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PHYSIOLOGIE – PHYSIOLOGY

Na-Dependent Phosphate-Influx into Mammalian Nerve Fibres

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Desheathed rabbit vagus nerves were mounted in a polyethylene tube in front of the window of a GM counter, and perfused with ^{32}Pi-Locke at 37 °C. The ^{32}Pi-uptake, measured and recorded continuously, proceeded linearly during at least 15 hours, for extracellular Pi concentrations between 0.08 and 3 m*M*. Plots of influx-velocities against extracellular Pi concentrations showed saturation kinetics of the Michaelis-Menten type. Replacement of extracellular Na by choline, Li, or K, lowering the temperature, or addition of arsenate, produced a rapid, reversible inhibition of Pi-uptake. A much slower inhibition was found in K-free or ouabain-containing Locke. In order to localize the ^{32}Pi taken up, the nucleotides of the nerves were extracted and separated by thin-layer chromatography. In different experimental conditions, the amount of labelled nucleotides paralleled the recorded ^{32}Pi-uptake, indicating that the ^{32}Pi had entered into the intracellular space. The results suggest that transmembranous Pi-transport in this tissue depends on the Na-gradient.

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Influence of Anoxia on the Membrane Potential of Retinula and Pigment Cells in the Retina of the Drone

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The drone retina is composed of numerous ommatidia, each formed by nine retinula cells surrounded by a sheath of about twenty pigment cells. Pigment cells have an average resting potential of −46 mV, retinula cells of −60 mV. Impaling a single retinula cell and a single pigment cell with two micropipettes and perfusing the preparation with a Ringer solution saturated with nitrogen leads in both cells to a decrease in membrane potential. As already shown (F. Baumann et al., Experientia *28*, 727, 1972) this effect of anoxia is the consequence of an efflux of potassium from the retinula cells and an accumulation of this ion in the extracellular space between retinula and pigment cells. After oxygen is readmitted the membrane potential returns to its original level rapidly in retinula cells but slowly in pigment cells. The slow recovery of the membrane potential of the pigment cell probably reflects the return to normal values of the extracellular potassium concentration. The fast recovery of the membrane potential of the retinula cell, however, might be due to the activity of an electrogenic pump located in the retinula cell membrane and capable of restoring a normal resting potential despite an increased extracellular potassium concentration.

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Influence of 1,25-Dihydroxycholecalciferol and Diphosphonate on Calcium Metabolism

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Disodium ethane-1-hydroxy-1, 1-diphosphonate (EHDP) when given at large doses in the rat decreases bone mineralization rate (Vo^+), net intestinal Ca absorption (Vna), Ca retention by the body (Vr) and increases urinary Ca (Vu). Very recently it has been shown that the same doses of EHDP impair Vitamin D_3 (D_3) metabolism by inhibiting the conversion of 25-hydroxycholecalciferol (25-OHD_3) into 1, 25-dihydroxycholecalciferol (1, 25-$(OH)_2D_3$). Furthermore very small amounts of 1, 25-$(OH)_2D_3$ can prevent the decrease in the intestinal Ca absorption capacity induced by EHDP treatment, whereas doses five hundred times larger of D_3 or 25-OHD_3 are uneffective. These observations indicate that the EHDP-induced reduction in Vna is directly related to the defective production of 1, 25-$(OH)_2D_3$. To assess whether such a defect also accounts for the effects of EHDP on the other variables of Ca metabolism, 1, 25-$(OH)_2D_3$ ($2 \times 13{,}5$ pmoles/day i.p.) was administered to rats receiving EHDP (10 mg P/kg day s.c.) and a Ca balance and ^{45}Ca kinetic study performed. In the EHDP-treated animals, 1, 25-$(OH)_2D_3$ prevented the fall in Vna but did not correct the reduction in Vr because of a conspicuous increase in Vu. The effect of EHDP on bone mineralization as estimated by the Vo^+ of ^{45}Ca kinetic, and by measuring the width of the epiphyseal plate of the tibia was not prevented by this dose of 1, 25-$(OH)_2D_3$. These results suggest that, contrarily to the diminished intestinal Ca absorption, the EHDP-induced reduction in bone mineralization may not be directly related to the diminished production of 1, 25-$(OH)_2D_3$.

Analoge Verteilungsstörung und Atmungsreaktionen nach Histamin-Aerosol, Ammoniakdämpfen (NH_3) und Zigarettenrauch (C.S.)

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Die Inhalation von NH_3 oder C.S. bewirkt u.a. eine Atmungsbeschleunigung mit Lungenvolumenzunahme. Diese Atmungsreaktionen, welche qualitativ durchwegs mit jenen des Histaminasthmas zu vergleichen sind, beruhen auf der Erregung sog. «lung irritant receptors» (Widdicombe). Die Erregungsbedingungen dieser vagalen Rezeptoren bilden Gegenstand vorliegender Versuche, die an tracheotomierten Meerschweinchen in Urethannarkose durchgeführt wurden. – Die verzögerte Argonauswaschung wie die Formanalyse exspiratorischer Argon-Partialdruckkurven zu Beginn der durch NH_3 oder C.S. ausgelösten Hyperventilation lassen auf eine ungleichmässige Belüftung infolge bestimmter lungenmechanischer Veränderungen schliessen. Derartige pulmonale Veränderungen (heterogene Zeitkonstanten mit Überdehnungen und Kompressionen) wurden im histaminbedingten wie anaphylaktischen Asthma bereits nachgewie-

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sen: sie bedingen die Auslösung des Lungenkollapsreflexes. Angesichts der analogen Verteilungsstörung und der vagalen Atmungsreflexe im Asthmaanfall sowie nach Inhalation von NH_3 oder C. S. erhebt sich die Frage: Sind «irritant receptors» Kollapsrezeptoren?

Effect of IDPN-Treatment on Oxygen Consumption and Growth of the Lung in Mice

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Three i. p. injections of β,β'-Iminodipropionitrile (IDPN) induce a permanent central nervous disorder with choreiform and circling movements. This study had to test whether IDPN-induced hyperactivity was a suitable model experiment for the investigation of the effect of increased oxygen consumption of the organism on the growing lung. About 30 mice were treated with IDPN after weaning and their growth curves, physical activity and oxygen consumption registered during the following weeks and compared to control values. The mice were then killed, their lungs prepared for morphometry; body surface area, body and organ weights were determined. In IDPN-mice the activity and the V 0_2/g body weight were increased by more than 40% as compared to controls. Body weight and body surface area were significantly reduced in IDPN-group, as were heart, kidney and liver weights. On the other hand the lung volume was at control level; consequently specific lung volume (LV/g body weight) was 20% higher in IDPN-group. We conclude: a) IDPN-treatment is a suitable experimental model to chronically increase oxygen consumption; b) previous findings of quantitative adaptation of lung structure to increased oxygen consumption of the organism during growth are confirmed.

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Angiographic Determination of Left Ventricular Muscle Mass in the Dog

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Left ventricular (LV) cinéangiocardiography in the right anterior oblique projection was carried out in 7 closed-chest anesthetized dogs of 18 to 30 kg of body weight. Calculation of LV muscle mass (LMM) was based on the determination of end-diastolic ellipsoidal chamber dimensions and of posterior wall thickness (h). Specifically, 3 different procedures of calculation were used: In method A end-diastolic volume (EDV) was determined according to the technique of Greene (Circulation *35*, 61, 1967); h was assumed to be equal at both ends of the long and the minor internal chamber axis. In method B systematic overestimation of EDV by Greene's method was corrected according to the regression equation proposed by Bentivoglio (J. Appl. Physiol. *33*, 365, 1972); h was the same as in A. In method C EDV was corrected as in B; it was however assumed that wall thickness on both ends of the long axis was only half of that at the minor axis (i.e. h/2). LMM was calculated in 56 instances under various hemodynamic conditions. The values obtained in an individual dog (4–11) were averaged. These average values were compared with the true LMM determined at autopsy. True LMM in the 7 dogs was 96 $\pm$ 16 g ($\pm$ 1 SD). Method A yielded 122 $\pm$ 23 g ($P < 0.001$), method B 109 $\pm$ 20 g ($P < 0.005$) and method C 91 $\pm$ 17 g ($P > 0.05$). In summary LMM is determined with reasonable accuracy (1) when monoplane EDV is corrected and (2) when h/2 is assumed at the long axis.

Influence of Ambient Temperature on Thermoregulatory Mechanisms during Muscular Exercice

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Experiments were designed to compare the rate of heat losses at rest and during exercice at 3 different ambient temperatures (Ta): 20, 25 and 30 °C. Six subjects, dressed in shorts, performed exercise on a bicycle ergometer inside a direct calorimeter at two work loads 40 and 90 W, for 50 min each. At rest, the mean total heat losses ($\dot{H}$) measured for 50 min at 20, 25 and 30 °C were respectively 116, 93 and 69 kcal. During exercise at 40 W, we found 138, 141 and 148 kcal, and during exercise at 90 W 249, 254 and 230 kcal. Thus, $\dot{H}$ during exercise was not significantly influenced by Ta (between 20 and 30 °C). However, the partition between radiative + convective ($\dot{R} + \dot{C}$) and evaporative ($\dot{E}$) heat losses was quite different: during exercise at 40 W, $\dot{E}$ represented 18% of $\dot{H}$ at 20 °C, 43% at 25 °C, and 71% at 30 °C. In addition, the skin blood flow increased during exercise at 40 W of 52 ml/m² at 20 °C, 229 ml/m² at 25 °C, and 473 ml/m² at 30 °C. Heat storage and metabolic rate were unaffected by Ta during exercise. These results show that if $\dot{H}$ is not influenced by Ta (between 20 and 30 °C) during exercise, the mechanisms of thermolysis, cutaneous vasodilatation and sweating, are stimulated differently in relation with Ta, the end result being similar heat losses.

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Régulation de la sécrétion amylasique du pancréas par une hormone duodénale

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Plusieurs hormones de la muqueuse duodénale agissent sur le pancréas exocrine et endocrine. Pour étudier le mécanisme de leur régulation, on réalise l'expérience suivante: une première série de rats reçoit une surcharge orale de glucose. La muqueuse duodénale de ces rats ainsi que des rats contrôle est prélevée et extraite. Ces extraits sont alors injectés dans le tronc coeliaque d'une deuxième série de rats dont on prélève le jus pancréatique. La concentration d'amylase mesurée par méthode radioimmunologique chez les rats de contrôle après injection ne diffère pas de façon significative de celle de la sécrétion pancréatique basale des rats n'ayant pas reçu d'injection (1 mg/ml). Mais on observe une importante sécrétion d'amylase après injection d'extrait de muqueuse prélevée 30 min après la surcharge de glucose (3,5 mg/ml).

Cette étude suggère que l'ingestion de glucose provoque une réponse pancréatique en stimulant la sécrétion d'amy-

lase et que la nourriture favorise l'activation ou la synthèse rapide d'une hormone duodénale qui a son tour stimule la sécrétion d'amylase pancréatique.

Redox Changes in Sympathetic Neurones after a Single Preganglionic Shock

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Sympathetic ganglia isolated from the rat have been perfused using a modified Krebs solution. The electrophysiological response to preganglionic stimulation has been recorded on the postganglionic nerve. Simultaneously the tissue fluorescence reflected after excitation by a 366 mμ light beam has been measured: its intensity is a measure of the amount of reduced pyridine nucleotides in the sympathetic neurones. In order to record the fluorometric response of the neurones to a short train of stimuli or to a single electrical shock given to the preganglionic nerve we have averaged the responses from the photomultiplier using a minicomputer PDP 11/20. The redox change thus measured after a single shock is an oxydation of the pyridine nucleotides appearing with a mean delay of 200 msec after the onset of the spike potential. This very early response to a single shock might be related to the action of acetylcholine on the postsynaptic membrane.

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Elektrische Reizung der Medulla oblongata während Respiratorbeatmung beim Kaninchen

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Wenn narkotisierte, nicht curarisierte Tiere mit einem Respirator so beatmet werden, dass die Frequenz nicht allzuweit von der spontanen Frequenz und das Inflationsvolumen nicht allzustark vom Zugvolumen abweichen, löst jede Saugphase eine Zwerchfellkontraktion aus. Die Tiere wurden indessen so rasch und mit so kleinem Inflationsvolumen beatmet, dass die Inspirationen nicht mehr dem Takt des Respirators folgten. Gleichzeitig wurde die Medulla oblongata mit kurzen Serien von repetierten Einzelimpulsen gereizt, je eine Reizserie pro Inflation und Deflation. Bei Lage der Elektroden in der Formatio reticularis lateralis war es möglich, die Inspirationen im Takt der kombinierten mechanischen und elektrischen Reizgebung auszulösen. Optimal wirksam waren Reizserien zu Beginn der Respiratorsaugphasen. Für jeden Punkt wurde zum Vergleich die Wirkung der elektrischen Reizung mit denselben Parametern auf die spontane Atmung untersucht. Von zahlreichen Punkten aus war die rein elektrische Inspirationsauslösung im Takt der Reizserien nicht möglich. Der allein unterschwellige mechanische und der ebenfalls unterschwellige elektrische Reiz führen also bei geeigneter Kombination beider zum Erfolg.

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Contribution to the Study of Direct Retinohypothalamic Projections in Rodents

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The idea of a direct retinohypothalamic connection was subjected to a controversal discussion. Only recently R. Y. Moore (Brain Research *49*, 402, 1973; J. Comp. Neurol. *146*, 1, 1972) using autoradiographic technic has demonstrated the retinal projection to the suprachiasmatic nucleus in several mammals.

After unilateral injection of 3H-proline in the posterior chamber of the eye (Cowan et al., Brain Research *37*, 21, 1972) of the woodmouse (*Apodemus sylvaticus* L.) we find a pronounced radioactivity in both suprachiasmatic nuclei, particularly at the contralateral side, and in the neighbouring accessory optic tract. Thus we confirm the existence of direct visual afferents to the hypothalamus in this animal, the behaviour of which is systematically studied in this institute.

The definition of the efferent fibers of the suprachiasmatic nucleus should precise its functional meaning. This is the aim of our next study.

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Dependence of Divalent Cation Transport on ATP in Yeast

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Divalent cations like Mg^{++}, Co^{++}, Zn^{++}, Mn^{++}, Ni^{++} and Ca^{++} can be transported into a non-exchangeable pool in yeast cells. The transport system displays saturation kinetics as well as competition between ion pairs and is dependent on fermentation reaction. For Mn^{++} uptake, the activation energy is 18,000 cal/*M*. The level of divalent cation uptake is low in starved yeast, but can be considerably enhanced (5–20 fold) by incubating such cells with potassium, glucose and phosphate. The induction of cation uptake is not altered by cycloheximide or 5-fluorocytosin, inhibitors of protein synthesis, which prevent yeast growth completely. In starved yeast cells, the ATP content is low. But after incubation, the ATP content is increased in parallel with the rate of divalent cation uptake. This is about the same under aerobic or anaerobic conditions. Metabolic inhibitors of fermentation reduce ATP and the rate of uptake of divalent cations without causing a measurable efflux of divalent cations or phosphate. Carbodiimide, which inactivates membrane ATPase, inhibits divalent cation uptake without effecting ATP content. These results suggest dependence of divalent cation transport on ATP and the presence of a membrane ATPase which is involved in transport.

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Swelling of Skinned Muscle Fibers in Relaxing Medium and its Reversal

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Single frog muscle fibers, skinned by removing the membrane mechanically under H_2O-saturated silicone oil, swell markedly upon transfer into aqueous relaxing medium (60 m*M* KCl; 3 m*M* $MgCl_2$; 3 m*M* ATP; 4 m*M*

EGTA; 20 m*M* Tris maleinate; pH 7.0). Fiber cross-sections calculated from optical measurements of top and side diameters, increase by 2.40 times (± 0.63 S.D.). Although these swollen fibers give normal isometric contractions in high Ca^{++} solutions, they do so only for about 30 minutes after skinning, whereas unskinned fibers respond to electrical stimulation for a day or more. Swelling is identical in relaxing media of pH 6 to 8 but can be prevented by adding 2.5 m*M* polyvinylpyrrolidone (PVP), a long chain polymer (M.W. 4×10^4), and fibers can contract normally in high Ca^{++}-PVP solutions for more than 90 minutes after skinning. Fibers in PVP-relaxing medium display normal sarcomere spacings as measured by laser diffraction and behave as perfect osmometers for millimolar PVP concentrations, indicating that swelling is likely due to the effect of osmotically active fiber constituents which are not free to diffuse away.

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Dendrito-Dendritic Connections between Spinal Motoneurones of a Teleost (*Tinca tinca* L.)

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In tench spinal cord, the motoneurones which respond to excitation of the ipsilateral Mauthner axon with latencies shorter than 1.0 msec (at 8–12 °C) comprise a distinct group of cells. The cell bodies, up to 300 μ apart, lie approximately in a column some 200–300 μ dorsal to the Mauthner axon. A large ventral dendrite can be traced to a point at which it is in close apposition to an ipsilateral Mauthner axon collateral, after which it gives rise to a myelinated motor axon. The present study is an attempt to determine the response characteristics of these so-called 'primary' motoneurones and to reconstruct their somato-dendritic complex histologically from 10 μ serial sections following intracellular iontophoretic injection of Procion Yellow. Experimental evidence suggests that the primary motoneurones may be responsible for passing on the Mauthner axon excitation to other 'secondary' motoneurones. Light microscopical findings reveals what appear to be dendrito-dendritic connections, not only between neighbouring primary but also between primary and secondary motoneurones.

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Free Water Reabsorption in the Remaining Kidney Following Contralateral Nephrectomy

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Changes in renal function immediately following uninephrectomy were studied in 29 rabbits. Contralateral nephrectomy induced compensatory adaptive changes in the remaining kidney: fractional excretion of Na and K increased, as well as glomerular filtration rate and effective renal plasma flow. Filtration fraction remained constant. These changes were already significant five minutes after elimination of the contralateral kidney. To define the site of this adaptation free water reabsorption during hypertonic saline diuresis was used as an index of sodium transport in the ascending limb of Henle's loop. The values observed in normal kidneys were compared to that observed in the remaining kidney following uninephrectomy and to those observed in furosemid treated animals. In normal rabbits infused with 2% NaCl at increasing rates, free water reabsorption rose continuously with increasing osmolar clearances up to 1.5 ml/min kidney. As expected inhibition of Na transport by furosemid depressed free water reabsorption to very low, and sometimes negative values. By contrast free water reabsorption in the remaining kidney immediately following uninephrectomy bore a linear relation to osmolar clearance with no evidence of a limit ($TcH_2O = 0.14 + 0.19\ C_{osm}$). This regression line was not different from that observed in normal animals. These observations suggest that contralateral nephrectomy depresses Na transport in the remaining kidney at a site which is not the ascending limb of Henle's loop.

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Elastische, schnelle und verzögerte Spannungsphasen in funktionell isolierten kontraktilen Strukturen von Skelettmuskelfasern

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Der Spannungsverlauf nach schnellen Entdehnungen (0,1–1% L_0, ausgeführt in 5 msec) von glyzerinextrahierten Skelettmuskelfasern des Frosches (m. sartorius) und von nach Julian (1971) kurz glyzerinierten Schildkrötenfasern (m. ileofibularis) zeigt a) einen elastischen Spannungsabfall synchron mit der Entdehnung, b) einen schnellen viskoelastischen Spannungsanstieg, c) eine Haltephase oder einen verzögerten Spannungsabfall, d) einen langsamen Spannungsanstieg zur isometrischen Gleichgewichtsspannung. Der entsprechende Spannungsverlauf trat nach Dehnung auf. Die schnelle Phase dauerte bei Schildkrötenfasern etwa 20mal länger als bei den Froschfasern und wurde nur bei Ca-aktivierten Fasern beobachtet, nicht bei erschlafften Fasern, nicht bei Fasern, die bis zum «Non-over-lap» von Aktin- und Myosinfilamenten vorgedehnt wurden, und nicht nach ATP-Entzug (Rigorzustand). Erhöhung der Temperatur beschleunigte die schnelle Phase. Die elastische und schnelle Phase wurden auf der Grundlage des Querbrückenmodells von Huxley und Simmons (1971) interpretiert. Demnach ist anzunehmen, dass die Rotationsbewegung der Querbrückenköpfe am Aktinfilament beim Schildkrötenmuskel langsamer als beim Froschmuskel ist und dass die Querbrückenköpfe im Rigorzustand nicht rotieren können.

Taurine – a Possible Transmitter in the Mammalian Central Nervous System

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Biochemical investigations suggest that taurine might be a transmitter in the mammalian central nervous system. In the present study we investigated the action of microelectrophoretically administered taurine on the firing rate and the membrane potential of neurones of the medulla oblongata of the cat. Taurine was a potent depressant of the spontaneous firing of brain stem neurones (H. L. Haas and L. Hösli, Brain Research *52*, 399, 1973). This depression was reversibly blocked by

strychnine, which also antagonized synaptic inhibition in the medulla oblongata.

The depression by taurine was accompanied by a hyperpolarization and an increase in membrane conductance. Using KCl-electrodes the hyperpolarization was sometimes reversed to a depolarization presumably due to the diffusion of chloride ions from the recording electrode. Autoradiographic studies showed that (^{35}S) taurine was taken up in cultures of rat medulla oblongata.

These results provide some evidence that taurine may be an inhibitory transmitter in the medulla oblongata.

Ionic Requirements of Vascular Smooth Muscle Excitation and Contraction in Bat Metacarpal Vein

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Conflicting evidence reported in different blood vessels whereby – a) some tissues become electrically active in Ca-free (EDTA) saline solution and inexcitable when Na is replaced by Tris (Graham and Keatinge Fed. proc. *2P*, 597, 1970), and vice versa; b) increases in $[Ca^{2+}]_0$ either depress or augment contracture tension; and c) $[Ca^{2+}]_0$ is needed or not for NE and high-K evoked contractions – leads one to imagine that different mechanisms may determine vascular smooth muscle excitation and contraction. In situ perfusion of pulsatile metacarpal bat veins is used to determine the role and relation between different ions and neurotransmitters in governing smooth muscle function and autoregulatory flow mechanisms. Sucrose-gap recordings and in vivo perfusion indicate respectively that these muscle cells remain electrically and mechanically active over long periods in Na-free Tris saline solution, while soon becoming inactive in absence of $[Ca^{2+}]_0$. The temporary restoration of normal vasomotion in Ca-free medium by NE^{-6} is immediately abolished in the presence of 5 m*M* EGTA (or EDTA).

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Spectral Analysis of Hippocampal 'Theta' EEG in the Rabbit during Consummatory Behavior

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Bipolar EEG recording electrodes were implanted chronically into the dorsal hippocampi of rabbits. Slow-wave EEG activity was correlated with components of alimentary behaviors, including the licking of water, and biting and chewing of foods differing in consistency. The EEG was subjected to autocorrelation and power spectral density analyses by computer. Licking of water and biting of solid foods were accompanied by high power 'theta' activity (4–7 Hz). 'Theta' during drinking was of lower frequency than during biting. Chewing was correlated with very low frequency activity (0.5–3 Hz) of high power and with 'theta' of lower power and frequency than during biting. 'Theta' frequency during eating of mash was between that accompanying drinking of water and biting of food. Hence, correlation of EEG with even a 'simple' behavior such as eating is meaningless unless the behavior is further parcelled into discreter components such as chewing and biting. It seems that high frequency and power 'theta' is correlated with goal-directed components of consummatory behavior such as licking and biting, but not with its terminal reflex-like components, such as chewing.

In vitro Lung Contraction and Hypoxia

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The contractility of rat, human and bovine pulmonary parenchyma, as well as of arterial and bronchial strips was tested in a microbath filled with Tyrode solution. Hypoxia was produced by bubbling gas mixtures containing 0%, 5% and 10% O_2 through this medium.

Hypoxia contracted the parenchyma and relaxed the bronchi.

Hyperoxia relaxed the parenchyma while the bronchi contracted. Both stimuli had no effect on arterial strips. Epinephrin contracted parenchymal strips substantially, arteries slightly, and relaxed bronchi. Serotonin contracted violently arteries and bronchi, while the contraction of parenchymal strips was insignificant.

It was concluded that contractile cells different from arterial and bronchial smooth muscle must exist in the lung. Ultrastructural studies revealed, in septal 'fibroblasts', bundles of parallel fibrils similar to those of smooth muscle cells. Immunofluorescence showed many interstitial cells to contain actin. The contraction of lung parenchyma in hypoxic media and the presence of 'contractile cells' in alveolar septa raise the question of a possible ventilation–perfusion autoregulation at the alveolar level. These findings might be a clue to hypoxic pulmonary hypertension.

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Versuche zur peripheren Blockierung der Asthma-Atmungsreaktionen am Meerschweinchen

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Im mittels Histamin-Aerosol ausgelösten Asthmaanfall tritt eine charakteristische Atmungsbeschleunigung mit Lungenvolumenzunahme auf. Diese Atmungsreaktionen sind Ausdruck der exspiratorischen Lungenkompression infolge der Verteilungsstörung; sie werden durch die vagalen Lungenkollapsfasern vermittelt. Versuche zur Blockierung des Lungenkollapsreflexes ergaben folgende Resultate: Procain, ins rechte Herz injiziert, vermag die Atmungsreaktionen vorübergehend zu blockieren bzw. deren Auslösung vorübergehend zu verhindern. Als Aerosol beeinflusst Procain die Atmungsreaktionen nicht wesentlich. – Isoprenalin, ins rechte Herz injiziert, vermag den Asthmaanfall zu verhindern. Während des Anfalls injiziert, bewirkt Isoprenalin eine raschere Normalisierung des Lungenvolumens und damit eine raschere Beendigung der Atmungsaktivierung. Als Aerosol vermag Isoprenalin die Atmungsreaktionen beim Meerschweinchen nicht wesentlich zu beeinflussen. Der Lungenkollapsreflex ist offenbar leichter über den Lungenkreislauf als über die Luftwege zu blockieren. Die Lokalisation der Lungenkollapsrezeptoren wird diskutiert.

