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PHYSIOLOGIE – PHYSIOLOGY

Increase in Phosphate Efflux by SH Reagents in Rabbit Vagus Nerve

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A Na-dependent ortho-phosphate transport system has recently been described in mammalian nerve fibres (Anner et al., J. Physiol. *232*, 47P, 1973). Using the same method for continuously recording phosphate fluxes, the effect of various SH reagents (p-chloromercuribenzoic sulfonic acid, N-ethylmaleimide, ethacrynic acid) has now been studied. These agents, applied to fibres loaded with labelled phosphate, were found to produce a dramatic increase in phosphate efflux; the effect, which was not due to a block of the phosphate influx, developed within 1–2 min after adding the agents at concentrations of 5 m*M*; with lower concentrations, the increase in efflux was somewhat delayed. Washing with Locke showed that the effect was irreversible; further, it could not be reversed by cysteine, dimercaprol, or dimercaptoethanol. The increased efflux was independent of extracellular phosphate (0.2–4 m*M*) and was strongly decreased by cooling from 37 to 10 °C, indicating that it was not due to an increase in passive leakage, but rather to a specific effect on SH groups of the phosphate transport system.

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A pressure Device for Intracellular Injection

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A pressure device has been developed which permits the injection of ionic and non-ionic substances into single cells, and allows continuous recording of the membrane potential of the impaled cell during injection. For injection of non-ionic substances, a double micropipette is used. One barrel is filled with the solution to be injected and connected to a sealed heating pipe. Upon heating, the solution is made to expand and is forced through the tip of the pipette. The second barrel is filled with 3*M* KCl to record the electrical activity of the impaled cell. A single micropipette connected to the heating device can be used when ionic substances are injected. Filled with 3*M* KCl, the micropipettes have a resistance of approximately 30 MΩ.

A further advantage of the pressure device is that the injection process itself does not modify the membrane potential, which therefore can be monitored during the injection.

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Extraction et Dosage biologique de l'Acétylcholine

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Pour doser biologiquement l'Acétylcholine totale extraite d'un seul ganglion sympathique supérieur cervical du rat, nous utilisons une sangsue commune dans le Léman (*Haemopis Sanguisuga*). Sa musculature plus puissante que celle d'*Hirudo Medicinalis* autorise l'emploi d'un transducteur isométrique qui permet une décontraction rapide du muscle et une ligne de base stable.

Le dosage est réalisé dans un microbain à perifusion continue et à injection automatique. Sensibilité maximale: 0,13 pmole d'Acétylcholine. L'extraction est faite selon une méthode, modifiée de Hebb et Whittaker.

Un ganglion fraîchement excisé contient 0,143 nanomoles d'acétylcholine. S'il est incubé dans du Krebs cette quantité augmente de 70% alors que, dans du Krebs sans glucose, elle diminue au-dessous de la limite de sensibilité de la méthode.

Crédit FNRS n° 3.457.70

Firefly Luciferase Purification and ATP Measurements

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In order to increase the specificity of ATP measurements in the presence of other nucleotides, after extraction from the superior cervical ganglion of the rat [F. Mir, M. Dolivo, S. Landolt, Experientia *28* (1972), 730], the crude firefly extract (FFT, Sigma) has been passed successively through Sephadex-G-10 and DEAE-Sephadex columns.

Sephadex-G-10 allows the isolation of luciferin [R. Nielsen and H. Rassmussen. Acta Chem. Scand. *22* (1968) 1757]. Mainly ATP, adenylate kinase, nucleoside diphosphate kinase and pyrophosphatase are separated from luciferase by DEAE-Sephadex.

The activity of the purified luciferase per gram of firefly tails (FFT, Sigma) equals the activity of the commercial crude soluble extract (FLE-50, Sigma).

Specificity has been verified by the measurement of ATP standard solutions, in presence of other nucleotides. ATP extracted from the superior cervical ganglion of the rat has been measured in parallel with the purified and the crude extract.

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Differential Effect of Illumination on Sleep Stages in the Rat

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In a previous study (Experientia *29*, 761, 1973, and paper submitted for publication) we have shown that the rat's motor and consumatory behavior is entrained by short light-dark (LD) cycles. In this study we recorded continuously by telemetry cortical EEG and neck muscle activity to study the effect of illumination on sleep. After a one-week control period, the animals were exposed to one-hour LD cycles for 1–2 weeks. Sleep stages and waking were scored for epochs of 24 seconds according to conventional criteria. During the light periods the rats exhibited significantly more slow-wave sleep than during the dark periods, whereas this differential effect was not evident for paradoxical sleep. Thus illumination schedules may be useful for dissociating the two sleep stages to study the underlying mechanisms.

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The Chromaffine Cells of the Cervical Ganglion of the Rat: Function Related to Structure

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In the superior cervical ganglion of the rat the chromaffine cells are mainly grouped around capillaries, fenestrated only in their immediate vicinity. These chromaffine cells contain typical adrenergic granules (800–1500 Å); cholinergic fibers make synapses on their soma. The functional role of these cells has never been really demonstrated.

In order to modify the storage of adrenergic material in the cells, rats have been injected i.p. with various drugs. After 3 days of treatment the sympathetic ganglia were excised and the excitability of the postsynaptic neurons has been tested in vitro by measuring the homosynaptic facilitation. The excitability was increased after the administration of drugs known for depleating the catecholamine storage and decreased after drugs that are increasing the catecholamine storage. The shape, size, and density of the granules of the chromaffine cells as measured by electronmicroscopy could not always be correlated to the changes in excitability.

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Determination of Mean Body Temperature Changes by Direct and Indirect Calorimetry During Exercise in Man

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Heat storage rate ($\dot{S}_m$) was measured continually by using both direct and indirect calorimetry in 10 subjects during exercise, at 2 intensities (40 and 90 W), and at 3 ambient temperatures (T_a: 20, 25 and 30 °C). At rest, $\dot{S}_m$ was negative at a T_a of 20 and 25 °C (−64.9 W and −25.1 W respectively), and slightly positive (+7.3 W) at 30 °C. After a 50 min exercise period $\dot{S}_m$ tends towards zero in the 3 ambient conditions: this shows that a precise balance between heat production and heat losses is reached, independently of T_a, whereas this equilibrium does not exist at rest. In addition, the energy stored during 50 min (corresponding to a change in mean body temperature: $\Delta \overline{T}_b$) was similar in the 3 environments. During thermal non-steady states, $\Delta \overline{T}_b$ obtained by thermometry (tympanic and skin temperatures measurements) was delayed by about 5 min in comparison with the values obtained by calorimetry.

These results show that during exercise (1) $\Delta \overline{T}_b$ depends on the work load and not on ambient conditions between 20 and 30 °C; (2) $\dot{S}_m$ tends towards zero after 50 min of a moderate exercise (40 and 90 W); (3) Calculation of $\dot{S}_m$ by thermometry is not applicable during thermal non-steady states, without taking into account the delay shown in this study.

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Potentiation of Brown Adipose Tissue Calorigenesis by Inhibitors of Hormone Binding

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The calorigenic response of rat brown adipose tissue (BAT) fragments to norepinephrine (NE) was measured during long periods of perifusion in a thermic flux differential microcalorimeter. The response proved to be a steady state one, or at least to end up as such after an initial transitory phase. Substances like Dopa or vitamin C at concentrations which have been shown to largely prevent NE from binding to microsomes obtained from BAT (Giacobino and Girardier, this meeting) induced small calorigenic responses when administered alone. When either of these drugs was added to the perifusion medium in combination with NE, the steady state calorigenic response was about eight times as large as the sum of the effects obtained by separate applications of the drug and the hormone. This potentiation effect suggests that Dopa and vitamin C may act as competitors of NE at sites where this hormone either has an inhibitory effect upon calorigenesis, or is destroyed. Nerve endings, although present and apparently still able to take up norepinephrine in our whole tissue preparations (Seydoux and Girardier, this meeting), do not appear to be involved in the potentiation mechanism.

