine. The effects of ACh on the precursor incorporation persisted when the generation of the action potential was blocked by tetrodotoxine. Depolarization with KCl had no effect on the RNA specific radioactivity. RNA electrophoresis showed that the increase in specific radioactivity was essentially localised on the heavy RNA fractions (>45 S). These results suggest that neuronal stimulation by endogenous or exogenous ACh increases the RNA synthesis and that the signal inducing the metabolic changes arises from the activated postsynaptic membrane.

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Measurement of Coronary Blood Flow by ^{133} Xenon and Electromagnetic Flowmeter

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Coronary flow (CF) in patients is usually estimated from the myocardial washout of inert radioactive gases such as ^{133}Xe. Although there is general agreement that diffusion time of ^{133}Xe is not a limiting factor for the estimation of CF at rest this may not be the case at increased flow rates. Therefore CF was determined in intact anesthetized dogs (average weight 22 kg) simultaneously by the ^{133}Xe technique (CFXe) and by a cannulating electromagnetic flowprobe introduced transvenously into the coronary sinus (CFEM). ^{133}Xe was injected selectively into the left coronary artery and precordial washout curves were recorded by a scintillation counter.

CF was increased by right atrial pacing, isoproterenol and carbochromen. The initial values of CFEM ranged between 30 and 96 ml/min. Up to +50% of the initial values of CFEM (mean +32%) CFXe increased +13%. Between +50 and +100% of CFEM (mean +76%) estimated CFXe showed an increase of +25%. Between +100 and +200% of CFEM (mean +150%) CFXe indicated an increase of +63%. In conclusion a definite underestimation of CF has to be expected with the ^{133}Xe technique mainly at increased flow rates.

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The Cardiac and Ventilatory Rhythms in the Dogfish *(Scyliorhinus canicula)*

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Recordings made over extended periods of the ECG and orobranchial pressure in unanaesthetized dogfish were analysed for the following parameters: a) cardiac and respiratory frequencies; b) interval histograms of ECG and ventilatory pressure; c) averaged pressure and ECG waveforms; d) event correllograms, i. e. position of ECG waveform in the orobranchial pressure cycle. Dogfish of a similar weight (500–800 gm) in running sea water (17–15°C) had a ventilatory frequency of 25–50 per min. Cardio-ventilatory coupling varied a) between individuals and b) for a single individual at different times. The overall distribution of the ECG occurrences was random. However, this is not because the two rhythms are independent. The coupling may be fairly close but the precise phasing between the two rhythms appears to be more randomly distributed. Although there are some indications of an increased coupling at times of stress (hypoxia or increased temperature) this is by no means certain. These results suggest that, although the two rhythms may be coupled, it is not yet possible to attribute a special significance to any particular phase relationship.

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Modifications of Membrane Potential of Frog Tibial Nerve by Aconitine

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The effects of aconitine on the resting and action potentials of frog myelinated nerve fibres were studied with the 'sucrose-gap' technique. Low concentrations (5.10^{-7} to 10^{-6} g/ml) had no effect on resting potential but produced a negative after-potential following repetitive stimulation. Increase of external Na slightly increased this effect. At higher concentrations (2.10^{-6} to 10^{-4} g/ml), aconitine greatly and irreversibily decreased the resting potential and suppressed nerve excitability. The aconitine induced depolarization could be partially antagonized by procaine or an increase in extracellular calcium and completely abolished by withdrawing Na or by application of tetrodotoxine. Furthermore, aconitine increased the normal hyperpolarization caused by withdrawal of the Na_e. These results are compatible with the notion that, once the membrane has become permeable to Na, aconitine prevents the normal return of Na permeability to its resting value and, at higher concentrations, increases the Na resting membrane conductance.

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Respiratory and Total Heat Losses in Resting and Exercising Men

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Cutaneous and respiratory heat losses (h_r) were separately measured by direct calorimetry in 6 men dressed in shorts during rest and exercise. Experiments were performed at two ambient temperatures (20 and 30°C) with a constant relative humidity of 30%. The data were obtained under steady state conditions.

At 20°C, the mean total body heat loss (H_T = cutaneous + respiratory heat losses) was 79 W/m², the mean h_r 6.2 W/m², and the ratio h_r/H_T expressed in per cent 7.8%. During an exercise of 80 W, H_T increased to an average of 150 W/m²; h_r reached a mean value of 15.5 W/m²; the ratio h_r/H_T was 10.3%.

At 30°C, the mean value of H_T of resting subjects was 47 W/m², h_r 5.7 W/m², and the ratio h_r/H_T 12%. During exercise of 80 W, H_T was 148 W/m², h_r 14.6 W/m² and h_r/H_T 10%.

Thus at rest, the fraction of the total body heat loss resulting from respiratory exchanges is lower at 20°C than at 30°C; this is mainly due to the elevated H_T at 20°C. During exercise this fraction becomes independent of the ambient temperature. This results from the fact that during exercise H_T is similar in both environments.

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Lungendehnungs-Rezeptoren (DR) und Verteilungsstörung im Asthma bronchiale

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Das afferente Vagusneurogramm des Meerschweinchens zeigt im mit Ovalbumin-Aerosol ausgelösten Asthma bronchiale die bekannte Aktivitätssteigerung, doch ist diese im besonderen auf die Exspirationsphase zu beziehen. Die Steigerung der afferenten Vagusaktivität resultiert aus dem veränderten Erregungsmuster der DR sowie aus der früher erörterten exspiratorischen Aktivierung der Kollapsafferenzen (Koller und Ferrer, Resp. Physiol., S. 172–183, 1970). Die einzelnen DR werden im Asthmaanfall sehr unterschiedlich beeinflusst, häufig bleiben sie auch exspiratorisch erregt. Die Impulszahl der inspiratorisch wie exspiratorisch aktivierten DR ist durch mässige Thoraxdehnung oder -kompression nur mehr gering veränderlich. Die Befunde widerspiegeln die ungleichmässige Belüftung mit lokaler Lungendehnung und -kompression auf Grund der intrapulmonalen Druckausgleichbehinderung im Asthma bronchiale.

Carbachol Effect on Mammalian Auricle and Its Suppression by Electrical Current

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Application of parasympathomimetic drugs to atrial fibres leads to a shortening of the action potential (AP) and a reduction in twitch tension (negative inotropic effect). It has been suggested that the action of these drugs is indirect, in that the shortening of the AP by

reducing the Ca inward current brings about the reduction in tension (Grossman, Furchgott, J. Pharmac. exp. Therap. *145*, 162–172, 1964). To investigate this problem further bundles of sheep or rabbit atrial fibres were used and the single sucrose gap (Wood, Heppner, Weidmann, Circulation Res. *24*, 409–445, 1969) employed to pass current so that the AP could be increased approximately to its normal value after the shortening induced by carbachol. The results showed that when the AP was so lengthened the twitch tension was increased to about its previous value. An additional finding was that with increasing concentrations of carbachol more current was required to lengthen the AP to the same degree, i.e. there was a decrease in membrane resistance. These findings support an indirect action of the drug.

Intracerebral Distribution of Carotid and Vertebral Irrigation in the Rat

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Simultaneous supravital perfusions of the carotid arteries and the vertebral arteries were made in conditions respecting the normal hemodynamic relations. Different media were employed to distinguish the basilar and carotic intracerebral distribution and the arteries from veins. Under normal conditions the carotico-basilar and left-right anastomoses do not work and the superficial blood distribution to the brain is relatively constant. Consequently, despite of the continuity of the capillary network, the arterial irrigation of the tissue allows the establishment of the demarcation zones, i.e. the zones of the hemodynamic equilibrium, between both principal blood sources. These zones are found in mesencephalon and diencephalon and are formed by the ramification of the terminal paramedian branches of the basilar stem. This paramedian group supplies the phylogenetically old periaquaeductal and periventricular regions as well as the most internal portions of thalamus. So the basilar terminal territory, wedged into the brain stem, represents an area of different hemodynamics in respect to the majority of the radially converging arterial branches supplying the rest of the brain stem.

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Zur Kritik eines hypnogenen Mechanismus im Thalamus

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An der wachen, freibeweglichen Katze wurde im intralaminaren Anteil des hypnogenen Areals des Thalamus (Hess, Helv. Physiol. Acta *2*, 305–344, 1944) mittels repetierter elektrischer Impulse (Frequenz 4 oder 8 pro sec) wiederholt in Abständen von 3 min jeweils 45 sec lang bis zum erfolgten Einschlafen gereizt. Der Haltetonus wurde auf seismographischer Basis registriert und die bioelektrische Aktivität kortikal und subkortikal abgeleitet. Als Kriterien des erfolgten Einschlafens dienten das Verhalten, ein herabgesetzter Muskeltonus und langsame bioelektrische «Wellen» (δ-Wellen).

In *akuten* Versuchen mit Versenken der Elektroden am Versuchstag liess sich auf elektrische Reizung in einigen Fällen eine geringe Verkürzung der Einschlafdauer nachweisen. In *chronischen* Versuchen mit implantierten Elektroden dagegen bewirkte elektrische Reizung innerhalb des intralaminaren Systems keine signifikante Verkürzung der Einschlafdauer gegenüber den Kontrollen mit spontanem Einschlafen. Die impulssynchron ausgelösten elektrischen Reizantworten waren in der Regel von einer Synchronisierung der kortikalen und subkortikalen bioelektrischen Aktivität begleitet, die im Rhythmus der Reizfrequenz erfolgte. Das Verhalten der Tiere wurde jedoch nicht beeinflusst.

A Double Sucrose Gap Method to Study the Slow Outward Current in Ventricular Muscle

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Using a single sucrose gap technique to voltage clamp small bundles of sheep or calf ventricular fibres (0.8–1 mm diameter) it was shown that during clamps of several seconds there is a slow increase in outward current (McGuigan, Experientia *26*, 682, 1970). The single gap has the disadvantage that it is difficult to keep the microelectrode in the cell for sufficient time to study the slow outward current in detail. To overcome this difficulty a double gap has been developed to clamp the bundles. The results with the double gap with both short and long clamps are similar to those obtained previously with the single gap. Action potentials lie between 80 and 100 mV and the method has the advantage of continuous recording for several hours. With the double gap it has been shown that the reversal potential of the slow outward current becomes more positive the longer the clamp pulse. These results suggest that K may be accumulating in a space near the membrane and contributing to the increase in conductance.

New Evidence of Humoral Transmission of Sleep

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The method of Koller, Monnier and Gamp (1964), Monnier and Hösli (1964), developed for dialysing a hypnogenic factor from cerebral blood and for humoral transmission of sleep in the rabbit has been improved by adding to the intravenous screening a new method of intraventricular screening (Monnier and Hatt 1970). The infusion of 0.05 ml into the third ventricle instead of 20 ml into the vein spares a great amount of dialysate for pharmacological and biochemical analyses.

Results. a) A group of 15 *sleeping donors* shows an increased amount of the delta activities (199%), symptomatic of sleep, during a dialysis of 60 min, instead of 95% in control donors. b) In 7 *recipients* the *intravenous* injection of hypnogenic dialysate increases the delta amount to 175% during a postinjection period of 60 min instead of 102% in control recipients. c) In 13 *recipients*, the *intraventricular* infusion of hypnogenic dialysate during 25 min increases the delta amount to 175%, instead of 98% in control recipients. d) In 5 *recipients*, the intraventricular infusion of *lyophilized* hypnogenic dialysate increases the delta amount to 145% instead of 95% in control recipients. These results confirm the humoral transmission of sleep by a hypnogenic factor.

Influence of Low Temperature on Contractility of Single Frog Muscle Fibres

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Lowering the temperature to 2°C has the immediate effect of increasing twitch tension 2 to 4-fold; the twitch duration is increased 2 to 5-fold. This may be due in part to a prolongation of the action potential, in part to a slower disappearance of free Ca^{++}. Tension produced by adding 190 m*M* K is slightly lower at 2° than at 23°; the plateau duration is 4 to 6 times longer at low temperature and the final tension decrease while still in 190 m*M* K occurs much more slowly. When the fibre is exposed to 190 m*M* K at 23° for 5 sec, then repolarized in Ringer for 5 sec, reapplication of high K for a second time produces much less tension than in experiments where the first contracture is performed at 2°, although both initial contractures have practically the same size. The recovery time from a K contracture is increased from 30 sec to 10 min at 2°C. Cooling prior to a contracture does not decrease the tension development in high K. These experiments suggest that both the liberation and the reaccumulation of Ca^{++} are affected in the same way and to a similar extent by cooling.

Changes in Muscle Activity During Sleep at Different Environmental Temperatures

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In unrestrained cats the electromyograms (EMG's) of neck, respiratory and limb muscles were studied. *Thermal neutrality.* Slow-wave sleep (SWS): postural activity is present in neck and also in intercostal EMG's; diaphragmatic and intercostal EMG's show respiratory activity; EMG's of limb muscles are silent. Fast-wave sleep (FWS): neck and intercostal postural activity disappears; intercostal respiratory activity is weak; respiratory activity of diaphragm persists. *Low temperature.* SWS: postural activity is present in neck and intercostal EMG's; shivering activity is observed in all EMG's; shivering intensity increases from waking to SWS; interaction occurs between respiratory and shivering rhythms. FWS: postural and shivering activities disappear. *High temperature.* SWS: neck and intercostal postural activity is weak; diaphragmatic and intercostal EMG's show panting activity; frequency of panting increases from waking to SWS. FWS: postural and panting activities disappear. Summing up, FWS is associated with changes in the regulation of postural, respiratory and thermoregulatory muscle activities.

Histochemical Demonstration of Na K ATPase in the Proximal Tubule

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The Wachstein and Meisel reaction fails to demonstrate the 'true' Na K ATPase in the intracellular region of kidney tissue. The reaction product ($PbPO_4$) is localized within the brush border, mitochondria and basal infolding membrane. To get a clear conception about the localization of Na K ATPase within the cells of the proximal tubule (PTC), this problem was investigated with quantitative histochemistry. Single PTC-segments were isolated from freeze-dried rat kidney sections. From each PTC (weighing 25 ng) two basal areas (each 3 ng) were separated. Total ATPase and Mg ATPase were determined in single PTC each, in both halves of one single PTC and in the basal area from one PTC using 'oil well'-technique, enzymatic P_i analysis and enzymatic cycling system. Total ATPase minus Mg ATPase yields Na K ATPase expressed in moles P_i/kg dry weight/h (= MKH). Na K ATPase was max. 1.7 MKH in the PTC and 8.8 MKH in the basal area. These findings reveal the site of localization of the enzyme within the basal infolding membrane of PTC. They are relevant to the polarization of the epithelial cell related to its transport properties with an active contraluminal and a passive luminal side.

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The in vitro Perfused Pig Liver (PPL) as Biological Research Tool

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The use of the PPL in physiological research implies adequate functional capacity of the isolated organ. This supposition, however, is as yet incompletely investigated. We therefore compared in 30 experiments the PPL with the intact pig (IP) in regard to quantitative tests of hepatic function (galactose elimination capacity, GEC, Tygstrup's method; biliary BSP-transport maximum, Tm). The kinetics of BSP were further studied using varying infusion rates. – In the IP the GEC ranged from 1.1 to 1.3 mmol/min. Mean GEC of PPL was reduced to 30–50% of the IP. A similar reduction of the biliary BSP-Tm was found (IP: 30–50 µmol/min, PPL 35–60%). The two function tests were highly correlated ($r = 0{,}95$, $p < 0{,}001$). The studies of BSP kinetics revealed a constant concentration ratio between plasma and liver. The excretion of stored BSP followed Michaelis-Menton kinetics during the first 4 h. – Even though function in the PPL was reduced compared to the IP the kinetics of BSP and the GEC/Tm ratio were normal. This suggests a reduction in number rather than a qualitative alteration of functional units of the PPL. With this restriction the PPL appears suitable as biological research tool.