Relationship between the Action of Carbachol on Mammalian Atria and Ca Ions

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Previous experiments on mammalian atrial trabeculae have shown that the negative inotropic effect of carbachol can be eliminated when the shortened AP was artificially lengthened by the application of current (Kubis, Experientia *27*, 721–722, 1971). The role of Ca ions in this process has now been investigated. The experiments were carried out in the presence of carbachol (5.10^{-6} *M*) and the Ca was varied stepwise between 0,9 and 9 m*M*. As the Ca concentration was increased the AP duration was further reduced (probably by an indirect effect of high Ca increasing the conductance of a depolarized membrane as suggested by Beeler and Reuter (J. Physiol. *207*, 191–209, 1970), but the twitch tension was increased. Prolongation of the AP by current application for a given time and to approximately the same potential also gave an increase in twitch tension as the Ca raised. These results can be explained if during an AP Ca ions flow into the cell. The negative inotropic effect of carbachol would be due to a reduction of inward Ca current caused by the AP shortening. An increase in Ca concentration or prolongation of the AP, by causing a greater Ca influx, would tend to modify this effect.

The Plasticizing Effect of ATP-Analogues on the Tension of Glycerol-Extracted Muscle Fibres

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Formation of actomyosin linkages and force generation may be the spontaneous processes of the crossbridge cycle in activated muscle while crossbridge detachment may be its energy requiring step. This is suggested by the following finding: Addition of ATP-analogues (β-γ-imido-ATP, pyrophosphate) which are not split by actomyosin (and which bind to myosin) induce relaxation of glycerol-extracted fibrillar muscle fibres immersed in salt solution. Removal of the analogue from the fibres generates tension and increases the number of angled conformations of crossbridges (observed by electron-microscopy) in the fibres. Tension is abolished by a release of about 7 nm per halfsarcomere. A 100% transformation of chemical free energy into mechanical work was obtained during the following reversible cycle: 1. Release of isometrically contracted fibres by 0.5% L_0 in a solution containing no analogue (static stiffness 22 m*N*/fibre). 2. Isometric relaxation by incubation in a 5 m*M* Mg-analogue solution. 3. Restretch in the analogue solution (static stiffness 11 m*N*/fibre). 4. Isometric contraction by removal of the analogue. The results of this study suggest that in muscles depleted of ATP force may be generated by a mechanism similar to that proposed by Huxley and Simmons (1971).

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Ouabain Induced Choleresis: a Result of ATPase Inhibition?

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Provided Na-K-ATPase located histochemically near the canalicular membrane plays a role in bile formation, ATPase inhibitors would be expected to inhibit active sodium transport by the hepatocytes with consequent decrease in bile flow. To test this hypothesis, ouabain a well known ATPase inhibitor was administered i.v. in a dose of 0.02 mg/kg b.w. to 8 dogs (18 to 22 kg; 15 experiments), cholecystectomized and equipped with a Thomas duodenal cannula. Bile salt loss was replaced by Na-taurocholate infusions at a rate of approximately 24 μEq/min. Ouabain resulted in a constant increase in bile flow averaging 52% of control. The ^{14}C-erythritol (Er) clearance showed a parallel increase, pointing to an enhancement of bile flow at the canalicular level. Since at the same time the bile-plasma ratio of Er decreased, and since Cl^- and HCO_3^- concentration increased (comparable to the effect of secretin, known to act at the ductular level), an additional effect on the duct system has to be postulated. These results are in contrast to work in the rabbit, where ouabain in a higher dose of 1.12 mg/kg b.w. produced cholestasis. This difference in effect could be either species or dose related. In any case, the precise role of liver ATPase in bile formation remains ill-defined.

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Energy-Supplying Metabolism and Transmembrane Potential of Rat Brown Adipose Tissue in vitro

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The metabolic requirement for the maintenance of the steady-state value of transmembrane potential in cells of rat brown adipose tissue in vitro has been investigated.

1. In our experimental conditions, the steady-state transmembrane potential averages -49.6 ± 2.8 mV.
2. Glucose removal from the oxygenated bathing medium or its replacement by pyruvate 11 m*M*/l or alanine 11 m*M*/l, as well as addition of inhibitors of glucose utilisation such as IAA 1–2 m*M*/l or 2-DOG 10 m*M*/l, resulted in a significant depolarisation to about half the initial value of the transmembrane potential, with variable delay from animal to animal (60–300 min).
3. On the other hand, treatments which are expected to severely deplete the total ATP content of the tissue, such as strong hypoxia or addition of various inhibitors of oxidative phosphorylation like rotenone 40 μM/l, oligomycine, 0.01–1 μg/ml did not cause any depolarisation, but rather a slight hyperpolarisation, provided glucose was present in the bathing medium.
4. These experiments suggest that, in the rat brown adipose tissue in vitro, the steady-state transmembrane potential is supported by an energetic supply, which seems exclusively of glycolytic origin in the non-stimulated state, and dependent on a continuous supply of exogenous glucose. This conclusion is discussed in relation to the calorigenic function of this tissue.

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Amino-Acid and Sugar Transport in the Mucosa of the Dog Colon

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Unlike that of many other mammalian species, the dog colon has been found to absorb sugars and amino-acids

actively when incubated in vitro in the presence of radioactive substrate. In addition, a net flux of these substrates from mucosa to serosa across the sheets of dog colon mucosa clamped between two flux chambers can be demonstrated. The transport systems are dependent on the presence of sodium and on the integrity of cellular metabolism. A kinetic comparison with the corresponding transport systems in the ileum has revealed that the km is greater in the colon and the V_{max} is smaller. At low substrate concentrations, distribution ratios of about 5 can be established, the corresponding value in ileal slices being 15. Like sodium transport across the mucosa, phenylalanine uptake by colonic mucosal strips is completely abolished by ouabain, partially inhibited by ethacrynic acid, but insensitive to *n*-butyl-biguanide. Thus the dog colon appears to be unique insofar as it possesses a sodium dependent amino-acid transport system, apparently similar to that existing in the small intestine, but of lower capacity.

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Dual Aspect of Inhibitory Function of the Pulmonary Stretch Receptor Afferents

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In rabbit with both vagi cut, short-burst (200/sec) stimulation applied to low-threshold, fast conducting vagal afferent fibres (A-Beta range) during the inspiratory phase a) curtails the inspiration in progress and b) hastens the onset of the next inspiration. The first reaction, i.e., the expiratory component of the Hering-Breuer reflexes, is clearly related to an inhibitory mechanism. As to the nature of the second, i.e., one of the inspiratory components of the Hering-Breuer reflexes, two main suggestions prevail. In one it is held that a stimulus-induced excitation of inspiratory innervation outlasts the concurrent inhibition, the latter remaining predominant during stimulation. The second explanation is that inspiratory innervation at its site of origin involves an inhibitory feedback mechanism which builds up with a certain time-lag during inspiration and decays with a similar delay during expiration; if the inspiration is prematurely suppressed by the vagal input, the critical level at which the following inspiration starts will be attained earlier. Experimental evidence supporting the second hypothesis will be presented.

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Extracellular K^+ Depletion in Mammalian Ventricular Muscle

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Mammalian ventricular muscle bundles were voltage-clamped using either a single- or double-sucrose gap method. During prolonged hyperpolarizing pulses up to −150 mV the inward current declined to approximately one-half of its initial value within 6–8 sec. The corresponding steady-state current-voltage relation shows inward-going rectification, but no negative slope. During hyperpolarization much of the current is probably carried by K^+, and this decline of inward current could be explained if during the pulse a diffusion limited space at the membrane was depleted from K^+ ions. In support of this idea is the finding that the zero current intercept of the instantaneous current-voltage relation (E_0) measured at the end of a hyperpolarizing pulse becomes more negative when either the amplitude or duration of the pulse is increased. Since E_0 depends upon external $[K^+]$ (Almers, J. Physiol. *225*, 33, 1972), this suggests a decrease in external $[K^+]$ during these pulses. In agreement with this, the action potential measured after switching off the hyperpolarizing clamp was slightly prolonged when compared to the action potential elicited before the clamp. While these results support depletion a concomitant conductance change cannot be excluded.

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Retino-Hypothalamic Pathway in the Avian Brain

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Stereotactic intraocular injection of ^{3}H-proline, ^{3}H-leucine or ^{3}H-fucose were performed in 8 pigeons (*Columba livia*) and 1 jackdaw (*Coleus monedula*). The labeled material which was transported within 24 hours from the retinal ganglion cells to the optic nerve terminals was studied by light microscopical technique. Three distinct zones of the contralateral hypothalamus showed a grain density equal to that of the optic tectum and 2.8 times higher than that of the adjacent optic tract. While the zones 1 and 2 seem to belong to the supraoptic nucleus, namely to its rostral and lateral aspect respectively, the zone 3 remains undefined. It contains large cells, lies some 700–1000 μm lateral to the wall of the third ventricle and extends caudally to the rostral border of the supraoptic decussation. The same results were obtained in both species and with either precursor.

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Calcium Activated Adenosintriphosphatase in Cultured Chinese Hamster Cells

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With the intention to study the influence of ionizing radiation to adenosintriphosphatase in mitotic active cells we investigated homogenates of cultured Chinese Hamster cells. They showed Ca activated ATPase activity, which was measured by determining the inorganic phosphate formed. This activity was found not to be Mg dependent and inhibitable by dicyclohexylcarbodiimid (about 80% inhibition at 10^{-5} *M*). Its activity is reduced in solutions of relative high ionic content ($\mu = 0.3$) and not changed by ouabain (10^{-4} *M*). After centrifugal fractionation most Ca activated ATPase activity was found in the mitochondrial fraction. During the reaction time a precipitation was formed which is dependent on ATP and Ca. This precipitation prevented a precise determination of the Ca activated ATPase and led to a disproportionality of activity against proteinconcentration.

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The Influence of Fatigue on Membrane Conductance in Frog Skeletal Muscle

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While the contractility of isolated single fibres is abolished within 2 min by repetitive twitching (1 Hz) in the presence of 1 m*M* IAA^- and 1 m*M* CN^-, membrane potentials more negative than −75 mV often persist longer than 1 h from the onset of rigor. Estimations of g_M by the 2 microelectrode technique show an increased g_M by a factor of 100–400 compared to resting conditions (Grabowsky et al., Pflügers Arch. *334*, 222–239). Removal of Cl^- and K^+ outside reduces the increased g_M to about 10% of its value. Monitoring of membrane potential during rapid changes of solutions shows: 1. Changes in $[K^+]_0$ with or without constant K·Cl product produce potential changes closer to E_K than in controls. 2. Changes in $[Cl^-]_0$ produce either mutually no change in E_M or induce slight changes in the opposite direction than normal. These findings can be explained on the basis of a substantially greater increase of g_K than that of g_{Cl}, possibly due to the rise in internal Ca^{++}-concentration in rigor (Krnjević and Lisiewicz, J. Physiol. *225*, 363–390).

Experiments performed at Yale University New Haven Conn. during the tenure of a Postdoctoral Fellowship by the SNSF.

Preoptic Temperature and Duration of Fast-Wave Sleep Episodes

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To test the hypothesis that the decrease in duration of fast-wave sleep episodes at low environmental temperatures is the result of the interaction between sleep and thermoregulatory processes, the influence of preoptic heating (0.75 MHz, 40–200 mW delivered by means of two electrode pairs) on the evolution of fast-wave sleep episodes was studied in freely moving cats at different environmental temperatures (20, 0, −10 °C). The mean duration of the episodes showed a statistically significant increase (50–150%, $P < 0.01$) on preoptic heating (1–2 °C above control preoptic temperature) at all environmental temperatures studied. Under such environmental conditions, moreover, heating of thalamic structures with the same intensity as that used for the preoptic region did not affect significantly the mean duration of the episodes. These results show that an optimum preoptic temperature exists which, by reducing the load error of the thermoregulatory system, protects the evolution of fast-wave sleep episodes in spite of deviations of environmental temperature from thermal neutrality.

Action Potential of *Stylonychia mytilus* (Ciliate Protozoa) in Ba-Free Solution

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In $CaCl_2$ solution, *Stylonychia mytilus* exhibits periodic transient reversal in the direction of ciliary power stroke. Each period of ciliary reversal is synchronous with a spontaneous depolarization (Machemer, Naturwiss. *8*, 398, 1970). The action potentials are of short duration (200–300 ms), but may also be suddenly interrupted during repolarization by a plateau phase lasting over several seconds. Though no 'overshoot' is evident, such depolarizations are of the 'all or none' type. Similar responses may also be simulated by outward current pulses where the 'latent period' is found to be a function of the intensity of stimulation. Both absolute and relative refractory periods may be recorded during the action potential, when the resistance of the membrane diminishes. Both Ca^{2+} and K^+ extracellular concentrations may influence the apparition of the plateau phase, as well as the normal amplitude and duration of the A.P. The conductile component of *Stylonychia mytilus* is more relevant than that of other ciliates, for whom Ba^{2+} is indispensable for such activity, e.g. *Paramecia* (Naitoh, Eckert, Z. vergl. Phys. *51*, 453, 1968).

Contribution of Direct Calorimetry to the Study of the Thermic Effect of Glucose and Amino Acids in Man

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In order to reinvestigate the classical concept of 'specific dynamic action' of food, the thermic effect of ingested glucose (50 g) and/or essential amino acids (50 g of Nesmida) was measured in 7 healthy male subjects dressed in shorts, using simultaneously direct and indirect calorimetry. Experiments were performed under conditions of thermal confort at 28 °C. Energy balance (heat production minus heat losses) was thus determined before and during 150 min following the nutrient ingestion. In all subjects, the energy balance was negative during the control period (mean heat deficit: -4.5 ± 0.2 kcal $h^{-1}m^{-2}$). Metabolic rate increased of $13.6 \pm 2.1\%$ after the glucose load, $17.4 \pm 2.5\%$ after amino acids, and $17.7 \pm 4.2\%$ after both glucose and amino acids: there was no thermic additive effect when both nutrients were given together. In contrast to the metabolic rate, heat losses were not significantly modified after nutrient ingestion; consequently, the energy balance became rapidly positive. These results show that a) the dietary induced thermogenesis, for a moderate caloric intake, is less dependent on the nature of the nutrients than classically admitted; b) this increased energy production mainly induces changes in heat storage rather than in heat losses.

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Effect of Divalent Cations on Na and K Permeability of Human Red Cell Ghosts

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Starved red cell ghosts which contained KCl as main osmotic constituent and EGTA-buffered free Ca concentrations (Ca_i) between 10^{-9} and $10^{-4}M$ showed a minimum of K permeability (P_K) with Ca_i around $10^{-6}M$. This effect was most pronounced at 0–4 °C. Na permeability (P_{Na}) was similar to P_K with Ca_i below $10^{-6}M$ but dropped sharply with higher Ca_i. Unlike, P_K, P_{Na} did not increase but remained small even when Ca_i was raised above $10^{-5}M$. The effect of Ca_i depended to some extent on the direction of the K and Na movements. If Na-

loaded cells were suspended in a KCl medium an increase in Ca_i beyond $10^{-6}M$ induced a decrease in both P_K and P_{Na}. Sr showed effects similar to Ca_i at somewhat higher concentrations. Also Mg_i ($10^{-3} - 3 \times 10^{-2}M$) reduced P_K; however, the discrimination between Na and K was much less pronounced. Hence, the potency of divalent cations in changing P_K and P_{Na} decreased in the order Ca > Sr > Mg. This corresponds to No. II or III in the Sherry series (Diamond and Wright, Ann. Rev. Physiol. *31*, 581). It is suggested that these effects are due to coulombic interactions between divalent cations and negative fixed charges near the inner surface of the membrane and that different pathways exist for transmembrane K and Na movements.

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Effects of LH-FSH Releasing-Hormone (LRH) and Sex Steroids on Gonadotropin Secretion in Normal Male Rats

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LH and FSH secretion is studied in 50 days old male rats, in vivo (160 rats) and in vitro (300 rats); gonadotropin levels are measured by RIA, using the NIAMD-kits for rat LH and FSH.

In vivo, in groups of 6 rats, a rapid i.v. injection of LRH induces a log-dose related rise of plasma LH, with a peak 10–15 min after LRH injection. 100 ng LRH/rat yields a 200% LH increase, but no constant FSH response. Pretreatment, 40 h before LRH injection, with Testosterone (T), Dihydrotestosterone (DHT) or Progesterone (P) shows a decrease of LH response to 100 ng LRH with 5,000 ng T, but no modification of the response with lower doses of T, DHT, P.

In vitro, pools of 6 half-antepituitaries are incubated in KRBG for 3 h, 37 °C, after 3 short preincubations; a log-dose response of LH release to LRH is obtained, and 10 ng LRH/pool yields a 200% LH increase in the medium, but no significant stimulation of FSH release. Low amounts of T (1 ng/pool) appear to increase the LH response to 10 ng LRH, but high levels of T and DHT reduce it; small doses of P show a direct stimulating effect on FSH release, but not on LH release.

In all these experiments, pituitary LH and FSH contents show no significant differences between control and treated rats.

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The Tonotopic Organization of the Medial Geniculate Body at the Unit Level

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The pars principalis of the medial geniculate body has a well structured organization in concentric laminae (D. K. Morest, J. Anat. Lond. *99*, 143–160, 1965). Single unit activity of this structure was recorded in anesthetized cats in response to acoustical signals delivered independently to both ears. Microelectrodes were advanced through a stereotaxically positioned chamber in a direction parallel to the plane of the laminae. Electrode penetrations done in external laminae showed cells responding to low frequency tones. More medially located laminae exhibited cells responding to higher frequencies. Units recorded at different depth of the same lamina responded to tones of about the same frequencies. Responses of 170 cells were recorded from 14 different penetrations in 8 animals. Temporal pattern of the response, ear dominance, intensity function and responses to broad band stimuli were not found to be simply related to the depth within a lamina.

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Rôle de la dépolarisation membranaire sur le niveau rédox des enzymes respiratoires du tissu adipeux brun de rat perfusé in vitro

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La fonction thermogénique du tissu adipeux brun est activée par le système nerveux sympathique et, si l'on soumet ce tissu à un pulse de noradrénaline, on observe une dépolarisation membranaire. Cette dépolarisation exerce-t-elle un contrôle sur la fonction de ce tissu ?

La thermogénèse résulte d'une augmentation du transport d'électrons le long de la chaîne respiratoire. Le niveau rédox de deux enzymes respiratoires a été suivi par la mesure des variations de la fluorescence de surface émise par les pyridines nucléotides [NAD(P)] et par les flavoprotéines. A ce niveau, l'effet d'une dépolarisation comparable a été étudié par l'adjonction de noradrénaline et de KCl au milieu de perfusion. Un pulse de noradrénaline ($5{,}10^{-8}$–$5{,}10^{-7}$ g/ml) a provoqué une réduction réversible des NAD(P)H et des flavoprotéines. Le KCl a également provoqué une réduction réversible qui se différencie cependant par une cinétique plus lente. Les effets de ces deux substances sur la réponse métabolique ainsi mesurée peuvent être dissociés en changeant les conditions de perfusion. En effet, en l'absence de bicarbonates, seule l'hormone a augmenté le niveau rédox des NAD(P)H. Or, nous avons montré que, dans ces conditions, ces deux agents n'ont provoqué aucune dépolarisation membranaire. Il sera montré que, dans nos conditions expérimentales, les variations de l'état rédox des enzymes respiratoires sont dues à un apport augmenté de substrat sous l'influence de l'hormone et non à un découplage de la respiration.

Ce travail a été effectué dans la Division du Dr B. Brauser, Institut für Physiologische Chemie und Physikalische Biochemie der Universität München.

Interactions of Diphenylhydantoin with Norepinephrine, Theophylline and Cyclic AMP in Frog Skin

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We reported previously that diphenylhydantoin (DPH) increases short circuit current (SCC) and potential difference (PD) across frog skin. This effect is additive to that of supramaximal doses of oxytocin or vasotocin and is completely blocked by amiloride. Additional experiments were carried out to characterize further the mode of action of DPH. After large doses of norepinephrine ($10^{-6}M$), DPH still increases PD and SCC, when no further increase could be obtained with oxytocin in the

control skin. While DPH increases PD and SCC like oxytocin and norepinephrine, the interaction with these hormones suggests that its effect is not mediated by cyclic AMP. To test this hypothesis, studies were carried out with theophylline and cyclic AMP. Exposure of frog skin to theophylline (10^{-3}–$10^{-2}M$) did not prevent the effect of DPH, while oxytocin was ineffective in the presence of large doses of theophylline ($10^{-2}M$). Similar results were obtained with large doses of cyclic AMP ($5.10^{-3}M$). To exclude more directly the mediation of DPH effects by cyclic AMP, measurements of the intracellular concentration of the nucleotide are being made in the isolated epithelium.

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Effects of Lanthanides on Transport Processes of Amphibian Epithelia

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The effects of lanthanides on potential difference (PD) and short circuit current (SCC) were studied on frog skin and toad skin. Pieces of abdominal skin were mounted in lucite double chambers with both sides of the epithelium exposed to identical aerated Tris-Ringer solutions. PD and SCC were monitored continuously by standard techniques. Addition of La^{+++} ($5.10^{-4}M$) to the external side of the skin resulted in a rapid, sustained rise in both PD and SCC. These effects mimicked but did not interfere with those of oxytocin, added to the internal medium before or after exposure to La^{+++}. Similar results were obtained with Ce^{+++}, Pr^{+++}, Nd^{+++}, Sm^{+++} and Eu^{+++}. Slightly higher doses ($10^{-3}M$) were needed for Yb^{+++}. Dose-response curves were obtained for each of the elements tested. Amiloride completely blocked the stimulation of PD and SCC produced by the lanthanides. Addition of La^{+++} to the internal medium bathing the skin had no conspicuous effect per se, but blocked the effects of oxytocin. It is suggested that lanthanides may interact with Ca^{++} at the external side of the epithelium, which results in an increased permeability to Na^{+} at sites distinct from those affected by the neurohypophyseal hormones.

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Morphophysiological Evidence for a Two Step Active Transport Path for Sodium in the Epithelium of the Frog Skin (R. Temporaria)

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Quantitative correlations between active asymmetric transport of sodium across the epithelium of the frog skin in vitro and simultaneous morphological changes have been described in previous reports (e.g. C. L. Voûte and H. H. Ussing, Exp. Cell Res. *62*, 375–383, 1970). It is however essential to apply an instantaneous fixation technique for topographic evaluation of rapidly reversible functional events at the cellular level. A new rapid freezing technique recently adopted by our laboratory brought further encouraging results:

1. Under constant short circuit conditions there is a linear relation between short circuit current (active sodium transport) and volume of the first cell layer below the str. corneum (C. L. Voûte and S. Hänni, *Structure and Function in Frog Skin*, A. Benzon, Symp. V, *Transport Mechanisms in Epithelia*, Munksgaard, Copenhagen, 1972, in press).

2. When applying under above conditions varying hydrostatic pressure gradients (inside positive) vesicles appear in the cytoplasm of this same cell layer visible in the lightmicroscope. There is a linear relation between their number per cell and the simultaneously measured asymmetric sodium transport.

Implications of these observations are discussed and a two step active transport path for sodium at the cellular level is proposed.

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The Efflux of TEA in Mammalian Ventricle

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Ventricular bundles of sheep and calf hearts were loaded with ^{14}C-labelled tetraethylammoniumbromide. The efflux of radioactivity into inactive solution was measured for periods up to 6 h by passing the muscles through a series of test tubes, the TEA being determined in a Packard Tricarb liquid scintillation counter. In a semilog-plot the decrease of the TEA-activity in the preparations could be fitted by the sum of three exponentials, with the following time constants: 2.7, 13.3 and 832 min. Experiments with connective tissue bundles showed only the first two components. From these results it is assumed that the first component is due to wash out of free extracellular TEA, the second due to bound extracellular TEA, and the slowest due to intracellular TEA. When $[K^+]_0$ was increased from 5.4 to 54 mM the transmembrane efflux of the cation TEA increased by a factor of about 2, while a factor of 4 would be expected from the decrease of the membrane potential. This discrepancy can be explained if P_{TEA} falls on depolarisation.

Régulation d'un facteur β cytotrope dans la muqueuse duodénale du rat

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L'existence dans la muqueuse duodénale d'un mécanisme contrôlant la réponse insulinique à une surcharge de glucose est connue depuis longtemps. On ignore pourtant encore tout de la régulation de la sécrétion de ces hormones par le duodénum.

Pour étudier cette régulation, une surcharge orale de glucose a été faite chez une première série de rats. Des extraits aqueux de la muqueuse duodénale de ces rats, prélevée à des temps différents, ont été injectés dans le tronc coeliaque d'une deuxième série de rats. Le sang a été recueilli dans la veine porte pour l'analyse du taux d'insuline. Des injections de sécrétine ont vérifié la sensibilité du système.