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Relationship between ATP Content and ATPase Activity in Vertebrate Nervous Tissues

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The total ATPase activities and the ATP level in tissues of different origin were compared. The effect of Mg, Ca, K and ouabain on ATPase activity was measured. Vagus rabbit nerve, cervical sympathetic ganglia and neurohypophysis of rat, electric organ and electric lobe of the Torpedo were used for the experiments. All of these tissues are of nervous origin, except for the electric organ of Torpedo whose electroplaques are homologues of muscle cells and are covered by a great number of cholinergic nerve terminals. The results showed a high level of ATPase and ATP in all tissues studied, the highest level of Na-K-Mg activated ATPase being found in the electric organ of the Torpedo and the lowest in vagus rabbit nerve. Mg was necessary for the enzyme activity. Ca enhanced ATPase activity in vagus nerves and inhibited it in the

other tissues. Since the ratio of ATPase ATP was almost constant in all tissues studied, 4 sec were sufficient, under optimal conditions, to split their ATP content. This indicates that the turnover of ATP in these tissues is much more rapid than in non-nervous tissues such as liver.

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Specific Hormonal Regulation by Food of the Pancreatic Enzymatic Secretion

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Several hormones of the intestinal mucosa have been described to act on the pancreas; little is known of their regulation. In order to study it, the following experiment was carried out: an oral glucose or Nesmida (protein hydrolysate) load was given to a first series of rats; the duodenal mucosa was removed and extracted in control rats and at several times after the oral load. These extracts were then injected into the coeliac trunk of a second series of rats, while collecting the pancreatic juice.

The mucosae extracts removed 45 min after Nesmida load induced a trypsin stimulation (1069.2 $\pm$ 63.9 µg/ml) which was significantly different from control rats (318.4 $\pm$ 55.6 µg/ml), while amylase concentrations did not differ from control rats. With glucose, the extracts removed 30 min afterwards elicited an amylase stimulation (3.43 $\pm$ 0.34 mg/ml), whole trypsin concentrations did not differ from control rats.

This study suggests that food promotes a rapid activation or synthesis of duodenal hormones which, in turn, stimulate the release of specific pancreatic enzymes.

Elasticity of Muscles in Contraction and Rigor

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The series elastic stiffness of muscle is largely located within the crossbridges and it depends on the number of bridges attached to actin at any one moment (Huxley and Simmons 1971). In rigor all bridges are presumably attached (Ready, Holmes and Tregear 1965; Huxley and Brown 1967). The ratio of stiffness between fibres in contraction and rigor should indicate the fraction of bridges attached to actin at anyone moment during contraction. This ratio was found to be approximately one to two in the following way: glycerinated fibres of rabbit psoas or tortoise iliotibialis were suspended in ATPsalt-solution (contraction) or in ATPfree-salt-solution (rigor). The fibres were then stretched and released by up to 1% within 1.1 msec by means of a ling-dynamics 101 vibrator which was controlled by a displacement and velocity dependent servo-amplifier and tension changes were recorded by means of a RCA-5734-transducer (resonance frequency 3500 Hz).

Respiratory Effects Caused by Intermittent Bulbar Stimulation in the Rabbit

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Spirogram and diaphragmatic activity were examined during electrical stimulation of regions of the medulla oblongata. One volley of repetitive stimuli per breath was applied. The volleys were triggered by the animal's own respiration. During inspiration volleys of 120 msec duration and 100 impulses per second caused and immediate and transient inhibition of the diaphragmatic activity. After the end of the volley, the inspiration continued. A rebound appeared: the tidal volume was increased. The inspiration was prolonged. The stimulation effects persisted when respiration was activated by an additional dead space or was partially inactivated by artificial hyperventilation or by inducing a metabolic alkalosis. The histological verifcation showed that inspiratory inhibition and rebound activation could be elicited from a relatively widespread area in the medulla oblongata. With afferent vagal stimulation with similar volleys, essentially the same effects were observed. Results suggest that the inspiratory rebound may be due to a delay in the activation by the respiratory centre of the central inhibitory feedback mechanism.

Angiotensin II in the Subfornical Organ (SFO)

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Recent investigations suggest that angiotensin II applied to the SFO surface elicits short-latency drinking behaviour (Simpson and Routtenberg, Science *181*, 1172, 1973). Therefore, we have studied the action of angiotensin II on single SFO unit discharges by applying this peptide with the aid of microelectrophoretic techniques. Angiotensin II produces a rapid acceleration of the discharge rate with a gradual recovery after withdrawal of the ejecting currents. The results of similarly applied acetylcholine were in agreement with earlier findings (Akert and Steiner, Current Research Neurosciences, Vol. 10, 1–14, Karger 1970), showing an increase in firing frequencies, which could be blocked by atropine sulphate. In contrast to these activating drugs, bradykinin, an angiotensin-like peptide, failed to show any alteration. The findings obtained with angiotensin II are consistent with the behavioural effects and call attention to the SFO as an important neural structure involved in the fluid control of the organism.

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Binding of Norepinephrine to Specific Sites in Brown Adipose Tissue (BAT)

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The interaction between norepinephrine (NE) and brown adipose tissue (BAT) cell membranes was studied. *In vitro* experiments showed that 7 ^{14}C-DL-norepinephrine binds with isolated BAT microsomes. The results obtained with specific inhibitors of neuronal uptake (desipramine; reserpine) and with microsomes isolated from denervated BAT or from isolated brwon fat cells show that the bulk of NE binding sites present in microsomal membranes have their origin in the brown adipocyte membranes. Studies with several structural analogues and Vit C determine the specificity of the binding sites. The following observations led us to conclude that the catechol binding sites (CBS) are not the β receptors: (a) the order of binding affinities of the structural analogues is: dopa = dopamine > epinephrine = isoproterenol; (b) NE binding to the CBS is not inhibited by propranolol; (c) D-NE and

L-NE have the same affinity for CBS; (d) an overload of dopa, sufficient to inhibit binding of NE to the CBS by 70%, unchanges the adenylate cyclase stimulation by a submaximal dose of NE.

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The Hydrosmotic Effect of Vasopressin: a Scanning Electron Microscope (SEM) Study

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SEM offers a particularly suitable technique for studying the surface topography of epithelial membranes. This is a report of experiments in which SEM has been used to investigate vasopressin-induced alterations in the luminal membrane of urinary bladders of toads *Bufo marinus*. Bladders were mounted in glass chambers and water fluxes were monitored with an optical method. Tissues were then fixed in 2% glutaraldehyde and processed for SEM. In control bladders, 3 types of cells were seen: large polygonal cells covered with blunt microvilli; smaller cells with long microvilli; and cells with a central orifice, occasionally showing a protruding mass of mucus. These correspond respectively to the granular, mitochondria-rich and goblet cells described with electron microscopy. Neither exposure of the bladders to a large osmotic gradient nor exposure to Pitressin in the absence of a gradient altered appreciably the epithelial surface. In contrast, the combination of Pitressin and an osmotic gradient resulted in a conspicuous diminution of the blunt microvilli of polygonal cells. However, the cells with long microvilli remained unchanged. These results suggest that the hydrosmotic effect of vasopressin is mainly exerted on the polygonal (granular) cells of toad bladder and that movement of water across their apical border is a prerequisite for the disappearance of the blunt microvilli.