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Two Sodium Pumps in the Dog Kidney

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Recent work on ion movements in guinea-pig kidney cortex slices has demonstrated the existence of two different sodium pumps in the proximal tubular cells (Whittembury and Proverbio, Pflügers Arch. *316*, 1, 1970). The Na^+-K^+-ATPase appears to be involved in the mechanism of only one of these pumps (Proverbio et al., BBA *211*, 327, 1970). The following results were obtained with slices of dog renal cortex: Glycine transport by dog kidney is entirely sodium-dependent, no concentration

gradient being established in the absence of sodium. Nevertheless, ouabain, even at very high concentrations, is unable to reduce the active component of glycine transport by more than 50–60%. 50% inhibition of the ouabain-sensitive component of glycine uptake occurs at the same ouabain concentration as that required to inhibit the Na^+-K^+-ATPase half-maximally. These results, like those obtained with the guinea-pig kidney, indicate the existence of a second pump, unconnected with the Na^+-K^+-ATPase, which continues to maintain the low intracellular sodium concentration necessary for glycine transport.

The Biphysic Electrical Response of Brown Adipose Tissue to Norepinephrine

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Norepinephrine depolarizes brown adipose tissue cells. Time analysis in vitro of this potential drop shows that it is clearly biphasic. The initial potential fall (IP) is followed by a late potential fall (LP). The IP is a phasic event which is blocked by propranolol and requires external calcium. It does not result from a change in K^+ gradient and the K^+ conductance is not significantly altered. IP is not secondary to the increased respiration or CAMP concentration (two well-known effects of catecholamines on this tissue) since none of the following substances depolarize the cells: octanoate, oleate, α-glycerophosphate, theophylline, dibutyrylcyclic AMP. Thus, the IP must directly result from the binding of the hormone to a β-adrenergic receptor. A well-known energy trapping agent, 2-deoxyglucose surprisingly prevents the norepinephrine depolarization. Thus, for the hormone-to-receptor reaction to trigger an IP, a supply of high energy phosphate seems to be required. The LP can be readily explained by a slow ionic shift: the internal $[K]^+$ decreases and the internal $[Na]^+$ increases. The two phases should be distinguished since they obviously result from different mechanisms. The question as to whether the LP is triggered by the IP or results from a direct effect of the hormone on membrane properties is not yet solved.

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Biological Properties of a Cutaneous Burn Toxin Produced by Thermal Injuries

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From skin of germ-free mice subjected to controlled thermal energy in vitro, a burn toxin has been isolated. This toxin was shown to be a polymer of a naturally occurring monomeric membraneous skin component. Injection of the isolated toxin had a lethal effect and caused a 5-fold increase of BUN, serum creatinine and a proximal tubular necrosis in the recipients. In vivo experiments were carried out protecting the animals' body from direct heat irradiation which made it possible to apply thermal injuries identical to those used in vitro. 14, 22 and 27% of the body surface were injured. The theoretical amounts of toxin produced were calculated to be 0.4, 0.53 and 0.71 mg/g body weight. All animals survived an injury of 14% while 27% body surface burned caused a 95% mortality. All animals survived scalds of 27%, confirming a highly specific mechanism for toxin formation. Active immunization with the toxin protected the animals from otherwise lethal burn injuries. A significant benefit of an immunserum obtained from rabbits with the toxin as antigen could be demonstrated. These results might be related to the events causing the late mortality in human burns.

The Gas Exchange Apparatus of the Smallest Mammal: *Suncus etruscus*

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The gas exchange apparatus of the Etruscan shrew *(Suncus etruscus)* was studied, the smallest mammal known (adult body weight of 2.5 g; Courtesy Dr. P. Vogel, Basel). This animal has an extremely high metabolic rate, eating 3 times its body weight daily in insects. In lightly anesthetized animals the heart rate was 1000–1300 per min and respiratory frequency 200–300 per min. Minimum O_2 consumption at rest was 0.25 to 0.5 ml · g^{-1} · min^{-1}, twice that of house shrew (10 g) and 8 times that of mice (23 g); it climbed to nearly 1 ml · g^{-1} · min^{-1} in mild exercise. The pulmonary gas exchange apparatus is strikingly dense, providing an alveolar surface of 0.2 m^2/cm^3 lung (mouse: 0.1, rat 0.075). The air-blood barrier is extremely thin. The capillary volume is relatively small, due to small capillary and red cell diameters. The diffusion capacity D_L per unit body weight calculated from morphometric data is 0.6 · 10^{-2} ml O_2 · min^{-1} · $mmHg^{-1}$ · g^{-1}, which is the same as for other mammals up to the dog. D_L is hence linearly related to body weight down to the smallest mammal.

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Die Reizwirkung von Mittelfrequenz-Impulsen als Funktion der Trägerfrequenz

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Die Reizwirkung von Mittelfrequenz-Impulsen nimmt mit der Trägerfrequenz ab. Das Ansteigen der Reizschwelle im Bereich zwischen 5 bzw. 18 und 82,5 kHz erfolgt für Längsreizung an Nerv *(N. ischiadicus)* und Muskel *(M. sartorius)* immer progressiv auf das 20- bis 30fache. Für Querreizung ergibt sich im Prinzip eine lineare Beziehung (Wyss und Boeckmann, J. Physiol. Paris *62*, Suppl. I, 228, 1970). Abweichungen gegen progressiven Verlauf sind auf Komplikationen der Querreizung durch Längskomponenten des Stromverlaufs und kapazitive Leitereigenschaften zurückzuführen. Messungen nach dem Stromkonstanzverfahren (grosser Serienwiderstand) ergeben eindeutig direkte Proportionalität zwischen Reizschwellen und Trägerfrequenz. Die Befunde lassen sich auf Grund der Annahme echter Querreizung der Ranvierschen Schnürringe erklären.

BIOCHEMIE – BIOCHIMIE – BIOCHEMISTRY

Studies on the Complement-Binding Site of Rabbit Immunoglobulin G. Effect of Chemical Modification

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Native IgG and its fragment Fc are capable of fixing complement when aggregated by different methods. Smaller fragments obtained by pepsin digestion of IgG are inactive when tested directly. However, they display an inhibitory effect on the anticomplementary activity of IgG preparations. Experiments on the purification of such peptides will be reported.

When IgG and Fc are reacted with 2-hydroxy-5-nitrobenzyl bromide (NBB), a reagent which specifically modifies tryptophan, they lose their anticomplementary activity. From our experiments it can be concluded that the complement-binding site of IgG is situated in the Fc region and that it includes at least one molecule of tryptophan. In order to locate the position of this tryptophan, modified IgG was subjected to pepsin digestion and the peptides labelled with NBB were isolated. The extent of labelling was determined spectrophotometrically at 410 nm. A significant amount of NBB was found only in the fragment known as PEP V (MW 5000) which is found in the N-terminal part of Fc. Between 0.5 and 1 molecule of tryptophan was modified per molecule of PEP V. Pepsin digestion of the modified Fc gave the same result. It seems therefore that the site responsible for the anticomplementary activity of IgG resides predominantly in the N-terminal region of fragment Fc and that tryptophan is involved.

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Gluconeogenesis, Glycolysis and Morphological Aspects of the Perfused Mouse Liver

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The use of a perfusion system adapted to mice is of obvious importance when investigating the anomalies of lipid, carbohydrate and protein metabolism, since there exist several strains of mice or hamsters with genetically-induced diabetes. The purpose of the present experiments is to describe a perfusion system adapted to the liver of the normal Swiss (nS) mouse, a first logical step in the study of abnormal livers. Livers from nS mice were perfused in situ, and their state of preservation was assessed by gross appearance, absence of K^+ leakage, and by electron microscopic appearance that was normal after 1 h of perfusion. In perfused livers from fed animals, glucagon (10^{-11} *M*), epinephrine (10^{-9} *M*) and cyclic-AMP (10^{-5} *M*) significantly increased glycolysis above control values. Gluconeogenesis was studied in livers obtained from 12–16 hour fasted animals. When used at saturing concentrations, fructose and dihydroxyacetone were very potent in increasing glucose production by, and glycogen deposition in the perfused livers. Substrates entering the gluconeogenic pathways at or 'below' the level of pyruvate (alanine, lactate, pyruvate) resulted in a marked increase in glucose output, although the gluconeogenic effect of these substrates was less than that of fructose and dihydroxyacetone. These substrates also produced a significant decrease in ketone body output when compared to livers perfused in the absence of substrates. The addition of fatty acid to livers perfused with lactate or pyruvate failed to alter the increased gluconeogenesis brought about by these intermediates. Finally, agents such as glucagon or cyclic AMP have not been found so far to stimulate lactate- or pyruvate-induced gluconeogenesis, suggesting that mouse gluconeogenesis might be regulated mostly by substrate levels.

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L-3-O-Methyldopa, a Biological Precursor of Dopa

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3-O-Methyldopa is a major metabolite of L-3,4-dihydroxyphenyl-alanine (L-Dopa). It has a biological half life of 13–15 h and markedly accumulates in the tissues including the brain. In the present work the metabolism of L-2-^{14}C-3-O-Methyldopa was studied in rats and men. The main metabolites found in tissues and urine were homovanillic acid (= 3-methoxy-4-hydroxyphenylacetic acid) and 3-methoxy-4-hydroxyphenyllactic acid. Besides small amounts of dihydroxylated compounds such as Dopa, dopamine and 3,4-dihydroxyphenylacetic acid as well as 3-methoxytyramine have been detected. Dopacetamide, an inhibitor of catechol-3-O-methyltransferase, administered before L-3-O-methyldopa enhanced the rise of dopamine and 3,4-dihydroxyphenylacetic acid but diminished that of 3-O-methylated metabolites such as 3-methoxytyramine and homovanillic acid. It is concluded that part of the L-3-O-methyldopa undergoes demethylation with intermediary formation of dopamine. This amine in turn is further metabolized by 3-O-methylation and/or oxidative desamination, the main metabolic end product being homovanillic acid.

Electron Microscope Studies on the Structure of Thrombosthenin A

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Thrombosthenin, the contractile protein of human thrombocytes, is built, like its analogue from muscle tissue, of two components: the actin-like thrombosthenin A (Th-A) and the myosin-like thrombosthenin M (Th-M). Although biochemical differences exist between thrombosthenin and actomyosin, they must be structurally very similar, as their components can cross-react and form active hybrids. The structure of thrombosthenin was studied by electron microscopy. In a previous publication (Thromb. Diath. haem. *22*, 431, 1969), it was shown that Th-A polymerizes as thin filaments and Th-M in spindle-shaped needles. In this presentation, Th-A, extracted as usual from human blood platelets and purified by gel chromatography (E. Probst, unpublished), is compared with actin prepared from rabbit muscle. A drop of solution containing either actin or Th-A was placed on perforated carbon films and contrasted with 1% uranyl acetate, so that the specimens are embedded in a thin layer of the negative stain extending over the holes. Th-A filaments seem very similar to actin: both form a beaded, twin-stranded cable, in which the strands wind around each other. The thickness of the filaments is 65,9 $\pm$ 1 Å for Th-A and 73,2 $\pm$ 1 Å for actin ($2p < 0.005$). Though structurally similar the two molecules differ in size.

Syncatalytic Conformational Changes in Aspartate Aminotransferase

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Transient alterations in side chain reactivity of the enzyme protein appear to be integral characteristics of the catalytic mechanism of aspartate aminotransferase (AAT α-subform of cytoplasmic enzyme from pig heart). Such catalysis-synchronous, i.e. syncatalytic, changes have been demonstrated by differential modification of the enzyme molecule in the resting state on the one hand and in the state of catalytic action on the other. In the absence of substrates, N-ethylmaleimide reacts with four sulfhydryl groups of the pyridoxal form of the enzyme. However, in the presence of the substrate pair glutamate and α-ketoglutarate six sulfhydryl groups are modified. The same catalysis-induced exposure of two additional sulfhydryl groups is evidenced by their reaction with tetranitromethane (TNM). Concomitantly with the syncatalytic modification of these groups enzymatic activity is abolished. Similar catalysis-dependent nitration by TNM has previously been reported for tyrosyl residues of AAT (Christen and Riordan, Biochemistry *9*, 3025, 1970). These syncatalytic modifications of two different side chains, cysteinyl and tyrosyl residues, are most consistent with the occurrence of transient conformational changes of the enzyme-coenzyme-substrate complex in the course of catalysis.

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Protection of Reactive Hemoglobin Sulfhydryl Groups in Erythrocytes

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Since erythrocytes have no protein biosynthesis they constitute an ideal system to study macromolecular aging. One of these aging products is believed to be hemoglobin A bound with a glutathione on each reactive β-93 SH-group as a mixed disulfide. In vitro synthesis of such a derivative (HbASSG) revealed it to be similar in electrophoresis and ion exchange chromatography to a naturally occurring minor component HbA_3. In contrast to HbASSG, HbA_3 has free SH-groups and differs in oxygen affinity ($p^1/_2$ O_2: HbA_3 4,0 mm Hg; HbASSG 1,5 mm Hg). These two components are *not* identical.

HbASSG is not detectable in normal hemolysates but is found in erythrocytes exposed to oxydizing conditions and possibly in congenital heinz body hemolytic anaemia. Reduction of HbASSG was studied under conditions similar to the ones inside erythrocytes. It was converted to normal hemoglobin A by reduced glutathione but *not* by the NADPH-dependent glutathione reductase. In view of the normally high concentration of reduced glutathione in erythrocytes of normal blood, the absence of HbASSG is readily explained.

Caractérisation de sérums de lapins anti-membranes de lymphocytes de souris

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Les membranes de cellules lymphoides provenant de la rate, des ganglions mésentériques et du thymus de souris (Swiss mice) ont été préparées selon Neville (Biochim. biophys. Acta *154*, 540, 1968). Les fractions membranaires ainsi que les homogénats cellulaires complets ont été utilisés pour l'obtention d'antisérums hétérologues chez le lapin. L'effet cytotoxique de ces antisérums mesuré par le relâchement du Cr^{51} par des cellules des 3 organes lymphoides étudiés, donne les résultats suivants: 1. La cytotoxicité de sérums anti-homogénats de thymocytes, très élevée à l'égard des cellules thymiques, se manifeste également, mais dans une mesure plus faible, à l'égard des cellules ganglionnaires et spléniques. 2. Les sérums anti-membranes de thymocyte présentent une activité et une spécificité encore accrues à l'égard des cellules thymiques. 3. Les sérums anti-membranes de lymphocytes de ganglions ou de rates, ne sont actifs que sur ces cellules.

L'étude in vivo de l'activité opsonisante des antisérums confirme ces observations. Ces résultats suggèrent que la présence d'antigènes spécifiques des cellules thymiques ou thymo-dérivées pourrait être révélée par des antisérums hétérologues. Des travaux sont en cours afin d'isoler ces antigènes et d'étudier leur répartition à la surface des cellules lymphoïdes.