Alors que le taux d'insuline ne croît que faiblement après les injections de contrôle (muqueuse de rats à jeun ou surchargés avec une solution isotonique), 5 minutes après la surcharge de glucose déjà, le taux d'insuline passe de 43 μU/ml à 420 μU/ml.

Cette étude suggère soit la présence sous forme inactive, soit la synthèse rapide d'un facteur hormonal à effet β cytotrope qui entre en action sous l'effet de la nourriture.

BIOCHEMIE – BIOCHIMIE – BIOCHEMISTRY

Fluorescence Properties of Octopine Dehydrogenase

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Octopine dehydrogenase (ODH) is one of the few monomeric dehydrogenases known up to date. The study of its physicochemical properties is therefore particularly interesting in view of the comparison with other protomeric dehydrogenases. Here we report on the fluorescence properties of ODH and its binary and ternary complexes. The intrinsic fluorescence of the protein is due to tryptophan. There is a remarkable enhancement of the NADH fluorescence intensity in the binary and ternary complexes, with a parallel blue shift of the fluorescence maximum. The fluorescence of the protein is quenched upon binding of both NAD and NADH and this permits to evaluate binding constants of binary and ternary complexes. The binding curves obtained by following the fluorescence quenching, exactly parallel those obtained by following the enhancement of NADH fluorescence. The tight binding in the ternary complex ODH·NADH·octopine permits an accurate titration of the enzyme active site concentration. The comparison of the various dissociation constants of coenzyme and substrate in binary and ternary complexes shows that the enzyme is characterized by a preferential order mechanism.

Critical Evaluation of Colloidal Gold as a Cytochemical Marker

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Colloidal gold has been used as a cytochemical marker in electron microscopy. Nevertheless non specific adsorption and lack of diffusion of the complexes into the tissue have to be carefully considered in the interpretation of the results. A basic study of the morphology of this colloid was undertaken using ferritin and antiferritin as a model system. Colloidal gold prepared according to Faulk and Taylor (Immunochemistry *8*, 1081, 1971) was labelled with ferritin (horse spleen) and antiferritin antibodies isolated by immunoadsorption from a bovine colostrum lactoserum. A vast number of electron micrographs indicated the following points.

The colloidal gold granules had an average diameter of 50–60 Å although some variations could be observed. At optimal proportions of ferritin and colloidal gold, the gold granules became adsorbed to the ferritin giving complexes with variable size due to aggregation. Well dispersed granules all coated with the antiferritin protein were obtained. The interactions of ferritin-antiferritin, ferritin-labelled antiferritin, labelled ferritin-labelled antiferritin have been studied. Rather heterogeneous complexes were observed in all cases.

In conclusion, care has to be taken in the interpretation of cytochemical experiments with this colloid.

Immunological Comparison of Red Cell Membrane Protein from Different Mammals

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Immunological studies of solubilized human red cell membrane proteins have detected a number of antigenic determinants associated with such proteins. Using rabbit anti-human red cell membrane proteins, comparative immunochemical studies were performed with red cell membranes from several mammals. The hemoglobin-free ghosts were solubilized with 0.5% NP 40 (Nonidet P 40 Shell), a non-ionic detergent, and tested in agar by Ouchterlony double diffusion. The results show that at least three protein antigens are shared by the different species studied. Components I and II are present in human, dog, bovine, sheep, goat, mouse and rabbit red cell membranes. Component I is also detected in horse and guinea pig. Both proteins are localized in the deeper layer of the membrane since they can be revealed by an antiserum absorbed with human red cells. Component III is detected in human, guinea pig, bovine and goat membranes using an antiserum specific for the major membrane glycoprotein isolated from human red cells according to Marchesi and Andrew (Science *174*, 1247, 1971). Antibodies against that protein can be absorbed by intact red cells indicating the presence of the antigen on the surface of the cell.

Isolation and characterization of these common proteins is in progress.

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Regulation of Pyruvate Metabolism in Liver Mitochondria by the ATP to ADP Ratio

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Pyruvate can be carboxylated to oxalacetate or oxidized to acetyl-CoA in liver mitochondria. Various regulation mechanisms are known for the purified enzymes and it was attempted to demonstrate which of these are operative in intact mitochondria. Rat liver mitochondria were incubated with ATP, Mg^{2+}, HCO_3^- and phosphate. Intramitochondrial ATP:ADP ratios were varied by addition of creatine, oleoyl-CoA and DNP. Solutions of various pyruvate concentrations were infused at constant rates. The amounts of pyruvate carboxylated and oxidized were calculated from the pyruvate used and metabolites formed. At a pyruvate concentration of about 60 μmolar, increasing intramitochondrial ATP/ADP ratios correlated on one hand with increasing amounts of pyruvate carboxylated and on the other hand with decreasing amounts of pyruvate oxidized. With high pyruvate concentrations (> 3 mM) the ATP:ADP ratio correlated only with pyruvate carboxylation whereas pyruvate oxidation was insensitive to ATP:ADP changes. At high or low pyruvate concentrations, no correlation of acetyl-CoA levels with either carboxylation or oxidation was obtained. These and other results show that with physiologically low

pyruvate concentrations the intramitochondrial ATP: ADP ratio directly regulates pyruvate carboxylation and oxidation.

Immunochemical Studies on Myosin from Rabbit Muscles

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Guinea pigs were immunized with myosin prepared from white muscles (m. long. dorsi) of the rabbit. The specificity of seven antisera showing a high titre with this myosin was checked by quantitative microcomplement fixation. Preparations of twelve proteins or protein systems of rabbit muscles were used as antigens. No reaction was found with myosin of the heart, a troponin (TN-I), AMP-deaminase (E.C. 3.5.4.6), adenylate kinase (E.C. 2.7.4.3) and creatine kinase (E.C. 2.7.3.2), a slight one with myosin from red muscle (m. ischiotibialis), actin, native tropomyosin and β-actinin and a very strong one with the relaxing protein system and a crude α-actinin preparation. Hence the strong reactions are not due to integral myosin. The question arises, whether they are caused by a contamination with light chains of myosin in these preparations or by impurities of unknown specification. In the latter more probable case all results on the specificity and localisation of myosin in the myofibril obtained with the aid of antisera hould be reevaluated.

Carbohydrate Analysis of Carcinoembryonic Antigens Containing Different Blood Group Specificities

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Carcinoembryonic antigen (CEA) was described by Gold as a proteinpolysaccharide complex present in digestive adenocarcinomas and in digestive organs of the foetus. We extracted CEA by perchloric acid (0.6 *M*) from liver metastases of large bowel carcinomas and purified it by Sephadex G200 and Sepharose 6B. The molecular weights of two preparations determined by the Yphantis sedimentation equilibrium method were 220,000 and 230,000 daltons. We have shown that several preparations can contain different blood group antigens in addition to their CEA specific determinants (Holburn et al., in preparation). The carbohydrates of five CEA preparations including one provided by P. Gold and one by F. Martin were analyzed by gas-liquid chromatography. The values represent the % of each monosaccharide per total carbohydrate (average from 2 to 3 determinations).

CEA-prep.	Blood group	FUC	MAN	GAL	GalNac	GlcNAc	SA
Gold	B	8	25	29	none	28	10
101	B	2	19	34	none	33	12
Martin	A	4	15	24	7	30	20
38	Lewis a^+b^-	4	18	26	none	38	14
105	none	3	19	22	none	36	20

It is interesting that the CEA with blood group A antigen seems to contain a small amount of GalNAc, and both preparations with blood group B antigen have a higher galactose content.

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Adherence of Entero-Pathogenic *E. coli* to Intestinal Cells: Effect of Hyperimmune Lactoserum

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In order to study the mode of action of a bovine anti-*E. coli* lactoserum, we have used a new test measuring the adherence of these bacteria on intestinal cells isolated from the small gut of rabbit according to Stern (Gastroenterology *51*, 855, 1966). The *E. coli* strains 0125 K70 (B15), 0124 K72 (B17), 0127 K63 (B8), 0128 K67 (B12), 055 K59 (B5), 0111 K58 (B4), 0119 K69 (B14), 028 K73 (B18) are cultivated in broth (48 h). One ml intestinal cell suspension (10^5 cells/ml) is incubated with 1 ml bacterial suspension (10^{10}–10^8 bact./ml) in the presence or absence of hyperimmune bovine lactoserum (1 g protein per 100 ml). After coloration, the number of bacteria adhering to the epithelial cells was counted under the microscope. Depending on the strain, and in absence of lactoserum, this number varies between 2 to 18 bacteria/cell. In four strains, the presence of lactoserum diminishes this number by a factor of 2 to 6. The strains agglutinating guinea pig red cells adhered to a larger extent to the cells suggesting the importance of fimbriae to the process of adhesion (Duguid, J. Path. Bact. *74*, 397, 1957).

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Some Properties of the Angiotensin Converting Enzyme from Human Seminal Plasma

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Angiotensin converting enzyme has been reported to occur in human seminal plasma[1]. We have investigated some properties of the enzyme from this source. Assays were performed on the supernatant of fresh human sperm centrifuged for 15 min at 2000 g, with *z*-phenylalanyl-histidyl-leucine as the substrate[2]. The seminal plasma was found to contain high concentrations of converting enzyme; they are about 100 times higher than those occurring in human blood plasma. The sperm enzyme resembles that of blood plasma and lung tissue in that it is also activated by chloride ions, inhibited by EDTA and has an optimum pH of about 8.

[1] D. W. Cushman and H. S. Cheung, Biochim. Biophys. Acta *250*, 261 (1971).

[2] Y. Piquilloud, A. Reinharz and M. Roth, Biochim. Biophys. Acta *206*, 136 (1970).

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Transport actif du myo-inositol chez *Aerobacter aerogenes*

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Aerobacter aerogenes possède un système de transport inductif pour le myo-inositol (MI). Les cyclohexanehexols transportés sont ceux qui ne diffèrent de MI que par une seule inversion. Le scyllo-inositol (SCY), qui n'est pas deshydrogéné par la MI-deshydrogénase et qui ne peut donc être métabolisé, a été utilisé comme substrat pour le transport. Le système présente pour SCY un K_M apparent de $5 \times 10^{-5} M$ et un V_{max} de 30 nM/mg/min. A l'équilibre la concentration à l'intérieur est de 100 fois celle de l'extérieur. L'énergie d'activation est de 8800 cal/M. Les réactifs du groupe SH comme l'iodoacétamide, le *p*-hydroxy-mercuribenzoate et la N-éthylmaléimide sont de très forts inhibiteurs; une protection contre l'effet de la dernière substance peut être obtenue par de fortes concentrations de substrat. L'influx et l'efflux se font par le même système. L'efflux du substrat accumulé peut fournir de l'énergie pour l'influx.

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Isolation and Purification of Extracellular $\beta(1 \rightarrow 3)$ Glucanases from the Myxomycete *Physarum polycephalum*

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The plasmodia of the acellular slime mould *Physarum polycephalum* have been found to produce two extracellular $\beta(1 \rightarrow 3)$glucanases in liquid culture, having a pH optimum of M 0.5 and a temperature optimum of 60 °C. The enzymes appeared in the growth fluid after 6 days and reached a maximum after 12 days. Hydrolysis of laminarin by the enzymes liberated a serie of $\beta(1 \rightarrow 3)$glucose oligomers indicating an endo-$\beta(1 \rightarrow 3)$glucanase activity.

The enzymes were isolated and purified from the growth fluid by cellulose chromatography and isoelectric focusing (isoelectric points 4.3 and 4.6, respectively).

Details of the purification and characterization of the enzymes will be presented.

Collagen Synthesis in Cultured Corneal Cells

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Synthesis of collagen is demonstrated in cultured corneal fibroblastic and epithelial cells of the rabbit. After exposing the cultures to ^{14}C proline for 22 h protein-bound ^{14}C proline and ^{14}C hydroxyproline were determined in the cells and in the nutrient medium in order to detect soluble collagen in the latter.

The total incorporation of proline is taken as a measure of protein synthesis while the radioactivity related to hydroxyproline indicates the rate of collagen synthesis.

The percentage of collagen synthesis in the fibroblastic cells was about 6.5% of the total protein synthesis; in the epithelial cells this rate was around 1%.

The capability of the epithelium to synthesize collagen may be important for the reconstitution of its basement membrane which contains collagenoid proteins. Electron microscopic studies, however, indicate that the corneal epithelium is also involved in the biosynthesis of native striated collagen fibrils, probably to support stromal wound healing.

Collagen synthesis has already been found in embryonic chick epithelium (neural crest and cornea) and in cultured cells of non-fibroblastic origin.

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Complementation in vitro by Restriction Enzymes from Mutant Strains *E. coli* K12

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The restriction endonuclease from *E. coli* K12 has previously been shown to a) bind and then cleave unmodified λ DNA in the presence of SAM, ATP and Mg^{++}, b) hydrolyze the ATP to ADP and inorganic phosphate in the course of the restriction reaction, and c) methylate unmodified λ DNA when only SAM is present. The restriction deficient mutant strains from *E. coli* K12, $r_K^- m_K^+$ and $r_K^- m_K^-$ have been shown to complement in vivo to yield a wild type phenotype, $r_K^+ m_K^+$. Using a binding assay based on complementation in vitro between the two mutant extracts, the restriction endonucleases from the two mutant strains were purified extensively. Complementation in vitro could be detected by specific binding and also by cleavage of the unmodified DNA when both mutant proteins were present. Either mutant protein by itself showed no activity. Studies on the methylase and ATPase activities of these mutant proteins, either alone or in combination are currently in progress.

Kinetics and Subcellular Distribution of ^{35}S-Taurine Uptake into Rat Cerebral Cortex Slices

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Taurine has been proposed as a potential inhibitory neurotransmitter in brain. A chief criterion for a potential transmitter is the presence of a high-affinity uptake mechanism. We have determined the uptake kinetics for ^{35}S-taurine at 22 taurine concentrations in the range 9×10^{-8} to $5 \times 10^{-3} M$, using rat cerebral cortex slices. Uptake was calculated in nM ^{35}S-taurine/min/g fresh weight. The uptake kinetics were analyzed by double reciprocal plotting. In the entire concentration range no K_m in the range $10^{-5} M$ (high affinity uptake) was found. Calculation of K_m by regression line gave a value of $3–8 \times 10^{-4} M$, indicating only a low-affinity, unspecific uptake mechanism. The synaptosomal fractions were prepared from homogenates of cortex slices after ^{35}S-taurine uptake by fractionation on a discontinuous sucrose gradient. Comparison with ^{3}H-glycine and ^{3}H-Gaba uptake showed very low concentrations of ^{35}S-taurine in the synaptosomes: 39.8% for ^{3}H-Gaba, 40.6% for ^{3}H-glycine and 27.4% for ^{35}S-taurine. Similarly, calculation of the uptake quotient Q (relative uptake from the medium) gave a low value for taurine: 1.05 for ^{3}H-Gaba, 0.31 for ^{3}H-glycine and 0.067 for ^{35}S-taurine. These results indicate that taurine is probably not a neurotransmitter in rat cerebral cortex.

Intestinal Glucose Transport in Diabetes

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D-glucose transport was investigated in isolated membrane vesicles from brush border of the small intestine. Alloxan-diabetic rats were compared to healthy rats – membranes being prepared with the same degree of purification. Transport properties for glucose are qualitatively similar: a) Uptake of D-glucose is faster than that of L-glucose; b) both isomers reach the same equilibrium uptake after prolonged incubation; c) D-glucose uptake is dependent on Na^+; d) phlorizin inhibits D-glucose transport. Incubation of membranes with D-glucose (1 mM) in the presence of a NaCl-gradient (100 mM) results in a transient uptake of D-glucose above equilibrium values during the first minutes, considered as 'active' transport. The ratio of maximum to equilibrium uptake was 1.47 $\pm$ 0.24 (medium $\pm$ range) in diabetic and 1.12 $\pm$ 0.02 in normal rats in 3 independent experiments. A similar change in the transport properties of this membrane is observed after in vitro perfusion of intestine with dibutyryl-cycAMP. These results suggest that the characteristics of the brush border membrane are altered in diabetes and that these may be responsible for the increased transport of intestine.

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Relationship between Renal Phosphoenolpyruvate Carboxykinase Activity and Gluconeogenesis during Fasting

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The effect of fast of varied duration on phosphoenolpyruvate carboxykinase (PEPCK) activity as well as on gluconeogenesis from α-ketoglutarate (α-KG) and from glutamine in rat kidney was studied. The enzyme activity was measured in the soluble fraction of kidney cortex homogenates by the rate of phosphoenolpyruvate formation from oxaloacetate. PEPCK activity rose from 72.9 $\pm$ 4.34 mU·mg $prot^{-1}$ in fed rats to 155.5 $\pm$ 1.89 in rats fasted for 36 h, with no further change after 72 h of fast (162 $\pm$ 4.14). Glucose production by kidney cortex slices incubated in Krebs-Henseleit medium with 10 mM α-KG increased from a fed value of 230 $\pm$ 9.2 μM·g dry $wt^{-1} \cdot 2\ h^{-1}$ to 329 $\pm$ 8.6 after 36 h of fast and was not further enhanced after 72 h (311 $\pm$ 10.4). By contrast, gluconeogenesis from 10 mM glutamine, which increased from 145 $\pm$ 7.9 to 219 $\pm$ 5.3 after 36 h, further rose to 274 $\pm$ 8.8 after 72 h of fast. Thus, the rate of gluconeogenesis from glutamine does not appear to be limited by PEPCK activity or to depend on the gluconeogenic flux from α-KG. The data suggest the existence of a control step between glutamine and α-KG in the pathway from glutamine to glucose.

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Anti-Idiotypic Antibodies and the Growth of MOPC-315 Plasmocytoma

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Lynch et al. (PNAS *69*, 1540, 1972) have reported that the growth in BALB/c mice of plasmocytoma MOPC-315 can be inhibited by prior immunization with large doses of the secreted myeloma protein. The protected mice had anti-idiotypic antibodies. Of the mice still producing tumors, most secreted light chains and not whole IgA molecules.

Repeating immunization with the same system, we also observed anti-idiotypic antibodies, but were unable to demonstrate any protective effect. These tumors all produced intact IgA. However, an immunization schedule using less antigen provoked slight tumor enhancement, which was reproducible.

It seems reasonable to assume that the effect of either sensitized lymphocytes or humoral antibodies would depend upon the presence of appropriate antigens on the surface of the tumor cell. We therefore evaluated the amount of IgA on the surface of the MOPC-315 cells by immunofluorescence and found very little if any. This could explain why we found no inhibition of tumor growth. It remains unclear why a low dose antigenic stimulation, whereby no anti-idiotypic antibodies were detected, gives rise to an enhanced tumor growth. The variability in density of surface bound antigen also could influence the enhancement.

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Enzymatic Degradation of the First Subcomponent of Complement (Clq)

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A highly purified preparation of human Clq was radioiodinated using lactoperoxidase according to Heusser et al. (J. Immunol., in press). Iodination was shown to occur only in the smaller of the two non covalently bound subunits of Clq. The effect of trypsin, chymotrypsin, elastase and collagenase on the hemolytic activity of Clq and its fixation to immune complexes has been studied and the fragments obtained were analyzed by SDS-polyacrylamide gel electrophoresis.

Trypsin and collagenase produced a rapid loss in the hemolytic activity of Clq, whereas its ability to fix to ovalbumin-antiovalbumin complexes decreased only slowly. Hemolytic activity was proportional to the amount of undigested Clq and was absent in the digested material. This finding is consistent with the requirement of an intact Clq (molecular weight 400,000 daltons) for the initiation of lysis.

In contrast, it was shown that one out of four fragments obtained after collagenase treatment (Clq has a collagen-like amino acid composition) retained its capacity to bind to immune complexes. This active fragment was detected by comparing the electrophoretic pattern of digested Clq before and after adsorption on immune complexes. It has a molecular weight of 40,000–50,000 daltons. This finding suggests that the ability of Clq to bind to immune complexes is confined to a restricted region in the molecule, which can be removed in active form by collagenase.

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Optimisation des conditions de préparation de matériel cytoplasmique, enzymatiquement actif de Lactobacilles et de Streptocoques lactiques

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Les microorganismes testés ont été le *Lactobacillus helveticus* H_2, le *Streptococcus lactis* ML, le *Streptococcus lactis* 274 MW, le *Streptococcus thermophilus* 24. Plusieurs méthodes d'ouverture des cellules ont été essayées: lyse enzymatique et ouverture mécanique (Hughes press, broyeurs à bille de verre). La méthode de broyage mécanique avec un broyeur à bille[1] a été trouvée la plus avantageuse. Une série de désintégrations par batch, à une température inférieure à 6 °C, à permis de déterminer les paramètres principaux (dimensions et quantité des billes de verre, vitesse circonférentielle de l'agitateur, concentration de la suspension microbienne, tampon, pH, stabilisateurs d'enzyme, etc.) permettant d'atteindre une ouverture complète des microorganismes avec une conservation maximale de diverses activités enzymatiques intracytoplasmiques. Ont été testées les enzymes suivantes: GOT, GPT, G-6-P-DH, MDH, les phosphatases acides et alcalines et surtout les LDH. Les conditions optimales ainsi définies ont été appliquées à un système de désintégration en continu avec le même appareil modifié: les différents microorganismes cités en suspension dense (5–12% de matières sèches) ont ainsi pu être broyés à 80–90%, avec un débit horaire continu correspondant à 20–30 fois le volume du récipient de broyage (4–6 litres/h, température env. 12 °C). Les activités des différentes enzymes testées ainsi libérées en continu sont restées constantes en cours du broyage et à un niveau très voisin de celui obtenu dans les conditions optimales observées lors des essais en système discontinu. Avec le même appareil, en continu, plusieurs désintégrations ont pu être effectuées dans des conditions stériles. Les parois cellulaires purifiées ont été en partie utilisées pour des essais de fixation de phages.

[1] J. Rehacek, *Continuous Disintegration of Microorganisms in New Laboratory Apparatus*, Experientia *27*, 1103 (1971).

Axoplasmic Flow of ^{3}H-Fucose Labelled Glycoproteins in the Retinotectal System of Pigeon

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The labelling of the prosthetic groups of glycoproteins after intraocular (i.o.) injection of ^{3}H-fucose reaches a plateau within 24 h, in contrast to the labelling of proteins, which reaches a plateau within 90 min after i.o. injection of ^{3}H-leucine. About 15% of ^{3}H-fucose labelled material is transported from the retina with the fast axoplasmic flow (in comparison: 3% after i.o. injection of labelled amino acids). There is no evidence for the transport of ^{3}H-fucose labelled material with the slow axoplasmic flow. A quarter of the transported radioactivity stays in the optic tract, the rest in the optic tectum. After subfractionation of tectal nerve endings (V. P. Whittaker et al., Biochem. J. *90*, 293, 1964) F and G fractions containing plasma membrane are mostly enriched in radioactivity. Turnover of transported prosthetic groups of glycoproteins is the same in different subfractions and it is substantially longer than that of the protein part. Ratio ^{3}H/^{14}C radioactivity in the tectum is the same 4 and 14 days after i.o. injection of ^{3}H- and ^{14}C-fucose, indicating that reutilization is not involved in this long turnover.

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Biochemical Analysis of Gross Virus Specific Tumor Antigens

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Using ultrasound, an antigenic fraction has been solubilized from mouse myeloma cells and characterized as follows:

- it inhibits specifically the cytotoxic action of an isologous anti-Gross tumor serum,
- it stays soluble after centrifugation at 105,000 *g*,
- SDS-electrophoresis of an immune-precipitate reveals 3 sugar containing components.

One of the 3 components has been identified as a viral envelope glycoprotein. Absorption experiments studying the origin of the other 2 components will be discussed.

Multiple Effects of Catechin and Quercetin on the Respiration of Mitochondria

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Experiments with the Clark oxygen electrode showed that coupled respiration of rat liver mitochondria was inhibited by 77% with 4.5 m*M* catechin and by 71% with 0.42 m*M* quercetin in the presence of 3-hydroxybutyrate as substrate and by 71% and 65% respectively with succinate as substrate. The uncouplers DNP and FCCP did not release the inhibited respiration with 3-hydroxybutyrate as substrate; however, with succinate a strong release of the inhibition was observed upon addition of the uncouplers. ATPase activity in the presence of DNP was inhibited by 42% with 5 m*M* catechin or with 0.57 m*M* quercetin. Furthermore, catechin and quercetin were found to be electron donors for cytochrom oxidase in the presence of added cyt c confirming earlier results obtained with liver homogenates by Horn et al. (Experientia *26*, 1081, 1970). The oxygen uptake in ngatoms/min in the presence of 0.8 m*M* of either compound amounted to 125 for catechin and 320 for quercetin. It is concluded that catechin and quercetin can act as electron donors as well as inhibitors. The inhibition appears to be strongest between NAD and CoQ, somewhat less on phosphorylation and is the least pronounced between CoQ and cyt c. Quercetin is an about 10 times more powerful inhibitor than catechin.

Sugar and Amino Acid Transport in the Small Intestine and their Interaction

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Glucose transport was studied in isolated microvillus membranes from rat small intestine. Uptake of D-glucose is inhibited by L-alanine, L-methionine and taurin, but

not by D-alanine. On the other hand, L-alanine stimulates the efflux of D-glucose from vesicles preloaded with this sugar. Both effects are dependent on NaCl in the medium. A competition of D-glucose and L-alanine transport for Na^+ is suggested by the finding that the lower the NaCl concentration the higher the relative inhibition of Na^+-stimulated D-glucose uptake. A direct measurement of the ^{22}Na-uptake in the presence or absence of the amino acids reveals no difference. The discrepancy between the apparent Na^+-dependency of the interactions and the unchanged ^{22}Na-uptake may be explained if most of the Na^+ enters the membrane vesicles as electrically neutral NaCl and the coupled glucose-Na^+ as well as amino acid-Na^+ entry are electrogenic. Consequently, replacement of Cl^- by SCN^-, a more lipophilic anion, results in a marked increase of D-glucose and L-alanine uptake. These results would indicate an electrical coupling of the Na^+-dependent uptake of D-glucose and L-alanine.