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Renal Acidification after Removal of one Kidney

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In dogs during metabolic alkalosis, uninephrectomy induces a progressive increase in the fractional excretion of H_2O, Na and HCO_3 by the remaining kidney. Renal HCO_3 reabsorption being linked to hydrogen secretion in the urine, it is possible that this adaptation is effected via an inhibition of hydrogen transport by the tubular cells. The present study defines the response of the remaining kidney during metabolic acidosis. Moderate or severe metabolic acidosis was induced in 15 rabbits. Exclusion of one kidney induced an immediate and transient increase in urine flow and GFR in the remaining kidney. There was also a marked transient increase in K excretion. Na fractional excretion did not change significantly. In severe metabolic acidosis, there was no significant change in urine pH, the excretion of titratable acidity and of ammonium. In moderate acidosis induced by sodium phosphate, there was no change in urine pH or NH_4 excretion but a significant increase in the excretion of titratable acidity. This could be accounted for by the increased filtered load of phosphate buffer. The absence of adaptive compensation by the remaining kidney during metabolic acidosis sharply contrasts with the adaptation which is observed during metabolic alkalosis.

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Central Control of Isometric Finger Contraction in Primates

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Recordings from single cells in the precentral motor cortex of the Java monkey were obtained during the performance of a precision grip of the hand in which careful control of the force exerted between the thumb and index was required. Animals were trained to produce for various durations sustained isometric forces within a selected range by squeezing a hand-held strain gauge. At the end of each cortical penetration, microstimulation through the recording microelectrode was used to evoke discrete muscle contractions via the cortico-spinal pathway, and to identify the cortical regions under study. Stimulation of the dentate and interpositus nuclei of the cerebellum were used to verify whenever possible whether a recorded cell received afferents from either of these two nuclei.

Results show that even in the production of finely regulated forces between the fingers involves the participation of cells in the part of the motor cortex controlling proximal musculature, probably related to postural stabilization of the arm.

Preliminary evidence suggests at least two types of cortical unit discharge patterns: cells which only discharge in relation to changes in force output, and cells which discharge in relation to both changes in force output and maintained finger pressure.

Electrophysiological Analysis of Cerebello-Thalamocortical Relations to Paroxysmal Discharges in the Motor Cortex (Penicillin Focus)

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Simultaneous recordings from the motor cortex (MC), n. ventralis lateralis thalami (VL), and medial thalamic complex (MT) have revealed that the paroxysmal activities in the MC following topical application of penicillin are preceded by prominent paroxysmal activites in VL and MT in the succinylcholine paralyzed, unanesthetized, encephale isolé cat. Large amplitude positive and diphasic spikes (100–300 msec duration) in the MC, and simultaneously occurring prominent negative waves (of similar durations) in VL and MT, were preceded by large amplitude positive waves (100–120 msec duration) at both thalamic recording sites. The positive waves in VL were detectable 60–80 msec sooner than the take-off of the neocortical spikes. The positive waves in MT were initiated posterior to the onset of those in VL but prior to the onset of the spikes in MC. Thalamocortical paroxysmal activities induced by topical application of penicillin to the MC were attenuated during archicerebellar, but abolished during neocerebellar stimulation. The data show that paroxysmal discharges in the penicillin focus in the MC reflect underlying thalamic events. Synaptic mechanisms in VL are temporally closer coupled to the spikes in the penicillin focus than those in MT. The neocerebellar cortical and brachium conjunctivum evoked activities suppress or enhance, respectively, the spiking in the penicillin focus by their effects on the underlying synaptic mechanisms in the dorsal thalamus.

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Zentrale Unterbrechung des Lungenkollaps-Reflexes beim Meerschweinchen

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Im Asthmaanfall des Meerschweinchens tritt mit Beginn der bronchialen Widerstandserhöhung eine Atmungsbeschleunigung mit Zunahme des Lungenvolumens auf. Diese inspiratorische Reaktion konnte auf die Erregung von vagalen Lungenkollaps-Afferenzen zurückgeführt werden; dem zentralen Verlauf dieser Fasern galt die vorliegende Untersuchung. An 100 spontan atmenden, mit Urethan narkotisierten Meerschweinchen wurden mittels Hochfrequenz-Koagulation Läsionen im Mittelhirn, Pons oder Verlängertem Mark gesetzt. – Läsionen, die den Tractus solitarius und/oder dessen vagale Afferenzen betrafen, vermochten die zuvor nachgewiesene histaminbedingte Asthmareaktion ohne Beeinträchtigung der Spontanatmung zu unterdrücken. Der Frequenzeffekt der afferenten Vagusreizung konnte nach erfolgreicher Läsion noch nachgewiesen werden. Damit ist eine allfällige zentrale Vagotomie ausgeschlossen und ein weiterer Nachweis geliefert, dass die für das Asthma bronchiale typische Atmungsreaktion durch Vagusfasern vermittelt wird, die von jenen für die «Selbststeuerung der Atmung» verantwortlichen zu unterscheiden sind.

Lymphocyte Uptake and Deiodination of Thyroid Hormones in Thyroid Disease

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The percentages of uptake and deiodination of thyroxine-I^{131} (T_4) and triiodothyronine -I^{125} (T_3) by normal human lymphocytes, incubated with both hormones for 2 hours in a protein-free medium, were 6.7% ± 0.5 SEM and 9.2% ± 0.6 respectively for each uptake, and 13.9% ± 2.9 and 7.7% ± 1.4 for each deiodiantion. These two parameters have been examined with lymphocytes from hyperthyroid patients before and during treatment and from hypothyroid subjects. With lymphocytes from hyperthyroid patients before treatment both parameters were increased, the uptake of T_4 to 10.3% ± 0.4 and of T_3 to 13.3% ± 0.5, the deiodination of T_4 to 34.2% ± 3.4 and of T_3 to 20.1% ± 2,2. With treatment these values returned to normal. Lymphocytes from patients with primary hypothyroidism showed an increase in uptake comparable to that of hyperthyroids, but a normal deiodination.

In all conditions the uptake of T_3 is higher than that of T_4, whereas in the case of deiodination that of T_4 is greater than that of T_3.

Thus lymphocytes seem to be adequate target cells for studying the peripheral metabolism of thyroid hormones.

Memory Consolidation as a 'Response' Directly Modifiable by Operant Conditioning?

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Short-term memory is often considered to be coded in form of active electrophysiological events. The possibility of direct control over electrical brain activity by operant conditioning procedures led to the hypothesis that short-term memory processes could be conceived of as 'responses' directly susceptible to operant reinforcement, whereby reward of memory would be manifested as an improvement in learning. This hypothesis was confirmed in an experiment with 927 mice, which were given one learning trial in a passive avoidance procedure (step-down task), and retested 24 h later. The experimental groups were given 1 min access to good reward at various times after the foot-shock. The control groups underwent exactly the same procedures, except that they received no food reward. The groups which received reward 20, 30, or 50 sec after the learning trial performed significantly better upon retest (had longer step-down latencies) than their controls. Reward had no effect when presented immediately (<5 sec) or later than 50 sec after the trial. We take these data as evidence that a labile post-trial period involving a process of memory consolidation is susceptible to reward. It follows that reward can determine learning not only by its contingency on the response to be learned but also by its contingency on the subsequent memory processes.

Proline as a Putative, Inhibitory Transmitter

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Histochemical data demonstrate a differential distribution of silver grains after injection of ^{3}H-leucine and ^{3}H-proline into the cat cerebellar cortex. After leucine injection, high grain density can be observed over Stellate, Basket, Golgi and especially Purkinje cells. In contrast, after application of ^{3}H-proline the silver grains are located primarily in the tissue adjacent to the Purkinje cells, mainly in the supraganglionic region and between the faintly labelled Purkinje cells. – Iontophoretic studies reveal a reversible depressant effect of proline on the spontaneous discharge of Purkinje cells. The failure of a specific interaction with strychnine and bicuculline leads to the conclusion that L-proline is not competing with the receptors for 'glycine-like' nor 'GABA-like' amino acids. These findings, together with other reported observations seem consistent with the hypothesis that proline may itself act as a neurotransmitter.