Chemical Isolation of Immunosuppressive Relevant IgG-fractions from Rabbit ATS

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Rabbit anti mouse-thymocyte serum (ATS) decomplemented at 56° for 30 min was precipitated by $(NH_4)_2SO_4$ (1.75 *M*). The precipitate was shown to contain 6 different IgG-fractions with an identical specific immunosuppressive activity, tested by tail skin grafting. The fractions were obtained by modifying the chromatographic procedure on DEAE-Sephadex A-50 as follows: the column (3.5 × 60 cm) was eluted with a very shallow gradient from 0.012 to 0.3 *M* phosphate (3.5 l). Every 25th tube (6 ml/tube) of the effluent was analyzed by disc-electrophoresis and the tubes with identical electrophoretograms were pooled. Electrophoresis on cellulose-acetate and immuno-electrophoresis were used for quantitative and qualitative analyses. Disc-electrophoresis, carried out in the presence and absence of SDS, showed a surprising increase of the relative mobility of the gammaglobulin bands in direct correlation to the increasing molarity of the elution gradient, i.e. small differences in size and/or charge were detected. Based on this observation, one pool containing pure IgG by all criteria was rechromatographed applying an even flatter gradient from 0.012 to 0.02 *M* (1 × 100 cm/2 l). One of the three IgG-fractions eluted, accounting for 2.8% w/w of the total IgG-content of the serum, exhibited a 30 × higher specific immunosuppressive activity than the loaded material. Moreover, all three fractions had no in vitro lymphocytotoxicity.

Immunochemical Analysis of Antilymphocyte Serum

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Highly immunosuppressive and non suppressive antilymphocyte sera were compared by quantitative microcomplement fixation. The skin graft survival coincided

with the microcomplement fixation properties using purified membranes of spleen cells as antigen. The immunochemical anti-membrane activity of the ALS was due to both, specific antigens of lymphoid cells and non cell-specific antigenic determinants. The results obtained immunochemically were confirmed morphologically by indirect immunofluorescent staining. The biological activity of ALS is discussed with respect to a co-operative action of specific and non cell-specific antibodies.

Biotin-Binding Proteins of Chick Oviduct

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Avidin, the biotin-binding protein, is commonly held to be produced in the magnum part of the hen's oviduct. However, using an assay with ^{14}C-Biotin, biotin-binding proteins were found in all parts of the oviduct. With antibodies against highly purified avidin, the identity of this biotin-binding capacity with avidin was tested. Crossreaction with avidin antibodies was found only in the infundibulum and magnum. Biotin-binding proteins other than avidin from oviduct and egg-white showed an electrophoretic mobility distinct from that of avidin. Sexual hormones seem to influence the rate of avidin production in the magnum only, not in the infundibulum.

Effet des variations des taux des acides gras libres (AGL) sur la tolérance au glucose, l'insulinémie et l'oxydation des glucides et des lipides

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Le but de ce travail a été de vérifier chez l'homme l'effet d'une élévation du taux des AGL sur l'oxydation du glucose et, de ce fait, de donner une explication à l'intolérance au glucose observée chez l'obèse. Une surcharge orale en glucose (OGTT, 100 g) a été effectuée chez des sujets normaux au cours d'une perfusion de lipides qui provoque une augmentation du taux des acides gras libres circulants. La glycémie s'élève davantage au cours de cette épreuve que lors d'un OGTT de contrôle (sans lipides).

La mesure de la consommation d'O_2 et du quotient respiratoire a permis de démontrer que l'adjonction de lipides induit une baisse de l'oxydation des glucides accompagnée d'une augmentation de l'oxydation des graisses. L'insulinémie au cours des OGTT est plus élevée lors des perfusions simultanées de lipides que lors des tests-contrôles. L'intolérance au glucose ainsi observée est le reflet d'une oxydation diminuée de ce substrat, malgré les taux plus élevés d'insuline circulante. Ces résultats montrent in vivo qu'une augmentation des taux circulants d'AGL entraîne une résistance à l'effet hypoglycémiant de l'insuline, accompagnée d'une diminution de l'oxydation du glucose.

Comparative Immunoreactivity of Rat and Pork Insulin

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The immunoreactivity of equipotent preparations of crystalline rat and pork insulin was compared, using the double-antibody radio-immunoassay (kit: Radiochemical Centre, Amersham). The potency of each preparation was checked by the fat pad bioassay (C-1 oxidation from glucose-1-C_{14}).

In a single assay, increasing doses of rat and pork insulin induced different decreases in the radioactivity of the (ox) insulin I_{125}-antibody complex. The difference between both standard curves varied, according to the batch of the 'insulin binding reagent' (insulin antibody and anti-gammaglobulin). With two of the three batches used, the decrease in radioactivity of the complex induced by increasing doses of rat insulin was less steep and approached an asymptote more distant from the abscissa (O radioactivity) than with pork insulin. With the other batch, small doses of rat insulin induced a slightly larger decrease in radioactivity of the complex than small doses of pork insulin, while higher doses of both insulins produced the same effect. In assays of serum insulin in rats, standardization with pork instead of rat crystalline insulin may therefore result in either a large underestimation, or in a slight overestimation at low concentrations, according to the batch of reagent used.

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Organspecific Histone Fractions in Rat Brain and Liver

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The histones act in vitro as repressors of DNA-dependent RNA synthesis. Their possible role as genetic repressors in vivo is still being discussed. So far, only few organ-specific differences in the composition of the histones have been found. As a basis for studies on nucleoprotein in ageing animals we have analyzed some of the major histone types in rat brain and liver. Nucleoprotein was prepared from nuclei of adult rat brain and liver by washing with Mg-Tris buffer, and the histones extracted by the standard method of Johns. The purified histones were fractionated on Sephadex G-75 with 0.02 *N* HCl, and the major fractions obtained were further analyzed by acrylamide electrophoresis. – The lysine-rich F1 histones from brain contained a large fraction (No. 5) of low molecular weight and high electrophoretic mobility totally absent in liver F1, which contained only the major fractions No. 2 and the minor No. 2a. – The intermediate histones F2b from brain also contained a component (No. 3a) absent in liver F2b. – Exhaustive extraction of the residual dehistonized nucleoprotein with 0.25 *N* HCl yielded a considerable further amount of acid-soluble proteins. These contained non-histone proteins with high acidic amino acid content (Fraction No. 1) in both brain and liver. In brain, these 'post-F2b' proteins contained the component No. 3a as the major fraction, which again was absent in liver 'post-F2b'.

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Small Intestinal Lactase and Phlorizin-Hydrolase

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Intestinal lactase is obtained in good yields and in homogeneous form from baby rats by papain solubilisation, Sepharose, Sephadex and DEAE-cellulose chromato-

graphy. Much of the phlorizin-hydrolase activity of the small intestine is associated with lactase. Except for the first steps of the purification, from both rat and hamster intestine, the lactase/phlorizin-hydrolase ratio remains constant (rat: ca. 50:1; hamster: ca. 8:1). Lactase and phlorizin-hydrolase show the same age-dependence in the small intestine of baby rats.

The heat stabilities of lactase and phlorizin-hydrolase activities are different; no mutual inhibition between the two substrates is observed. It is concluded that lactase and a phlorizin-hydrolase are associated in a complex, in much the same way as sucrase and isomaltase. Like sucrase and isomaltase, lactase and phlorizin-hydrolase seem to be subjected to the same (or similar) biological control.

The extent of inhibition of sugar uptake in the small intestine correlates strongly with the phlorizin-hydrolase activity present in the tissue, which indicates that not phlorizin itself, but a derivative of it, may be the actual inhibitor of intestinal sugar transport.

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Effect of Reduction and Iodination on the Biological Activity of Clq

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Clq is the first component of the 11 complement proteins which is able to react with antigen-fixed immunoglobulin to initiate the sequence of complement reactions. Together with Clr and Cls, it forms a macromolecular complex which exhibits, during complement activation, an enzymatic activity in the Cls component. Purified Clq has been shown to have a molecular weight of about 400,000 and to consist of at least five subunits. In this study, an attempt was made to observe separately the IgG combining activity and the hemolytic activity, the latter requiring an additional site necessary to elicit proteolytic activity in Cls. In order to study the direct interaction of Clq with antigen-fixed IgG, the purified Clq was radioiodinated. It was observed that Clq was rendered more susceptible to denaturation and that the hemolytic activity of the labelled Clq preparation was lost more rapidly than its ability to combine with antigen-antibody complexes, suggesting the existence of two functional sites on the Clq molecule. Reduction of Clq with dithiothreitol (> 0.1 *M*) resulted in complete loss of hemolytic activity. In the absence of alkylating agents, as much as 50% of this activity was slowly restored by reoxidation in air at 0–4°C.

Subcellular Distribution Patterns in Rat Cerebral Cortex Using Different Concentrations of H^3-labelled Transmitters

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Aliquots of nuclei-free supernatant from rat cerebral cortex slices incubated (10 min, 25°C) with 10^{-7} and 10^{-5} molar, H^3-1-noradrenaline (NE), H^3-dopamine (DA) and H^3-serotonin (SE) were separated by centrifugation (2 h, 60,000 g) on a 12.7 ml discontinuous sucrose density gradient (0.3, 0.6, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.7 *M*). Radioactivity was determined in 33 fractions. With 10^{-7} *M* concentrations the synaptosomes separated into 3 main peaks with 2 different patterns. H^3-SE labelling was found mainly in the lighter synaptosomes, peak $2 > 3 > 1$. H^3-NA and H^3-DA were in the heavier synaptosomes, labelling sequence peak $2 > 1 > 3$. Using 10^{-5} *M* concentrations radioactivity was approximatively $10\times$ higher. Relative activity was shifted. For H^3-SE an increase of the label was seen in peak 1 and 2, indicating a shift to heavier fractions; this can be interpreted as H^3-SE entering catecholaminergic neurones. Typical for H^3-NA is a distinct decrease in peak 2 and for H^3-DA an increase in peak 1. Electronmicroscopic autoradiography studies are in progress.

Polyacrylamid Gel als Immunoadsorbens für Anti-DNP-Antikörper

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Die Isolierung von Antikörpern mit Hilfe von Adsorption an ein entsprechendes Antigen, das in eine unlösliche Form gebracht wurde, ist zuerst von Campbell et al. beschrieben worden. Solche Immunoadsorbentien beruhen auf einer chemischen Bindung von Antigenen an eine inerte Trägersubstanz. Eine neue Art von Immunoadsorbens, basierend auf der mechanischen Fixierung von Protein in den Maschen eines hochgradig vernetzten Polyacrylamid Gels, wurde von Carrel et al. entwickelt. Diese Methode wurde modifiziert, um auch Anti-Haptен-Antikörper isolieren zu können. Eine Kolonne, gefüllt mit Polyacrylamid Gel, in das Dinitrophenyl (DNP) substituiertes bovines Serumalbumin einpolymerisiert wurde, gestattet eine selektive Adsorption und anschliessende Elution von Antikörpern gegen DNP-Gruppen. Mit diesem Immunoadsorbens wurden präzipitierende Kaninchenantikörper IgG gegen DNP-humanes Gammaglobulin wie auch nicht präzipitierende Mäuseantikörper IgG gegen DNP-Haemocyanin isoliert. Das von Eisen et al. beschriebene und als IgA mit Anti-DNP-Aktivität bekannte murine Myelom-Protein MOPC-315 konnte auf diese Weise ebenfalls gereinigt werden. Diese Methode ist einfach und die Ausbeute relativ hoch.

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Lipoprotein-Mediated Transport of Phylloquinone in the Thoracic Duct Lymph of Rats

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The appearance of phylloquinone or its derivatives in the thoracic duct lymph was investigated. An emulsion containing proteins, carbohydrates and tritiated vitamin K_1 (0.5 µmole) in 4 or 16% monoolein and saline, was given by stomach intubation to thoracic duct-fistulated rats. Lymph samples were collected hourly and protected from strong light. For lipoprotein studies the samples were directly subjected to agarose-gel electrophoresis. For the determination of radioactivity by liquid scintillation spectrometry the whole lymph and the different lipoprotein fractions were extracted with ethanol and isopropylether, 2:1.

Within 12 h after feeding phylloquinone in the 4% monoolein emulsion about 15% of the administered radioactivity could be recovered in the lymph. However, after administering of the vitamin in the 16% monoolein emulsion only 5% of the radioactivity was recovered within the same time interval. Thus, the absorption of phylloquinone seems to be influenced by the amount of lipid in the diet.

In humans (R. Blomstrand and L. Forsgren, Int. Z. Vit.-Forsch. *38*, 45, 1968) vitamin K_1 was found to be carried almost exclusively by chylomicrons. In the rat about 80% of the radioactivity is transported by very low density lipoproteins.

Thin-layer and glass-fiber paper chromatography revealed that in total lymph as well as in the different lipoproteins two radioactive compounds occurred, one of which behaved like phylloquinone.

Biguanide Effects in Normal and Streptozotocindiabetic Rats

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Antidiabetic biguanides have been shown to improve the glucose tolerance after oral but not after parenteral administration in normal human subjects and in the dog.

In the rat, in vitro, the glucose transport across the intestinal wall (everted sacs of small intestine) is inhibited by about 50% in the presence of 3×10^{-4} *M* phenethylbiguanide (I) and *n*-butylbiguanide (II) or 10^{-2} *M* dimethylbiguanide (III) respectively. A similar effect is also achieved shortly after pretreatment of the intact rats with 60 μmoles/kg of (I) and (II) and 600 μmoles/kg of (III) orally. In the normal rat the hyperglycemia after oral glucose administration is reduced maximally 2 h after intubation of biguanides (50% inhibition after 100 to 300 μmoles/kg of [I] and [II] and 1000 μmoles/kg of [III]). After 40 h of starvation, however, the same doses of the biguanides do not influence the blood glucose of normal and streptozotocindiabetic rats. After refeeding, streptozotocindiabetic rats, in contrast to normal animals, do respond to the biguanides which again show a similar order of potency.

The present experiments support the assumption that at least part of the antidiabetic properties of biguanides may be explained by their inhibitory action on intestinal absorption.

Tryptophan Fluorescence Quenching in Dehydrogenases

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The intrinsic tryptophan fluorescence of horse liver alcohol dehydrogenase (LADH), yeast alcohol dehydrogenase (YADH), pig muscle lactic dehydrogenase (LDH), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (GPDH), is quenched by binding of NADH and/or NAD^+. The maximal quenching is approximately the same for the two coenzymes, and is about 50 to 60% in LADH and 90% in YADH and LDH, though the latter enzymes contain a much larger number of tryptophan residues per subunit. The quenching of tryptophan fluorescence in the aforementioned dehydrogenases allows for an exact titration of the enzyme active sites (in the case of a tight binding). Furthermore, it provides a fast routine method to determine enzyme—coenzyme binding constants and to study subunit interaction over a wide range of experimental conditions. The quenching of tryptophan fluorescence can not solely be ascribed to an energy transfer mechanism (as NAD^+ does not meet the necessary spectroscopic requirements). It is also unlikely to be due to a conformational change that, in all the cases investigated, brings the fluorescent tryptophan residues into a quenching environment. The other possibility is that of a direct interaction between coenzyme and tryptophan at the active site, and this re-opens the problem of the role of tryptophan in dehydrogenases.