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Physical and Chemical Characterization of Wheat Germ Agglutinin

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Lectins are a group of plant proteins, some of which have useful properties for the study of cell surfaces and cell division. Wheat germ agglutinin (WGA) is such a lectin which has been isolated and crystallized in our laboratory. Crystalline WGA has been prepared from unprocessed wheat germ by a new purification procedure. Its molecular weight as determined by sedimentation equilibrium is about 34,000 at neutral pH, but at lower pH, the molecular weight is approximately 17,000. Gel electrophoresis in the presence of sodium dodecyl sulfate also shows a molecular weight of around 17,000. The amino acid composition of this protein is unusual; it contains high amounts of glycine and half-cystine. In contrast to semi-pure WGA, the 3 × crystalline protein is devoid of neutral sugars. Equilibrium dialysis experiments using N-acetylglucosamine-^{14}C indicate that the binding of the sugar is highly specific, with a dissociation constant of $1.3 \times 10^{-3} M$; glucosamine and N-acetylgalactosamine show almost no competing effect on the binding of N-acetylglucosamine. Di-N-acetylchitobiose and tri-N-acetylchitotriose, di- and tri-saccharides of N-acetylglucosamine respectively, show higher affinity for the agglutinin binding sites than does N-acetylglucosamine, an observation somehow reminiscent of the affinity site characteristics of lysozyme.

Structure-Activity Relationship for Substrates and Inhibitors of a Purified Human Epoxide Hydrase

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Intermediate oxiranes are now recognized as proximal agents responsible for mutagenic, carcinogenic or cytotoxic effects of several aromatic or olefinic compounds. Epoxide hydrase, which transforms such oxiranes to much less reactive vicinal diols, was solubilized from human liver microsomes and purified fourtyfold. The specificity of this human epoxide hydrase was studied and compared to the specificity of epoxide hydrase purified from rat and guinea pig liver. Monosubstituted oxiranes with a lipophilic substituent larger than a propyl-group (*t*-butyl, *n*-hexyl, phenyl) readily interacted with human epoxide hydrase, and so did in a series of styrene oxides monosubstituted, as well as 1,1- and cis-1,2-disubstituted oxiranes, but not trans-1,2-di-, tri- or tetra-substituted oxiranes. Qualitatively the same results were obtained with rat and guinea epoxide hydrase. It is concluded that human hepatic epoxide hydrase is very similar to the enzyme of rat or guinea pig and that studies on this enzyme in the latter two species are significant with respect to man.

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Study on Degradation of Poly A with a Poly A Polymerase

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We reported a new poly A polymerase, extracted from *E. coli* cell debris, which is a subunit of RNA polymerase. The enzyme preparation polymerizes ATP to poly A, liberating orthophosphate and also monomerizes poly A into ATP in the presence of phosphate ion. The later reaction was observed by aminoacylation of tRNA as well as by bioluminescence of luciferin. This preparation also catalyzes a reversible reaction ADP $\rightleftharpoons$ Poly A + Pi. Further details of the above mechanisms and the biological significance will be discussed.

Detection of Monomers in Several Species of Human Hemoglobin

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Whether or not reversible dissociation of liganded hemoglobin into $(\alpha\beta)$ dimers under physiological conditions is followed by dissociation into single chains has been the object of numerous studies which led to the belief that monomerization only occurs at extreme pH values and very low protein concentration. However, we have detected and isolated Hb A formed in mixtures of Hb S and β_A chains under mild conditions (4 °C: 0.01 *M* phosphate buffer pH 6.0 and 7.0 with and without NaCl 0.5 *M*: Hb S and β_A concentrations, 0.1–0.2 m*M* heme). Increasing amounts of Hb A were detected by starch gel electrophoresis as early as 3 h after mixing Hb S and β_A chains. Low pH (6.0) and high ionic strength (0.5 *M* NaCl) mildly enhanced Hb A formation. To exclude microheterogeneity of Hb S as responsible for Hb A formation, Hb S was allowed to hybridize with β_A chains for 3 days in the conditions mentioned and subsequently separated on CM Sephadex from Hb A and β chains. Pretreated Hb S was then reincubated with β_A chains and the amount of Hb A formed was comparable to the one formed in mixture of non-pretreated Hb S and β_A chains. Hb A also appeared in mixtures of β_A chains with Hb A_2 and Hb ZH ($\beta^{63\,his-arg}$). These data suggest that newly formed Hb A results from the recombination of β_A chains with α chains yielded by the monomerization of hemoglobin S, A_2 or ZH.

Activation of N^{α} -Glycyl-glycyl-glycyl-(8-lysine)-vasopressin ('TGV') in the Cat: Plasma and Urine Levels of Immunological and Biological Activity

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TGV is thought to act as a 'hormogen', releasing lysine vasopressin (LVP) by enzyme action in vivo. After injection of TGV, cats or rats excrete LVP in the urine (Kynčl and Rudinger, J. Endocrinol. *48*, 157, 1970). An antibody against LVP (Edwards et al., J. Endocrinol. *52*, 279, 1972) crossreacting with TGV permitted us to measure the sum of LVP and TGV in plasma and urine samples by radioimmunoassay (RIA) while the rat antidiuretic (AD) activity of samples is essentially due to LVP. Three nembutal-anaesthetised cats in mild mannitol diuresis were given equipotent (in terms of rat AD activity) i.v. doses of TGV (1.3 mg) and LVP (0.02 mg). RIA titres in plasma decreased exponentially with a half-life of 2–4 min after both peptides. Plasma AD activity after LVP decreased similarly but after TGV it rose to a maximum during 30–50 min and then decreased, being still detectable after 2 h. The ratio of RIA to AD titre (LVP equivalents) was about 1:50 shortly after injection and 1:10 at the highest level of plasma AD activity. RIA and AD titres of urine fractions indicated excretion over 2 h of about 0.5% of the TGV as active hormone and 2% as unchanged TGV (and/or RIA-positive, AD-negative metabolites). Urinary recovery of AD and RIA activity after LVP corresponded to 7–15% of the injected dose. These results are in accord with the proposition that TGV acts as a 'hormogen'.

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Structure and Fragments of Bovine IgG Immunoglobulins

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The kinetics of the proteolysis of bovine IgG1 and IgG2 by trypsin and pepsin has been studied (de Rham and Isliker, Experientia *28*, 738, 1972). Important differences have been found both with respect to the number of amino groups released and the relative amounts of intact protein and fragments produced during proteolysis. IgG1 was more resistant to trypsin and more susceptible to pepsin as compared to IgG2.

In order to characterize and explain the nature of these variations, larger quantities of IgG1 and IgG2 antibody directed against ferritin have been digested with pepsin, trypsin and papain and the fragments have been isolated. They have been characterized by their behavior in immunoelectrophoresis, ^{125}I-ferritin fixation, gel filtration, ion-exchange chromatography and electrophoresis in SDS-polyacrylamide gel.

According to their size, the fragments have been assigned to three categories. These correspond essentially to fragments $F(ab')_2$ (or Fab), Fc and Fc'. Except for minor variations in electrophoretic mobility, little or no differences have appeared between IgG1 and IgG2. No essential structural differences are evident when comparing bovine IgG to human and rabbit IgG. However, heterogeneity appears especially in the case of the IgG1 subclass, as demonstrated by the different positions of proteolytic degradation, the length of bands obtained in immunoelectrophoresis, and the kinetics of proteolysis.

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Cell Wall Lipopolysaccharide of *E. coli* K12, and the Specific Receptor Site for Bacteriophage C21

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E. coli K12 is a rough organism having an incomplete cell wall lipopolysaccharide (LPS), containing glucose, galactose, rhamnose, heptose, 2-keto-3-deoxyoctonate (KDO) and glucosamine. L-rhamnose is a major serological determinant of this LPS although, contrary to what is usually found in *Salmonella*, it is not present in O-antigenic side-chains but in the core of the LPS. Bacteriophage C21 attacks *E. coli* and *Salmonella* mutants whose inability to synthesize UDP-glucose and/or UDP-galactose, or to transfer glucosyl or galactosyl units onto the nascent polysaccharide results in formation of an incomplete LPS. In bacteria having a complete LPS the C21 receptors are present, but are masked by the external sugars of the polysaccharide, and these cells are resistant to the phage. LPS isolated from bacteria sensitive to C21 can specifically and irreversibly inactivate this phage. Using LPS from K12 mutants variously affected in LPS structure, correlations could be established between the degree of defectiveness of the polysaccharide and its ability to adsorb C21; this adsorption was much decreased by the presence of small amounts of galactose or rhamnose in the LPS. The C21 receptor is contained in the inner core of the K12 LPS, but KDO is not an essential part of the phage receptor.

Kinetics and Na-Dependence of Riboflavin Absorption by Intestine in vivo

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The intestinal absorption of riboflavin is measured in vivo by perfusion of a proximal segment of the jejunum in a rat. The absorption of water was measured by weighing the amounts of water both entering and leaving the loop. The calculations for the measurement of riboflavin absorption are based on this technique.

The transport of riboflavin across the intestinal mucosa follows saturation kinetics (K_t = 3 μM; V_{max} = 0.26 nM/min/g tissue).

The absorption of riboflavin is strongly inhibited (as much as 40% of the control) when NaCl in the perfusion medium is replaced by isotonic mannitol. The absorption of D-glucose, measured in parallel, is found to be lowered to the same extent. Finally, it was noted, that there was a complete blockage of water absorption under these conditions. When NaCl is replaced by LiCl there is also an important reduction in the absorption of riboflavin and water.

It is concluded, that the absorption of riboflavin by the intestine necessitates the presence of a specific carrier and that the system is Na^+ dependent. The role of Na^+ in the absorption of riboflavin and water will be discussed.

Interaction of Immune Complexes Containing Normal and Tryptophan-Modified Antibodies with Complement

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Previous studies have shown that modification of 5 to 6 tryptophanyl residues per IgG with 2-hydroxy-5-nitrobenzyl bromide (NBB) abolishes the anticomplementary activity of IgG aggregates (Allan and Isliker, Experientia *28*, 733, 1972).

We have studied NBB-modified anti-ovalbumin combined with ovalbumin at equivalence with respect to: a) the precipitin reaction; b) the binding of Clq; c) the hemolytic activity of bound Clq, and d) the binding of whole complement. Clq was labelled with ^{125}I using lactoperoxidase as described by Heusser et al. (J. Immunol., in press). Results indicate that the binding of Clq by immune complexes at a ratio of 20 moles IgG/mole Clq is not significantly affected by NBB-treatment. However, at the lower molar ratio of two IgG per Clq, a modification of 3 tryptophanyl residues per IgG resulted in a reduction of over 50% Clq binding. The same effect was shown using whole complement system. Clq was found to remain hemolytically active when bound to NBB-treated immune complexes, whereas no significant activity is found with non-modified complexes.

Attempts have been made to determine the change of Clq binding by antibody due to NBB-treatment. The association constant of Clq to normal immune complexes was of the order of 5×10^8 l/mole. The valence of IgG for Clq was found to be approximately 0.5, supporting the concept of a requirement of two IgG molecules to fix one molecule of Clq.

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The Structure of an Isoacceptor Serine tRNA from Rat Liver

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The structure of serine tRNA I coding for UCU, UCC and UCA has been reported previously. Another isoaccepting species, serine tRNA III, with coding properties for AGU and AGC has been isolated by partition chromatography, reversed phase chromatography and BD-cellulose. The primary sequence has been determined by partial hydrolysis with various enzymes. The structure shows the following differences in comparison with serine tRNA I.

1. The three bases in the anticodon are different.
2. The base next to the anticodon is methyl-N-(purinyl-6-carbamoyl)threonine instead of isopentenyladenosine.
3. 9 of the 16 base pairs in the stems of the amino acid arm, the TψC arm and the extra arm are different.
4. ψ in the base paired region of the anticodon arm is not O-methylated.

The DHU arm is the only part identical in serine tRNAs I and III. This might suggest its role in the recognition by the corresponding tRNA synthetase.

Isolation of θ-Alloantigen and Mouse T-Lymphocyte Specific Xenoantigen (MTLA) from Mouse Thymocyte Membranes

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By immunization of rabbits with mouse thymocyte membranes, antisera can be obtained which are cytotoxic for 100% of thymocytes and 40% of spleen lymphocytes. The simultaneous use of two in vitro tests whereby both humoral and cellular immune responses can be demonstrated in the same animal with one alloantigen, shows that the xenoantigen detected is specific for mouse thymus-derived T-lymphocytes. Pretreatment of mouse thymocytes with pepsin fragment $F(ab')_2$ of rabbit Ig anti-MTLA, inhibits the cytotoxicity of isologous mouse anti-θ serum. This steric hindrance shows that both xeno and alloantigens are closely located on the cell surface and might be different antigenic determinants on the same molecule.

In an attempt to isolate these antigens, thymocyte membranes prepared according to D. Allan and M. Crumpton (Biochem. J. *120*, 133, 1970) were solubilized with 0.3 *M* lithium diiodo-salicylate and extracted with 50% phenol/water. The waterphase soluble proteins were separated on a Sephadex G-200 column packed in 1% SDS. The four fractions obtained were dialyzed against water, lyophilized and tested for their capacity to inhibit the cytotoxicity for thymocytes of heterologous anti-MTLA or isologous anti-θ. The activity was concentrated in a protein fraction showing one band in electrophoresis on 5.6% acrylamide gels in the presence of SDS, with an apparent molecular weight of 15,000 to 20,000 daltons. The purification and characterization of that fraction is currently attempted.

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Effect of a High-Fat Diet on Glyceride Synthesis by Rat Diaphragm in vitro

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Immediately after weaning, groups of male Wistar rats were fed ad libitum during 3–4 weeks a high-fat carbohydrate-poor diet, or a control carbohydrate-rich diet. The glyceride content of diaphragm of fat-fed rats was 2–3 fold increased compared to control tissues. Hemidiaphragms were incubated with the following ^{14}C-labelled substrates: glucose, pyruvate or acetate. Fatty acid synthesis was reduced by the high-fat diet to 7% of the control value with glucose, 25% with pyruvate and 83% with acetate as a substrate. In contrast glyceride glycerol synthesis from glucose and pyruvate was respectively increased by 44% and 89% and was negligible in the presence of acetate. These results suggest that the high-fat diet decreases fatty acid synthesis at the level of pyruvate dehydrogenase. An important turnover of muscular glycerides is shown in normal tissue, which is further increased by fat diet.

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Mechanism of Action of the Exoplasmodial Acid Phosphomonoesterase of Physarum polycephalum: Water and Alcohols as Acceptors

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The common mold Physarum polycephalum is a primitive organism of remote origin. Among the numerous enzymes secreted by its plasmodes, the acid phosphatase appears already endowed with a highly complex catalytic organization equivalent, e.g. to human phosphatases. It hydrolyzes nucleotides much faster than any other natural P-ester, with a marked preference for 3'CMP. Glycoproteins and non ionic polyOH-detergents fully protect purified Pase solutions against surface-inactivation or freezing damages, and raise thermal denaturation by 30°. A search for simple alcohols as stabilizers proved that Pase remains active in MeOH up to 15 *M*, 9 *M* glycerol or 8 *M* EtOH, while such alcohols do function in part as phosphoryl-acceptors. This opened the way for extended mechanistic investigations. Full activity stays up in aqueous EtOH up to 3 *M*, and 4 *M* with the others, the overall rate (cytidine release) being constant. Downward curvature beyond 4 *M* in glycerol assigns the onset of denaturation. Rate in P_i formation, with CMP in 10% glycerol, is only 4 fold that of transphosphorylation, while collision probability toward the active site is 50:1 for H_2O. Plots for transfer reactions are all sigmoidal and saturation demonstrates the actual binding of the acceptor to Pase, raising the alternative of a separate H_2O site or direct solvolysis. Types of inhibition by products, in absence of alternate acceptors, point out to a sequential mechanism, the OH-moiety coming off first followed then by P_i. Since velocities vary widely for different nucleotides, the rate-limiting step occurs before P_i. In the hypothesis of a phosphoryl-enzyme intermediate, competition by alternate acceptor would initiate sigmoid plots.

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ORD and CD Properties of Heme Depleted Hemoglobins

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Interchain-contact forces are responsible for the functional behaviour of Hb. Isolated α or β chains do not show cooperativity or Bohr effect. ApoHb has a lower content of ordered structure than Hb. IC_{II}, a Hb carrying heme only on its α chains has an intermediary structure, but its rudimentary $\alpha\beta$ contacts endow the molecule with $\sim 1/2$ of the normal Bohr effect and give the α chains an increased stability against denaturants. The O_2-affinity is lower than that of free α chains but cooperativity is close to O. The optical rotation at 233 nm of Hb depends on the conformation of each subunit; unliganded Hb has 8% more than liganded. In IC_{II} this difference was 4% as was also obtained for $\alpha_2\beta_2^h$. A Hb with only one heme per tetramer gave $\sim$ 2.5% difference in ORD when liganded, as did isolated chains. On this basis, we conclude that the gain in ordered structure upon binding of heme leads to the formation of interchain-contacts sufficient for the conformational changes needed for Bohr effect and lowering O_2-affinity, but not the appearance of cooperativity. The nature of these interchain-contacts are presently under investigation.

Purification of *Drosophila* Xanthine Dehydrogenase

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The appearance of XDH in *Drosophila* is under the control of at least three genes, *rosy*$^+$ (*ry*$^+$), *maroon-like*$^+$ (*ma-l*$^+$), and *low xanthine dehydrogenase*$^+$ (*lxd*$^+$). Evidence indicates that *ry*$^+$ is a structural gene for XDH but the *ma-l*$^+$ and *lxd*$^+$ genes are both also required for the appearance of active enzyme. One approach we have taken towards understanding the function of these genes has been to attempt purification of active XDH and of the *ry*$^+$ gene product and then to subject these purified substances to structural analysis. Differences observed between the XDH and the *ry*$^+$ gene product should provide information on the function of the *ma-l*$^+$ and *lxd*$^+$ genes. XDH has been purified to homogeneity on polyacrylamide disc electrophoresis by ammonium sulfate fractionation, heat precipitation, and chromatography on DEAE cellulose, hydroxylapatite, and Sephadex G-200. Characterization of the purified product is in progress.

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Separation of Human Urinary Erythropoietin from Glycoproteins of Similar Size Using Concanavalin A-Sepharose

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Separation methods based on charge and size have failed to achieve purification of urinary erythropoietin (epo) to homogeneity, and new methods of affinity chromatography will be required for further progress.

We report here, firstly, a simple assay in which mouse bone marrow cells cultured in methyl cellulose give rise within 48 h to epo-dependent erythroid colonies. The assay simultaneously detects granulocyte colony stimulating activity (CSA), a contaminating urinary glycoprotein.

Secondly, we have successfully applied Concanavalin-A-Sepharose to the further purification of a preparation of human urinary epo already selected for glycoprotein content by benzoic acid adsorption, and for uniformity of size by two passages through Sephadex G-100. Material non-adherent to the Con-A-Sepharose column contained all the initial erythropoietin activity. In contrast, almost all the initial CSA activity adhered to the column and could be eluted with methyl-α-glucopyranoside.

We conclude that CSA possesses exposed terminal α-linked glucose or mannose like sugar, while epo does not, and that affinity chromatography using lectins can allow selective elimination from epo preparations of contaminating glycoproteins of similar size.

One-Step Isolation of Clean Ribosomal Subunits from Chicken Liver

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It has recently been reported that rough microsomes can be non-destructively dissembled into ribosomes and stripped membranes by incubation with puromycin in a medium of high ionic strength (M. R. Adelman, D. D. Sabatini and G. Blobel, J. Cell Biol. *56*, 206–229, 1973).

We have used these conditions as a basis for the preparative isolation of ribosomal subunits from chicken

liver in a simple procedure which avoids detergents, multiple high salt washes and extensive centrifugation.

Subunits prepared in this way are active in the poly (U) directed synthesis of polyphenylalanine and have undetectable levels of the elongation factors, EF and EF_2.

Stability of Colchicine Binding Protein from Neuroblastoma Cells

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The half-life of colchicine binding protein from neuroblastoma cells can be increased 50-fold to about 200 h by 0.8*M* sucrose or 4*M* glycerol. In crude preparations, addition of exogenous GTP has no further effect. Both stabilizing reagents prevent precipitation by vinblastine or calcium, but do not interfere with colchicine binding. Sulfhydryl reagents do not affect the half-life, although the binding activity is reversibly abolished by Ellman's reagent. This stabilization, the greatest so far reported by a non-specific reagent, will enable purification and characterization of the relatively small amounts of colchicine binding protein available from tissue culture.

TSH-Releasing Hormone (TRH); Specific Uptake in Various Tissues, Including the Thyroid, in vivo

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In a previous study (Experientia *28*, 737, 1972), a peripheral effect of L-pyroglutamyl-L-histidyl-L-proline-amide (TRH) on the thyroid could be shown in hypophysectomized rats.

Recently we observed, that tritiated TRH is not only accumulated in the pituitary but also in the thyroid. TRH-^{3}H (specific activity 34.8 Ci/m*M*) competed in fact with TRH at the binding sites in the pituitary, the thyroid and possibly the hypothalamus. This binding seems to depend on the integrity of the tripeptide. However, some other tripeptides are able to compete with TRH-^{3}H also, namely e.g. L-prolyl-L-histidyl-L-proline-amide (PHP). It was shown that this substance induces a rapid accumulation of TSH in the pituitary but no release (Schweiz. med. Wschr. *102*, 1270, 1972). The present work shows that it has a high affinity for the TRH binding sites in the pituitary, but *not* in the thyroid, in contrast to TRH, Diiodohistidyl-TRH or L-pyroglutamyl-L-histidyl-L-tryptophane-OH. The TRH composing aminoacids and iodinated tyrosine did not displace TRH-^{3}H.

The question remains, if specific binding of a substance is paralleled by a specific cellular response. The results of in vitro experiments will be given.

Energy Dissipation by Ca Recycling in Rat Liver Mitochondria

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Previous studies with intact rat liver mitochondria incubated in the presence of pyruvate, bicarbonate, ATP and phosphate revealed the existence of energy dissipating processes. From experiments with atractylate, an inhibitor of the adeninenucleotide translocase, it was concluded that about 30% of this energy dissipation was due to extramitochondrial ATPase activity as well as to the maintenance of an asymmetric distribution of ATP and ADP across the inner membrane. Furthermore, the total energy dissipation was reduced by about 20% when the energy dependent uptake of Ca into the matrix was inhibited with ruthenium red. In addition, measurements with suitable electrodes showed a net release of Ca into the medium and a diminished respiratory rate. The endogenous intramitochondrial Ca content decreased from 20 to 4 n ions/mg protein. It is proposed that the leakage of Ca out of the matrix which is compensated by an active reuptake of this ion is a cyclic process that dissipates energy. Since the effects of atractylate and ruthenium red on energy dissipation were almost additive, this recycling of Ca across the inner membrane may be another of the processes responsible for state 4 respiration of the mitochondria.

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Modification of the *E. coli* Envelope upon T4 Infection

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Cell envelopes of *E. coli* are damaged by infection with the virulent bacteriophage T4. They are temporarily repaired by phage coded functions and later degraded by phage lysozyme and additional viral gene products. Of the proteins which are synthesized with delayed-early kinetics, we have found two that are tightly associated with the envelope. Using deletion mutants, these proteins could be identified as the products of the rIIA and rIIB cistrons. The lengths of their respective polypeptide chains correspond to those predicted from the genetic map. Later in infection, a protein with a subunit mass of 95,000 daltons appears in the soluble cell fraction. Upon infection of a non-permissive host with an amber mutant in the viral 't' gene, known to play a role in cell lysis, this polypeptide is not made. In the permissive host, its peak synthesis is between 40 and 50 minutes. Simultaneously with the appearance of this protein, one of the major host envelope proteins is transferred to the soluble cell fraction. Such a transfer is also seen upon lysozyme-EDTA treatment of uninfected cells. Possible correlations of these phenomena are currently under investigation.

Association of Polyoma DNA with the Cellular DNA in the Lytic Infection and in Transformed Cells

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The investigations on a possible integration of polyoma DNA into the chromosomal DNA of permissive cells were continued using the same method described earlier (Experientia *28*, 752, 1972). Because some control experiments gave positive results too, the experiments concentrate now on the detection of polyoma DNA bound covalently to the cellular DNA.

With the same hybridization assay, which permits to detect 0.5 polyoma genome equivalents per cell, we also tested some polyoma transformed cell lines and cells isolated from a polyoma induced hamster tumor. The

results indicated the persistence of 1 to 3 genome equivalents polyoma DNA in these cells. However also with two established 'normal' mouse cell lines (A9 and A9 HT) obtained from G. Klein (Stockholm) hybridization results significantly above those of mouse DNA were found. Further analyses with DNA from several transformed and untransformed cell lines are being done to reveal possible variations in the hybridization to polyoma complementary RNA.