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Possible Relationship between $[Ca]_i$ and P_K in Ventricular Muscle

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In various excitable tissues it has been demonstrated that an increase in $[Ca]_i$ produces an increase in P_K. A similar effect for ventricular muscle was proposed by McGuigan (J. Physiol. in Press). To test this idea, use was made of the relationship $[Ca]_i \propto [Na]_i^2\ [Ca]_0/[Na]_0^2$ (Glitsch et al., J. Physiol. *209*, 25–43) and the effect of the altered $[Ca]_i$ studied on the action potential. In these experiments alteration of $[Ca]_i$, by increasing $[Na]_i$ (cooling on ouabain) or variation in the external concentration of Na or Ca gave results that supported the idea $P_K \propto [Ca]_i$. Since an increase in $[Ca]_i$ can also be induced in voltage clamp experiments (Bassingthwaighte and Reuter, Electrical Phenomena in the Heart, ed. de Mello: Academic Press) the outward current was measured at the end of a clamp of fixed duration and amplitude, at a low (6/min) and at a high (60/min) frequency. At the high frequency more outward current was measured, and this was proportional to external [Ca]. This frequency dependent increase was reduced by blocking the Ca inward current by Verapamil. However at the higher frequency $[Na]_i$ would also be ex-

pected to increase. These results also support a connection between $[Ca]_i$ and P_K. Such a relationship could explain rate-induced shortening of the AP as well as some effects of metabolic inhibitors.

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Visual Deficits in Pigeons after Unilateral Forebrain Lesions

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Fifty-five pigeons were trained monocularly to discriminate two patterns presented simultaneously. When criterion was reached by each eye individually, unilateral lesions were made either in nucleus rotundus (rt), nucleus opticus principalis (op), or in the wulst. In some cases, the supraoptic decussation was also sectioned. Postoperatively, discrimination performance was impaired in most pigeons when the eye projecting to the lesioned side was tested: Large lesions including both rt and op produced permanent deficits; no pattern discrimination was ever learned to criterion, whether presented preoperatively or not. However, a simple light-dark discrimination was performed perfectly. Smaller lesions in rt and op resulted in impaired retention and slower learning of both an easy and a difficult pattern task. Lesions in op or in the wulst produced slower learning only of a difficult discrimination. Performance of the second eye was normal. The results suggest a critical role in visual dicrimination tasks of at least two different pathways to the telencephalon.

Imidazole Inhibition of Hormone-Induced Sodium Transport

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Inhibitors of phosphodiesterase (PDE) have been widely used to investigate the stimulus-effect coupling of hormones in a variety of cells. In contrast, there is a dearth of data on experiments with activators of PDE, particularly in intact cell systems. We have obtained data with theophylline supporting the view that stimulation of sodium transport in frog skin by oxytocin or nor-epinephrine is mediated by cAMP. Studies were subsequently designed to test the impact of imidazole – an agent known as an activator of PDE – on the biological effects of these hormones. The ventral skin of frogs *Rana ridibunda* was mounted in lucite double chambers and exposed to identical aerated Tris-Ringer solutions. Potential difference (PD) and short circuit current (SCC) were monitored by standard techniques. Substitution of imidazole for Tris (40 mM) resulted in a biphasic effect, with a rise in PD and SCC, followed by a fall of both parameters to values below those prior to imidazole exposure. The salient feature of the imidazole action, however, was a marked inhibition of the stimulation of PD and SCC by oxytocin or nor-epinephrine, even in the presence of supramaximal amounts of the hormones. Preliminary experiments with NH_4Cl (20 mM), another activator of PDE, gave comparable results. These findings suggest that activators of PDE stimulate the enzyme in both acellular and intact cell systems and antagonize the biological effects of the hormones by decreasing the intracellular levels of cAMP.

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Structure and Biochemical Function of Human Skeletal Muscle after a 100 km Run

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In order to investigate the effects of an exhaustive physical exercise on structure and biochemical function of skeletal muscle, needle biopsies were taken from 11 trained subjects before and after a 100 km run.

Electron microscopy revealed an almost complete diminution of the cellular glycogen and triglyceride stores, while mitochondrial fine structure was well preserved.

The activities of the glycolytic enzymes hexokinase, glyceraldehyde-3 P-dehydrogenase, and total malate-dehydrogenase on the one hand and the activity of 3-hydroxyacyl-CoA-dehydrogenase involved in the β-oxidation of free fatty acids on the other were significantly decreased by 19 to 25%. The enzymes of the citric acid cycle seem to be less affected: the activity of succinate-dehydrogenase was 9.9 ± 2.9 mmoles/kg · min before and 8.9 ± 2.2 mmoles/kg · min after the run, this 10% difference statistically not being significant.

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Analogue Model for Colour Discrimination

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Having in mind technological applications, an electronic model was designed to allow the study of colour discriminating networks. Its technology derives from that of special purpose analogue computers. Its photoreceptive matrix has 3 categories of receptors having a maximal sensitivity response at one of the following wavelengths: 515,550, and 620 nm. The analogue signals coming from the matrix are analyzed in parallel through 3 processing stages, the last one indicating the recognized colours. The device allows the identification of 8 spectral colours besides black and white. The processing logic is inspired from the known neurophysiological mechanisms of colour vision in primates. Intersting functional comaprisons can be made between the electronic networks thus obtained and certain neuronal networks found in the visual system.

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Purine Metabolism during Experimental Disturbances of the Homeostasis

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With the intention to study the influence of the disturbances of the homeostasis on the development of the disorders in the purine and lipid metabolism a small animal model was developed allowing evaluations of pharmacological activities in different experimental situations. In a series of experiments (male Wistar rats) the influence of (1) acid-base changes, (2) osmotic load, (3) carbohydrate osmotic load was investigated. The hyperuricemia and hyperuricosuria was produced by application of potassium oxonate (2,4-dihydroxy-1,3,5-triazine-6-carboxylic acid). The induced acidosis in the hyperuricemic and hyperuricosuric rats decreased the urine elimination of uric acid, lowered the uric acid level in the kidney and elevated serum uric acid levels. Sodium chloride load increased the uric acid excretion without changes of serum and liver uric acid contents. The application of fructose stimulates the

excretion of uric acid; in addition fructose potentiates the hyperuricemic effect of potassium oxonate. The hyperuricosuric effect of fructose was accompanied by the increase of serum triglycerides. The results obtained with the experimental model prove the validity for the investigations of metabolic disturbances in humans.

Some Properties of the Isolated, Perfused Mammalian Eye

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The isolated eye of the cat, perfused with a serum-enriched, oxygenated salt solution, is suitable for the study of the electrophysiology of the retina. Variations in the flow rate, oxygenation, temperature, and pH of the perfusate elicit prominent alterations of the electroretinogram (ERG) and the compound action potential in the optic nerve (CON) to photo stimulation. – Under slow flow rates of perfusion, a-waves are established in the ERG. Gradual increase of the flow rate elicited progressive increments of the b-waves on the ERG; pari passu the CON increased in magnitude. Stepwise reduction of the partial pressure of the oxygen in the perfusate elicited pronounced decrements of the b-waves. Reduction of temperature of the perfusate from 39 to 26 °C (1) decreased the amplitude, (2) increased the latency, and (3) increased the peak time of the b-waves. Changing the pH of the perfusate from 7.4 to 7.76 and 7.27 considerably reduced the amplitude of the b-waves and unmasked large a-waves. All these effects were reversible. The data indicate, that the oxygen mass flow is a crucial parameter for functioning of the retina in the isolated eye. Equally important that this preparation is extremely sensitive to slight changes in pH and temperature.