Utilisation of Hexoses and Polyols by the Normal and Streptozotocindiabetic Rat

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Continuous $^{14}CO_2$ exhalation was recorded after intravenous administration of similar load doses of ^{14}C-labelled glucose, fructose, sorbitol and xylitol. After a period of 6 h normal rats exhaled approx. 35% of the given dose of the substrates. In streptozotocindiabetic rats 12–16% of the four sugars had been exhaled after 6 h. As earlier experiments in our laboratory have shown, the rat converts large loads of these substrates almost quantitatively into glucose. Because the diabetic rats had 3 to 4-fold increased blood glucose values, the oxidation of these sugars is not markedly influenced by insulin. This does not mean, however, that the utilisation of fructose, xylitol and sorbitol is insulinindependent but rather that CO_2 production from glucose is relatively independent of insulin. This suggests that glucose is a fuel of minor importance for the insulinsensitive tissues and that it is mainly oxidized by insulinsensitive tissues such as brain. As these results may have practical implications in parenteral therapy, similar studies are being carried out in men.

Considérations théoriques sur la synthèse des acides gras

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Différents modèles mathématiques représentant la biosynthèse des acides gras à partir de l'acétate ont été examinés dans une étude théorique. Cette étude théorique est une analyse compartimentale, et consiste à suivre l'évolution d'un système à l'état stationnaire dans lequel on a introduit de l'acétate radioactif. Les paramètres des modèles sont choisis de manière à ce que les résultats des calculs correspondent à certains résultats expérimentaux présentés dans la bibliographie.

L'intérêt de la méthode est de montrer que les concepts classiques d'élongation et de condensation doivent être redéfinis en termes plus cinétiques. D'autre part, l'absence apparente des intermédiaires de synthèse peut s'expliquer par leur faible concentration et leur courte durée de vie.

Préparation d'extraits en vue de l'analyse des nucléotides du ganglion cervical isolé du rat

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Dans le but d'étudier les modifications du pool des nucléotides lors de l'activation fonctionnelle du tissu, nous avons analysé comparativement les différentes étapes

conduisant à l'obtention d'une fraction contenant ces constituants. L'efficacité de l'homogénéisation du tissu a été testée par étude des modifications ultrastructurelles en résultant. Trois paramètres ont été choisis comme témoins de la qualité et de la reproductibilité des différentes méthodes d'extraction examinées: 1. Le rendement en protéines tissulaires séparées de la fraction contenant les nucléotides et dosées par la technique de Lowry. 2. Le rendement en ATP, dosé au moyen de la technique basée sur la production d'une émission photonique proportionnelle à la quantité du nucléotide par le système luciférine-luciférase extraits d'abdomens de Photinus Pyralis. 3. La migration des nucléotides séparés par chromatographie bidimensionnelle sur couche mince de polyethylèneimine-cellulose.

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Rabbit Intestinal Sucrase-Isomaltase Complex: Separation of the Isomaltase

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The sucrase-isomaltase complex (SI) was isolated from rabbit small intestine by papain solubilisation, ammonium sulfate precipitation and Sephadex G-200 chromatography. The $S_{20^0,w}$ at $\mu = 0{,}0125$ (K phosphate buffer, pH 6.8) was 9.9; $\bar{v}$ was 0.739; mol.wt. was 200,000. At $\mu = 0.3$ it aggregated to a mol.wt. of ca. 406,000, assuming that $S_1/S_2 = (M_1/M_2)^{2/3}$. Increasing the pH to 9.2 reversed the aggregation. At intermediate μ and pH values both forms of the SI were present. Treatment of SI at 37°C, pH 9.6, for 30 min destroyed sucrase activity with the appearance of a 6.6 S component (mol.wt. approximately 120,000) and of higher aggregates; the mixture could be separated on Biogel P-200 into an inactive fraction (mol.wt. $\gtrsim$ 300,000), residual SI, and the isomaltase moiety (mol.wt. 130,000 both by gel filtration and by SDS-electrophoresis). The specific palatinase activity of the intact IS was 1.3–1.5; that of the isolated isomaltase was ca. 2.7.

The SI was rich in acidic aminoacids, aminosugars (7.8%), and neutral sugars (ca. 8%: D-glucose, D-galactose, mannose and fucose). The isomaltase moiety had a higher lysine/histidine ratio, and contained less glucosamin than the intact SI.

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Heterogeneity of Mitochondria in Different Rat Brain Regions

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The brain regions cortex, cerebellum, striatum, hippocampus, mesencephalon with diencephalon, pons with medulla oblongata, colliculus posterior were homogenized in 0.3 *M* sucrose and centrifuged (10 min, 200 g). 1 ml of the supernatant – corresponding to ca. 100 mg of tissue – was separated by centrifugation (2 h, 60,000 g) on a (27 ml) discontinuous sucrose density gradient (0.3, 0.8, 1.3, 1.35, 1.4, 1.45, 1.7 *M*). In addition to the myelin and synaptosomal fraction 4 main peaks were obtained [F_1 (heaviest) – F_4 (lightest)], which contained mainly mitochondria as checked by electron microscopy. In all regions F_1 showed higher glutamate-dehydrogenase (GDH), F_2 higher succinate-dehydrogenase (SDH), F_3 higher cytochromeoxidase (Cyt. ox.) and F_4 higher monoamine-oxidase (MAO) activity. The distribution of the various enzymes was similar for all brain regions except for the striatum (higher activity of SDH, Cyt. ox., GDH in F_1 and MAO in F_3), cerebellum (relatively lower activity of SDH, GDH in F_4) and pons with medulla oblongata (relatively higher activity of Cyt. ox. in F_4).

Effects of Phenobarbital on Rat Liver Regeneration

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Rats were partially hepatectomized and injected i.p. with a phenobarbital solution or water immediately after surgery. At various time intervals thereafter, animals were injected i.m. with the labelled thymidine analogue, ^{131}iododeoxyuridine (^{131}IDU) and sacrificed 2 h later. Radioactivity retained in formalin-fixed liver tissue was determined as a measure of DNA synthesis at the time of ^{131}IDU administration.

In control animals, ^{131}IDU incorporation into liver began to increase between 14 and 16 h after partial hepatectomy. Injection of 0.1 mg phenobarbital per g of body weight resulted in a delay of the increase in ^{131}IDU incorporation by several hours, and the subsequent increase in mitotic activity was also delayed or occurred more slowly than in controls. These effects of phenobarbital on the time course of DNA synthesis and cell division were, however, smaller than the reported delay of microsomal enzyme induction following phenobarbital administration in partially hepatectomized, as compared to control rats (Chiesara et al., Lab. Invest. *22*, 329, 1970).

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Mitochondrial Pyruvate Metabolism: Measured and Computer-Simulated Fluxes

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Rat liver mitochondria were incubated in a medium containing pyruvate-2-^{14}C, HCO_3^-, ATP and phosphate. Metabolic fluxes through individual enzymatic steps of pyruvate metabolism including the citric acid cycle were calculated from the measured amounts and from specific radioactivities of the products obtained. These calculations revealed that the overall ratio of total ATP formed to NADH and FADH oxidized in this system amounted to only about 0.6. Experiments with oligomycin and atractylate showed that neither uncoupling nor an extramitochondrial ATPase was responsible for this low value. A computer model of mitochondrial pyruvate metabolism involving 54 reactions and 39 metabolites was developed. A close fit of the experimental and of the computer simulated data could only be obtained when an intramitochondrial ATPase was included in the computer model. These findings suggest the existence of unknown intramitochondrial energy requiring processes. The hypothesis will be discussed whether the transport of anions out of the mitochondria is such an energy requiring process.

Selectivity of Enzyme Induction in Sympathetic Ganglia by Nerve Growth Factor (NGF)

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NGF, isolated from mouse salivary gland, stimulates both growth and differentiation of sympathetic neurons. Intraperitoneal injection of new-born rats for 10 days with 10 μg/g of NGF resulted in varying changes in the activity of enzymes involved in the synthesis and metabolism of norepinephrine. The increase in total activity (product formed/pair of ganglia) amounted to 1800% (controls 100%) for tyrosine hydroxylase (TH), 1300% for dopamine-β-hydroxylase (DBH), 400% for dopa-decarboxylase and 300% for monoaminoxidase. The corresponding values for the increase in 'specific activity' (product formed/mg protein) were 650%, 500%, 150% and 120%. Since, in nontreated controls, the neurons represent $\sim {}^3/_4$ of the total volume of the superior cervical ganglion, the increase in enzyme activity cannot be explained by a relative increase in size and number of ganglionic cells. It is concluded that NGF produces a selective induction of TH and DBH which are exclusively located in adrenergic neurons. Similarities between the effect of NGF and trans-synaptic induction of enzymes by increased sympathetic activity are discussed.

Preservation of 2,3-diphosphoglycerate in Stored Blood by Exclusion of Oxygen

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The concentration of 2,3-diphosphoglycerate (DPG) in blood decreases upon storage. Low levels of DPG in erythrocytes are correlated with an increased oxygen affinity of hemoglobin. Since this may be responsible for the tissue hypoxia observed after multiple transfusions with stored blood, preservation of DPG is of potential clinical interest.

On storage of blood under anaerobic conditions loss of DPG was diminished as compared to normal storage under air. Concentrations of DPG in blood after 3 and 7 days of storage both under nitrogen and under air, given in percent of the initial value (6.7 micromoles of DPG per ml of packed red blood cells) were as follows:

Form of storage	% DPG at time 0	% DPG after 3 days	% DPG after 7 days
Under air	100	31	2
Under nitrogen	100	81	54

The effect of partial preservation of DPG is explained in molecular terms by the preferential interaction of DPG with deoxyhemoglobin.

Non-Competitive Inhibition of Human Erythrocyte and Electric Eel Acetylcholinesterases

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According to a recent model acetylcholinesterase (EC 3.1.1.7) has a tetrameric structure with a composition of $\alpha_2 \beta_2$ subunits. One of the subunits (α) contains the catalytically active center and the other (β) is thought to be the cholinergic receptor. Since the enzyme is activated by mono- and divalent cations the effects of chelating agents on the catalytic activity were determined. No significant inhibition could be observed when EDTA was added to the partially purified enzymes from human erythrocyte membranes and from the electric organ of Electrophorus electricus. However, these enzymes were inhibited in a non-competitive manner by a variety of chelating agents such as o-phenantroline, 8-quinolinol, salicylaldehyde and 1-phenyl-butane-1,3-dione. The inhibition constants, as determined graphically, varied between 0.1 m*M* for o-phenantroline and 2.0 m*M* for salicylaldehyde.

PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

Compartmental Analyses of the Gastrointestinal Absorption of a Sulfonamide in Man

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Chemical determinations of non-metabolized and total sulfonamide were performed in plasma and urine of 9 human subjects after receiving a single oral dose of 1 g sulfamethoxazole. Three subjects were control, 3 were pretreated with an anticholinergic drug (propantheline bromide) and 3 were gastrectomized patients. The data were submitted to compartmental analyses, focussing on the absorptive phase of the process.

The ascending part of the blood level curve could not be adequately solved by assuming an unique compartment for the entry of the drug. On the other hand, three distinct compartments were identified, each with a specific first order kinetic constant.

The absence of the 2 first compartments in gastrectomized subjects and the persistance of the drug in these 2 first compartments in patients pretreated with the anticholinergic drug leads us tentatively to identify these 2 compartments with the gastric absorption of the drug, whereas the third compartment of entry appears to be purely intestinal.

Amphetamine Hyperthermia in the Rat

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Amphetamine increases intraperitoneal temperature and enhances motor activity in the unrestrained rat kept at an ambient temperature of 30°C. We blocked neuromuscular transmission by curarizing the animal, and still obtained a dose-dependent hyperthermia after administration of amphetamine. Spinal transection at C1 diminished,

but did not abolish the dose-dependent hyperthermic response. Pretreatment with the alpha-blocking agent azapetine or with the beta-blocking agent propranolol abolished amphetamine hyperthermia in the curarized preparation with and without spinal transection. In the unrestrained rat, the blocking agent did not prevent motor stimulation and hyperthermia produced by amphetamine. We conclude that neither the increase in motor activity nor direct effects on supraspinal sites of the CNS can fully account for the amphetamine hyperthermia. Both alpha and beta receptors seem to be involved in the hyperthermic response observed in the curarized rat.

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Applications of Telemetry in Neuropharmacology

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Physiological and behavioral parameters can be recorded continuously from entirely unrestrained rats by multichannel telemetry. We measure body temperature and ECG with transmitters implanted beneath the skin or in the peritoneal cavity, and EEG or evoked responses with transmitters attached to the skull. Simultaneously, motor activity, and food and water intake are recorded at controlled ambient temperatures. Details of implantation procedures, experimental set-up, and some pharmacological applications will be illustrated with video-tape recordings.

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Effect of Glucagon in Rats: Liver Cyclic AMP, Liver Glycogen, Plasma Glucose

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Glucagon increases cyclic 3′, 5′-adenosine-monophosphate (cAMP; measured by a modification of the isotope dilution method of BROOKER et al., Biochemistry 7, 4177 (1968)) in liver, but not in brain of fed rats. The hepatic cAMP level reaches a maximum 5 min after s.c. injection of glucagon and is almost back to normal (2×10^{-9} mol/g) after $^1/_2$ hour. The increase is dose-dependent: 10 µg/kg cause an increase of 50% and 500 µg/kg of 600%.

Plasma glucose shows a rise of 15–25% for 5–30 min after 30–500 µg glucagon/kg and thus a similar time course as the cAMP in liver. The glucagon-induced decrease of glycogen in liver (around − 35% after 30–60 min) behaves initially similar to the changes in cAMP and blood glucose but persists somewhat longer. The concomittant changes of cAMP, glycogen and glucose support the second messanger theory of SUTHERLAND et al.

The glucagon-induced decrease of α-amino nitrogen in blood plasma is pronounced (− 40% after 30–60 min) and its time course almost identical to the drop of hepatic glycogen. Gluconeogenesis from amino acids in vivo might thus have a greater importance for the glucagon-induced hyperglycemia that hitherto considered.

Wirkung einiger trizyklischer Antidepressiva auf Cortex-Synaptosomenzyme

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Aus homogenisiertem Cortex von Meerschweinchen wird durch diskontinuierliche Dichtegradientzentrifugation die Synaptosomenfraktion isoliert und an dieser der Einfluss von Imipramin, Desipramin, Chlorimipramin und Opipramol auf die Aktivität der Acetylcholinesterase, der (Na^+-K^+)-aktivierten ATPase und der Mg^{2+}-aktivierten ATPase untersucht.

Bei In-vitro-Versuchen zeigt sich eine generelle Hemmung aller untersuchten Enzyme bei höheren Konzentrationen dieser Pharmaka, wobei Chlorimipramin die relativ stärkste Wirkung aufweist. Opipramol hemmt nur die Mg^{2+}-aktivierte ATPase und nicht die (Na^+-K^+)-aktivierte ATPase.

Bei In-vivo-Versuchen (Dosis = 10 mg/kg i.p.) werden die genannten Enzyme in den nachträglich isolierten Synaptosomen nicht deutlich beeinflusst.

Die Gehaltsmessung von ^{14}C-Imipramin ergibt eine maximale Anreicherung 10 min nach der Injektion. Der Anteil der verabreichten Substanz beträgt zu dieser Zeit: im Gesamtgehirn 0,15%, im Cortex 0,04% und in der kortikalen Synaptosomenfraktion 0,0065%, d.h. $^1/_6$ des Gehalts im Cortex.