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Creatine Kinase and Aldolase Isoenzyme Patterns in Cultures of Myogenic Cells

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During chick skeletal muscle development in situ there are characteristic transitions in the isoenzymes of creatine kinase and aldolase: the MM form of creatine kinase replaces the BB form and A_4 aldolase replaces forms containing predominantly C subunits. The same transitions are seen in primary cultures of chick embryo skeletal muscle. Creatine kinase and aldolase specific activities increase during in vitro muscle differentiation for many days after the percent of nuclei in fused cells has reached a maximum (of 75 to 90% at 60 h in culture). The activity and pattern changes of both enzymes are prevented in low calcium medium or in media containing 5-bromodeoxyuridine ($0.2–7 \times 10^{-5} M$), conditions in which cell viability is maintained but myoblast fusion is prevented. Cells grown in 0.1 mM dibutyryl cyclic AMP showed the same enzyme changes and fusion kinetics as control cultures. These results indicate that the changes in activity and isoenzymes pattern of both enzymes are dependent on prior myoblast fusion. The possibility that levels of creatine kinase and aldolase isoenzymes are coordinately controlled in chick muscle development is discussed.

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Microheterogeneity of Alkaline Phosphatase Due to Glycosylation

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Alkaline phosphatase has been purified from microvillar membranes of pig kidney. It exists in discrete multiple forms with different degrees of glycosylation at polypeptide chains which appear to be identical. These multiple forms have been characterized. They form a regular series with different degrees of stability, charge, molecular size and pH-optima for extractibility from the membrane by butane-1-ol.

They showed no differences in catalytic properties and antigenic sites. The multiple forms, therefore, cannot be considered to be true isoenzymes.

Localization of Creatine Kinase at the M-Line of Skeletal Muscle Myofibrils

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Immunofluorescence studies show that a part of the creatine kinase (CPK) of skeletal muscle is bound to the contractile apparatus. Antiserum prepared against chicken MM-CPK binds specifically to the M-line region of myofibrils isolated from chicken skeletal muscle. The bound CPK can be dissociated at low ionic strength and purified to homogeneity by a procedure employed previously in purifying an M-line protein of unknown function. Protein samples purified according to a standard MM-CPK purification scheme and according to the M-line protein procedure had similar CPK specific activities, behaved identically in two electrophoretic systems and reacted identically in a serological test with anti-MM-CPK antiserum. These results, taken together with published information on the properties of the two proteins, provide conclusive evidence for the identity of M-line protein and MM-CPK.

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Possible Circadian and Circannual Variations in Monoamine Uptake in Rat Brain

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Neurotransmitter uptake in vitro ($10^{-7} M$, 10′ at 37 °C) was investigated in brain slices from paired littermates kept under controlled conditions of lighting (6^h–18^h) and minimal handling or stress, killed at 8^h or 20^h. The difference in uptake (T/M ratio = cpm:g/cpm:ml) was analysed by the Wilcoxon test for matched pairs. In ovariectomised rats ($n = 14$) a significantly higher ($p < 0.05$) dopamine (DA) uptake at 8^h than at 20^h was found in cortex (median T/M $8^h = 16$, $20^h = 10.3$), striatum (62.5, 52.5) and midbrain (14.5, 13.0), whereas serotonin (5HT) uptake showed no significant variation. In male rats ($n = 14$) a significantly higher uptake at 8^h than at 20^h was found for: DA in striatum (65.2, 52.2) and hypothalamus (17.6, 15.8); 5HT in hippocampus (16.8, 13.8); noradrenaline in hypothalamus (19.6, 17.3). No variation was found in cortex, thalamus, midbrain, pons and medulla, or in GABA uptake in any region. In addition, a seasonal rhythmicity in T/M values was noted in the ovariectomised rats, DA uptake sharply increasing in March–April, with a minimum in October–November, whereas 5HT uptake showed the reverse pattern. Without further longitudinal studies, alternative causative factors for this latter observation cannot be eliminated.

Kinetic Studies of the Interaction between *E. coli* RNA Polymerase and Rifampicin

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A fast and sensitive assay using dextran coated charcoal has been used to study the kinetics of the interaction between *E. coli* RNA polymerase and rifampicin. The rate of dissociation (k_d) of the enzyme-antibiotic complex follows first-order kinetics with a half-life of 8 h at 0° and 8 minutes at 37°. The rate of association of enzyme and rifampicin follows second-order kinetics with the rate constant for association (k_a) being $6 \times 10^4 M^{-1}\text{sec}^{-1}$ at 0° and $1.3 \times 10^6 M^{-1}\text{sec}^{-1}$ at 37°. From the ratio k_d/k_a the equilibrium constant (K_{eq}) has been calculated to be 4×10^{-10} at 0° and 10^{-9} at 37°. The binding forces involved in the association seem to be mainly lipophilic, since in

the presence of dimethylsulfoxid (24%) the K_{eq} increases 20-fold, whereas it is not affected by sodium chloride concentrations as high as 3*M*. By measuring the UV-spectrum of the complexed rifampicin the involvement of the naphthoquinone ring system in the binding to the enzyme could be directly demonstrated.

Subunit Synchronization in Aspartate Aminotransferase

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Cytoplasmic aspartate aminotransferase is a dimer of identical subunits and catalyzes the transamination between aspartate and 2-ketoglutarate obeying Michaelis-Menten kinetics. During the reaction the coenzyme molecule on either subunit shuttles between pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP). Following removal of the substrates three forms of the enzyme dimer may theoretically exist with respect to the coenzyme: PLP/PLP, PMP/PMP, and the hybrid PLP/PMP. In a reaction mixture adjusted with glutamate and 2-ketoglutarate to contain equal proportions of enzyme-bound PLP and PMP independent action of the subunits would result in an amount of hybrid equal to the sum of the PLP/PLP and PMP/PMP dimers. However, by means of isoelectric focusing a distribution of (PLP/PLP):(PLP/PMP):(PMP/PMP) = $1:<0.1:1$ was found. In contrast, if half of the internal aldimine bonds of the PLP/PLP dimer was reduced (RED) with $NaBH_4$ a binominal distribution was obtained, (PLP/PLP):(PLP/RED):(RED/RED) = 1:2:1. The results demonstrate a catalysis-dependent coupling of the two subunits which synchronizes their active sites during transamination.

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PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

'Compensatory Adaptation' of the Natriuretic Effects of Furosemide and Acetazolamide after Unilateral Nephrectomy in Rats

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Fractional excretions of water and electrolyte from the remaining kidney increase within 1 to 2 h after unilateral nephrectomy. We investigated the natriuretic effect of furosemide (F) and acetazolamide (A) 2 h after uninephrectomy ($^1/_2$-Nex) compared to that in sham-operated controls. Unanesthetized rats, infused with saline at 50 µl/min, were given i.p. supramaximal doses of F (45 mg/kg) or/and A (20 mg/kg). After F or A fractional excretion of Na^+ ($FE\text{-}Na^+$) increased in both groups but was always twice as high in $^1/_2$-Nex than in controls. The effect of the diuretics on Na reabsorption was thus doubled in the remaining kidney. Simultaneous administration of F and A induced an additive effect on $FE\text{-}Na^+$ which, again, was twice as high in $^1/_2$-Nex than in controls. A similar 'compensatory adaptation' of the effect of a supramaximal dosis of A was found when the drug was given after massive volume expansion. It was concluded that the two drugs act downstream from the part of the nephron in which inhibition of reabsorption occurs after unilateral nephrectomy.

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Release of Free Acetylcholine by Stimulation of the Nerve-Electroplaque Junction

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In the electric organ of *Torpedo*, acetylcholine (ACh) is stored in two compartments. Most of bound ACh is present in synaptic vesicles, it represents about half of the total ACh found when the tissue is extracted with trichloracetic acid. Free ACh is the fraction hydrolyzed by the esterases when the nerves terminals are disrupted. Transmission through the nerve-electroplaque junctions fails when the nerves are given about 1,500 succesive stimuli. During the exhaustion of the descharge an important amount of ACh is released from the free compartment; there is also a large but transient increase in the synthesis of new ACh which reaches the free compartment and can be released at once. The amount of bound ACh and the number of synaptic vesicles are only modified when stimulation is continued over 10,000 stimuli. It is concluded that the transmitter located in the compartment of free ACh is immediately available for release on stimulation.

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Behavioral Effects of One-Hour Light-Dark Rhythms in the Rat

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Motor activity, feeding, drinking, body temperature as well as various other parameters in the rat exhibit a circadian rhythmicity which is largely due to the changes in light intensity. We investigated to what extent one-hour rhythms of light and dark are able to influence motor and consumatory behavior. Light-dark schedules of 60/60 min, 80/40 min, and 40/80 min were maintained each for one week, and compared to the 12/12 h control schedule. Under all experimental conditions feeding, drinking and motor activity were more intensive during dark than during light. The behavior of the rats was rapidly entrained by the one-hour rhythms, although the original circadian rhythm remained initially superimposed. Experimental data will illustrate some applications of these schedules to neuropharmacological problems.

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A Slide-Rule: A Simple Approach to the Correct Dosage of Drugs

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A pharmacokinetic slide-rule based on the generally accepted models of pharmacokinetics is presented. It enables the physician to calculate a more exact individual dosage regimen for his patients as follows:

1. Using the renal clearance of a certain patient, the proportionality constant characterizing the renal excretion of a certain drug (a) and the non-renal rate constant of elimination of this drug (k_{nr}), the rate constant of the total elimination (k_e) can be calculated.

2. With k_e, the apparent volume of distribution (V_d) and the desired final mean concentration of a drug ($\bar{c}\ \infty$), exact data for the loading dose (D^*), the dosage schedule consisting of the maintenance dose (D) and the dosing interval (τ) and also the infusion rate for intravenous administration can be calculated.

3. In addition, the slide-rule provides information about the rate at which $\bar{c}\ \infty$ is reached by only administering D at τ, and the fluctuation of the concentration around $\bar{c}\ \infty$ to be expected during τ. Using this calculation, the slide-rule facilitates the decision whether a loading dose should be given, and which dosage schedule is optimal for the therapeutic problem.

Thus, an individually exact dosage regimen, even for patients with excretory disfunctions, can be calculated. In addition, the pharmacokinetic slide-rule might help to convey insight into pharmacokinetic phenomena to the physician.

Inhibition of Drinking Induced by Intrahypothalamic Renin by an Inhibitor of 'Converting Enzyme'

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Intrahypothalamic injection of renin in rats induces drinking responses comparable to those elicited by intrahypothalamic angiotensin II (AT II)[1]. If this effect of renin is due to formation of AT II, it should be depressed by inhibition of the enzyme converting AT I into AT II. SQ 20,881 (Squibb, New Brunswick) a nonapeptide-inhibitor of converting enzyme, in doses from 10 to 10,000 ng injected intrahypothalamically 2 min before renin, caused a dose dependent inhibition of drinking after 6 mU of partially purified hog renin (National Biochemical Corporation, Philadelphia Pa) injected at the same site. Drinking after 1,000 ng of val_5-AT II amide (Hypertensin® Ciba) was not depressed by the same dose of SQ 20,881. The inhibition of the drinking response to intrahypothalamic renin does not appear to be due to the known converting enzyme inhibiting effect of SQ 20,881[2]. Drinking after intrahypothalamic $ileu_5$-AT I, 162 or 1,300 ng, was not depressed by preinjection of 10,000 ng of SQ 20,881. SQ 20,881 in the rat hypothalamus thus appears to inhibit the generation of AT I under the influence of renin. When injected into the hypothalamus, renin generates AT I from cerebral substrate different from plasma angiotensinogen.

[1] J. T. Fitzsimons, Physiol. Rev. *2*, 52 (1972).
[2] S. L. Engel et al., Proc. Soc. Exp. Biol. Med. *140*, 240 (1972).
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Possible Influence of Endogenous Cyclic AMP on Serotonin and Dopamine Turnover in Rat Brain in vivo

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Administration of dibutyryl-cAMP into the brain ventricles of the rat enhances the turnover of cerebral serotonin (5HT) (Tagliamonte et al., Fed. Proc. *29*, 236, 1970). However, no evidence exists that changes in synthesis or catabolism of endogenous cAMP have an influence on the activity of aminergic neurons. In the present experiments, adenylcyclase stimulating compounds (epinephrine, NaF) were injected by intraventricular route into the rat brain or inhibitors of phosphodiesterase (theophylline, SQ 20,009) were administered intraperitoneally. After various intervals 5HT and dopamine (DA) and their main metabolites, 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA), respectively, were measured fluorometrically in the brain. All the above mentioned compounds, but not butyric acid or 5-AMP, increased the cerebral levels of 5HIAA and HVA without affecting those of 5HT and DA thus indicating an enhanced turnover of these amines. In addition, the concentration of cerebral endogenous cAMP was found to be increased by intraventricular injection of epinephrine (Burkard, J. Neurochem. *19*, 2615, 1972) and NaF.

These results suggest that endogenous cAMP influences the 5HT and DA turnover and might therefore be involved in the regulation of the activity of the corresponding neurons.

Sinus Nerve Stimulation and Blood Pressure in Hypertensive Animals

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It is not clear whether baroreceptor resetting in hypertensive individuals is due to an increased stiffness of the vascular wall in the surroundings of the baroreceptors or to changes in the central or efferent structures of the baroreceptor reflex. In the present experiments, the influence of vascular wall properties on baroreceptors was circumvented by electrical stimulation of the sinus nerve (SN). Stimulation frequency was stepwise doubled from 1 to 32 shocks/sec resulting in a gradual decline of systolic blood pressure (BP). The depressor effect of SN stimulation in 12-week-old genetic hypertensive rats (GHR) was smaller at high stimulation rates than in age-matched normotensive rats (NR). In 17-week-old GHR stimulation with 1 and 2 shocks/sec caused an increase in BP and the depressor responses to 4 and 8 shocks/sec were lower ($p < 0.01$) than in NR. A separation of chemoreceptor and baroreceptor fibres was attempted in dogs. The reduction of BP and perfusion pressure of the perfused hind limbs due to baroreceptor fibre stimulation was smaller in renal hypertensive than in normotensive dogs. Thus, alterations in the central or efferent structures of the baroreceptor reflex contribute to the process of 'baroreceptor resetting'.

Cellular Calcium Stores and Contraction in Vascular Smooth Muscle

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In contrast to the contraction by KCl, the noradrenaline-induced contraction in strips of rabbit main pulmo-

nary artery (RMPA) depends only partially on extracellular calcium (Ca_0) and is independent of the membrane potential (MP) of the vascular smooth muscle (VSM) cells (Haeusler, 1972). The present experiments on RMPA with simultaneous recording of contraction and of MP through intracellular microelectrodes revealed a similar independence on Ca_0 and on MP for contractile responses to 5-hydroxytryptamine (5-HT). The ionophore X-537A (Ro 2-2985) which possesses the property of transporting divalent cations across membranes caused concentration-dependent contractions in strips of RMPA and rat diaphragm. In RMPA but not in rat diaphragm these contractions were reduced in calcium-free solution and abolished after removal of Ca_0 with lanthanum. This indicates differences between VSM and striated muscle with regard to the accessibility of the cellular calcium stores to the ionophore. The results support the hypothesis (Haeusler, 1972) that in VSM contraction to noradrenaline and 5-HT is caused predominantly by a release of calcium from the cell membrane.

The Action of Diazoacetylcholine on the Active Site of Acetylcholine Acetyl-Hydrolase E.C. 3.1.1.7

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Cholinergic receptors and the active site of acetylcholinesterase (acetylcholine acetyl-hydrolase E.C. 3.1.1.7) represent small sections of macromolecules with chemically similar behavior. In collaboration with J. Frank and R. Schwyzer (Experientia *26*, 1207, 1970) we synthesized diazoacetylcholine and used this substance for affinity labelling of the cholinergic receptor of motor endplates (P. G. Waser, A. Hofmann and W. Hopff, Experientia *26*, 1342, 1970). One disadvantage using diazoacetylcholine is the fast reaction of the liberated carbene, which might react with other than the specific groups of the forementioned proteins. We observed that the affinity of diazoacetylcholine to the active site of acetylcholinesterase is much lower than the affinity to the cholinergic receptor. Conclusion: In the original form diazoacetylcholine is sterically hindered from adequately fitting the esteratic center, and thus, cannot combine with the active site of the enzyme. Only when the carbene is already formed the molecule can fit in the esteratic center and react there. Nonspecific binding of the carbene easily explains why we need higher concentrations of diazoacetylcholine for trapping the cholinergic receptor than would be expected theoretically.

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The Effect of Scillirosidine on the (Na^+K^+)-ATPase of the Cat Brain in vitro and in vivo

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Grey matter of cat's brain was prepared. In homogenates of this material we determined the activity of the Na^+K^+ATPase. This enzyme was inhibited by ouabaine and scillirosidine.

We determined a 50% inhibition with $5 \times 10^{-7} M$ ouabaine and $7 \times 10^{-8} M$ scillirosidine indicating a 7 times higher reaction due to the last named. According to the Dixon-plot, we obtained a Km value of $1.77 \cdot 10^{-6} M$ for ouabaine but no exact result for scillirosidine. The inhibition due to ouabaine was not competitive. With scillirosidine the curve obtained was not a straight line. Therefore, no inhibition pattern could be deduced, since the inhibition due to scillirosidine was more effective than ouabaine.

After injection of the drugs by isolated canulation of a branch of the arteria cerebri media, we obtained an enzyme inhibition with both drugs. 100 μg/kg ouabaine applied to cats ($\sim$ 0.17 μM/kg) did not decrease the ATPase activity in the cat brain significantly. On the other hand, using 75 μg/kg ($\sim$ 0.12 μM/kg) scillirosidine, the enzyme was inhibited significantly by 50%.

It is possible that different enzymes exist, which are similarly inhibited by ouabaine but no by scillirosidine.

Central Dopaminergic Actions of Ergotoxine Alkaloids and some Derivatives

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Recent evidence has indicated that Ergocornine and 2-Bromo-α-ergokryptine stimulate dopamine receptors in the CNS (H. Corrodi, K. Fuxe, T. Hökfelt, P. Lidbrink and U. Ungerstedt, J. Pharm. Pharmacol. 1973, in press). We have extended these observations to include other Ergotoxine alkaloids, using the stimulation of locomotor activity in mice, and production of stereotyped behaviour in rats as models of increased functional dopamine.

At 10 mg/kg s.c., in both models, α-Ergokryptine is the most and Ergocristine the least potent alkaloid. Ergocornine and β-Ergokryptine show similar activity being intermediate in potency to the other two alkaloids. 2-Bromo-α-ergokryptine is more active than the α-Ergokryptine in mice, and as active in rats. In contrast, 2-Bromo-ergocornine is less potent in both species than the respective parent alkaloid.

Our results suggest that the Ergotoxine alkaloids reported here have properties typical of dopaminergic stimulants. Quantitative and qualitative differences indicate strict structural requirements within this chemical class.

Enhancement of 3-Methoxy-4-hydroxy-phenylethyleneglycol in Rat Brain by Neuroleptic Drugs

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Various neuroleptic drugs considerably increased the concentration of 3-methoxy-4-hydroxy-phenylethyleneglycol (MOPEG) – the major metabolite of cerebral noradrenaline (NA) – in rat brain. This increase is probably the consequence of a feed-back activation of noradrenergic neurons, and thus NA turnover, due to blockade of NA receptors by the neuroleptics. The potency of various neuroleptics in increasing the concentration of cerebral MOPEG declined in the following order: methiothepin, haloperidol, clozapine, thioridazine, chlorpromazine, pimozide. The neuroleptics (except pimozide and chlorpromazine) also caused a significant reduction of the levels of endogenous cerebral NA, possibly as a result of an insufficient rate of hydroxylation of dopamine.

It is concluded that a) the determination of cerebral MOPEG represents a suitable and relatively simple method for detecting and comparing changes of NA turnover in the brain such as those induced by neuroleptic drugs; b) the neuroleptics studied markedly differ in their ability to activate noradrenergic neurons.

Zur Frage der Sensibilisierung strahleninduzierter Schäden durch Reverin bei Zellkulturen

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Reverin (Pyrrolidinomethyltetracyclin), ein äusserst wirksamer Sensibilisator von mit geringen Strahlenmengen induzierten embryonalen Störungen bei Säugern (H. Fritz-Niggli und Ch. Michel, im Druck), wurde auf zellulärer Ebene auf einen möglichen Sensibilisierungseffekt für strahleninduzierte Chromosomenaberrationen und Zelltod getestet. Als Material dienten Dauerkulturen embryonaler Fibroblasten des Chinesischen Hamsters. In beiden Experimenten wurden Deckglaskulturen während 1–4 Std. mit 275 µg/ml Reverin behandelt. Bestrahlt wurde während der Reverinbehandlung mit 25 r und 50 r (38,5 r/min) für die Chromosomenaberrationen, mit 9000 r (3000 r/min) für Zelltod. Die Chromosomenaberrationen wurden anhand von 200 Metaphasen pro Experiment quantitativ und qualitativ erfasst, die mutmasslich toten Zellen mit dem Trypanblau-Farbtest bestimmt. Während 25 r bereits 8,5%, 50 r 32%, Reverin allein 4% Chromosomenaberrationen in mittlerer S-Phase erzeugten, förderte eine kombinierte Behandlung den Effekt nicht (25 r + Reverin: 12%, 50 r + Reverin: 41,5%). Auch die chromosomale Reunionsrate ergab keinen eindeutigen Unterschied. Im Gegensatz zum Kriterium Aberrationen wird der «Zelltod» nach Bestrahlung mit hohen Dosen in Kombination mit Reverin gefördert: zunehmende Sensibilisierung der Zell-Letalität bis 2 Tage nach Bestrahlung (Sensibilisierungsfaktor 1,8 nach einem Tag; 3,6 nach 2 Tagen), während nach 3 bzw. 4 Tagen keine deutlichen Unterschiede hervortreten (1,3 nach 3 Tagen; 1,2 nach 4 Tagen). Möglicherweise sensibilisiert Reverin durch Repairhemmung.

The Effect of Clonidine (Catapres®) and other Hypotensive Agents on Plasma Renin Activity (PRA) in Rats

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PRA of rats anaesthetized with pentobarbital (40 mg/kg i.p.) was measured by incubating 0.2 ml plasma with an excess of purified rat renin substrate and pressor assay in nephrectomized rats, before and 15 min after i.v. injection of different drugs. Results are expressed as ng A II-equivalents/ml plasma generated within 4 h.

Results: *Clonidine* (C): 10–300 µg/kg induced a non dose-dependent fall of PRA to less than 50% of the initial value. BP after 10 or 30 µg/kg was purely hypotensive, whereas after 100 and 300 µg/kg an initial short rise of BP was followed by an initially smaller hypotensive response. *Pentolinium tartrate* (3 mg/kg) lowered BP to the same extent as 30 µg/kg C and produced a similar fall of PRA. *Nitroglycerine* or *sodium nitroprussiate* (NaNP) dosed to obtain a similar lowering of BP produced a significant large rise of PRA. Propranolol 1 mg/kg did not affect BP and did not prevent the rise of PRA under NaNP, but depressed PRA in normal controls. It was concluded that agents which depress betasympathetic activity depress the resting renin secretion of rats, even if they lower BP, but that the increase of PRA in response to a fall of BP induced by NaNP may not be mediated by the sympathetic nervous system.

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Transplacental Passage of ^{3}H-Ergotamine in the Rat, and Determination of the Intra-Amniotic Embryotoxicity of Ergotamine

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Tissue radioactivity was measured 60 minutes after i.v injection of ^{3}H-ergotamine (2.5 mg/kg, c. 300 µC/kg) to pregnant rats on day 14 p.c. Based on these determinations, an alkaloid concentration of 0.3 µg/g was calculated in the blood, with a three times greater concentration in the uterus, placenta and yolk-sac (1.0 to 1.2 µg/g). Very low activity was detected in the amniotic fluid and the fetal tissues (0.05 and 0.07 µg/g fresh weight). In previous investigations (Grauwiler and Leist, 1973) it has been shown that ergotamine (2.5 mg/kg i.v.) reduces the transplacental passage of ^{3}H-l-leucine for at least 3 hours, and that this effect can be antagonized by simultaneous administration of phenoxybenzamine. At the same time the embryolethal effect of ergotamine after 3 hours is distinctly reduced (from 63.2% to 39.6%) by phenoxybenzamine.

In a second series of experiments it was found that the intraamniotic dose of ergotamine necessary to produce a similar embryolethal effect (LD_{50} = 3.25 µg/g fresh weight of the fetus) is 50 times greater than the amount reaching the fetus following maternal intravenous administration.

The low transplacental passage of ergotamine demonstrated by these studies can be explained partly by its vasoconstrictive action (i.e. alpha-receptor stimulation of the vessels of the uterus and placenta which is antagonized by phenoxybenzamine), but the known uterotonic effect of ergotamine (also mediated by alpha receptors) could produce an additional reduction in placental flow.

Studies on Midbrain Dopamine (DA) Neurons in Morphine-tolerant Mice

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In previous microfluorimetric studies, morphine induced a rapid increase in the intensity of midbrain DA neurons similar to that seen after electrical stimulation or acute cold exposure. During tolerance development, the intensity of DA neurons in the substantia nigra of male mice remained unchanged, but the intensity response to a single dose of morphine disappeared. On the other hand, the fast reaction to acute cold exposure was still elicited, thus indicating that the neurons remained responsive to certain functional changes. Therefore, we looked for possible changes in the input to the DA neurons, especially with regard to direct or indirect interactions with cholinergic systems. Physostigmine induced a typical biphasic intensity response which resembled the one

observed in tuberal DA neurons after transsynaptic stimulation. In early tolerance this response was not completely suppressed but showed a clearly protracted course. This could indicate that an input to the DA neurons resulting from cholinergic activity might change during the development of tolerance. Experiments with cholinergic blocking agents did not yet yield clearcut additional information.