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An Early Optical Signal in Isolated Single Fibres of Frog Skeletal Muscle

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Changes in transmission of polarized light in single fibres of frog muscles between excitation and onset of contraction were measured by a photodiode through a water immersion ojective (NA 0.75, field diameter 500 μ). When the area closest to the cathode was observed, stimulation of the fibre caused a decrease in transmitted light of 0.1–0.2% lasting 2 to 4 msec. This light signal had a maximal amplitude at an angle of 45° between plane of polarization and longitudinal axis of the fibre. It was minimal at 0 and 90°. Blocking contraction by hypertonic Ringers reduced the amplitude of the optical response by a factor of 50–100. Measurement of transmembrane action potential within the area of light measurement showed that in normal and hypertonic Ringer onset of optical signal ocurred within 0.5 msec after the action potential reached 20% of its height. Temporal proximity of optical and electrical events in hypertonic solutions suggests structural changes of the surface or tubular membranes as the origin for the optical signal. The higher amplitude of the optical signal in normal Ringers indicates early conformational changes in other structures involved in EC-coupling.

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Ear Skin Temperature Changes During Fast Wave Sleep in Cats

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Ear skin temperature during fast wave sleep was studied in freely moving cats under different environmental conditions. Ear skin temperature increased at thermal neutrality (20 ± 2°C) and still more at low environmental temperature (0 ± 2°C). The observed changes in ear skin temperature depend on passive adjustments of ear skin blood flow to actual systemic arterial pressure as a result of a decrease in sympathetic outflow occurring in fast wave sleep. Preoptic heating (0.75 MHz, 50–90 mW, delivered by means of two electrode pairs), producing a steep increase in ear skin temperature during slow wave sleep at thermal neutrality and low environmental temperature, did not affect the slope of spontaneous ear skin temperature changes characterizing fast wave sleep. These results suggest that an alteration in hypothalamic autonomic control may underlie the decrease in sympathetic outflow during fast wave sleep.

Effects of Changes in Preload on Mean Velocity of Fiber Shortening in the Dog

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The cineangiographically determined mean velocity of circumferential fiber shortening ($\overline{V}cf$) has been used as a measure of basal cardiac contractility in man. In order to test whether acute changes in preload affect the magnitude of $\overline{V}cf$, dextran was administered i.v. in steps of +2, +4 and +6% of body weight to 8 closed-chest anesthetized dogs. Prior to infusion cardiac reflex adjustments were minimized by 0.5 mg/kg propranolol i.v. and by cutting the vagi. $\overline{V}cf$ was estimated from left ventricular (LV) cinefilms in the right anterior oblique projection. At +2 and +4% of dextran $\overline{V}cf$ remained unchanged as compared to control. At +6% $\overline{V}cf$ decreased from 1.57 circ/sec (control, i.e. after autonomic blockade) to 1.24 circ/sec ($P < 0.01$). $LV_{max}\ dP/dt$ increased significantly up to the step of +4% and decreased then slightly despite a further significant increase of LV end-diastolic pressure. In additional 6 dogs the largest volume load (+6%) was applied as the first step after control. Under these conditions there was no significant change in $\overline{V}cf$. In conclusions, $\overline{V}cf$ appears to be essentially independent of changes in preload. Its usefulness as a measure of contractility is however limited by the negative inotropic effect of repeated injections of contrast dye.

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Kinetics of Bile Salt Transport by the Perfused Rat Liver

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Hepatic transport of bile salts from the blood into the bile, one of the main mechanisms of bile formation, involves two sequential steps, namely transport through the

sinusoidal and the canalicular membrane. Although the over-all excretion of bile salts has been well characterized no information is available on the kinetics of each of the two steps. The kinetics of 14-C-taurocholate (TC) uptake by the perfused rat liver were therefore investigated by a multiple indicator dilution technique (Goresky, Amer. J. Physiol. *207*, 13, 1964) using 51-Cr-labelled erythrocytes and 99-Tc-m-labelled albumin as reference substances. TC uptake obeyed Michaelis-Menten kinetics and could be characterized by a V_{max} of 32.5 nmol/s · g liver and a K_m of 90.6 nmol/g liver. The excretory transport maximum determined by constant TC infusion was found to be only 3.2 nmol/s.g liver. These findings indicate that TC uptake is dependent on a saturable process compatible with carrier-mediated transport or diffusion with subsequent binding to an intracellular protein, but is not rate-limiting in the over-all bile salt transport from blood to bile.

Isolation of Neural Multi-Unit Activity with a Four Window Discriminator

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Isolation of different neuronal spike shapes within the electrical activity recorded with a single microelectrode has been approached by diverse methods. We propose here a discrimination based on amplitude and duration. A continuously variable time window can be adjusted at a chosen trigger level to eliminate wave shapes of longer duration than the chosen spikes. Two positive and two negative trigger levels are available, all associated with an independent time window. An amplitude window is provided between the low and high levels of a same polarity. Four output lines are available. On two of them pulses are fired whenever the signal falls into the amplitude and time windows associated with the low levels, on the other two, whenever the signal excesses the high levels but falls within their time window. To check for the quality of the spike isolation, the signal (to which the different pulses may be added) can be passed through a delay line and visualised on a scope whose time base is triggered by the discriminating pulses. Application of this technique to a microelectrode study of the thalamic auditory relay nucleus is illustrated.

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Counter-Transport of Amino-Acids and Sugars in the Guinea-Pig Intestine

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Counter-transport, the acceleration of substrate movement across a membrane by an elicitor, substrate or homologue, at its opposite face, may be explained by two models: (A) inhibition of recapture of the transported molecule by the carrier; (B) acceleration of movement of the carrier on loading. Efflux of amino-acid or sugar from enterocytes can be accelerated by an elicitor, but influx cannot be stimulated by preloading. Efflux of amino-acids from guinea-pig intestinal sacs is more rapid into a Na^+-free medium than into a medium with Na^+, and is accelerated by an amino-acid in both cases; sugar acceleration only occurs in the presence of sodium. Acceleration of efflux is independent of external volume. The sodium gradient across the membrane must remain intact for counter-transport. These results comply best with model A, provided an unstirred layer is postulated at the intestinal surface. Model A is also consistent with a kinetic analysis of the effect of different elicitor concentrations, and, decisively, with the fact that phloridzin also accelerates sugar efflux, although it cannot cross the membrane.

Precocious Puberty in Rats After Stimulation of the Brain

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Precocious puberty in female rats can be induced by pregnant mare serum (PMS), hypothalamic electrolytic lesions and administration of estrogen or androstanediol. We here report on the induction of puberty by electrochemical stimulation (i.e. iron deposition from steel electrodes) of the hypothalamus. Five to 10 mC of direct anodal current was applied uni- or bilaterally to 23-day old rats. Three distinct groups of induced sexual maturation could be distinguished with vaginal opening (VO) occurring on days 26, 27 and 28 of life. The day 26 group had high ovarian estrogen and progestin (as determined by radioimmunoassays); a full set of tubal ova was present the following day. The day 27 group had low ovarian progestin, but also ovulated the next day. Animals with VO on day 28 had similar ovarian steroids as the day 27 group but did not ovulate. VO in untreated controls occurred around day 33. Like PMS-induced superovulation or spontaneous ovulation in the adult, the first ovulation provoked by brain stimulation could be blocked by Pentobarbital or Reserpine injected before the 'critical period' for LH release. We propose that the discharge of LRF caused by hypothalamic stimulation elicits release of gonadotrophins which, like PMS and exogenous steroids, provoke further activation via a positive feedback effect of ovarian steroids on the brain.