Hypertension in Rats and Vascular Supersensitivity to 5-Hydroxytryptamine (5-HT)

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Vascular supersensitivity (SU) to 5-HT has been observed in genetic (New Zealand strain) and renal hypertensive rats (RHR) (McGregor and Smirk, Am. J. Physiol. *219*, 687, 1970). In DOCA/saline and RHR but not in genetic (Japanese strain) hypertensive rats (GR) increased pressor responses to 5-HT were found in pithed rat preparations.

Mesenteric arteries (MA) isolated from all these animals were supersensitive to the vasoconstrictor effect of 5-HT to a varying extent. This SU was much more pronounced than that to noradrenaline or KCl. It seems, therefore, difficult to explain SU to 5-HT only by structural changes of the vessel wall. Since isolated gastric strips and renal arteries of normotensive and hypertensive rats responded equally to 5-HT, SU to 5-HT is not a feature common to all types of smooth muscle or to all arteries of hypertensive rats. Methysergide, which blocked the pressor response to 5-HT in pithed rats, did not influence blood pressure in GR. This finding questions a causal relationship between vascular SU to 5-HT and the maintenance of this type of hypertension.

Depressant Amino Acids and Possible Antagonists on Medullary Reticular Neurones.

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Both glycine and γ-aminobutyric acid (GABA) depress the firing of bulbar reticular neurones, glycine being more effective than GABA. The depressant action of glycine, but not that of GABA, is blocked by strychnine

(Hösli and Tebēcis, Exp. Brain Res. *11*, 111, 1970). In continuation of these studies, we tested other possible antagonists on the depression caused by these amino acids. All substances were applied microelectrophoretically to neurones of the bulbar reticular formation of decerebrate cats. Bicuculline, a convulsant which blocks synaptic inhibition in various areas of the brain, often reversibly reduced the depressant action of GABA. It sometimes also affected the depression by glycine, although to a lesser extent. The GABA derivative, β-(4-Cl-phenyl) GABA (Lioresal®, Ciba-Geigy AG), usually had no action on the firing frequency, and rarely reduced the depressant actions of GABA (5/18 neurones) and glycine (2/22 neurones). Extracellular administration of glycine to neurones from which simultaneous intracellular recordings were made revealed that the depression by this amino acid was accompanied by a hyperpolarization (3 cells) and an increase in membrane conductance (1 cell). Both glycine and GABA are likely to be inhibitory transmitters in the medulla oblongata of the cat.

Catecholamine Content of Tuberal Nerve Cells and Serum LH after Electrical Stimulation

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By means of a microfluorimetrical technique, the intensity of the catecholamine fluorescence was determined in nerve cells of the arcuate and periventricular hypothalamic nuclei of ovariectomized rats pretreated with estrogen and progesterone. Intermittent electrical stimulation of the arcuate nucleus or of the medial preoptic area caused an acute increase in the fluorescence intensity of these nerve cells within 5 min, and high intensities were found after 10 and 30 min of stimulation. The initial increase was followed by a decrease below control levels, as observed after 60 min. The increase in fluorescence intensity was completely prevented by pretreatment with a tyrosine hydroxylase inhibitor and therefore appears to be due to an enhancement of amine synthesis. This type of change in cellular amine concentration appears to be characteristic for an acute increase in neuronal activity. The neuronal response to preoptic stimulation was accompanied by a fall in pituitary luteinizing hormone and a rise in serum LH, which indicates that the tubero-infundibular dopamine neurons may be involved in the induction of LH discharges from the preoptic region. A similar influence on LH was seen after arcuate stimulation.

Lysolecithine Content of the Membranes of Monoamine Storage Organelles

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Lysolecithine which has been found in the chromaffine granules of bovine adrenal medulla is thought to be involved in the catecholamine release by exocytosis. It was therefore of interest to compare the phospholipid pattern of the isolated membranes of these granules with that of the membranes of 5-hydroxytryptamine organelles and of the cytoplasmatic membranes of rabbit blood platelets. The phospholipids were separated by mono- and bidimensional thin layer chromatography, further characterized by staining and quantitatively analyzed for phosphorus.

All the three membranes contained phosphatidylcholine, phosphatidyl-ethanolamine, sphingomyeline, phosphatidyl-inositol and phosphatidyl-serine in similar relative amounts. Lysolecithine was detected almost exclusively in the membranes of the chromaffine granules. Its content amounted to about 17% of that of the total phospholipids. Therefore, if lysolecithine is essential for the process of exocytosis, the present results would indicate that amine release from platelets, unlike that from the adrenal medulla, cannot occur by this mechanism.

Affinity labelling of cholinergic receptors of the motor endplate

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Two derivatives of acetylcholine and two compounds of similar nature were used for affinity labelling of the cholinergic receptor of endplates in the mouse diaphragm.

With diazoacetylcholine and irradiation by light (Hg high pressure lamp) irreversible fixation and blocking of neuromuscular transmission were obtained, through photolysis of the molecule, formation of a reactive carbene, and subsequent covalent bonding in the vicinity of the receptor. This radioactive compound was concentrated 2 to 10-fold in the endplate region.

Iodacetylcholine was effective through an electrophilic substitution near a receptor group (probably -SH) while simultaneous hydrolysis by acetylcholinesterase was diminished only when the concentrations of the compounds were increased at least tenfold, which demonstrates their specific action on the cholinergic receptors.

We failed to fix 2-Trimethylamino-aethylisothiocyanate-iodide (TAI) and 1-Cyclohexyl-3-(2-morpholinoaethyl)-carbodiimid-metho-*p*-toluol-sulfonate (CMC) to the receptors in other attempts at covalent bond formation. These last two compounds behaved as weak curarizing and as depolarizing compounds respectively. They ceased their action immediately after washing of the nerve-muscle preparations.

^{14}C-Choline-Uptake in Endplates under the Influence of Different Drugs

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The uptake of ^{14}C-choline into endplates of the diaphragm of mice was investigated with the autoradiographic technique after intravenous injection. Choline was concentrated within 2 min in the endplate region proportional to the injected dose (0.1–1.0 μg/g) and remained there for over 20 min. Cutting of the phrenic nerve diminished the radioactivity by 40% within 14 h, demonstrating the presynaptic location of the transport mechanism. Only traces of ^{14}C-choline remained in the terminal nerve axons. The uptake into the synaptic axon was diminished by hemicholinium-3, decamethylendicholine, tetra-ethylammonium, triethylcholine and chlorpromazine. The muscle tissue was not much influenced in its choline content by these compounds. Cocaine, imipramine and reserpine had no significant influence on choline uptake in endplates or muscle.

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

Incorporation of ^{3}H-Thymidine in Arteries after Removal of Endothelium

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Endothelium of rabbit iliac artery was removed by means of a balloon catheter; the other iliac artery served as control. Sequentially, platelets, granulocytes and monocytes adhered to the denuded subendothelial surface. Proliferating cells were first observed on this surface at day 2. After 2 weeks the new intima was in the average nearly as thick as the media. In order to determine the site of proliferative activity, ^{3}H-thymidine was injected i.v. 3 h prior to fixation by perfusion of glutaraldehyde. Incorporation of ^{3}H-thymidine in smooth muscle cells of media increased till day 4, and reached control level again after 2 to 4 weeks. On the luminal side of internal elastic lamina (IEL) incorporation was first observed at day 2, reached a peak after 1 to 2 weeks and still exceeded that of media after 6 weeks. These findings are consistent with the observation that smooth muscle cells of media migrate from the media into the intima starting at day 3, and that they form the main component of the new intima. However, the source of the new endothelium remains to be established.

Recovery of Rat Hepatocytes after Phenobarbital Treatment; a Morphometric Study

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We have extended the morphometric study of Weibel et al. and Stäubli et al. (J. Cell Biol. *42*, 68, and *42*, 92, 1969) to include the 7 days after the last of 5-daily injections of 100 mg/kg of phenobarbital (PB). Orrenius and Ericsson (J. Cell Biol. *28*, 181, 1966) reported a 5-fold increase in *o*-demethylase activity of rat liver microsomes after PB treatment which returned to control levels 5 days after the drug was withdrawn. Electron microscopy suggested that the PB induced ER membranes disappeared only after 15 days with no apparent increase in autophagic activity. – Our results suggest that the surface area of the ER follows more closely the changes in enzyme levels. After the 5-daily injections of PB, the surface area of the ER is 51 m^2/100 g body weight when, according to Orrenius, the demethylase activity is at a maximum. The ER returns to the control level, 39 m^2/100 g body weight, already by the fourth day after the last injection. We also found an increase in autophagic activity. These findings suggest that there is a close relationship between changes in enzymatic activity and ER surface area, and that membranes are removed from the cytoplasm of the hepatocyte by autophagy.

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RNA-Dependent DNA Polymerases from Bacterial and Eukaryotic Cells

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An enzyme catalysing RNA-dependent DNA synthesis was prepared from E. coli by partition in an Albertsson system, chromatography on DEAE cellulose, phosphocellulose and DEAE Sephadex, and sucrose gradient centrifugation. Thymidylate incorporation was nil without template, and 240 nmoles/mg and 10 min with nicked, double-stranded Qβ RNA as template. Unnicked double-stranded Qβ RNA (a mixture of RI and RF) was inactive. Since double-stranded DNA (calf thymus) stimulated DNA synthesis to about the same extent as the nicked RNA duplex, the enzyme may be identical with one of the known DNA polymerases of E. coli (DNA polymerase I or II). A similar enzyme activity was also observed in a purified polymerase preparation from AMV virus as well as in extracts from AMV-infected myeloblasts and normal fibroblasts.

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Formation of Initiation Complex with Purified Mammalian Ribosomal Subunits

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It is not known if initiation of protein synthesis in eukaryotic cells involves 80 s ribosomes directly or if the 40 s subunits alone can form an initiation complex with the initiator codon AUG and met-t-RNA$_F$. We have used mouse plasmocytoma ribosomal subunits prepared under mild conditions to test the ability of purified subunits to form such a complex. Met-t-RNA$_F$ and met-t-RNA$_M$ were separated by reverse-phase chromatography.

Preliminary results indicate that purified 40 s subunits bind met-t-RNA$_F$ at 5 mM Mg^{++} in the presence of AUG and of 'crude initiation factors'. The reaction is specific for met-t-RNA$_F$. Under the same conditions, 60 s subunits do not bind met-t-RNA$_F$.

Such results suggest that initiation in mammalian tissues can take place, as it does in the case of bacteria, in a stepwise manner, beginning with the formation of a complex involving the small ribosomal subunit.

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Elektronenoptische Darstellung amyloider Strukturen bei Wänden von Pilzsporen

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Als Modell für die Versuche wurde *Lactarius mitissimus* Fr. gewählt, da er leicht beschaffbar ist und seine Sporen grosse, von einer amyloiden, in Jodlösung dunklen Masse bedeckte Oberflächenskulpturen aufweisen. Die elektronenoptische Darstellung beruht auf der Fällung des locker in der Masse gebundenen Jodes mit einem Schwermetallsalz. Von 20 Varianten ergab folgende Methode erfolgversprechende Resultate: Die Sporen wurden in 1 ml Glutaraldehyd 1% aufgeschwemmt, nach 20 min wurde 1 ml Jodlösung (H_2O 10 ml, KI 0,3 g, I_2 0,1 g) zugegeben, und nach weiteren 5 min zentrifugiert. Nach zweimaligem Waschen wurden die Sporen in 1% $HAuCl_4$ für 5 min in Dunkelheit gestellt, danach gewaschen, in Agar eingebettet, entwässert und in Epon eingeschlossen. Die Dünnstschnitte zeigten ohne weitere Kontrastierung die amyloide Masse dunkel gegen die helle Zellwand. Eine lichtoptische Kontrolle war möglich, da die Goldfällung dunkel erscheint.

Structure and Antigenic Properties of Lipoproteins Isolated from Mouse Skin

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From mouse skin exposed to thermal energy, a lipoprotein with toxic activity has been isolated. A non-toxic precursor was derived from normal skin. A spin labelled derivative of stearic acid was incorporated in both lipoproteins. Identical spectra were obtained for both compounds, typical for fast anisotropic motion of the label, suggesting a limited number of highly ordered, fluid lipid regions. IR-spectra of both lipoproteins in KBr were identical. However, the IR-spectrum of the toxic compound differed from that of the precursor when dissolved in lipid active solvents. An increase of the ratio amide-I (1650 cm^{-1}): C = 0 stretching vibration (1735 cm^{-1}) was observed for the toxin, suggesting a progressive lipid-protein dissociation. The toxin showed a progressive shift of the maximum at 1650 cm^{-1} to 1630 cm^{-1}, indicating a transition from alpha and/or random to beta-conformation with progressive lipid solubilization. A difference of the antigenic properties was shown by rising an antitoxic serum with the toxin and an immunosuppressive serum with the non-toxic compound. The spectroscopic results suggest only minor differences in the molecular structures causing dramatic differences of the biological properties.

Mouvements subcellulaires de l'Acétylcholine dans l'organe électrique de la Torpille

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L'Acétylcholine est la substance neurotransmettrice du couple nerf-électroplaque chez *Torpedo Marmorata*. Lors d'une brève stimulation répétitive une importante quantité d'ACh est libérée et convertie en choline. La fraction «libre» d'ACh diminue alors ou disparaît. Mais il reste une importante fraction, l'ACh «stationnaire» qu'une activation même prolongée ne peut libérer. Cette dernière est liée aux vésicules «synaptiques». Le venin de la Veuve Noire (*Latrodectus mactans tredecimguttatus*) exerce sur la répartition subcellulaire d'ACh des effets différents de ceux de l'excitation.

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Photochemical Affinity Probes for Cholinergic Receptors and Acetyl Cholinesterase

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We have synthesized diazoacetyl choline bromide (Experientia *26*, 1207, 1970), and *p*-azidophenyl trimethyl ammonium iodide in order to evaluate their potentialities as affinity labels for investigations of the active sites of acetyl cholinesterase and acetyl choline receptor.

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'Schiff-type' Electron Stains in Ultrastructural Cytochemistry

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26 differents stains were tested by fluorescence and electron microscopy for possible 'Schiff-type' properties. Tissues were fixed in either aldehyde or permanganate and embedded in various plastics; Feulgen-type reactions were then carried out on tissue-sections (Gautier, Schreyer and Cogliati, Experientia *26*, 693, 1970; Gautier and Schreyer, Proc. 7th internat. Conf. E.M. *1*, 559, 1970). The specificity of these reagents for tissue aldehydes was tested in following experiments: a) with or without acid hydrolysis, b) with or without SO_2-bubbling of the stain and c) after treatment with Aniline-HCl as blocking agent for aldehydes, or with NaCl–HCl as control (modification of the Oster-Mulinos histochemical procedure as recently described by Peters and Giese (Proc. 7th internat. Conf. E.M. *1*, 557, 1970).

At least five of these stains were shown to be satisfactory at the ultrastructural level, with regard to specificity and intensity of DNA staining.