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Effects of Autonomic Drugs on Cyclic AMP Level in Rabbit Parotid Slices

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Cyclic AMP levels in rabbit parotid slices were measured by using a prelabeling technique (cyclic AMP turnover) and a saturation assay method (endogenous cyclic AMP). Control values were 53 $\pm$ 2.1 cpm/mg protein and 11.7 $\pm$ 0.9 picomoles/mg protein respectively, in presence of $5 \times 10^{-3} M$ theophylline. The effects of autonomic drugs on cyclic AMP concentration were investigated under conditions of a-amylase secretion. Noradrenaline, adrenaline and isoproterenol ($10^{-5} M$) caused a 7-fold increase of radioactive cyclic AMP and a 10- to 15-fold increase in the absolute amount of cyclic AMP, suggesting that in our system both methods are not exactly comparable. These increases were blocked by the β-antagonist propranolol at $2 \times 10^{-4} M$, the α-antagonist phentolamine being inefficient. In contrast, carbachol, betanechol, pilocarpine and physostigmine up to $10^{-4} M$ did not cause the cyclic AMP concentration to rise. These results indicate that the secretory process mediated by cholinergic agents in rabbit parotid gland in vitro is independent of cyclic AMP.

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Induction of Choline Acetyltransferase in the Preganglionic Sympathetic Neuron

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Treatment of rats with reserpine (7.5 mg/kg), which leads to a reflex increase in the activity of the peripheral sympathetic nervous system, produced a marked increase in the in vitro activity of choline acetyltransferase in the superior cervical ganglion, where the total activity of this enzyme is located in the preganglionic cholinergic nerve terminals. 24 h after reserpine treatment the enzyme activity amounted to 152 $\pm$ 8% of controls and after 48 h to 160 $\pm$ 21%. This increase appears to be specific since total protein concentrations did not change significantly ($0.4 > p > 0.3$). If enzyme preparations of controls and reserpine treated animals were combined, the activity was additive. Moreover, the increase of choline acetyltransferase activity could be blocked by cycloheximide (0.9 mg/kg every 6 h), indicating that this increase was due to an enhanced synthesis of enzyme protein. Thus, the induction of enzymes as an adaptation to increased transmitter utilization is not confined to the terminal adrenergic neuron but a similar induction takes place in the preganglionic cholinergic neuron.

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Tyrosine Hydroxylase Induction: Time Requirement for Completion of Transcription

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In previous experiments it has been shown that a prolonged increase in the activity of the sympathetic nervous system leads to a marked increase in the synthesis of tyrosine hydroxylase (TH) in the terminal adrenergic neuron. This transsynaptic induction is a slow process and no consistent increase in TH activity can be observed earlier than 18 h after the beginning of conditions leading to an increased preganglionic activity. However, in recent experiments it has been shown that an intense stress of 2 h is sufficient to lead 48 h later to a consistent and statistically significant increase of TH activity of about 30% in the cell bodies of the adrenergic neurons and of about 40–50% in the adrenal chromaffin cells. Treatment of rats with a transcription inhibitor, actinomycin D (0.8 mg/kg) immediately before or after the stress completely prevented this increase in TH activity. If actinomycin D was injected 6 or 12 h after the stress, the increase in TH was reduced but not abolished whereas treatment with actinomycin D 24 h after the stress had no effect. However, increased synthesis of enzyme protein continued for at least another 24 h. It is concluded that it takes more than 12 but less than 24 h until the phase of transcription is completed and that the TH messenger-RNA must have a slow turnover.

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Some Aspects of the Action of Diazepam (D) and Benzoctamine (B) on the Cat Spinal Cord

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Both D and B in the dose-range studied (0.05–1 mg/kg i.v.) depressed mono- and polysynaptic reflex discharges in ventral roots (VR) of decerebrated and high spinal cats, the aequieffective doses being about 3 times higher in the spinal preparation. In reducing the spontaneous gamma-motoneuron activity D was approximately twice as potent as B in decerebrate cats; in spinal cats both drugs were only slightly less potent. D increased and prolonged the dorsal root (DR) potential produced by peripheral afferent stimulation in spinal and decerebrate cats in equal doses, B was inactive. DR potentials produced by brain stem stimulation (dorsolateral part of caudal medulla) in decerebrate cats were also augmented by D. The results demonstrate some similarities but also marked qualitative differences between the actions of D and B on the spinal level. Furthermore, they show that the spinal cord is an important site of action of D; the active dose-range for depression of spinal activities is identical with that previously found for depression of intralimbic evoked potentials.

Synthesis and Degradation of Uric Acid in Rat Renal Cortical Tissue

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Slices of rat renal cortex incubated for 5 minutes at 25 °C in oxygenated phosphate buffer (pH = 7.4) without

adding substrate produced 21.8 ± 2.3 ($n = 5$) mcg (mean ± SE) urate per 100 mg of fresh tissue. Urate production by slices was not inhibited by allopurinol. When urate synthesis was studied in renal cortical homogenates it was completely inhibited by allopurinol ($5.10^{-4}M$). Bowman and Weiner (personal communication) recently observed urate degradation in the isolated perfused rat kidney. Renal cortical homogenates also destroyed urate when neosynthesis was suppressed by adding allopurinol: 55 ± 2.4% of 2 or 5 mcg/100 mg of fresh tissue were destroyed within 1 h ($n = 6$). In 5 pentobarbital anesthetized rats the balance of urate across one kidney (sum of urinary excretion and amount leaving by renal vein minus amount entering by renal artery) varied from −9 µg/min (net degradation) to +11 µg/min (net neosynthesis). Urate concentrations were measured by the Praetorius UV spectrophotometric method.

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Renal Excretion of N¹-Methylnicotinamide in the Rat

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The renal transport of an organic base, N¹-methylnicotinamide (NMN) was investigated in the rat utilizing both clearance and micropuncture techniques. In clearance experiments the maximum secretion of NMN (U/P_{NMN}: U/P_{Inulin} ranged from 2 to 3) occurred at plasma concentrations of approximately 0.50 mM/l. Higher concentrations resulted in some depression of secretion. The addition of a second organic base, mepiperphenidol (Darstine®) 5 mg/kg resulted in an inhibition of NMN secretion. Ultrafiltration of plasma showed that NMN is bound to plasma proteins, depending on plasma concentration. At 0.50 mM/l approximately 50% of NMN was protein bound. Free-flow micropuncture studies showed net proximal secretion of NMN (TF/P_{NMN}: TF/P_{Inulin} ranged from 2 to 4) uncorrected for protein binding. The final urine clearance ratio was lower indicating reabsorption of NMN in distal portions of the nephron. Studies are in progress to further localize these sites of secretion and possible reabsorption of NMN.

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Free-Flow Micropuncture Study of Renal Uric Acid Excretion in the Rat

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In male Sprague-Dawley rats anesthetized with pentobarbital and infused (0.05 ml/min) with a buffered (pH = 7.4) solution containing 5 percent mannitol, 4 percent inulin and 0.1 percent uric acid urine samples as well as free-flow samples of proximal or distal tubular fluid were obtained. Urate concentrations were measured by an ultramicroadaptation of a fluorimetric method[1]. Plasma uric acid concentration was 1.1 ± 0.1 mg % in 20 infused animals (0.5 mg % in the absence of urate infusion). In 15 samples proximal fluid, TF/Purate was 1.7 ± 0.2 (mean ± SE), TF/Purate: TF/P inulin was 1.3 ± 0.1 and significantly ($p = 0.01$) different from unity. In 5 samples of distal fluid, TF/Purate was 1.2 ± 0.4, TF/Purate: TF/P inulin was 0.39 ± 0.02. As in results published by Greger and al.[2], net secretion was thus observed in proximal tubules, and extensive reabsorption between proximal and distal tubules. Under similar conditions and with the same analytical methods, in Cebus monkeys net reabsorption has been demonstrated to occur in proximal tubules[3].

[1] P. Bloch and G. Lata, Analyt. Biochem. *38*, 1–19 (1970).
[2] R. Greger, F. Lang and P. Deetjen, Pflügers Arch. *324*, 279–287 (1971).
[3] F. Roch-Ramel and I. M. Weiner, Am. J. Physiol. (in press). Supported by SNSF, grant 3.44.68.

Carcinostatic Activity of GP 48989 against DMBA-Induced Mammary Carcinomas in Female Sprague-Dawley Rats

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Mammary tumours were induced by a single oral administration of 15 mg DMBA to 50 days old female Sprague-Dawley rats. Animals with 1–2 tumours of 8–12 mm diameter (85% carcinomas) were used for chemotherapeutic experiments. Oral doses of 6.25 to 400 mg/kg of GP 48989, a thiazolidinonylidene-hydrazono-thiazolidinone derivative, were administered by stomach tube for 5 days per week. Complete regression was seen in 80 and 62% of the tumours after 30×25 and 15×100 mg/kg, respectively. In 6 different experiments with 53 tumour-bearing rats altogether, 56% of the tumours regressed completely, 31% partially and 13% were found to be resistant to treatment with 15×100 mg/kg. With exception of transient slight weight loss, concurrent with reduced food consumption, the compound was well tolerated. Except for decreased uterine size and increased lymphopoiesis in the spleen, no changes were found. In view of the findings observed additional studies appear justified, including evaluation in man.

Enhancement of Striatal Acetylcholine Turnover by Neuroleptic Drugs

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Based on the observation that anticholinergic drugs ameliorate both Parkinson's disease and neuroleptic-induced parkinsonism, it has been proposed that, in addition to impaired dopaminergic transmission, a cholinergic mechanism is involved in the genesis of extrapyramidal symptoms. However, no direct evidence of this cholinergic involvement has yet been provided. In the present experiments acetylcholine (ACh) released from the caudate nucleus of curarized or chronically prepared, unrestrained cats, was collected by means of a push-pull cannula and measured radioenzymatically. Chlorpromazine (10 mg/kg i.v.) or haloperidol (2 mg/kg i.v.) markedly increased the release of ACh without affecting its tissue concentration. This indicates an enhanced ACh turnover. Promethazine, a non-neuroleptic phenothiazine, was ineffective. At these doses, the neuroleptics had no effect on ACh-esterase activity. It seems therefore that the increase of ACh turnover is due to dopamine receptor blockade by the neuroleptics. This view is supported by the finding that apomorphine, a dopamine receptor stimulating compound, completely reversed the effect of neuroleptics on ACh. The present findings suggest that a

cholinergic mechanism in the striatum is under the influence of a tonic dopaminergic inhibitory input. They provide the first direct evidence that this cholinergic mechanism – possibly involved in the genesis of parkinsonism – is activated by neuroleptic drugs.

Retrograde Axonal Transport of Nerve Growth Factor

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The protein Nerve Growth Factor (NGF) has a potent growth promoting effect on the peripheral sympathetic nervous system and produces a selective induction of tyrosine hydroxylase and dopamine β-hydroxylase, key-enzymes in the synthesis of the adrenergic transmitter norepinephrine. Recent studies have indicated that NGF may exert its trophic action on the adrenergic neuron from the periphery rather than by a direct effect on the cell body. We have now demonstrated a retrograde axonal transport of Nerve Growth Factor from the adrenergic nerve terminals in the eye to the superior cervical ganglion after the injection of ^{125}I-NGF into the anterior chamber of the eye. The rate of this transport was calculated to be approximately 2.5 mm/h. This transport could be blocked by prior injection of colchicine into the eye and by transection of the postganglionic nerve fibres.

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Natriuretic Action of some New Vasotocin Analogues

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The synthetic neurohypophysial hormone analogue, [Leu^4]-arginine-vasotocin: Cys-Tyr-Ile-Leu-Asn-Cys-Pro-Arg-Gly-NH_2 (*1*) is strongly natriuretic (Cort et al., Experientia, *29*, 173 (1973). Its Lys^8 analogue (*2*) and Leu^3 analogue (*3*) and the desamino derivatives of *1*, *2* and *3* have now been found to have a similar natriuretic activity in the chloralosed cat preparation (Cort et al., Am. J. Physiol. *215*, 921 (1968): 30 μg/kg i.v. approximately doubled total sodium excretion. The Phe^2, Phe^3 and Phe^4 derivatives are appreciably less active. The N$^\alpha$-glycyl derivative of *1*, constructed as a 'hormonogen', had somewhat lower but considerably more protracted natriuretic activity.

The most potent analogues of this series in the cat assay, namely *1*, and its desamino and the N$^\alpha$-glycyl derivative, have also been tested in dogs and rats. In conscious dogs all three analogues at a dose of 30 μg/kg i.v. increased the sodium excretion 3 to 5 times during a period of 3 to 4 h. In conscious saline-loaded rats the mean sodium excretion during a 5-hour collection period was doubled in a dose range of 30 to 100 μg/kg s.c.

Pharmacokinetics and Metabolism of Hydrallazine: Specific Affinity for Blood Vessels

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The pharmacokinetics of hydrallazine were investigated in the animal (mouse, rat and dog) and in man after the administration of ^{14}C-labelled substance. Distribution studies in the mouse and the rat, including whole-body autoradiography and radiometric determinations in 30 different organs and tissues, revealed that the drug displays a specific affinity for the blood vessels. The concentrations were slightly higher in the arteries than in the veins. Radioactivity was measurable in the blood vessels for at least four times as long as in the other organs. On the basis of these findings, experiments were performed to ascertain whether there was a correlation between the pharmacological effects of hydrallazine and the concentrations reached in the arterial wall. The apparent half-life of the drug is longer in human than in animal blood. Hydrallazine is rapidly eliminated from the body: in the rat and the dog and in man, 85–90% of the radioactive dose administered was excreted within 24 h of administration, mainly in the urine. In human urine, 7% of the radioactivity was accounted for by unchanged hydrallazine, the rest being attributable to metabolites, four of them were identified.

Peripheral Neuropathy in Hexachlorophene (HCP)-Treated Rats

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Female rats received 5, 10, and 15 mg/kg HCP p.o. twice daily, 5 days per week for 5 weeks. Disturbance of neuromuscular functions as measured with the rotating rod test was found with 10 and 15 mg/kg beginning between the fifth and fifteenth dose. A slight to moderate reduction of temperature sensitivity (hot plate test) developed after 5 administrations of 10 and 15 mg/kg. Rectal temperature, food and water intake and taste (2 bottle test) were unchanged. Spongiform encephalopathy (Kimbrough and Gaines, Arch. Env. Health *23*, 114, 1971) was not found in rats treated with 5 mg/kg. With 10 and 15 mg/kg it was first observed after 9 doses; it was moderate after 15 and severe after 21 and more doses. The n. ischiadicus of rats treated with 15 or more doses of 15 mg/kg showed focal degeneration of single nerve fibers with marked ballooning, occasionally also fragmentation of myelin sheaths. Fat-laden macrophages, located near fragmented nerve fibers, were scarce. Axis cylinders were mostly unchanged even in nerve fibers with marked swelling of myelin sheats. Disruption of axis cylinders was seen in nerve fibers showing severe degeneration and globular clumping of myelin.

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

A Study of the Structure of the T-Layer of Bacillus Brevis

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Low pH dissociated subunits of the external protein layer (T-layer) of bacillus brevis were reassembled in vitro with and without treatment with pronase to give cylindrical and sheet structures respectively. Subsequent SDS gell electrophoresis showed that the treated and untreated subunits had molecular weights of 125 KD and 140 KD ($\pm$ 7 KD). Electron micrographs were obtained of the two reassembled forms from which the pitch of the cylindrical form was measured to be 39° $\pm$ .5° and the lattice constants of the two forms found to be the same ($\pm$ 2%) and equal to 130 Å $\pm$ 5 Å. Optical and computer filtering techniques were then applied and reconstructions to 30 Å were obtained, however, these failed to show structural differences significant at this resolution.

τ-Particles: a Precursor to Phage T4 Head

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τ-particles produced by mutants in gene 21 or 24 of bacteriophage T4 are morphologically similar, but functionally different. In 21^- infected cells, τ-particles are abortive. With mutants in gene 24, they can be transformed into phage heads. In this case, the major head protein P23, which is uncut in τ-particles, is cleaved after temperature shift to the permissive temperature. Pulse labelled τ-particles, produced at non permissive temperature in heavy medium, are transformed into heavy capsids after temperature shift down and transfer into light medium. This proves the conservative nature of this transformation. Therefore it is concluded that 24^- τ-particles are – or can at least act as – precursor particles to normal phage heads.

Differential Analysis of Inactivation of Yeast Cells by X-Rays, ^{4}He and ^{16}O Ions

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The survival of haploid yeast (S. pombe) was investigated with x-rays, α-particles and heavy ions. The cells were cultivated in liquid medium, harvested, washed and irradiated (at G2 stage) in monocellular layers in isotonic NaCl on membrane filters. Criterion for survival was the division-probability (DP) of the first three post-irradiation mitotic divisions and microcolony forming ability. In experiments with charged particles (1 MeV/AMU) where the track segment method was employed, LET_∞ varied from 132 to 1090 keV/μ. Independent of radiation quality and milieu during irradiation, the first postirradiation mitotic division was less affected than the second one, DP did raise again after the second one, but replication instability was observed for at least 8 generations. Survival-curves were exponential for DP of the first two divisions, but not so for microcolony forming ability, where some of the curves had shoulders, implying that a differential analysis of inactivation is necessary. OER was reduced from 2.2 for x-rays to approx. 1.0 for heavy ions, depending on the criterion.

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Stereological Model for the Guinea Pig Pancreas

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In order to study morphologically membrane movements associated with the secretion of zymogen in the pancreas, a stereological model has been developed which provides detailed quantitative information on the structure of this organ. The model first considers volume densities of tissue components, and then volume and surface densities of exocrine cell organelles and membranes. The volume of exocrine cells, accounting for 82% of the pancreas, consisted of 54% cytoplasmic matrix, 22% RER, 8.3% nuclei, 8.1% mitochondria, 6.4% zymogen granules, and 0.7% condensing vacuoles. And for the surface area of membranes: 60% RER, 21% mitochondria, 9.9% Golgi, 4.8% plasma membranes, 2.6% zymogen granules, and 0.4% condensing vacuoles. In analyzing the data, particular emphasis was placed on finding the minimum sample size by testing for regional homogenities, and by determining the number of tissue samples required per individual animal and animal population.

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Use of Uranyl Formate Staining for the Electron Microscopic Visualization of DNA-Protein Complexes

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Uranyl formate has been frequently used for the negative staining of viruses and proteins. The present communication shows the application of uranyl formate for the positive staining of nucleic acids in bright field and dark field electron microscopy, particularly in view of the visualization of protein-nucleic acid interactions.

Purified DNA was adsorbed on positively charged thin carbon films and stained with an aqueous solution (0.5%) of uranyl formate. Double stranded stained DNA molecules appeared very regularly with a thickness of 12 to 15 Å. It is possible to visualize at the same time positively stained DNA and negatively stained protein molecules fixed on it.

Micrographs will show examples of: a) DNA in bright field and dark field, b) λ-repressor fixed on the operator region of the DNA, c) the complex of host modification enzyme and λ-DNA.

Characterization of Avian Myeloblastosis Virus Proteins Labelled in vivo and in vitro

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Avian Myeloblastosis Virus (AMV) grown on chick embryo fibroblasts in tissue culture was labelled with ^{35}S-methionine. Unlabelled AMV was purified from sera of leukemic chickens and labelled chemically with ^{125}I by the chloramine T method. This reaction results in the labelling of tyrosine residues and is carried out after oxidative unfolding of the proteins. Labelled viral polypeptides were separated by polyacrylamide gel electrophoresis, eluted, and digested with trypsin. Two-dimensional fingerprints of the major viral polypeptides (MW 24,000, 19,000, 12,000 and 11,000) have been obtained, each giving a characteristic pattern. The two different labelling techniques give different characteristic peptide maps. This preliminary work serves two main purposes: 1. The fingerprints will enable us to identify polypeptides made in an in vitro protein synthesizing system. 2. The in vitro labelling technique will allow us to characterize minor components of the virus that can not be sufficiently labelled in vivo.

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Resolution of Three Distinct Populations of Nerve Endings from Rat Brain

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Conditions have been established for the fractionation of subcellular components of rat brain homogenates by zonal isopycnic equilibration in continuous sucrose density gradients using a B-XIV rotor. Starting from postnuclear supernates of forebrain homogenates, it has been possible to resolve three distinct populations of nerve endings from each other, as well as from free mitochondria and myelin fragments. The three types of nerve endings differ in their apparent specific gravity, their biochemical properties and their ability selectively to accumulate exogenous transmitter substances in vitro. There are indications to suggest that the three types of nerve endings also differ morphologically. These three particle populations are likely to represent, in order of increasing modal equilibrium density, a) cholinergic nerve endings (characterized by their high contents in acetylcholine), b) the endings of inhibitory GABA neurons (high glutamate decarboxylase activity and accumulation of exogenous GABA), c) catecholaminergic nerve endings (high monoamine oxidase activity and accumulation of both dopamine and noradrenaline).

Presumptive Late Polyoma Messenger RNA

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In primary cultures of mouse kidney cells infected with polyoma virus, several size classes of polyoma-specific RNA can be found late after infection (Acheson et al., PNAS *68*, 2231, 1971). We attempted to localize them in the cell by fractionating infected cultures labeled with ^{3}H-uridine; RNA was then extracted and analyzed by sedimentation velocity in sucrose gradients. Polyoma-specific RNA was located in the gradients by hybridization of each fraction to an excess of purified polyoma DNA.

The results show that polyoma-specific RNA molecules the size of the viral genome and larger (with sedimentation coefficients $\geqslant$ 26 S) are only present in the nucleus, and do not appear in the cytoplasm. The cytoplasm contains viral RNA with lower sedimentation coefficients: a major peak at about 16 S and a shoulder at about 19 S. The same viral RNA species were found in isolated polyribosomes. These species are displaced from the polyribosome region of a gradient when polyribosomes are dissociated with EDTA, suggesting that they may represent functional polyoma messenger RNA during the late phase of infection.

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Thymus-Specific Surface Antigenic Determinants of the Mouse, Cross-Reacting with Anti-Polyclonal μ- and α-Ig Sera

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Direct evidence is presented that thymocytes contain cell specific μ and α antigenic determinants. 23–30% thymocytes from Swiss mice were killed by 4 different anti-polyclonal μ sera induced in rabbits and guinea pigs and 9% by antipolyclonal α serum in a cytotoxic assay. Anti-monoclonal μ serum (MOPC 104) did not react with thymocytes but killed 25% spleen cells. Absorption of anti-μ and α with thymus abolished the reaction with thymocytes but not with spleen cells; absorption with spleen abolished the reaction with spleen cells but not with thymocytes. Absorption with brain did not diminish the reactivity with both, spleen and thymus cells. The reactivity of anti-μ with thymus- and spleen-cells was abolished by absorption with polyclonal IgM. Thus thymus and spleen contained different, cell-specific μ antigenic determinants. 4 days after antigenic stimulation with sheep erythrocytes, thymocytes containing μ and α determinants had proliferated selectively: 67% cells were killed by anti-μ and 25% by anti-α. Absorption experiments with membranes of immunized and normal thymocytes showed that after antigenic stimulation new thymus-specific μ and α determinants appeared on thymocyte membranes.

Cell Wall Structure of the Fission Yeast *Schizosaccharomyces pombe*

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The taxonomic position of a fungus is reflected to a certain extent in its cell wall structure. Thus fission yeasts, which are intermediate forms between true yeasts and mycelial yeasts may have an intermediate type of cell wall structure. Some indications of this have already been found, but a complete structural analysis of the walls of these yeasts is lacking.

The cell walls of a fission yeast, *Schizosaccharomyces pombe*, have been analyzed by enzymic and chemical techniques. Three main cell wall components have been demonstrated and structural studies have shown them

to be an $\alpha(1 \rightarrow 3)$glucan (45%), a $\beta(1 \rightarrow 3)$glucan (40%) and a galactomannan (10%) containing $(1 \rightarrow 2)$, $(1 \rightarrow 3)$ and $(1 \rightarrow 6)$ linkages, most of the galactose being in non-reducing terminal positions. Although the use of a specific fluorescent marker indicated that chitin was present in the cross walls of the yeast, this was not confirmed by glucosamine determination after cell wall hydrolysis.

The wall of *S. pombe* does therefore appear to be intermediate between the true yeast cell wall which contains approximately equal amounts of β-glucan and α-mannan and the mycelial yeast or fungal wall which contain both glucans and chitin.

The Proteins of Polyoma Virus

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Polyoma virus proteins were radioisotopically labeled by propagation of the virus in primary mouse kidney cells maintained in depleted Eagle's medium supplemented with ^{3}H or ^{14}C amino acid mixture. The virus was purified from the infected cell lysates by the classical procedures of enzyme treatment, sedimentation, sucrose gradient sedimentation and isopynic centrifugation in CsCl. Highly purified preparations of polyoma virions, pseudovirions and capsids were obtained by sedimentation velocity centrifugation in shallow (1.27–1.32 gm/ml) CsCl gradients. The proteins of the above viral preparations were dissociated to their respective polypeptides by heating in the presence of 2% SDS and 5% mercaptoethanol and subjected to gel electrophoresis in 10% SDS polyacrylamide gels. It was found that the polyoma virion and pseudovirion preparations revealed six distinct polypeptides (I–VI). However, the capsid preparation was found to lack polypeptides IV, V, VI. In all instances the major capsid polypeptide (I) consisted of 80 to 83% of the virion protein. Purified polyoma virions and capsids were also degraded to capsomeres by treatment with a reaction mixture consisting of Clelands reagent, EDTA and pH 9.0. Electron microscopy revealed that the degraded virions consisted of 72 capsomere subunits. Polyoma capsomeres were isolated by centrifugation in glycerol gradients (10–30%); and when analyzed on SDS-gels demonstrated the presence of polypeptides I, II, III.