Evidence for Two Receptor Areas in Brown Adipose Tissue (BAT)

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Giacobino and Girardier (this meeting) have shown that brown adipocytes contain specific catechol binding sites (CBS) and that the bulk of CBS does not represent the β-receptor. On the other hand Chinet and Girardier (this meeting) have shown that the displacement of norepinephrine (NE) from CBS by Dopa produces a remarkable potentiation of NE calorigenic effect. An early response to NE in BAT is a membrane depolarization. This response is not modified by Dopa but desipramine, an inhibitor of neuronal NE re-uptake, increases the sensitivity to the hormone, producing a displacement of the dose-response curve of at least one order of magnitude. The same displacement can be observed after surgical denervation. A model of the adipocyte membrane organization is proposed. β receptors responsible for depolarization are segregated in the region close to the nerve terminals, whereas β receptors responsible for controlling calorigenesis are distributed in the remainder of the membrane. NE responses of the two areas are separately and differently modulated by the mechanisms of neuronal re-uptake and binding to CBS.

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Extrahypothalamic Control of Alimentary Behavior: Effects of Limbic and Neocortical Spreading Depression and Electrical Stimulation

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Single waves of spreading depression, triggered in the hippocampus, caudate nucleus or cortex of rats by application of 0.5–2.0 µl of 25% KCl induces consumatory behavior. Whereas cortical spreading depression induces mostly eating, but also drinking behavior hippocampal depression evokes primarily eating. Electrical stimulation (10–100 µA, 60–80 Hz, sinusoidal waves, at 1.0 sec train duration) of the same structures induces eating with similar onset latencies (about 4 min) and response topography as after spreading depression.

It is well known, that electrical stimulation induces after-discharges followed by postictal depression. The invariant parameter for both treatments is likely to be neuronal depression, whereby the amygdala might be a critical focus for the elicited behavior. These results lead to the hypothesis that the post-stimulation eating resulting from stimulation of various subcortical areas, as well as the eating induced by various pharmacological agents, which cause amygdaloid-hippocampal seizure activity, may all be subsumed by a common mechanism linked to our depression-induced eating.

Retrograde Axonal Tracing of Thalamo-Telencephalic Connections

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Although the basic mechanisms underlying retrograde axonal transport of exogenous substances are unknown, the phenomenon itslef seems to provide an interesting new tool for tracing connections in the nervous system [K. Kristensson et al., Brain Res. *32* (1971) 399–406; J. H. LaVail et al., Brain Res. *58* (1973) 470–477]. After unilateral injection of either horseradish peroxidase or Evan's blue-albumin into the wulst of young chicks, adult chickens and pigeons labeled neuronal cell bodies are found in both the ipsi- and the contralateral nucleus opticus principalis thalami. On the ipsilateral side labeled neurons mainly occur in the ventral portion of this nucleus, whereas on the contralateral side they seem to be confined to the dorsal part. These ata confirm the ones of R. E. Meier et al. (Exp. Brain Res, 1974, in press) obtained by means of the retrograde degeneration method. There are indications that the contralateral projection crosses via the supraoptic decussation.

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Aldosterone Effect in the Epithelium of the Frog Skin – A New Story about an Old Enzyme

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It is well established that aldosterone stimulates active epithelial sodium transport. There is also increasing evidence that thix effect on transport is mediated through an intermediate step of protein-synthesis. So far morphobioelectric results of our laboratory point to just one specialized cell in all epithelia studied, namely the mitochondria rich cell or MR cell (C. L. Voûte et al. J. Steroid Biochem. *3*, 161, 1972). This latter cell responds in a characteristic pattern to the hormone with respect to induction and protein synthesis. – We could now show that carbonic anhydrase activity is not only selectively located in the MR cells but that its activity pattern parallels our earlier morphological observations. – As there is evidence that sodium selectivity of the outer membrane in frog skin is strongly pH dependent (W. Zeiske and B. Lindemann, in prep.) we tentatively propose that epithelial transport regulation by aldosterone is mediated through carbonic anhydrase (CA) and that CA is induced, synthetized and secreted by the MR cells. CA might control the pH in the extracellular compartment next to the outer membrane and thus regulate the sodium permeability of the latter.

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Modulation alimentaire d'un facteur duodénal contrôlant la réponse insulinique

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Si le concept d'un contrôle entéro-humoral de la sécrétion d'insuline est déjà ancien, le facteur hormonal contrôlant la réponse d'insuline à la nourriture n'a pourtant pas encore été identifié. Une approche de sa régulation par les différentes classes d'aliments a été faite à l'aide du système suivant: des extraits duodénaux aqueux provenant de rats surchargés par voie orale avec divers aliments, sont injectés dans le tronc cœliaque d'autres rats chez qui on dose l'insulinémie portale avant puis jusqu'à 15 minutes après l'injection.

Le glucose stimule de façon biphasique l'activité de ce ou ces facteurs (5 et 45 minutes après l'ingestion). Un hydrolisat de protéines (Nesmida) ne change en rien l'activité de ce facteur. L'arginine et non la lysine stimule de façon monophasique ce facteur 5 minutes après son ingestion.

Ce facteur a été partiellement purifié par adsorption sur poudre de verre et élution Acide-Acetone, montrant qu'il s'agit d'un petit polypeptide.

EKG-Veränderungen des Gesunden bei akuter Hypoxie

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50 gesunde, 20–25jährige Studenten wurden im Liegen bei simulierter, schrittweiser Höhenexposition (Unterdruckkammer) untersucht. In Schritten von 1000 m wurden Herzfrequenz und Blutdruck gemessen sowie die Standard- und unipolaren Brustwandableitungen aufgenommen. Die maximale Höhenexposition betrug 7000 m (302 mm Hg). – Mit zunehmender Höhe steigen Herzfrequenz und Blutdruckamplitude; während der systolische Blutdruck leicht ansteigt, fällt der diastolische geringgradig ab. Im EKG ist die P-Welle in II und III erhöht, das PQ-Intervall verkürzt. Die QRS-Alteration spricht im Sinne einer geringgradigen Drehung der elektrischen Herzachse in der Frontalebene und einer Verschiebung der Übergangszone nach links. Hervorzuheben sind die Änderungen der ST-Strecke und der T-Welle: Mit zunehmendem Sauerstoffmangel wird die ST-Strecke gesenkt, das T flacher, breiter und symmetrischer, bis – auf 7000 m – die ST-Strecke in den Standard- und den links-praecordialen Ableitungen unter die Null-Linie absinkt, die T-Welle stark erniedrigt wird und präterminal negativ werden kann.

BIOCHEMIE – BIOCHIMIE – BIOCHEMISTRY

Determination of the Levels of NAD, NADH, NADP and NADPH in Polymorphonuclear Leucocytes (PMN) at Rest and During Ingestion with Heat Killed Staphylococci Albi

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The levels of the coenzymes NAD, NADH, NADP and NADPH have been determined in PMN from human blood and from guinea-pig peritoneal exsudates using a kinetic method involving enzymatic cyclisation as described by Nisselbaum and Green [Anal. Biochem. *27*, 212 (1969)]. For these two types of cells, the studies of the effects of incubation at 37 °C and of S. albi phagocytosis have led to the following observations:

Human PMN incubated in *the presence of serum:*

- a tendency for the reduction in NADP levels during the incubation, which increases again during phagocytosis;
- an increase during phagocytosis of NAD;
- no significant changes in NADH levels;
- 'de novo' synthesis of coenzymes sum during phagocytosis.

PMN from guinea-pig exsudates incubated *without serum:*

- phagocytosis leads to an increase in NADP without changes in the other coenzymes;
- during the incubation NADH levels increase whilst NADP and NADPH decrease. These results represent a disagreement with those of DeChâtelet [Infec. Immun. *6*, 302 (1972)] who observed a diminution in NAD and NADP levels in incubated guinea-pig PMN which he attributed to a NAD(P) glycohydrolase.