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Eine mit Zeitraffer verfolgte Synthese von Glykogen in kultivierten Myoblasten

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Die sogenannten Asphaltflecken (Gross und Riedel 1969) wurden durch histochemische (Krompecher, Krompecher-Kiss und Gross 1969, Müller, Krompecher und Gross 1970) und elektronenmikroskopische Untersuchungen (Gross und Müller 1971) als Glykogen diagnostiziert. Es ist eine Ansammlung lose aneinanderliegender β-Teilchen. Diese Glykogendepots sind in der lebenden Herzmuskelzelle mit dem Phasenkontrastmikroskop deutlich in Erscheinung zu bringen. Als unregelmässig begrenzte Flecke ändern sie während der Kultivierung stetig ihre Form. Bei der sich ausbreitenden Zelle erscheint als «Asphaltfleck» das Glykogen, das schon im Hühnerembryo während der Entwicklung im Ei gebildet war. Während der Vergrösserung der Zellen in den ersten zwei Tagen der Kultur wachsen auch die Flecke. Diese Energiereserven werden aber bei der Pulsation der Zellen in weiteren 48 h aufgebraucht. Nach Fütterung und der Invasion des Nährmediums in die Zellen durch Pinocytose bilden sich neue Flecke. In 3 Zellen ist diese Synthese vom Zeitrafferfilm festgehalten.

Duration of Cell Cycle Phases and Diploid DNA Content in Four Vertebrate Species

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The last maturation cycle of erythroblasts has been examined in order to study the possible relationships between phase length and diploid DNA amount per nucleus. DNA content has been determined in erythrocyte nuclei using Feulgen microspectrophotometry and fluorometry. Various phases of the cell cycle have been analysed

in vivo by the labelled mitoses method following a single injection of ^{3}H-TdR as well as by the double ^{3}H-TdR and ^{14}C-TdR labelling technique; both methods have yielded comparable results. The duration of S is approximately equal to 41 h in *Triturus cristatus* ($2n = 24$; DNA/nucleus = 136 AU), to 26 h in *Rana esculenta* ($2n = 26$; DNA/nucleus = 39.6 AU), to 15.4 h in *Lacerta viridis* ($2n = 38$; DNA/nucleus = 8.8 AU) and to 6.9 h in *Gallus domesticus* ($2n = 78$; DNA/nucleus = 6.75 AU). The duration of G_1 and M do vary according to the same trend. The minimal value of G_2 is similar for all four species. These results indicate a direct but non-linear relationship between DNA amount per nucleus and the duration of synthesis, and bear on the arrangement of the DNA molecule within chromosomes.

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Cytophotometrical Evaluation of the Hale Reaction in Two Ascites Tumors

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The surface of most mammalian cells carries a net negative charge. Placed in an electric field, such cells demonstrate an anodic mobility which is dependent on the quantity of negatively charged groups. The bulk of these groups consists of the terminal carboxyl groups of sialic acid. In order to complement the physical technique of cell electrophoresis, a cytochemical procedure, the Hale reaction was chosen. It is based on the reaction of negatively charged groups with colloidal iron which, in turn, is visualized by the Prussian blue reaction. After complete standardization of the Hale reaction, attempts to evaluate it quantitatively by cytophotometry were performed. Two types of ascites tumor cells (M.Mel. No. 1 and TA3) were examined. Testing method was the evaluation of the effect of neuraminidase on the negatively charged surface groups. The two tumor cell systems reacted differently. When M.Mel. No. 1 cells had been fixed before treatment with neuraminidase, the reduction in staining intensity was minimal, whereas treatment before fixation resulted in a decrease of 20%. In contrast both fixation groups of TA3 cells showed the same reduction of 50%. These findings run parallel with those of cell electrophoresis.

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Charakterisierung der Proteine von Polyoma Virus und von SV40

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Polyoma-Virus und SV40 wurden in Gewebekultur mit S^{35}-Methionin markiert. Bei der Virusreinigung durch Gleichgewichts-Zentrifugation im CsCl und durch Sedimentation im Saccharose-Gradienten ging etwa die Hälfte der Infektiosität verloren. Die Viren wurden auf verschiedene Arten aufgebrochen und die resultierenden Polypeptide durch Elektrophorese in 7,5% Polyacrylamid-Gelen in Gegenwart von 0,1% SDS getrennt. Für SV40 wurden Polypeptide mit folgenden Molekulargewichten beobachtet: 42000, 35000, 25000, 16000, 12500 und 9300, wobei das grösste Polypeptid ungefähr 70% der Radioaktivität enthält.

Die Gelanalyse von Polyoma gab ähnliche Resultate. Die Molekulargewichte der Polypeptide sind 43000, 30000, 26000, 21000, 16000 und 13000, wobei das erste wiederum etwa 70% der Radioaktivität enthält. Die Polypeptide wurden mit Trypsin verdaut und die resultierenden Peptide durch Elektrophorese auf Cellulose-Dünnschicht separiert und durch Autoradiographie nachgewiesen. Für jedes Polypeptid ergab sich daraus ein charakteristisches, von der anderen grundsätzlich verschiedenes Bild.

Nachweis von sauren Polysacchariden mit Acriflavin und Phosphorwolframsäure

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An fixiertem hyalinem Knorpelgewebe wurden verschiedene Möglichkeiten einer zweistufigen Blockfärbung mit Acrilflavin und PTA ausgetestet. Neben der allgemeinen und bekannten Kontrastierung durch PTA konnte nach Färbung bei pH 5 und 7 in der Matrix des Knorpels ein dichter, körniger Niederschlag beobachtet werden. Versuche mit enzymatischem Abbau verschiedener Gewebekomponenten zeigten, dass der Niederschlag für die im Knorpelgewebe vorhandenen sauren Mucopolysaccharide spezifisch ist. Weitere Versuche (Variation des pH, der Konzentration) und die Färbung von Wurzelspitzen von *Allium cepa* und Hefeprotoplasten ergaben eine Spezifität der Acrilfavin-PTA-Methode für saure Polysaccharide.

Interferon Assay and Action Kinetics

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Purification of Interferon (IF) is limited by extremely small amounts of active material and tedious assay. Our new assay is based on *inhibition of synthesis* of the *first* easily detectable viral material: viral *RNA*. *1. Optimal viral RNA synthesis in chick fibroblast cell culture:* Cell sheets are infected during 60 min with a multiplicity of 0.1 of Semliki Forest Virus (SFV), then treated in the following manner: Eagle's MEM Medium at + 1 h, 5 µg per culture of Actinomycin D at + 2.5 h, 3 µC uridine-^{3}H per culture at + 3 h, followed by solubilization of cells with SDS and precipitation with TCA at + 9 h. RNA synthesis is exponential within this time range and $1–1.5 \cdot 10^{4}$ cpm/µC/9 h ± 1000 are incorporated in absence of IF. *2. Assay:* IF is added to the medium for 2–4 h before infection. Sensitivity in terms of reduction of RNA synthesis is in the range of 1–110 µg/ml IF (semipurified); with a linear log dose response curve in the range of 5–90 µg/ml. A precision at least as high as in plaque assay could be achieved. The minimum time lapse between the application of the sample and the final result is 13–14 h. *3. Kinetics of action:* Maximum effect is observed after about 6–10 h of cell contact dependent upon concentration. An increase of activity can be blocked during this time with Actinomycin, but the effect starts as early as 15 min after cell contact even at low doses.

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The Resynchronisation of in vitro Qβ RNA Synthesis Using a Ribosome as Stopper

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Sequence analysis of Qβ RNA using sequential RNA synthesis in vitro requires synchronous elongation (Billeter et al., Nature *224*, 1083, 1969). Since synchronicity is largely lost after several hundred nucleotides we searched for a method of resynchronisation. Qβ RNA with a ribosome bound to the coat protein initiation site serves as template for Qβ replicase. However, synthesis stops at the ribosome yielding a minus strand about $^2/_3$ the normal length (Kolakofsky and Weissmann, Nature, in press). The ribosome-blocked synthesizing complex was EDTA-treated to detach the ribosome; after Sephadex chromatography and readdition of substrates, unfinished minus strands were completed and released. For resynchronisation replicase was run up to the ribosome, using unlabelled substrates. After ribosome removal, synthesis was restarted with α-^{32}P-nucleoside triphosphate. After 4″, 8″, 12″ and 16″ at 20°C, samples were analyzed by Sanger's techniques. The simple fingerprints indicate synthesis of a unique labelled RNA segment, which appears to be complementary to the region extending from the coat initiation site toward the 5′ end of the RNA. Sequence analysis should reveal the structure of the intercistronic region between the A protein and the coat cistron.

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Zur Lokalisation von Phospholipiden in Cytomembranen

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Ein Teil des Kontrastes, der bei OsO_4-Fixation entsteht, geht auf die Reaktion des OsO_4 mit den ungesättigten Fettsäuren der Phospholipide zurück. Durch die Eliminierung der Doppelbindungen und die dadurch bewirkte Änderung des Kontrastes versuchen wir Aufschluss über die Lokalisation von Phospholipiden in den Membranen von Saccharomyces cerevisiae zu erhalten. Die Eliminierung der Doppelbindungen erfolgt unter milden Bedingungen über die Bildung eines Quecksilberadduktes und anschliessende Reduktion des Hg mit Natriumborhydrid. Dabei entsteht ein sekundärer Alkohol, der mit OsO_4 nicht oder nur sehr langsam reagiert. Anhand von Aufnahmen verschiedener Membranen werden die Resultate diskutiert.

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G-Methylation of tRNA by Rat Liver Methylase

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G-derivatives methylated in position 2 occur at two specific sites in tRNA: N^2-methylguanosin (m^2G) between stem and dihydro-U arm and N^2-dimethylguanosin (m^2_2G) between dihydro-U and anticodon arm. By column chromatography on DEAE cellulose and hydroxylapatite an enzyme fraction was isolated which upon incubation with ^{14}C-S-adenosyl methionine methylated E. coli tRNA yielding 0.1–0.2 residues m^2G and 0.05–0.1 residues m^2_2G per tRNA molecule. The ratio of the two methylated bases remained constant during enzyme purification. This indicates that the G-methylation observed is due to one enzyme which modifies the G between the dihydro-U and anticodon arm to m^2_2G. In agreement with this hypothesis is the fact that total rat liver tRNA having already a full complement of m^2_2G is not methylated by the enzyme. Furthermore, no methylation occurs with rat liver serine-tRNA which carries an unmodified G residue between stem and dihydro-U arm. It therefore seems that the enzyme isolated is only responsible for the formation of m^2_2G and that the m^2G found is an intermediate in that reaction.

Modification of Proliferation and Specific Cell Functions in Mastocytoma Cultures

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Murine mastocytoma cells were incubated in various semisynthetic media in order to study the effects of reducing the concentration of a single amino acid (L-leucine, L-histidine or L-tryptophan, respectively). In media with a reduced concentration of leucine or histidine, cell multiplication was impaired and an increase in the relative number of dead cells was observed, whereas omission of tryptophan had no appreciable effect on cell proliferation during at least 96 h.

After incubation of cells in these media during 4 days, the mean cellular histamine and 5-hydroxytryptamine (5-HT) contents, as well as the capacity to incorporate $^{35}SO_4$ into TCA-insoluble cellular material were determined. The mean cellular content of histamine or 5-HT was reduced in media containing low concentrations of the corresponding precursor amino acid. On the other hand, appropriate reduction of the leucine level in the culture medium resulted in an increase of the mean cellular 5-HT content and an increased $^{35}SO_4$ incorporation.

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Enhancement of Abnormal Cell Proliferation in Lung Explants after Marijuana Cigarette Smoke

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To the best of our knowledge, there are no publications on experimental studies of effects of marijuana cigarette smoke on cells of the respiratory tract. Using the same model system which we developed for exposing lung explants to puffs of fresh cigarette smoke under standardized conditions (Exp. Cell Res. *62*, 1970), we now report on comparative cytological and cytochemical results after exposure of lung explants from Snell's and C57 Black mice to cigarette smoke with and without marijuana. Addition of marijuana to cigarettes evoked morphological and cytochemical alterations in epitheloid cells to a significantly higher degree than did smoke from cigarettes without marijuana. After marijuana cigarette smoke larger sizes and atypical shapes of nuclei and cells were frequently observed. There was also an increase in mitotic index, DNA content (microspectrography), and

DNA synthesis (^{3}H TdR), which was significantly higher after cigarette smoke *with* marijuana than after that *without* marijuana. The higher the content of tetrahydrocannabinol (THC), the more pronounced were the cytological and cytochemical alterations.

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Ultrastructure and Electrophysiology of Retina and Optic Nerve after Intraocular Xylocaine Administration

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Adult rabbits were examined for Electroretinogram (ERG), Visual Evoked Response (VER) and ultrastructural changes of retina and optic nerve after intravitreous administration of 0,01 g of Xylocaine (Lidocaine). Retinal pigment epithelium and photoreceptor cells showed a normal ultrastructure, the a-wave of ERG stayed unaltered. Synapses in the outer plexiform layer, bipolar and horizontal cells showed reversible ultrastructural changes; b-wave and oscillatory potentials of ERG were found abolished $^1/_2$–2 h after the injection, with complete recovery 4–7 h later. Retinal ganglion cell layer and optic nerve fibres of large calibre showed irriversible cellular and axonal degeneration; the first component of the biphasic part of VER was abolished $^1/_2$–2 h after the injection; partial recovery occured 6–12 h later. The discrete temporal recovery of functional as well as ultrastructural changes allows a correlation of these two factors.

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Juvenile Hormone Mimics: Metabolism of Farnesol and Farnesal in Drosophila

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It has long been known that insect development is governed by various hormones. But little is known about the mechanisms that regulate the observed, stage-specific titers of these hormones. Drosophila possesses several enzymes such as alcohol dehydrogenase (ADH), octanol dehydrogenase (ODH), aldehyde oxidase (aldox), and the esterases, whose stage-specific concentrations would control the titer of at least one developmentally important hormone: the juvenile hormone (JH). Since both farnesol and farnesal are JH mimics and farnesol is a known starting material in organic JH synthesis, we wondered whether these substances might serve as substrates for the respective enzymes. Crude homogenates of various Drosophila stocks differing in ADH, ODH, and aldox phenotype were electrophoresed on agar-gels. The gels were stained for the respective enzyme activities with 2-butanol, *n*-octanol, benzaldehyde, farnesol and farnesal. The results showed that farnesol is used as a substrate by ADH and ODH, farnesal by aldox.

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A Morphometrical Study on the Nexus of Rat Cardiac Muscle

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A morphometrical study on the nexus of rat cardiac muscle has been carried out, using (A) permanganate immersion fixation, (B) osmium tetroxide immersion fixation, and (C) glutaraldehyde perfusion fixation, followed by immersion fixation in osmium. The values for the nexus surface relative to the total surface of the intercalated disk are (A) 11%, (B) 14% and (C) 9%. Though these mean values are close X^2 tests show that the three groups of observations are inhomogeneous, indicating a dependence of the nexus surface from the fixation method employed. The wide distribution of the values is probably explained by a systematic fault in the counting procedure, which can be estimated by means of goniometry: the nexus surface is underestimated by about 50%. Generally a hexagonal subunit structure of the nexus is assumed. A nexus surface of 47.0 μ^2 of one ntercalated disk yields 6.7×10^5 hexagons, each presumably the equivalent of one intercellular channel. These values have been examined for their consistency with the concept of the nexus as a low-resistance pathway between cardiac muscle cells.