These studies on the components of the purified polyoma virion are being continued with the hope of better understanding the complexities of in vivo virion assembly.

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Préparation de spécimens biologiques pour la microscopie électronique en fond noir

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Pour être observé de manière satisfaisante au microscope électronique, les spécimens biologiques délicats doivent être protégés contre les effets du séchage et du faisceau d'électrons.

A partir d'une idée de E. Kellenberger, nous avons pu en grande partie éliminer les artefacts de séchage en remplacant l'eau par un liquide à basse tension superficielle (Ether par exemple). De telles préparations, ressemblent, y compris par leurs artefacts aux préparations obtenues par la méthode du point critique et de la lyophilisation.

Pour adapter au fond noir la méthode de coloration négative nous avons fait appel à des matériaux plus légers. Nous avons d'excellents résultats avec le silicate de sodium et le fluorure de béryllium. Ce dernier produit est moins diffusant que la matière organique et les images montrent la structure biologique fortement contrastée enrobée dans une couche de matériel peu diffusant.

Localization of Lipids in the Cell Nucleus Using Ultrathin Epon and Frozen Sections

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Mouse and rat liver labeled for 4 h in vivo with cholinemethyl-^{3}H, and cultured mouse cells (Px 815) labeled during growth for 15–18 h, were used. After glutaraldehyde fixation the specimens were washed for up to 24 h in a phosphate or cacodylate buffer and either enveloped in gelatine for cryoultramicrotomy (W. Bernhard, A. Viron, J. Cell. Biol. *49*, 731, 1971), or post-fixed with osmium, dehydrated (G. H. Cope, M. A. Williams, J. Microsc. *90*, 31, 1969) and embedded in Epon. Both Epon and frozen sections were processed for electron microscope autoradiography. Preliminary results show that in Epon sections the nuclear radioactivity is localized on the periphery of the nucleus, in the nucleolar region and in the nucleoplasm. In the frozen sections the label is found particularly either over the nucleolar region or over the nucleoplasm. Controls to determine the amount of lipid extracted by each method show about 20% loss during processing for Epon embedding, and practically negligible extraction during preparation and sectioning for cryoultramicrotomy.

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Sequence of Polyoma Virus Induced Chromosomal DNA Synthesis in Primary Mouse Kidney Cell Cultures

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Studies on the sequence of chromosomal DNA synthesis in several kinds of normal and neoplastic mammalian cells led to the conclusion that the DNA replicated at the beginning of S phase has a higher G + C:A + T ratio than DNA replicated later in S phase.

Mouse chromosomal DNA contains a minor species of AT rich DNA (light satellite DNA; S = 1,689) which can be separated from the bulk DNA in CsCl equilibrium density gradients. It was found that this satellite DNA replicates in the latter $^{1}/_{2}$ of S phase.

In this report we present the results of a study on the sequence of polyoma virus induced chromosomal DNA synthesis during infection of primary mouse kidney cell cultures under standard conditions of infection and under conditions where the onset of virus induced DNA synthesis was synchronized by the use of FUdR.

The results lead to the conclusion that the sequence of polyoma induced DNA synthesis is analogous to that described by other authors for the sequence of chromosomal DNA synthesis in uninfected mouse cells.

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Purification of 14 S mRNA of Immunoglobulin Light Chains and its Transcription into Complementary DNA

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RNA prepared from membrane-bound polysomes of an L-chain producing mouse myeloma (MOPC-41) was fractionated by poly(dT)-cellulose chromatography and a poly(A)-containing RNA fraction was obtained. Successive sucrose gradients and gel electrophoresis allowed the purification, from that fraction, of a 14 S RNA which behaves as a single peak with an estimated M.W. of 380,000 (1,100 nucleotides). Purified 14 S RNA is translated in a cell-free system into a single protein which is identical to MOPC-41 L-chain except that it is about 20 amino acids longer by SDS-gel electrophoresis and has 2 additional tryptic peptides. 14 S RNA is therefore the mRNA of what appears to be a precursor of secreted immunoglobulin L-chains. In *Xenopus laevis* oocytes, the translation product of the same 14 S RNA is of the exact size of L-chain, suggesting cleavage of the precursor in that system.

With reverse transcriptase from AMV, purified 14 S mRNA can be transcribed into DNA. This DNA is fully complementary to 14 S RNA and hybridises to no other RNA tested. The availability of highly radioactive DNA complementary to purified mRNA of L-chains makes now possible experiments on the dosage of immunoglobulin genes and on amplification mechanisms.

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Abrogation of Sensitization to Marrow Grafts in Dogs

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In patients with aplastic anemia or acute leukemia, the success of allogenic bone marrow grafts after conditioning with cyclophosphamide or radiation may be jeopardized by prior transfusions with whole blood. In dogs, marrow grafts may be rejected even after transfusions between donors and recipients which are littermates compatible for the major dog leucocyte antigen locus.

Attempts were made to abrogate sensitization with an immunosuppressive protocol derived from experiments designed to induce tolerance to skin allografts in mice. With unrelated, mismatched pairs of dogs (Beagles), the recipients were immunized with two infusions of 100 ml whole blood from the prospective bone marrow donor. Prior to the total body irradiation with 1,200 r and the marrow graft, the recipients received for six days a regimen of alternating doses of procarbazine and ALS. In the experimental group, 7 out of 8 dogs had marrow takes as contrasted to 0/6 in the untreated control group ($p < 0.01$).

Ultrastructural Cytochemistry: A New Specific Stain for DNA and Polysaccharides

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Among the numerous Schiff-type reagents tested up to now in the electron microscope (Gautier et al., Experientia *27*, 735, 1971, and J. Micr. *14*, 48a, 1972), a newly synthetized inorganic Osmium^{IV}-Ammine was shown to possess simultaneously specificity, intensity and fineness, both in Feulgen-type and in PAS-type reactions. After acid hydrolysis of thin sections of aldehyde-fixed tissues, only DNA is revealed by the reagent used for section staining; after periodic acid treatment of similar sections, only polysaccharides are stained. Analogous but less specific results are obtained if similar reactions are applied to thin sections of tissues fixed with aldehyde-OsO_4 or OsO_4 alone, with or without peroxidation of the sections.

If the same reagent is added to both aldehydic and osmic fixatives, like Ruthenium Red in the Luft's procedure (Anat. Rec. *171*, 347, 1971), extracellular polysaccharide moieties are strongly stained, the light and electron microscopic appearances being similar to those obtained with Ruthenium Red.

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Activity of the Single-Strand-Specific Nuclease S_1 on Double-Stranded DNA

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The single-strand-specific nuclease S_1 extracted from *Aspergillus oryzae* degrades both single-stranded RNA and DNA. We studied the action of this nuclease on intact and nicked double-stranded DNA from polyoma virus.

– Nuclease S_1 can cut at least 90% of double-stranded supercoiled polyoma DNA (20S) to unit length rods, which contain no internal nicks.

– Nuclease S_1 can also degrade into rods double-stranded circular polyoma DNA which contains one or more nicks (16S not supercoiled, generated by X-rays, pancreatic DNase or secondary effects of P^{32} decay). Under appropriate conditions of salt and temperature, the nuclease can recognize most of the single-strand nicks and cut the opposite DNA strand.

These results suggest that nuclease S_1 can recognize a short single-stranded region exposed by transient melting of the free ends generated by a nick on the opposite strand. From the fact that S_1 does not attack rod shaped double-stranded molecules, but does cut supercoiled polyoma DNA, we conclude that this DNA contains at least transiently single-stranded regions.

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Retrograde Axonal Transport as a Method of Mapping for Spinal Motoneurones

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Retrograde axonal transport was examined as a new method of mapping the motoneurones of the spinal cord in rats. Albumin labelled with the fluorescence dye Evans Blue was injected into various muscles of the anterior appendage. In some cases adjacent muscles were denervated to prevent them interfering. After 24 h the spinal cord was investigated by fluorescence microscopy. According to the injection side a specific group of motor pericaryons was stained. After injection into the triceps, neurones in the lateral part of the ventral horn in segments C6 to C8 were marked; whereas the lower leg and the hand are represented by cell bodies lying more dorsally, the neurones of the radial nerve were lateral to

those of the ulnar and median nerve. Segmental distribution: ulnar: C7-T1; median C6-T1 (hand: C8 mainly). These results conform in general to the maps made using other methods, especially chromatolysis. In addition they show that transport really is axonal.

Ultrastructural and Histochemical Study of the Thyroid Gland of Gunn Rats

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In Gunn rats with hereditary UDP-glucuronyl transferase insufficiency, conjugation and excretion of thyroxin by the liver are decreased (Lüders, Z. Kinderheilk. *113*, 129, 1972). The morphology of their thyroid gland seemed therefore worth to be investigated.

In young Gunn rats (older than 4 weeks) and in adults the thyroid is macroscopically brownish-black. Light microscopically the follicles appear small, the colloid diluted, the follicular cells high and filled with brown granules. On the ultrastructural level these granules are shown as dense intracellular secretion droplets associated with abundant ER. The histochemical analysis suggests that these granules contain a melanin-like pigment. Impaired hepatic thyroxin excretion may therefore induce thyroxin retention in the follicular cells where thyroxin and/or its precursor may be partly converted into a melanin-like pigment.

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Tissue-Unspecificity of Skin Extracts Tested for the Presence of Chalones

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Crude extracts prepared from dorsal epidermis or whole back skin of adult male mice inhibited the ^{3}H-thymidine incorporation into mouse ear epidermis kept in organ culture. The inhibition was time- and dose-related. Ethanol and acetone fractionation of epidermal extracts and gel filtration of a whole skin extract with Sephadex G-100 revealed the presence of inhibitory activities in most of the fractions. Neither trypsinization nor heating of the whole skin extract diminished its inhibiting effect. After Sephadex G-100 chromatography of the heated material, every fraction was active. Thymidine incorporation into primary mouse kidney cell cultures or multiplication of murine P-815 mastocytoma cells in vitro were inhibited by all extract preparations, and strong, dose-dependent cytotoxicity often accompanied the inhibition. In vivo, a crude epidermal extract inhibited the incorporation of injected thymidine into spleen and intestinal wall, but not in the ear epidermis. As to the postulated tissue-specificity of the epidermal chalone(s), the results disagree with those published so far.

Dissimilar Effects on Thymidine Incorporation and DNA Synthesis of the Podophyllotoxin Derivative VP 16-213

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VP 16-213 is a new glycosidic derivative of podophyllotoxin. Cultures of P-815 X2 mastocytoma cells were incubated with VP 16-213 (1 or 10 μg/ml) and aliquots were withdrawn at intervals of 1.5 h. Cell multiplication, mitotic index, and incorporation of various labelled precursors were determined. In addition, total DNA, RNA, and protein content was measured. VP 16-213 inhibited cell multiplication rapidly and prevented the cells from entering mitosis. Incorporation of ^{3}H-thymidine into the TCA-insoluble fraction of pulse labelled cells was inhibited by 50% within 3 h with 1 μg/ml of the drug. Measurements of mean DNA content per cell showed, however, that DNA synthesis continued at an increased rate. Pulse-label experiments with ^{3}H-uridine and ^{3}H-leucine as well as photometric determinations indicated a continuing synthesis of RNA and protein. We conclude from our results that VP 16-213 primarily stops the cells in G2 phase, and that the alterations of the precursor incorporation are not directly relevant to the inhibition of cell division.

Separation of Two Types of Granules from Human Polymorphonuclear Leukocytes

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Post-nuclear supernates from homogenates of purified polymorphonuclear neutrophil leukocytes (PMNs) from human blood were fractionated by means of zonal sedimentation and isopycnic equilibration. Two distinct populations of granules, which were resolved almost completely from each other in both systems, were characterized biochemically and morphologically. The first population sediments rather fast and has a modal equilibrium density of 1.23 gxml^{-1}. It contains all the myeloperoxidase of the preparation, a major proportion of four lysosomal hydrolases, and about half of the lysozyme. The second population sediments approximately three times more slowly than the first and equilibrates at the density of 1.19 gxml^{-1}. It contains the remainder of the lysozyme, but no myeloperoxidase and little if any acid hydrolase. Unexpectedly, it also appears to be devoid of alkaline phosphatase. The main components of the first population can be identified by electron microscopy as the azurophil granules, while the main components of the second population resemble the specific granules of the intact PMN.

A Structure Representing the Chromosomal DNP from Interphase Cells

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The chromosomal deoxynucleoprotein (DNP) is released from mouse cells (line P815) by the non-ionic detergent Nonidet P-40 in medium of low ionic strength (0.7 m*M* salt). The DNP is released as a structure which conserves the form and ultrastructural characteristics of chromatin within the cell. The nuclear envelope cannot be detected by electron microscopy, and the content of choline-containing phospholipids is only one-tenth of that in nuclei. We conclude that the nuclear envelope has been removed, and that the compact peripheral region of the chromatin is responsable for maintenance of the nuclear form in these structures.

DNP prepared by shearing these structures has the same content of DNA, histone, non-histone proteins and

RNA as DNP prepared by classical methods. This procedure is suited for routine, quantitative isolation of chromosomal DNP, especially the DNP containing nascent DNA (Fakan, Turner, Pagano and Hancock, PNAS *69*, 2300, 1972). Preliminary studies show that it is also applicable to other cell lines.

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Ribosomal Proteins Involved in Interaction with Elongation Factor G and GTP

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Elongation factor EF-G is involved in the translocation of peptidyl-tRNA from the donor site to the acceptor site on the ribosome. Although its mode of action cannot be studied directly during this process, two partial reactions characterize its interaction with the ribosome. One reaction is the formation of the ribosome-EF-G-GDP complex, the other the hydrolysis of GTP dependent on both ribosomes and EF-G.

Antibodies prepared against 50 individual ribosomal proteins were tested for their ability to interfere with both complex formation and GTP hydrolysis. With 70S ribosomes, only antibodies to proteins L7 and L12 were inhibitory, inhibiting completely both complex formation and GTPase. However, when individual subunits were treated, antibody to protein L19 inhibited complex formation, while those antibodies to L14, L19, L23, L27, S9 and S11 inhibited GTPase.

The results indicate that several proteins become exposed after subunit disassociation, and permit the delineation of spatial relationships between ribosomal proteins.

Cellular Origin of the Basic Proteins in SV40 and Polyoma Virus Particles

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Polyoma and SV40 were grown in the presence of S^{35}-methionine and purified by centrifugation in CsCl and sedimentation through sucrose gradients. The particles were disrupted by boiling in the presence of SDS and mercaptoethanol and the polypeptides separated by electrophoresis in SDS-polyacrylamide gels. The gel pattern of the small polypeptides is identical for polyoma and SV40: three bands (S^{35}-met labeled) with molecular weights between 10,000 and 15,000 daltons. These polypeptides are not found in empty capsids. After elution from the gels the polypeptides were digested with trypsin. The resulting peptides were separated by electrophoresis on thin layer cellulose at pH 3.5 and detected by autoradiography. Distinct fingerprints were obtained which are virtually identical for Polyoma and SV40. Extracts of the basic proteins of the nuclei of unifected host cells gave three main bands on gels with the same mobility and similar fingerprints as the described small viral proteins. This confirms the work of Frearson and Crawford.

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Etude structurale des hémoglobines et myoglobines reconstituées

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Précédemment Antonini et al. (Biochim. Biophys. Acta *79* [1964]284) ont développé une technique permettant de séparer l'hème de la globine et de recombiner ensuite ces deux composés. Ils ont ainsi remplacé l'hème natif (protohème) par des groupes hémiques chimiquement modifiés tels que mesohème et deuterohème. L'hémoglobine reconstituée contenant l'hème natif montrait des propriétés fonctionelles normales, contrairement aux hémoglobines, portant le deuterohème ou le mesohème, où les effets allostériques étaient fortement diminués.

Puisque la spectroscopie RMN est un détecteur sensible des changements de structure aux alentours de l'hème, on a appliqué cette méthode pour voir si de tels changements accompagnent lesdites altérations de fonctionnement. Dans notre expérience, on a examiné les spectres RMN des hémo- et myoglobines reconstituées chacune avec proto-, meso- et deuterohème. L'hémoglobine portant le protohème avait un spectre semblable à celui de la protéine native, tandis que celles avec deutero- et mesohème avaient des spectres différents. Cela met en évidence la relation entre la conformation des alentours de l'hème et la fonction de la protéine.

Two Pathways Yielding Plaque Forming Particles in Bacteriophage

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Gene D comprises 20% of the phage λ head's protein mass. Lysates of bacteria infected with λ, mutant in gene D, contain, besides other structures, defective particles, that can be isolated and complemented to infective particles by adding purified gene D product. The defective particles contain all head gene products including gene W product and gene F product as well as the phage tail. They adsorb to λ-sensitive bacteria, however, they do not inject their DNA until gene D product is added. Thus, it might be that gene D product functions to loosen the connection of DNA to the capsid. An alternative pathway was established by in vitro experiments (Casjens, Hohn & Kaiser, J. Mol. Biol. *64* (1972) 55), according to which a prehead already containing gene D product is converted to phage by addition of gene W product, gene F product and tail.

Scanning Electron Microscopy of Isolated *Chironomus* Salivary Gland Chromosomes

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Chromosomes were isolated, by micromanipulation, in Glancy's Medium, placed on a piece of coverslip, fixed in glutaraldehyde, washed, and lyophilized at 5×10^{-6} Torr and –100 °C. The preparation was then coated with 200 Å of gold or platinum and placed into a scanning EM. The body of the chromosome resembles a sponge of various pore sizes. Fibrils are packed more densely in bands than in interbands. The nucleolus appears as a compact body with a more or less soft surface. In Balbiani rings, fibrils are arranged more radially, and are often covered with

spherical particles of approx. 0.25 μ diameter. Addition of a small amount of Mg^{2+} to the fixative results in a much finer network of fibrils. Thin sections of scanned chromosomes show a marked change in ultrastructure as compared with thin sections of freshly dissected chromosomes. The nature of these apparent artifacts is being investigated by critical point drying with nitrous oxide and thin sectioning at various stages of the preparation.

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Adaptation of Muscle Cell Structure in Men Trained to High Performance

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In order to test whether the increased performance of muscle in highly trained men is associated with a structural adaptation of muscle cells, needle biopsies were secured from nine untrained men (NT, max. $\dot{V}_{02}$ 61 ml/min · kg) and from five well-trained long-range runners (LT, max. $\dot{V}_{02}$ 76 ml/min · kg). Morphometric analysis of 60 electron micrographs per biopsy revealed an increase in volume density of mitochondria from 5.2 ± 0.7% in NT to 7.3 ± 0.9% in LT. The subsarcolemmal mitochondria were increased threefold, the interfibrillar mitochondria only 1.3 fold. The surface of mitochondrial cristae was 1.6 times greater in trained muscles. No change was found in surface or volume density of sarcoplasmic reticulum, but fat droplets were increased 2.5 fold in trained muscles.

The changes in mitochondrial parameters correlated significantly with maximal $\dot{V}_{02}$ and with biochemical findings on enzymes of the Krebs cycle.

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Characterization of the RNA Polymerase Complex Found in Influenza Virus Infected Cells

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Two RNA-dependent RNA polymerase activities are detected in the cytoplasm of Influenza Ao/NWS infected cells. They are distinguished mainly by different requirements for monovalent and divalent cations. Both polymerase activities are associated with a heavy complex which sediments slightly faster than monosomes on a sucrose gradient. The polymerase complex is not bound to ribosomes, since treatments which dissociate ribosomes do not change its sedimentation properties.

The polymerase complex can be specifically labeled with radioactive uridine and amino acids in infected cells treated with actinomycin D. The isolated complex contains viral RNA and one main protein which corresponds to the nucleoprotein of the virus.

Direct Demonstration that Giant Nuclear RNA from Erythroblasts is an Informational Precursor to Globin mRNA

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Highly purified mRNA which directs the synthesis of three duck globins in a cell-free protein synthesising system was copied into antimessenger DNA (amDNA) by the RNA-dependent DNA polymerase. This DNA was hybridized to giant nuclear pre-mRNA (50–100 S) from the same cell isolated on DMSO gradients. C_0t curves show that $1–3 \times 10^{-4}$ of the giant RNA consists of globin specific sequences. A quantitative comparison done by hybridizing individual RNA fractions from polyacrylamide gels in amDNA excess reaction shows that a significant proportion of the nuclear globin mRNA sequences are contained in pre-mRNA of M of more than 1.5×10^6 Daltons.

We conclude that the globin genes are first transcribed into giant molecules which give rise (through metabolism) to functional mRNA.

Cooperativity of Hemoglobin pH Dependence in Presence of Inorganic Phosphate

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Organic phosphates, notably 2, 3-diphosphoglycerate, influence the affinity of hemoglobin for oxygen. A similar, but less pronounced, effect is observed for inorganic phosphates. The co-operativity of ligand binding of 2.8 in normal human adult hemoglobin, as measured by the n of Hill, is independent of pH between 5.5 and 9. When measured in borate buffer (0.1–0.4 M) alone, no change of cooperativity was observed between pH 7.5 and 9. However, in a mixture of 0.2 M borate and 0.1 M phosphate the values of n increased to 3.25 at pH 8.25 in the case of 'stripped hemoglobin'. Concomitantly, the log pO_2 ($^1/_2$) increased from 0.12 to 0.35. Hemoglobin not freed of intracellular 2, 3-DPG exhibits, under the above conditions, an n value of 3.45. In this case the log pO_2 ($^1/_2$) increased from 0.18 to 0.4. Maximum effect was observed at 1.5×10^3 molar excess of inorganic phosphate. At this ratio the effect of 2, 3-DPG was additive. It thus appears that organic and inorganic phosphate have distinct binding sites and that the latter, in view of the sharp pH dependence, interacts with only one kind of amino acid residue.

Hybrid-Labeling of Paternal DNA in Mouse Zygotes

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Specific labeling of the paternal part of the genome in fertilized mouse ova has been obtained. Germinal cells of males were exposed to tritiated thymidine during the last rounds of premeiotic DNA-synthesis. Matings with unlabeled superovulated females were performed on the day of peak production of labeled sperm. Zygotes were recovered from oviducts at early stages of cleavage (pronuclei to 16 cells). Nuclei were observed on autoradiographs of air-dried whole cells. Labeled metaphases have been obtained which show a straight mendelian 1/1 distribution of labeled and unlabeled chromosomes, with evidence of semiconservative replication through isolabeling of chromatids as well as of sister-strand somatic exchanges. Interphase nuclei demonstrate a non-random distribution of the paternal material. This preferential localization at interphase of part of the genome originating from either parent is confirmed for labeled prophases.

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Regulation of Acetate Metabolism in *Neurospora crassa*

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A study of the acetate pathway in *Neurospora* was based on the separation of the glyoxysome-like particles (GLPs) from the mitochondria, and the determination of their respective enzyme complements. The GLPs carry two of the glyoxylate cycle enzymes, but the lack of malate dehydrogenase and citrate synthetase precludes the glyoxylate cycle from operating within the GLPs. In order to ascertain whether a limiting step of the Krebs cycle might accumulate the substrate (s) required for gluconeogenesis, the oxidative activity of mitochondrial preparations was measured. With acetate present in the growth medium, the rate of oxidation of all the intermediates tested increases markedly. However, the oxidation of isocitrate is elicited much more than that of α-KG. This imbalance results in an in vivo accumulation of α-KG, as evidenced by ion-exchange chromatography, TLC and enzymatic analysis. Since the GLPs contain a NADP-dependent isocitrate dehydrogenase coupled with the isocitrate lyase and malate synthetase, it is suggested that part of the α-KG produced by the mitochondria might be driven to malate via a reversal of the dehydrogenase step taking place in the GLPs.

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Molecular Weight Determination of Sendai RNA by DMSO Gradient Sedimentation

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The molecular weight of the large RNA of Sendai virus has been determined by sedimentation analysis in sucrose gradients containing 99% DMSO to be 2.3×10^6 daltons. Since Sendai RNA which was recovered from a DMSO gradient by ethanol precipitation was found to co-sediment in ordinary sucrose gradients with non denatured Sendai RNA it is unlikely that this lower molecular weight determination was due to fragmentation of the Sendai genome. The value of 2.3×10^6 daltons is considerably smaller than the estimates of 6.7×10^6 daltons determined under non denaturing conditions (Barry and Bukrinskaya, J. Gen. Virol. *2*, 71, 1968) suggesting a unique structure for Sendai RNA.

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In vitro Morphogenesis of the Tail of Bacteriophage λ

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The morphogenesis of the tail of bacteriophage λ is controlled by 11 genes, called Z, U, V, G, T, H, M, L, K, I and J (Parkinson 1968). With exception of conditional lethal mutants in gene U, which produce 'polytails' under nonpermissive conditions, no visible structures were found in defective lysates of tail mutants (Kemp et al. 1968). In vitro complementation studies between different tail mutants were, as far, unsuccessful (Weigle 1966, Parkinson 1968).