Fluorescence Changes of Nicotinamide $1,N^6$-Ethenoadenine Dinucleotide (ε NAD) upon Binding to Some Dehydrogenases

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NADH fluorescence has been greatly exploited to obtain information about the enzyme-coenzyme interactions of many dehydrogenases. In order to obtain analogous informations for the oxidized coenzyme, we investigated the fluorescence of εNAD when bound to Octopine DH, horse-liver ADH, yeast- and lobster muscle GAPDH. εNAD is a coenzyme analog having a fluorescent tag on the adenine ring, and provided with a biological activity and an affinity which compare well with those of NAD. In all enzyme systems studied we found a blue shift of the εNAD fluorescence maximum, and a remarkable increase (by a factor 13 ± 1) of fluorescence intensity. Interestingly, this enhancement is close to that found when the two aromatic moieties of the free coenzyme (which exists in solution as an internally stacked conformation) are enzymatically cleaved. These data are taken to indicate that: (a) the adenosine moiety interacts with hydrophobic regions of the proteins, and therefore is involved in the binding process; (b) the binding is accompanied by a conformational change of the coenzyme from a 'closed' to an 'open' conformation.

Structural Studies of Human Liver Alcohol Dehydrogenase (HuLADH) Isoenzymes

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Isoenzymes of HuLADH are dimers composed of subunits A, B, B′ and C. Two phenotypes of isoenzyme BB ('normal' and 'atypical') with different pH optima and specific activity but identical electrophoretic mobility can be isolated from individual liver homogenates, suggesting the existence of a genetic variant subunit [T. Schenker, Eur. J. Biochem. *24*, 271 (1971)]. pH rate profiles show that the variant isoenzyme (pH opt. 8.8) contains subunits B_1 and B_2, the usual isoenzyme (pH opt. 10.2) only B_1. The peptides of tryptic digests of the variant isoenzyme were compared with the usual ones. The primary structures of subunit B_1 and B_2 show a high degree of homology with subunit A from horse liver [H. Jörnvall, Eur. J. Biochem. *16*, 25 (1970)]. The sequence of one peptide from the usual isoenzyme (B_1B_1) is Phe-Ala-Lys, corresponding to positions 229 to 231 in the coenzyme binding site of the horse enzyme. In the variant isoenzyme, the corresponding sequence for the B_2-subunit is Phe-Pro-Lys. The results indicate that most 'atypical' individuals genetically represent heterozygotes.

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Gluconeogenesis from L-Serine

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In our studies on the gluconeogenic fate of L-serine in the perfused livers of fed and fasted rats, we observed an increase in the rate of glucose synthesis as the duration of fasting was increased. In the presence of quinolinic acid, an inhibitor of phosphoenol pyruvate carboxykinase, there was a marked inhibition of glucose synthesis and this inhibition increased with fasting. However, quinolinate did not inhibit the radioactivity incorporation from L-serine-3-^{14}C in the case of fed rats. An assay of the activities of hepatic serine pyruvate transaminase (SPT) and serine dehydratase (SDH) revealed that while SPT remained unchanged both in the fed and fasted state, SDH was increased severalfold by fasting. This increase could be supressed by Actinomycin D.

These results suggest that gluconeogenesis from L-serine does not proceed through one pathway alone.

Changes in Activation of Adenylate Cyclase and of Dopamine Turnover in Rat Striatum During Prolonged Haloperidol Treatment

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Dopamine (DA) activates the adenylate cyclase in homogenate of striatum; this effect is antagonized by DA receptor blocking agents, e.g. haloperidol. Therefore, the adenylate cyclase in the striatum might be connected with the DA receptors (Kebabian et al., Proc. Nat. Acad. Sci. *69*, 2145, 1972).

In rats treated for 1 to 21 days with haloperidol (1 mg/kg daily, p.o.) a progressive enhancement of the activation of striatal adenylate cyclase by DA was observed. Conversely, the haloperidol-induced rise of striatal homovanillic acid markedly declined during prolonged treatment as compared to a single administration of the neuroleptic drug.

The increase in striatal homovanillic acid caused by neuroleptic agents probably indicates a compensatory feed-back activation of DA neurons triggered by the blockade of DA receptors (Andén et al., Life Sci. *3*, 149, 1964). It is therefore suggested that the enhanced effect of DA on striatal adenylate cyclase during prolonged haloperidol treatment reflects a supersensitivity of DA receptors which might be responsible for the reduction of the feed-back activation of the dopaminergic neurons.

Purification of Angiotensin-Converting Enzyme from Human Seminal Plasma

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Following the finding by Cushman and Cheung (Biochim. Biophys. Acta *250*, 261, 1971) that human seminal plasma is rich in converting enzyme, we have partially purified the enzyme from this source, obtaining a 500-fold increase in specific activity. The procedure involved ammonium sulfate fractionation, gel filtration on Sephadex G-200, dialysis at pH 4.7, CM-cellulose batch filtration and chromatography on DEAE cellulose. From experiments on Sephadex G-200, the molecular weight was estimated to be 168.000, which correlates with the finding of Lee et al. (Arch. Biochem. Biophys. *142*, 548, 1971) for the enzyme of human blood plasma. In addition, a second fraction of higher molecular weight (about 420.000) was observed. Both fractions are inhibited by the nonapeptide SQ 20.881. Three substrates were investigated on the different purification steps. Their susceptibility to the enzyme was consistently in the order Z-Phe-His-Leu > angiotensin I > hippuryl-His-Leu.

Teneur et répartition des activités enzymatiques dans les éosinophiles de cheval

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Les activités enzymatiques des éosinophiles isolés selon la nouvelle méthode ont été déterminées dans des homogénats de ces cellules et de leur substructures cellulaires. On a noté l'absence totale d'histidine décarboxylase, des monoamine et diamine oxydases. D'autres oxydases trouvées normalement dans les péroxysomes n'étaient pas présentes non plus. Les cellules sont très pauvres en flavoenzymes, mais riches en NAD-Deshydrogenases. Le système enzymatique des mitochondries est décelable, mais peu important. Par contre, les éosinophiles sont riches en enzymes protoplasmiques nécessaires à la glycolyse. Ces résultats confirment de nombreuses observations histochimiques antérieures. Trois enzymes en rapport avec la régulation humorale ont pu être décelés: l'acétylcholinestérase, l'adénylate cyclase et un enzyme dégradant l'adrénaline et la noradrénaline. L'étude de la répartition de l'activité enzymatique a mis en évidence une accumulation des péroxydases, de la catalase et de certaines hydrolases qu'on trouve soit dans les membranes soit dans les lysosomes.

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Relation between Divalent Cation Transport and Divalent Cation Activated ATPase in Yeast Plasma Membranes

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In decreasing order of affinity the divalent cations $Mg^{++} = Co^{++} = Zn^{++} > Mn^{++} > Ni^{++} > Ca^{++}$ are taken up into yeast cells by a transport system which is dependent upon ATP derived from fermentation. Sr^{++} is not taken up. La^{+++} strongly inhibits the uptake of the divalent cations. The order in which divalent cations activate ATPase in isolated yeast plasma membranes is the same as for transport in intact yeast cells. Mg^{++}, Co^{++}, Zn^{++} and Mn^{++} have the highest affinity for transport and show highest activation of the ATPase in plasma membranes. Ni^{++} and Ca^{++} have a low affinity and activate only slightly, while Sr^{++} is neither taken up nor does it activate. La^{+++} acts as a strong inhibitor in both systems. The pH optimum for Mn^{++} uptake and for Mn^{++}-activated ATPase in plasma membranes lies near pH 7. Neither the Mn^{++}-activated ATPase nor the Mn^{++} uptake were inhibited by oligomycin or ouabain, whereas ATPase and uptake were inhibited by carbodiimide (EDAC). The good correlation between effects on transport and on ATPase activity in the plasma membrane suggests that the divalent cation activated ATPase in yeast plasma membranes is involved in the transport of these cations in intact yeast cells.