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Ultrastructural Study of Mouse Lymphoid Cells Bearing Surface Immunoglobulins (IgG)

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Surface IgG has been recently described on lymphoid cells of various species. An ultrastructural study of CBA mouse lymphoid cells has been carried out, using peroxidase-labelled antibodies to detect surface IgG. When a suspension of lymphoid cells is incubated with a rabbit anti mouse IgG antibody followed by a sheep anti rabbit IgG antibody coupled to peroxidase (both antibodies being obtained by purification on an immunoadsorbent) peroxidase activity can be detected on the surface of some cells indicating specifically the presence of mouse IgG, as shown by the absence of any peroxidase activity when the cells are incubated with normal rabbit IgG instead of anti mouse IgG antibody. About 20% of lymph node cells bear surface IgG, mostly focally, on one or several spots. 'Stained' cells are mostly small lymphocytes; plasma cells usually do not have surface IgG. When cells of thymic origin bearing the θ antigen are killed by prior incubation with anti θ serum and rabbit complement, the percentage of peroxidase-labelled cells increases, suggesting that most if not all the cells bearing surface IgG are not thymus derived.

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Modifications ultrastructurales de l'hypophyse du têtard de Crapaud après un traitement au PTU

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Il est connu depuis longtemps que l'administration de goîtrigènes à des têtards de Batraciens provoque une inhibition de la métamorphose par manque d'hormones

thyroidiennes. La microscopie optique avait permis de voir, au niveau de l'hypophyse, une dégranulation et une hypertrophie de cellules glycoprotidiques (basophiles).

Les modifications ultrastructurales de la pars distalis de têtards de Crapaud maintenus dans une solution de propylthio-uracile (PTU), sont importantes. Les cellules hypophysaires qui réagissent le plus fortement ont les mêmes caractéristiques que les cellules glycoprotidiques de type II décrites chez le Crapaud adulte. Rapidement, dès la deuxième semaine de traitement, ce type cellulaire se dégranule et se transforme en cellule de thyroïdectomie.

D'autre part, les cellules indifférenciées, très nombreuses chez les têtards témoins, ont disparu. Il semble donc que toutes les cellules hypophysaires disponibles ont été sollicitées pour produire l'hormone thyréotrope.

Enfin les mitoses s'observent fréquemment, aussi bien dans les cellules granulées que dans les cellules «chromophobes».

Travail réalisé grâce au FNRS.

Tissue Culture of Chicken Erythroblasts Infected with Avian Erythroblastosis Virus (AEV)

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Avian erythroblastosis virus (AEV) is a RNA tumor virus and, when injected intravenously, causes either an anemia, or an erythroblastemia followed by severe anemia; in either situation the disease is lethal. Leukosis-free chickens (Villejuif strain) were injected with virulant plasma. Nine to thirteen days after infection, animals containing large numbers of erythroblasts in their peripheral blood were bled under sterile conditions. Blood was centrifuged and the erythroblast-rich fraction was washed off with Puck's saline. The final pellet contained 50–70% erythroblasts. It was plated either in petri-dishes containing coverglasses or in Falcon plastic bottles. Cells were grown in the medium NCTC supplemented with 30% fetal calf serum and 3–4% plasma from chickens rendered anemic by injections of phenylhydrazine. Cultures were maintained at 38°C and in a 5% CO_2 + 95% air atmosphere.

Within the first five days of tissue-culture, cells attached strongly to the substratum and formed colonies which contained erythroblasts. These colonies rapidly increased in size and number and partially underwent cell fusion. At the end of the first month, giant cells containing as many as 20 cell nuclei were found. Cultures have been maintained for 4 to 5 months. The pattern of incorporation of ^{3}H-thymidine and ^{3}H-uridine was studied by autoradiography and will be discussed.

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In situ Detection of Single Strand Breaks in DNA of Fixed Cell Nuclei

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Calf thymus terminal deoxynucleotidyl transferase utilizes free 3'-OH ends of oligodeoxynucleotides or denatured DNA as initiators and synthesizes poly-deoxynucleotide homopolymer. Thick sections of ethanol-fixed chick lens were incubated with this enzyme and ^{3}H-dATP and the incorporation was localized and measured by quantitative autoradiography. As terminally differentiating lens fiber cell nuclei degenerate, disappear and lose DNA (Modak and Perdue, Exp. Cell Res. *59*, 43, 1970), they incorporate increasing amounts of ^{3}H-dAMP probably due to increase in the number of free 3'-OH ends (Modak and Bollum, Exp. Cell Res. *62*, 421, 1970).

We have now studied the time-course of terminal transferase-catalyzed incorporation of ^{33}H-dAMP in various nuclear populations. The data show that as fiber nuclei degenerate, they indeed contain increasing numbers of free 3'-OH ends in their DNA reflecting appearance of single-strand breaks between 3'-OH and 5'-P. The biological significance of these findings will be discussed in the light of the levels of RNA synthesis during fiber cell differentiation (Modak and Persons, Exp. Cell Res. *64*, in press, 1971).

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Isolation of a Messenger Ribonucleoprotein Complex from Duck Erythroblasts

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Milligram amounts of messenger ribonucleoprotein complexes (mRNP) have been isolated from immature duck red blood cells. The main steps in its preparation are: 1. Preparation of polyribosomes from duck erythroblasts. 2. Dissociation of these polyribosomes with EDTA. 3. Centrifugation on a computer-calculated isokinetic gradient in a zonal rotor. 4. Pooling of the mRNP region and concentration by membrane ultrafiltration. 5. Repurification by isokinetic sucrose gradient centrifugation. This isolated mRNP has a sedimentation coefficient of 15–20 S, a low buoyant density in CsCl gradients compared to ribosomes or ribosomal subunits, and its RNA can be separated on polyacrylamide exponential gels into 4 major bands, 3 of which migrate in the region where the globins mRNA are expected. These facts, together with the specific localization in the cell at the polyribosome level, suggest that these structures contain the mRNA for the synthesis of the duck globins. The characterization of their proteins and the comparison with the proteins of the nuclear complexes that contain messenger-like RNA are now in progress.

Die Abbildung von Schwermetallatomen im Elektronenmikroskop

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Triacetoxymercuryaurin (TAMA) ist ein chemisch klar definiertes Molekül. Wenn es möglich ist, einzelne Schwermetallatome mit dem Elektronenmikroskop abzubilden, würden wir für TAMA-Moleküle, die senkrecht zur Bestrahlungsachse stehen, ein Bild erwarten, das 3 Punkte zeigt, die in den Ecken eines gleichseitigen Dreiecks von 8 bis 9 Å Seitenlänge stehen. Unsere Ergebnisse lassen uns glauben, dass einzelne Quecksilberatome mit dem Elek-

tronenmikroskop abgebildet werden können. Es wird gezeigt, dass diesem Ergebnis folgende Hauptpunkte zugrunde liegen: Herstellung von sehr strukturarmen Aluminiumoxidträgerfilmen, optimale Abbildungsbedingungen im Elektronenmikroskop und Kontrolle der Kontamination im Elektronenmikroskop.

Unterstützt durch NF, Projekt 3.273.69.

Template Requirement of RNA-Dependent DNA Polymerase from AMV

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The RNA-dependent DNA polymerase from Avian Myeloblastosis Virus (AMV) (Temin and Mizutani, Nature *226*, 1211, 1970; Baltimore, Nature *226*, 1209, 1970) was purified by chromatography on DEAE Sephadex and phosphocellulose, and sucrose gradient centrifugation. The template-dependent enzyme was stimulated by double-stranded calf thymus DNA or AMV RNA. Heating AMV RNA at increasing temperature (2 min, 0.1 × SSC) decreased its template activity, with an apparent Tm of about 55°. Single-stranded Qβ RNA and undamaged, double-stranded Qβ RNA (RI) were inactive, while nicked, double-stranded Qβ RNA was the most efficient template. DNA synthesized in the AMV RNA stimulated reaction banded with its RNA template in a Cs_2SO_4 density gradient. After heat denaturation it banded between RNA and DNA; after heat denaturation followed by RNase treatment it was found in the DNA position. This suggests that the newly synthesized DNA chains are covalently attached to RNA segments, forming *end-to-end* RNA-DNA hybrids. The requirements of the enzyme are accounted for if both template and primer are necessary for DNA synthesis, as with the Kornberg DNA polymerase. The stimulatory property of native AMV RNA could be explained if it consisted of long RNA strands hydrogen-bonded to short segments of complementary RNA which serve as primer.

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Zur Darstellung von sauren und basischen Gruppen in Gewebedünnschnitten

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Wird bei der kombinierten Uranyl-Plumbit-Färbung der pH der Uranyllösung gesenkt, so vermindert sich der Kontrast. Carboxy-Methylierung verhindert die Färbung überall, ausser dort, wo viele Phosphat- und Sulfatgruppen vorkommen (z.B. Kern, Basalmembran). Daraus folgt, dass diese Kombinationsfärbung *saure Gruppen* selektiv darstellt. Trotz der Reaktion basischer Stickstoffverbindungen mit den Fixationsaldehyden wird Wismutjodid (BiI^-_4) an viele der *kationischen Gruppen* des Gewebes gebunden. Dieser Schluss folgt aus dem Verhalten von BiI^-_4 in vitro und aus der Unterdrückung der Färbung durch Amino-Acetylierung. Der schwache Kontrast der BiI^-_4-Imprägnierung muss durch Behandlung mit Uranyl und Plumbit verstärkt werden, wodurch aber anionische Komponenten gleichzeitig zur Darstellung gelangen. Letzteres wird durch Carboxy-Methylierung oder pH-Senkung der Uranyllösung auf 1–1,5 weitgehend vermieden. Die dabei verbleibende Elektronendichte zeigt, dass die erzielte Kontrasterhöhung nicht auf reiner Addierung der Färbungen kationischer und anionischer Gruppen beruht, sondern vor allem auf einer Anlagerung von Uranyl und Plumbit an im Gewebe fixierte BiI^-_4-Moleküle.

Unterstützt durch NF, Projekte 3.133.69 und 3.134.69.

Quantitative Cytofluorometric Determination of Feulgen DNA by Conventional Schiff's Reagent

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The Feulgen reaction obtained by conventional Schiff's reagent (Pararosaniline-SO_2) excited in green light emits a red fluorescence which permits DNA cytofluorometric determination. Cytofluorometry presents important advantages: a) higher sensitivity with respect to absorption cytophotometry; b) absence of distributional and cytometric errors; c) simple instrumentation and fast measuring rate. However, possible sources of error, such as photodecomposition, autoabsorption and partial lack of linearity between fluorescence intensity and fluorochrome concentration have to be eliminated. The principles of cytofluorometry, instrumentation and technical measuring procedures have been previously described by us (G. Prenna, Mikroskopie *23*, 150–154, 1968). For measurements, Leitz MPV histophotometer and Leitz-Microspectrograph (vibrating mirror oscilloscopic system), both equiped with Ploem's illuminator, were used. Excitation and emission spectra, photodecomposition and emission intensity recovery were measured. The optimum concentration of Schiff's reagent cytofluorometric measurements was established. Comparison of cytofluorometric and absorption measurements demonstrates the value of conventional Feulgen reaction cytofluorometry as a quantitative method for DNA nuclear content determination. Experiments on the quantitative determination of Feulgen DNA in single chromosomes are in progress.

Quantitative Cytofluorometric Determination of PAS Reaction by Conventional Schiff's Reagent

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The red fluorescence emitted from PAS reaction excited in green light permits quantitative cytofluorometric determination of cellular content of polysaccharides.

Human leucocytes and isolated hepatic rat cells were employed. Parallel measurements of PAS positive substance in absorption and in fluorescence were performed. For absorption measurements Deeley's Microdensitometer and for cytofluorometry Leitz MPV histophotometer and Leitz-microspectrograph (vibrating mirror oscilloscopic system), both equipped with Ploem's illuminator, were used. Linearity between fluorescence intensity and fluorochrome concentration, excitation and emission spectra, photodecomposition and emission intensity recovery were studied. The optimum concentration of Schiff's reagent for PAS cytofluorometric measurements was determined. The results obtained demonstrate that cytofluorometry presents several advantages and is a reliable method for quantitative determination of PAS-positive substances.

Effet de la Vincristine sur le lobe nerveux de l'hypophyse in vitro

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Des hypophyses postérieures de rats ont été maintenues in vitro dans une solution de Locke. La stimulation électrique de la tige pituitaire provoque la libération d'ocytocine dont le taux est mesuré à l'aide d'un test biologique sur la rate allaitante. On a incubé les glandes en présence de Vincristine à la concentration de 10^{-5} *M* pendant 15 à 180 mn. Cette incubation n'a pas modifié la libération d'octapeptides hypophysaires. La microscopie électronique a permis de montrer que la Vincristine provoque dans plusieurs types cellulaires la formation d'inclusions cristalloïdes dans lesquelles sont impliqués les microtubules (Bensch et Malawista, J. Cell Biol. *40*, 65, 1969). Au niveau des fibres nerveuses de l'hypophyse postérieure de telles images ont été observées après une heure d'exposition. Leur fréquence et leur taille augmentent avec le temps d'incubation. Les cristaux semblent être le résultat d'une réorganisation des microtubules. Simultanément apparaissent des autophagosomes comprenant un grand nombre de granules neurosécrétoires. Les pituicytes montrent également des images cristalloïdes et des phénomènes de phagocytose.

Travail réalisé grâce à l'aide du FNRS.

Renouvellement des protéines des segments externes des photorecepteurs rétiniens du pigeon

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Le renouvellement des protéines des segments externes des photorécepteurs a été étudié chez le pigeon en utilisant l'autoradiographie en microscopie électronique après injection intra-oculaire de 1-leucine ^{3}H. 18 h A.I. (= après injection), la radioactivité était concentrée en bande à la base des segments externes des bâtonnets. 3 jours A.I., la bande radioactive était retrouvée près de l'extrémité des segments externes. 7 jours A.I. quelques grains pouvaient encore être observés. 14 jours A.I., aucun marquage n'était détecté. Au niveau des segments externes des cônes, les grains étaient diffusément répartis dès 18 h A.I. La radioactivité était retrouvée identique 3 jours A.I., puis devenait plus faible à 7 jours pour disparaître complètement à 14 jours A.I. Comme chez d'autres vertébrés, les disques des bâtonnets semblent bien être formés à la base des segments externes par des protéines synthétisées dans le segment interne. Un segment externe est donc renouvelé complètement en 3 à 4 jours. Ceci implique la synthèse de 9 à 12 disques à l'heure. A l'opposé, le renouvellement des protéines des disques des cônes semble se faire au niveau de la membrane elle-même.

Travail soutenu par le FNRS, 3287.69.

Spin Label Studies of Membraneous Structures

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Spin labelled molecules are highly sensitive probes for the investigation of the molecular organization of membranes. A series of spin labelled fatty acids has been incorporated into liquid crystalline model systems and isolated membrane units such as sarcoplasmic vesicles. The spin labels in the sarcoplasmic membrane display a fast, anisotropic rotation. The first 7 carbon-carbon bonds adjacent to the carboxyl group of the spin labelled fatty acid experience a highly ordered environment, whereas for $n>7$ a pronouced increase in the flexibility of the hydrocarbon chain is observed although the system is still more ordered than pure lipid dispersions. The fluidity of the membrane was shown to be due to its lipid constituents, since removal of the lipids leads to spectra characteristic for immobilized spin labels. The comparison with other model systems leads to the assumption that a direct lipid-protein interaction must play an essential role in the organization of the membraneous lipids. The effect of this interaction is to increase the stiffness of the hydrocarbon chains of the lipid moieties. The enzymatic activity of a lipid deficient membrane is restored by the addition of oleic acid as demonstrated for the case of Calcium dependent ATPase. The epr spectra show that oleic acid assumes a physical state similar to that of the natural membraneous lipids.