By studying nonsense mutants in tail genes U, V and J the following results were obtained:

1. Concentrated lysates of mutants in genes U, V and J can complement each other with high efficiency.
2. In vitro complementation between mutants in genes U and V is about 100 times less efficient than between mutants in genes U and J or V and J – suggesting that polytails are abortive and cannot be reutilised for in vitro morphogenesis of λ tail.
3. In sucrose gradients slowly sedimenting precursors are found which act very efficiently as donors of gene products U, V and J for in vitro complementation.

Radiation Effects on Molecules in Aqueous Solution as Detected by Spin Trapping

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Spin Trapping is a recently developed method for the detection and identification of low concentrations of free radicals in reacting systems. By addition of a special type of scavenger products are formed which are detectable by ordinary esr spectroscopy. It is shown in our laboratory, that certain short living intermediates which are produced by ionizing radiation can be trapped successfully. After irradiation (100 kV Röntgenrays, 225,000 R) of dilute aqueous solutions of Glycine in the presence of 2-Methyl-2-nitrosopropane as spin trap and strict exclusion of Oxygen the following radicals could be identified: $\dot{C}H_2COOH$ dominant in neutral, $NH_2 \cdot \dot{C}H\text{–}COO^{\ominus}$ in alcaline solution. Other spin adducts were present, partly to be associated with radiolysis products of the spin trap. The intensities are rather poor, they correspond to very low G-values.

In some cases maximum heigth of signal is reached only some time after irradiation.

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The Control of Aldolase Isoenzyme Levels in Chick Brain

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We determined the roles of synthesis and degradation in the control of the levels of aldolase tetramers and subunits in chick brain. The proportions of tetramers are: C_4 (81%), AC_3 (17%), A_2C_2 (2%), reflecting random combination of A (5%) and C (95%) subunits from a single subunit pool. We isolated the two predominant activities and measured their rates of intracellular degradation with the aid of radioisotopes. The rate of decline of specific radioactivity was used to calculate an apparent half-life of about 4 days for the AC_3 heterotetramer. The double-labelling technique was then used to measure the relative rates of degradation of the homo- and heterotetramers. The $^{14}C/^{3}H$ ratios observed were the same as those predicted for enzymes with identical half-lives. The relative rates of degradation of A and C subunits isolated from the AC_3 hybrid were also determined. Different aldolase tetramers and subunits have very similar rate constants for degradation. Thus, the highly specific aldolase patterns observed in vertebrate tissues may result predominantly from differential subunit synthesis.

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ATP Mediated Subunit Exchange between Triose-PO_4 Dehydrogenase Isoenzymes

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Recently, Deal and others have demonstrated a marked effect of adenine nucleotides on the structures of yeast and rabbit muscle triose-PO_4 dehydrogenases (TDH). Unlike most higher vertebrates, trout tissues contain isoenzymic forms of TDH. Several of these activities have been partially purified from trout muscle. Heteromeric forms of trout TDH, when incubated with physiological concentrations of ATP (1 to 10 m*M*) at 0 to 23 °C, readily generate 'new' isoenzymes. This ATP effect was completely prevented by the coenzymes NAD and NADH. The present observations suggest that subunit exchange between TDH tetramers may occur in vivo and, if so, that the exchange processes are highly dependent on the intracellular environment. These findings are in contrast to those on aldolase isoenzymes which appear to have very stable quaternary structures. The implications of in vivo subunit exchange, or lack of it, will be discussed in relation to the possible mechanisms of construction and destruction of isoenzymes and their subunits within the cell.

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In vitro Synthesis of SV40 and Polyoma Proteins

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E. coli RNA polymerase was used to prepare complementary RNA from SV40 and polyoma form I DNA's. The RNA was 90 per cent asymmetric as judged by ribonuclease resistance after annealing. The RNA stimulated amino acid incorporation up to ten fold over background in a high efficient mammalian cell free protein synthesis system.

^{35}S-methionine labeled product was analyzed on polyacrylamide slab gels. The largest portion of the incorporation was into polypeptides less than 10,000 *MW*, but there are distinct bands of 50,000; 27,000; 23,000 and 19,000 *MW* for SV40 and 20,000, 15,000 and sometimes 33,000 *MW* for polyoma.

A tryptic fingerprint analysis of the product is in progress.

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Non Equivalence of Chains in Hemoglobin Oxidation

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Auto-oxidation of Hb A at pH 7.2 and 37 °C was shown to be biphasic. The fast and a slow phase differ in rate by a factor of 10. By a special rapid chain separation technique it was shown that the rapidly oxidizing components are the α-chains ($\varphi = 0.035\ h^{-1}$), whereas the β-chains oxidize more slowly ($\sigma = 0.004\ h^{-1}$). Lowering the oxygen pressure to about $^1/_3$ of atmospheric O_2, pressure hastens the rate of the oxidation reaction for both chains but more so for the β than the α. This observation indicates that in oxyhemoglobin α-chains have a higher affinity for O_2 than β-chains. The chemical oxidation (by $K_3[Fe(CN)_6]$) of non-liganded Hb is about 80 times more rapid than that of the liganded form. If a partially liganded hemoglobin (say 40% oxygenated) is oxidized by sufficient amounts of potassium ferricyanide to oxidize only 60% of the heme groups present, of course preferentially the non-liganded hemes will be oxidized in view of their much higher oxidation rate. In experiments of this nature β-chains were more oxidized than α-chains. We therefore conclude that in partially liganded hemoglobin the ligands are more bound to α-chains than to β-chains.

Evidence Suggesting that 'Early' Virus-Specific RNA May Contain Information Necessary for SV40-Induced Chromosome Replication and Mitosis

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SV40 induces in 'contact-inhibited' mouse kidney tissue culture cells an abortive infection which leads to the appearance of intranuclear SV40-specific tumor (T-)-antigen, followed by the replication of the mouse cell chromatin and mitosis while no viral progeny DNA or capsid protein are produced. Synthesis of 'early' SV40-specific RNA ('19S RNA') begins a few hours before the appearance of T-antigen and appears to be switched off soon after the onset of chromatin replication. As the most simple working hypothesis, which can account for the experimental results presently available, we assume that early SV40 RNA contains information necessary for the production of T-antigen and that the latter (or an as yet unknown early virus-specific function which would simply parallel the appearance of T-antigen) activates or de-inhibits a cellular regulatory element which governs chromosome replication and mitosis. The experimental results are in accordance with the idea that SV40 acts primarily as a mitogen.

Astrocyte Induced Morphological Differentiation in Neuroblastoma Cells

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Conditioned medium by astrocyte culture can induce the morphological differentiation of neuroblastoma cells in tissue culture. The factor secreted by the astrocytes can be obtained in a serum free medium, concentrated with a UM-10 or UM-20 E Diaflo membrane and tested in presence of 10% fresh serum. The morphological differentiation is linearly dependent upon the amount of factor used. It is not correlated with an increase in cyclic AMP level and, unlike other methods used to induce differentiation, it does not affect the growth rate of the neuroblastoma cells.

Chromosomal Deoxynucleoprotein during the Growth Cycle of Synchronised CHO Cells

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We are studying the chromosomal DNP of chinese hamster cells synchronised by mitotic selection. The buoyant density is CsCl (reflecting the protein/DNA ratio) of DNP prepared in low salt concentrations (R. Hancock, these abstracts) is essentially identical through the S phase and in G2. In metaphase, the buoyant density is slightly higher. The ratio of non-histone protein to DNA

is essentially the same in DNP from cells in metaphase and in interphase. Studies of the synthesis and turnover of the non-histone proteins during the growth cycle will be presented.

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Free and Membrane-Bound Polysomes in 3T3 and Py3T3 Cells

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Investigations carried out in several laboratories indicate that free polysomes differ in their function from membrane-bound polysomes and that normal cells contain considerably higher amounts of polysomes attached to the endoplasmic reticulum than their transformed counterparts. Previous work from this laboratory showed that defined changes in cell membranes occur when 3T3 cells are transformed by polyoma virus. Therefore, in the current study we investigated whether these membrane changes are accompanied by and correlated to changes in the pattern of free and membrane-bound polysomes.

A system was established for quantitative separation and analysis of free and membrane-bound polysomes. The amount of free and membrane-bound polysomes in 3T3 and Py 3T3 cells was determined at one day intervals during the growth cycle until two days after the cell density had reached saturation. Rather unexpectedly, the results show no significant difference in the ratio of free to membrane-bound polysomes between normal and transformed cells. Hence, it is tentatively concluded that in this system transformation is not connected with a mechanism which first alters the amount of polysomes attached to the endoplasmic reticulum. To what degree such a change is however required for the expression of the transformed state remains to be investigated.

The Sodium-Dependence of Calcium Efflux from the Neurohypophysis

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Hormone release from the neurohypophysis is dependent on the influx of Ca^{2+}, whose intracellular level thus rises temporarily. In order for the neurosecretory axons to maintain a large, inwardly-directed concentration gradient for Ca^{2+}, calcium which has entered the cells during depolarization must ultimately be pumped out. We have loaded isolated rat neurohypophyses with ^{45}Ca, and have studied its extrusion. After a 2 h washout period, the fractional rate of loss of ^{45}Ca stabilizes at about 0.005 per min. Calcium efflux does not seem to depend directly on the availability of energy-rich phosphate compounds, since it has a low $Q_{10} \sim 2$, and since efflux is not reduced but rather increased in the presence of cyanide and dinitrophenol. In contrast, Ca efflux is markedly reduced when the external Na concentration is lowered. At least 40% of the Ca efflux was found to be Na-dependent, in the presence as well as in the absence o external Ca. Thus, as in squid axon (Blaustein and Hodgkin, J. Physiol. Lond. *200*, 497–528, 1969), Ca^{2+} appears to be expelled from the cells in exchange for external Na^+.

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Structural Basis for Intercellular Communications between Cells of the Islets of Langerhans

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The remarkable secretory coordination between islet cells in their critical roles of regulation of fuel homeostasis is compatible with an intercellular system which guides the responses of individual cells in a manner appropriate to the net secretory response of the entire endocrine organ.

In a variety of tissues, gap junctions seem to mediate intercellular communications (ionic and metabolic coupling). To determine whether such specialized pathways exist between islet cells, we have made an electron microscopic study of replicas of freeze-fractured islets of rats, mice and Chinese hamsters. The freeze-fracture technique allowed the identification of intermembrane differentiations characteristic of the gap junctions (plaque-like aggregation of 85 Å membrane particles) and has helped to unmask a frequent close association of minute gap junctions with the linear branching and anastomosing elements of focal tight junctions. The demonstration of such membrane specializations in islet cells provides a structural basis for coupling capabilities of these cells. It is possible that some of the flow of informations which leads to the initiation and maintenance and/or cessation of secretory activity could occur by way of these intercellular communications.

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Quantitative Determination and Location of Newly Synthesized Virus-Specific RNA in Chicken Cells Infected with Rous Sarcoma Virus

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A sensitive and quantitative nucleic acid hybridization assay for the detection of radioactively labeled avian tumor virus-specific RNA in infected chicken cells has been developed. In our experiments we have made use of the fact that DNA synthesized by virions of avian myeloblastosis virus in the presence of actinomycin D (AMV DNA) is complementary to at least 35% of the sequences of 70S RNA from the Schmidt-Ruppin strain of Rous sarcoma virus (SRV). Annealing of radioactive RNA (either SRV RNA or RNA extensively purified from SRV-infected chicken cells) with AMV DNA followed by ribonuclease digestion and Sephadex chromatography yielded products which were characterized as avian tumor virus-specific RNA-DNA hybrids. The amount of viral RNA synthesized during pulse labeling with ^3H-uridine could be quantitated by the addition of an internal standard consisting of ^{32}P-labeled SRV RNA. This quantitative assay was used to determine that, in SRV-infected chicken cells labeled for increasing lengths of time with ^3H-uridine, labeled viral RNA appeared first in a nuclear fraction then in a cytoplasmic fraction, and still later in mature virions.

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DNA Dependent-RNA Polymerase of *Neurospora crassa*

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DNA dependent RNA polymerase from vegetative hyphae of wild type *Neurospora crassa* has been solubilized and partially purified. The enzymes can be separated by DEAE cellulose chromatography. The mixed enzymes are inhibited by KCl concentrations above 0.04 *M*. α-amanitin insensitive (up to 50 μg/ml) fraction is stimulated by NaCl up to 00.7 *M* and NH_4Cl up to 00.5 *M*; higher concentrations of both being inhibitory. Ammonium sulphate above 0.3 *M* incorporated in the assay mixture partially inhibits the enzyme activity. The enzyme shows dependence on added template and transcribes both salmon sperm and calf thymus DNAs.

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Photooxidation of L-Rhamnulose 1-Phosphate Aldolase from *Escherichia coli*

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L-rhamnulose 1-phosphate aldolase (I) of *Escherichia coli* consists of four identical subunits and two g-atoms Zn/mole. Exposure of I to light in the presence of air and rose bengal resulted in irreversible loss of enzyme activity concomitant with destruction of a number of the histidine residues of the molecule. The pH profile of the inactivation followed the titration curve of histidine. The reaction of all twelve sulfhydryl groups of I with $HgCl_2$ yielded an inactive enzyme (II), which could be completely reactivated by 2-mercaptoethanol. II was photooxidized as described; enzymic activity and the amino acid composition of the protein were determined after treatment with 2-mercaptoethanol. The rate of activity loss was essentially linear until 50% of initial activity remained, at which time four of the original twenty histidine residues, but no other amino acids, had been destroyed. Subsequent activity loss proceeded at a slower rate. The substrates L-rhamnulose 1-phosphate and DHAP, as well as the competitive inhibitor, L-rhamnitol 1-phosphate partially protected II from photooxidation. The substrate analogue, fructose 1-phosphate, did not protect. The enzyme retained its full complement of zinc upon photooxidation. The data suggest that while histidine is involved in the catalytic activity of I, the catalytically active histidine residues do not bind zinc. Furthermore, the sulfhydryl groups likewise have no role in zinc binding.

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A Mutant of *Escherichia coli* which Blocks the Injection of Phage λ DNA

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We have isolated a new mutant of *E. coli*, called *pel*$^-$, which blocks the growth of phage λ. From rare λ plaques formed on *pel*$^-$ hosts, one can isolate λ mutants (called λ *hp*) which regain plaque forming ability. Some λ *hp* are temperature sensitive in growth on *pel*$^+$ and *pel*$^-$ hosts. Mapping experiments place the λ *hp* mutations in the tail gene cluster of the λ chromosome.

When λ infects a *pel*$^-$ host, adsorption is normal but about 5% of the cells either produce phage with a normal burst size or become lysogenic. The cells which do not produce phage are not killed by infection. Double infection of *pel*$^-$ with λ and λ *hp* does not enable λ to grow, thus the defect in λ growth cannot be complemented in trans. After induction of λ from *pel*$^-$ lysogens the phage grow normally, in contrast to their poor growth after infection.

One explanation for these results is that λ fails to inject its DNA into *pel*$^-$ bacteria. Direct biochemical tests confirm this hypothesis. In addition, electron micrographs of λ infected cells show that *pel*$^+$ cells have empty phage particles attached to the surface but that *pel*$^-$ cells have mainly phage whose heads still contain DNA attached.

Ultrastructural Changes in Kupffer Cells of Rat Liver Induced by Streptozotocin

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Streptozotocin (N-methyl-N-nitrosoglucosamin), an oncolytic and diabetogenic (B-cytotoxic) drug, is also known to induce tumors after prolonged administration. We studied its effects on the ultrastructure of rat liver. Four weeks after a single injection (35 mg/kg, i.v.), many Kupffer cells appear filled with numerous cytoplasmic vacuoles of varying size containing pale and finely granular material. These vacuoles, which tend to be confluent, resemble those found in mucopolysaccharide storage diseases. Several months (3–12) after a single injection, similar alterations occur in almost every Kupffer cell. Streptozotocin seems therefore to be an useful tool in establishing an experimental model for the study of mononuclear phagocyte 'reactivity'.

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Isolation of Two Nucleoprotein Complexes Involved in Polyoma Viral DNA Replication

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In primary mouse kidney cells lytically infected with Polyoma virus, the viral DNA replicates in close association with the host cell chromatin. From there, two virus specific nucleoprotein complexes could be dissociated: Complex A, sedimenting at approximately 105 S, and complex B, sedimenting very homogeneously at approximately 75 S. Both complexes band, after fixation with formaldehyde, at a density of 1.48 g/cm^3 in CsCl.

Analysis of the DNA from the two complexes by sedimentation, ethidium-bromide CsCl gradients and hybridization showed that complex A contains exclusively viral DNA, predominantely in its replicating form, while complex B contains the newly replicated, superhelical viral DNA molecules.

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Studies of a Deoxynucleoprotein Complex in Cells Replicating SV40 Virus

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Cells replicating SV40 virus contain viral DNA as a 44S deoxynucleoprotein complex (Eason and White, J. Virol. *8*, 363, 1971). We have used formaldehyde fixation (Hancock, J. Mol. Biol. *48*, 357, 1970) to study this complex, using CsCl equilibrium gradients to estimate the protein/DNA ratio. The complex contains a major component (> 95%) with a protein/DNA ratio of about 0.9, and a minor component (> 5%) with a ratio of 0.05.

With increasing ionic strength, conformational changes in the complex are detected below 0.6 *M* NaCl; above this concentration the protein/DNA ratio decreases. Certain temperature sensitive SV40 mutants produce complexes of lower protein/DNA ratio at the non-permissive temperature.

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A Method of Determining Rates of DNA Synthesis in Cell Cultures

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In cultures of a murine mastocytoma (cell line P-815-X2), endogenous synthesis of thymidine phosphates, as determined by the incorporation of ^{3}H-deoxy-uridineinto DNA, was reduced within 10 min to less than 2.5% of control values by the addition of amethopterin (0.01 m*M*) in combination with hypoxanthine and glycine. If ^{3}H-thymidine and unlabeled thymidine were added simultaneously with amethopterin, the increase with time of radioactivity in cellular DNA, after a lag period of 15 to 30 min, was linear for at least 1 h, indicating that during this time interval intracellular thymine nucleotides had the same specific activity as exogeneously supplied ^{3}H-thymidine. This permitted calculation of the amount of thymidine incorporated per min into 10^6 cells. In conjunction with previously reported data on the base composition of mouse DNA, these results were used to calculate rates of DNA synthesis. Rates of DNA synthesis thus obtained were within 10% of those derived from the DNA content of diploid mouse cells and from measured cell multiplication rates.

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Electron Microscopy of Potato Spindle Tuber Viroid

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The term 'viroid' has been introduced to denote a novel class of subviral agents which are characterized by the apparent absence of a dormant phase (virions) and by genomes that are much smaller than those of known viruses (Diener 1971). In spite of the small amount of genetic information that viroids introduce into their hosts, they are able to replicate and to incite disease in certain organisms.

Since essentially pure potato spindle tuber viroid (PSTV) is now available (Diener and Smith 1973), direct length measurements by electron microscopy appeared feasible. Four different main experiments were carried out using the protein monolayer spreading technique (Kleinschmidt and Zahn 1959):

From these data we conclude that PSTV is a double stranded RNA with a molecular weight in the order of 10^5 daltons.

T. O. Diener, Virology *45*, 411 (1971).
T. O. Diener and D. R. Smith, in press (1973).
A. K. Kleinschmidt and R. K. Zahn, Z. Naturforsch. *14b*, 770 (1959).

Immunogenicity of Enzyme Anti-Enzyme Specific Antibody Complexes

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The immunogenicity of specific antigen antibody complexes has been reported with various antigen systems. Immunoenzymological techniques allow the tracing of active enzyme anti-enzyme specific complexes and the detection of the newly-formed humoral and cellular anti-enzyme antibody. Horse radish peroxidase (HRP) anti-HRP complexes, prepared at equivalence or in antigen excess, are immunogenic and induce an early germinal center formation without detectable circulating antibody. These newly-formed germinal centers are characterized by a persisting deposition of the enzymatic HRP activity within the dendritic intercellular network. HRP anti-HRP complexes induce with time the appearance of specifically sensitized cells which upon a second injection of fluid antigen will be the precursors of antibody-producing elements within the germinal centers and the medullary cords of the lymph nodes regional to the sites of injection. Large antigen excess conditions, as determined in vivo by the second fluid injection, may well be instrumental in the onset of the specific antibody production. The relative proportions of antigen and antibody, within the complexes at that germinal center strategic location may influence the temporal course of the specific immune response.

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Turnover of Nuclear Pre-mRNA in Duck Erythroblasts

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The nuclear pre-mRNA which has been shown to represent the precursors to cytoplasmic mRNAs' consist of a heterogeneous population of RNA species with sedimentation values from 20 to 80 S. The synthesis and decay of nuclear RNA of different size classes from duck erythroblasts have been investigated by electrophoretic analysis on polyacrylamide gels. Incorporation of ^{3}H-Uridine into the nuclear RNA reaches steady state after approximately 80 min. At this time 40 to 50% of the radioactivity is found in RNA species heavier than 45 S. During a cold uridine chase the RNA heavier than 45 S decays with a half life of the order of 30 min, whereas the smaller RNA species are more stable and may decay with

half lives of the order of 2 to 3 h; the longest half lives being observed in the smaller RNA species (S < 28) and representing approximately 15% of the total nuclear radioactivity. We conclude that there exist two principle pools of pre-mRNA: 1. The largest molecules representing the immediate transcription products, which break down as fast as they are synthesised with a half life of up to 30′. 2. A fraction of smaller (20–45 S) pre-mRNA which is relatively very stable and may constitute a nuclear reserve. No 9 S mRNA peak was observed.

Differential Translational Capacities of Duck and Rabbit Globin Messenger RNAs

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The relative abilities of purified 9 S RNAs from duck and rabbit reticulocytes to be translated into the corresponding globins was measured using a number of eukaryote cell free systems. In a lysate system from rabbit reticulocytes, the duck RNA was translated with very low efficiency whereas the rabbit RNA in a duck reticulocyte lysate was translated to such an extent that the translation of the endogenous duck messenger RNA was almost completely depressed. In a partially purified cell free system consisting of mouse liver ribosomes, rabbit reticulocyte initiation factors and rat liver pH 5 fraction (M. Schreier and T. Staehelin, J. Mol. Biol., in press), both duck and rabbit 9 S RNA when added alone were translated equally well. However, when both were added together in saturating amounts, only the rabbit messenger was translated. We would propose that our experiments indicate a basic difference between the relative abilities of duck and rabbit globin mRNA to be translated in cell free systems. We are at present continuing investigations to determine whether, as with rabbit α and β globin mRNAs (H. Lodisch and M. Jacobsen, J. Biol. Chem. *247*, 3622, 1972) there are also translational differences between the mRNAs which code for each of the 3 or 4 duck globins.

The Subunits of the Thermophilic Aminopeptidase I from *Bacillus stearothermophilus*

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The thermophilic aminopeptidase I (API_{Th}) from *Bacillus stearothermophilus* contains two different subunit types (α, β), which can combine in different ratios. Enzymes with α/β ratios of 1, 2 and 5 are found in the bacillus extract. In addition an aminopeptidase composed only of α subunits can be prepared. Up to now we never were able to reactivate an aminopeptidase containing only β subunits.

The α subunit is obviously responsible for the degradation of peptides with aminoterminal lipophilic amino-acids. Peptides with an aminoterminal asp or glu are also rapidly degraded by aminopeptidase I but not by the α subunit enzyme. The hydrolysis rate of peptides like asp gly is proportional to the β subunit content of the degrading enzyme.

Structural and kinetic investigations suggest that aminopeptidase I is a multienzyme complex composed of two subunit types with aminopeptidase activity but different specificity.

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The Specific Origin of the Proteins of Chromosomal Deoxynucleoprotein

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The origin of the histones and non-histone proteins of purified chromosomal deoxynucleoprotein (DNP) was examined using DNP prepared from a mixture of two populations of cells, one containing DNA distinguishable by a density label and the other containing radioactively-labelled proteins. DNP was prepared by a lysis procedure in which the cell membrane and nuclear envelope are instantaneously removed, so that each species of DNA was equally accessible to radioactive cellular proteins. Labelled proteins were associated exclusively with DNA of normal density, and not with dense DNA, and vice versa. The proteins associated with DNA in DNP prepared by this procedure were thus already specifically associated with DNA in vivo, and other proteins are not bound non-specifically to DNA during the preparation of DNP.

When a mixture of DNP molecules thus prepared is precipitated in 150 m*M* NaCl and redissolved, some labelled histones migrate onto other (dense) DNA molecules. This migration may occur via the formation of intermolecular aggregates between the 2 species of DNP.

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Formation of Avian Oncornavirus Proteins: Identification of a High Molecular Weight Precursor

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Using antibodies prepared against disrupted virions of avian myeloblastosis virus (AMV) we have been able to precipitate and identify, on SDS-polyacrylamide gels, labelled viral polypeptides from AMV-infected cells in tissue culture. After a ten minute pulse a non-virion protein with a molecular weight of 75,000 daltons is labelled while relatively little radioactivity is incorporated into virion proteins. After a 60 minute incubation in non-radioactive medium, however, radioactivity appears to be chased from the 75,000 molecular weight protein into the virion proteins. We observe the same pulse-chase pattern in Rous sarcoma virus transformed cultures.

Trypsin digestion of the isolated S^{35}-methionine labelled 75,000 mw protein gives the same fingerprint pattern as the tryptic peptides of S^{35}-methionine labelled AMV virions.

These results imply that the 75,000 mw protein is a precursor to avian oncornavirus proteins.

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