Messenger RNA and Cytoplasmic mlRNA in Duck Reticulocytes

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We found recently (Spohr et al., Eur. J. Biochem. *17*, 296, 1970) that in the cytoplasm of Hela cells as much messenger-like RNA (mlRNA) exist in free ribonucleoprotein particles (mlRNP's) as there is true messenger in polyribosomes. Thus, a pre-translational regulatory mechanism discriminating RNA quantitatively or qualitatively may exist. In order to check such a hypothesis the situation in the highly differentiated avian erythroblast was examined. – Duck reticulocyte RNA was labelled for 40 min. The cytoplasm was fractionated in polyribosomes and subribosomal particles. The subribosomal fraction contain mlRNP's with 10–60 S and buoyant densities of 1.4 to 1.48 g/cm^3. By polyacrylamide gel electrophoresis, RNA bands of 4, 6 and 9 S were found n the 10 to 20 S mlRNP. The 20 to 60 S mlRNP's contained mlRNA ranging from 12 to 16 S. Pure polyribosomes showed a predominance of label in the 9 S-Hemoglobin-mRNA and in the 4 S band. Thus, free mlRNP contain in addition to 9 S a spectrum of heavier RNA molecules. – This may indicate a mechanism selecting preferentially globin messenger RNA to function in translation.

Fixation et apport de contraste par les ions UO_2^{++} et Pb^{++} en l'absence de OsO_4

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Un apport de contraste par les cations UO_2^{++} sur des blocs ou des coupes de tissus préalablement fixés par OsO_4 est devenu une méthode de routine dans beaucoup de laboratoires de microscopie électronique.

Il nous est apparu récemment que si les blocs de tissu sont traités par des solutions aqueuses renfermant les

cations UO_2^{++} ou Pb^{++} après fixation à l'aldéhyde glutarique mais sans traitement subséquent au OsO_4 certaines structures subcellulaires sont sélectivement contrastées, ainsi p.ex. les organelles stockant des amines biogènes dans les plaquettes sanguines, dans les terminaisons nerveuses adrénergiques et dans les cellules de la médullosurrénale. En plus les ions UO_2^{++}, mais non les ions Pb^{++}, contrastent certaines membranes cellulaires. En outre dans bien des cas les cellules contiguës présentent un contraste général très différent l'une de l'autre ce qui permet de différencier clairement d'éventuelles intrications cytoplasmiques. Ces phénomènes de contraste sélectif dépendent des conditions expérimentales précises, tel que le PH, la présence d'autres ions dans la solution, etc. Les mécanismes d'action probables de ces contrastants vont être discutés.

Mise en évidence des transmetteurs chimiques adrénergiques au microscope électronique

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Il sera présenté une courte revue d'ensemble des possibilités actuelles ainsi que des problèmes non encore résolus dans ce domaine. Seul les trois principaux transmetteurs adrénergiques, à savoir, la noradrénaline, la dopamine et la sérotonine seront considérés. Ces amines biogènes peuvent être mises en évidence de façon précise par la microscopie électronique dans certains neurones centraux et périphériques, avant tout dans leur terminaisons axonales, mais aussi dans de nombreuses autres cellules, où elles y sont sythétisées et stockées.

Les principaux aspects discutés seront les suivants: 1. Problèmes concernant la conservation «in situ» de ces amines pendant la fixation et/ou pendant leur mise en évidence par un contrastant plus ou moins spécifique tel que OsO_4, $KMnO_4$, $Cr_2O_7K_2$, UO_2^{++}, Pb^{++}, etc. 2. Possibilités et limites d'utilisation de techniques cytopharmacologiques p.ex. la réserpine et l'α-methyl-métatyrosine. Localisation de «faux transmetteurs». 3. Possibilités et limites de l'autoradiographie. 4. Spécificité, résolution et sensibilité des diverses méthodes comparées entre elles.

The Formation of Polyoma Pseudovirions

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Polyoma pseudovirions are polyoma virus particles containing linear fragments of host (mouse) chromosomal DNA instead of circular polyoma DNA molecules. They are found in all polyoma preparations in varying amounts, i.e. 10–90%. The time course of the degradation of cell DNA to low molecular weight fragments (10–14s) and that of the formation of pseudovirions were studied in polyoma infected mouse kidney cell cultures. The amount of 10–14s DNA and the proportion of pseudovirions increased with time after infection. The formation of pseudovirions, but not of 10–14 s DNA, was enhanced by partial inhibition of cellular and viral DNA synthesis, e.g. by mitomycin or by an incomplete release of an FUdR block. These and earlier results lead to the hypotheses that 1. the 10–14 s DNA is formed by a host enzyme activated or released after viral DNA synthesis and 2. pseudovirions are formed mainly after the preferential formation of infectious virus by a random encapsidation of 10–14 s DNA. Pseudovirions are potentially capable of transducing genes of animal cells and may be useful to study the mechanisms involved in virus assembly.

This work was done at the Swiss Institute of Experimental Cancer Research, Lausanne. Supported by SNSF, grant 3.291.69.

Visualization of Extracellular Lining Layer of Lung Alveoli by Freeze-Etching

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The extracellular material representing the surfactant system of the lung consists of an aqueous hypophase and a surface film of adsorbed phospholipid molecules. Conventional preparation methods for EM by necessity eliminate the hydrophobic air-surfactant interface at one stage or another. By freeze-etching of unfixed air-filled lungs we succeeded in demonstrating an intact air-surfactant interface: the lining film was seen as the smooth boundary of the hypophase on fracture planes and could be examined as a threedimensional surface from the alveolar side. Our results are on the whole in agreement with those obtained in thin-sectioning after fixation by vascular perfusion. The new findings are: 1. the surface film covers the hypophase smoothly; 2. spherical and tubular myelin figures are not the result of a fixation artifact.

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Gene-Enzyme Systems in Drosophila as Tools for the Study of Animal Development

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More than twenty enzymes have now been mapped within the Drosophila genome, largely through the use of enzyme variants differing in their electrophoretic mobility. The resulting genetic maps promise insight into the question of clustering of related genes in a higher organism. Some electrophoretic markers have also been useful for the investigation of cell-lineage relationship during metamorphosis. In these studies, enzyme localization through biochemical and histochemical methods were combined with transplantation and culture experiments involving tissue primordia and host organisms of differing genotypes. In at least two cases, these combined methods made possible the unambiguous demonstration of cell-autonomous enzyme synthesis.

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Studies on the Interaction between Q β Replicase and Q β RNA

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Qβ RNA specifically binds to Qβ replicase and thereby acquires the property of being retained by Millipore filters. After short treatment of the complex with RNase T_1 about 1% of the RNA remained on Qβ replicase and was recovered by Millipore filtration. It was resolved into 2 major and 3 minor fractions by gel electrophoresis and/or homochromatography. The RNA fragments were

from about 30 to 100 nucleotides long. The two main fragments are partly overlapping segments from the same RNA region since they contain identical nucleotide sequences. Both fragments appear to comprise the left-hand part of the ribosomal binding site of the coat protein cistron. The replicase binding site defined by these fragments is therefore located between the first and second third of the viral RNA (Hindley et al., P.N.A.S. *67*, 1180, 1970). A partial overlap between the replicase and the ribosome binding site explains why attachment of $Q\beta$ replicase to $Q\beta$ RNA prevents ribosome binding (Kolakofsky and Weissmann, Nature, in press). From mechanistic considerations one expects that replicase should also bind the 3' terminus of $Q\beta$ RNA; the corresponding sequences are being sought. Possibly, however, the 3' terminus cannot be isolated by our method if binding to replicase is not sufficiently tight.

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Studies on the Rifampicin-RNA Polymerase Complex

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It has been shown that rifampicin forms a very stable complex with DNA-dependent RNA polymerase of E. coli (W. Wehrli et al., Proc. nat. Acad. Sci. U.S.A. *61*, 667, 1968). We have now studied the properties of this complex and its role in the inhibition of the enzyme. Kinetic experiments have shown that even at 0 °C over 90% of the complex is formed in less than 30 seconds. The stability of the complex depends on the purity of the enzyme: the purer the enzyme the less stable is the complex. This result indicates that during enzyme purification the properties of the complex-forming site change.

Furthermore, we have studied the complex formation with rifampicin during the various enzymatic steps of RNA synthesis. It is known that RNA polymerase is protected against inhibition by rifampicin during RNA chain elongation. It has now been shown that the complex-forming ability of such a protected enzyme is less than 30% of that of free enzyme.

Thus it seems that complex formation and enzyme inhibition are closely related.

A Structure Resembling Proteoglycans in Preparations of Mitochondrial DNA from Mouse Liver

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The presence of polysaccharides as a common contaminant in phenolextracted DNA has been reported by various authors. After isopycnic centrifugation in CsCl density gradients this material accumulates together with M-DNA at a buoyant density of 1.68–1.71 g/cm^3. During electronmicroscopical studies on M-DNA prepared by the protein monolayer technique we found at a frequency of about 0.5% structures resembling in configuration but not in dimensions proteoglycans from bovine nasal cartilage. Although chemical characterization of our material has not yet been attempted, these macromolecules deserve attention, since they may represent proteoglycans of cytoplasmic or even intramitochondrial location. They consist of a central filament, 50 Å thick, and unbranched side chains, 80–110 Å thick, which are fully extended and insert at the central filament at intervals of 200–300 Å. The side chains have a rather constant length of 1574 $\pm$ 135 Å (S.D.). On the other hand, the length of the central filaments of 26 well measurable molecules varies from 790 to 6220 Å, but there is a close correlation between the length of the central filament and the number of attached side chains.

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The Influence of Hypertonicity of the Growth Medium on Polyribosome Structure in HeLa Cells

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In 1970 Robbins et al. (J. Cell Biol. *44*, 400) showed that incubation of HeLa cells in hypertonic growth medium leads to a reversible breakdown of a large part of the polyribosomes inside the cell. We studied this process in more detail. The results are the following: 1. The breakdown of polyribosomes is quantitative with a reduction of polyribosome bound ribosomes to 50% of the control value in about one minute. During breakdown there is no intermediate stage of accumulation of small polyribosomes. The same effect is seen also in cells pretreated with low or large doses of Act. D, to suppress specifically rRNA synthesis or all RNA synthesis respectively. 2. The process is totally inhibited by preincubation of the cells with Cycloheximide or Emetine. 3. During the dissociation the polyribosomes are converted to 80 S ribosomes free of mRNA. The mRNA set free does appear as part of the cytoplasmic mlRNP. 4. After restoration of isotonic growth conditions the dissolciation reaction is rapidly reversible even if RNA synthesis is blocked by high doses of Act. D. – The types of intermediates, the kinetics and the influence of drugs lead us to think that hypertonic growth medium leads to a rapid block of initiation of protein synthesis in the HeLa cell. The impossibility to isolate a complex of a ribosome or a ribosomal subunit with the mRNA suggests that one of the very early steps of initiation is inhibited under our conditions.

Bidirectional Chromosome Replication in *Escherichia coli*

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Sueoka and Yoshikawa have demonstrated a gradient of gene frequency from origin to terminus in growing cultures of *B.subtilis* and concluded therefrom that replication begins at a fixed origin and proceeds to a fixed terminus. A new technique for measuring the frequency of a particular region of the chromosome of *E.coli* has been devised. Several strains lysogenic for bacteriophages λ and μ had μ integrated into a different region of the chromosome whereas the location of λ remained fixed. The quantity of μ specific DNA relative to λ DNA found in rapidly growing cultures of each strain provides the relative frequency of the region into which μ is integrated. To make this measurement, a mixture of μ and λ DNA, each labeled with a different radioisotope, was hybridized to DNA extracted from each strain growing rapidly in rich

medium. Results indicated that the origin of replication (region with highest μ frequency) is near ile (280°) on the circular map and the terminus (region with lowest μ frequency) is near try (100°) on the map. The gradient of gene frequency appears to go symmetrically in both directions indicating a bidirectional replication. The sequence of replication of markers in cells synchronized by amino acid starvation confirmed these conclusions.

Control of Chromosome Replication and Cell Division by Integrated Episome

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A temperature-sensitive mutation (T46) affecting the initiation of DNA replication in *E. coli* is not complemented by an F factor. If, however, temperature-resistant revertants are isolated from a $T46^{s}F^{+}$ strain most are Hfr, with F integrated at various sites. These cells still contain the $T46^{s}$ mutation. They are sensitive to acridine orange and ethidium bromide at 42°C, but not at 30°C, suggesting that, at the restrictive temperature, they are under F replicon control. Similar results were obtained in a partial diploid strain carrying the F'lac factor. In many Hfr t.r. strains formed, F'lac had integrated in the chromosomal lac region, but Hfr t.r. strains also arose by integration of F'lac elsewhere. 'Integrative suppression' (Int. sup.) by F'lac was also observed for another temperature-sensitive mutation affecting the initiation of DNA replication (T83). It requires recA gene function. Int. sup. does not take place for mutations affecting continuation rather than initiation of DNA-replication. Several episomes (RTF, col factors, Pl) are capable of int. sup. We conclude that, in the Hfr t.r. strains, chromosome replication and cell division are under F control. It seems that in these cells the chromosome has become part of the F replicon.

Heterogeneity of Plaice Body Muscle Acetylcholinesterase

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As shown previously (S. J. Lundin, Acta Chem. Scand. *22*, 2183 (1968)) an acetylcholine splitting esterase was isolated from the body muscle of plaice (Pleuronectes platessa). This enzyme hydrolyses acetylcholine faster than butyrylcholine and accordingly should be classified as an acetylcholinesterase (EC 3.1.1.7). However, no inhibition is observed at high substrate levels thus deviating from the properties of other acetylcholinesterases. This anomaly can be either explained in terms of heterogeneity at the enzyme level or in terms of differences in properties of the catalytic site. To investigate the possible heterogeneity of the enzyme, it was subjected to isoelectric focusing and found to consist of mainly two enzymatically active fractions which in turn contain mixtures of acetylcholinestersae of different isoelectric points. The kinetic parameter K_A, V_1 and optimal substrate concentration for the different enzyme species will be discussed.

CONSTRUCTIONES

Eruopean Training Awards in Brain and Behaviour Research

In cooperation with the Organization for Economic Cooperation and Development, a group of European Scientists have initiated an experimental schema under which younger scientists working on Brain and Behaviour can apply for awards to enable them to acquire training in a specialized area. The money to finance this training program has been provided by the Max-Planck-Gesellschaft. Successful applicants will receive travel and living expenses to enable them to study in selected laboratories. The normal duration of an award will be three months, but some longer term awards can be made.

Eligibility. To be eligible for an award, a candidate must already by undertaking research in the field of Brain or Behaviour in a laboratory situated in a member country of O.E.C.D. Applicants must produce evidence that their own research will benefit by the training for which they apply. In making the awards, preference will be given to candidates applying for a type of training that will assist them to follow an interdisciplinary approach in their own research. Candidates are expected to return to their original laboratory at the expiry of their training.

Nature of training courses. Some of the training programs incorporate formal course work, others involve the learning of techniques whilst undertaking closely supervised research on a particular problem. Training programs exist in the following subjects: Animal behaviour, brain biochemistry, brain modelling, ethology, experimental psychology, histochemistry, morphology, neuroanatony, neuropharmacology, neurophysiology etc.

Method of application. Further details of the scheme (including a list of laboratories participating in the training programs) and application forms can be obtained from:

The Executive Office, Foundation FUNGO,
Laan van Meerdervoort 53D, Den Haag (The Netherlands).