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Detection of Tryptic Peptides on Thin-Layer Cellulose Plates by Fluorescamine

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Tryptic digests of fibrinogen derivatives (following plasmic degradation) were examined by two-dimensional thin-layer electrophoresis (pyridine: acetic acid: water 20:7:973) and chromatography (tert. butanol: formic acid: water, 695:10:295). The plates were dried and sprayed with 0.05% fluorescamine (Fluoram®, Roche) in acetone and viewed or photographed under long-wave ultraviolet light. Subsequently, the same plates were sprayed with ninhydrin. Fluorescamine clearly revealed several spots which were not detectable by the ninhydrin reaction. However, certain spots reacted more strongly with ninhydrin. The comparison of the differential patterns obtained with both reagents facilitates the identification of peptides on fingerprints.

Effects of Fluorinated Compounds on Acetylcholinesterase

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It is generally accepted that an acyl-enzyme intermediate is formed during the action of acetylcholinesterase (AChE), the acylation presumably involving the formation of a tetrahedral intermediate on going from the E-S complex to the acyl-enzyme. Substrate-like compounds being able to form tetrahedral adducts might be expected to strongly interact with AChE. Thus pentafluoro-ben-

zoylcholine (F_5-BzCh), 3,3-dimethylbutyl-trifluoroacetate (F_3-DmbAc), ethyl-trifluoroacetate (F3-EtAc) and trifluoroacetone (F_3-A) were tested as substrates and inhibitors of AChE. The results were compared to the effects of the parent, non-fluorinated compounds (listed in parenthesis). F_5-BzCh was a poor substrate for AChE from plaice body muscle; V_{max}/Km = 0.2 and (V_{max}/Km = 25) but inhibited the eel enzyme; Ki = 90 μM, (Ki = 800 μM). DmbAc was a substrate for the eel enzyme (Km = 10 mM); F_3-DmbAc, although very labile in aqueous solutions, was a strong inhibitor; Ki ≃ 40 nM. Compounds with less resemblance to the substrate also inhibited eel AChE. F_3-EtAc had a Ki = 100 μM, (Ki = 25 mM) and F_3-A a Ki = 200 μM (Ki ≃ 100 mM). IR- and NMR-spectroscopy suggested that the fluorinated compounds readily form hydrates, the resulting tetrahedral structure possibly being responsible for the strong inhibition of AChE. Thus these compounds might be regarded as transition state analogue inhibitors of AChE.

Synthesis of Glycosaminoglycans by Corneal Epithelium in Vitro

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It is now an established fact that the epithelium of the avian and rabbit cornea synthetizes collagenlike proteins and even fibrils. Hitherto collagen and glycosaminoglycans (GAG) were assumed to be of mesodermal origin only. Therefore the question arises whether corneal epithelial cells are also capable of producing GAG. Epithelial cultures of the rabbit cornea and cultures of corneal fibroblasts were incubated either with D-glucosamine-6-^{3}H or $Na_2{}^{35}SO_4$. Following the exposure the cells and the nutrient medium were digested with pronase, thoroughly dialysed against Na_2SO_4/distilled water and treated with TCA and ether. GAG-fractions were eluted from cellulose columns with seven salt solutions of increasing ionic strength. The epithelium incorporated the precursors, but the shape of the fractionation curves differed from the curves of the fibroblasts. In the latter the peaks of CPC-soluble and CPC-insoluble fractions resembled the ones found in cultured chick corneal fibroblasts. Epithelial synthesis of GAG may implicate a regulation of fibril diameters in newly formed collagen in the embryonic stage or in wound repair. It has been demonstrated in other systems that fibrillogenesis is influenced by GAG.

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Characterization of Human Clq with Respect to its Biological Activity

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Clq, the first component of complement reacts with immune complexes; after its fixation Clq becomes modified and activitates Clr (and indirectly Cls) and thus triggers the complement activation sequence.

Two Clq-activities were measured; the hemolytic activity of Clq in presence of RClq and the binding of ^{125}I-Clq to ovalbumin-antiovalbumin complexes, which was dependent on the ratio of IgG per Clq. Under the conditions tested 100% binding occurred at molar ratios over 13, and 50% at a ratio of 3 IgG per Clq (nonspecific binding: 5%). No specific fixation could be detected using immune complexes prepared with $F(ab')_2$ instead of normal IgG. Binding activity was found to be less susceptible to chemicals ($NaIO_4$, I_2, DTT, urea), as well as to proteases (trypsin, chymotrypsin, collagenase) than hemolytic activity and thus may be due to a more restricted region within the molecule.

Incubations of Clq from pH 4.5 up to 10 (at 4 °C) and temperature up to 45 °C (at neutral pH) did not affect either activity. An activation effect was induced at 35 °C.

The pI of Clq was determined to be 9.9 at 4 °C.

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The Readthrough Protein A_1 is Required for in vitro Reconstitution of Infectious Qβ Particles

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The capsid of group I phages (MS2, R17) consists of about 180 molecules of coat and one of maturation protein. Both protein species are essential for infectivity of the particle [Roberts and Argetsinger-Steitz, Proc. Natl. Acad. Sci. U.S.A. *58*, 1416 (1967)]. The group III phage Qβ contains an additional protein (II b or A_1) which arises by readthrough at the end of the coat cistron. In order to establish whether all Qβ capsid components are required for infectivity, the three viral proteins were purified by chromatography on hydroxyapatite in the presence of SDS. Infectious virus particles could be reconstituted by mixing solutions of all protein components in 6M guanidinium hydrochloride with QβRNA and dialyzing against a buffer containing 0.15 m NaCl. Specific infectivities of 4×10^3–1.3×10^5 pfu/μg RNA were attained. Omission of any one component reduced the infectivity below 10–50 pfu/μg RNA showing that all proteins, including the readthrough protein, were required for the reconstitution of infectious virus.

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Inhibition of Adenine-Uptake into Isolated Fat Cells

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The uptake of adenine-^{14}C (initial concentration 8.4 μ*M*) into rat fat-cells incubating in Krebs-Ringer-bicarbonate-buffer with serum-albumin and glucose, was progressively inhibited with time: the disappearance-curve, log(adenine) vs. time, was concave-up. $ACTH_{\beta\ 1-24}$ (Synacthen®), 10^{-7}*M*, further increased this inhibition. Less than 1 per cent of the total adenine-prelabelled cell-pool of purine derivatives was released as adenine during incubation with or without ACTH. ATP-content of fat-cells was neither changed significantly after 12 and 30 minutes of incubation in the absence of ACTH, nor after 12 minutes in its presence. A 15 per cent decrease in ATP-content was observed after 30 minutes of incubation with ACTH. Hence, a change in availability of ATP for the synthesis of P-ribosyl-PP (PRPP) is not likely to account for the observed inhibition of adenine-uptake if one assumes a homogeneous ATP-pool. Progressive release and accumulation of adenosine, inosine and hypoxanthine in the incubation medium was positively correlated with the inhibition. These compounds are known to influence PRPP-

synthesis as well as the reaction PRPP + adenine → AMP + PP_i. The latter step is considered to be responsible for the uptake of adenine into cells.

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Effect of Adrenergic Blockers on the Activation of Brain ATPase by Noradrenaline

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Cat brain ATPases (A) are activated by noradrenaline (NA) under particular ionic conditions ($K^+ > 5$ mM; $Mg^{2+} > 2$ mM) see IRCS: Biochem. Neurobiol. Neurophysiol. Pharmacol. *2*, 1182 (1974). The influence of different NA-concentrations (10^{-5} –$10^{-3}M$) upon Na^+-K^+-ATPase (AN) and Mg^{2+}-ATPase (AM) was determined in homogenates of brain cortex and of subfractions obtained by sucrose density gradient centrifugation. Whereas AM showed only moderate activation by NA in all subfractions, an activation on AN could be clearly detected. The increase of AN by NA, strongest in the myelin fraction was 20% lower in the synaptosomal, in the supernatant fraction and in the whole homogenate. α- and β-blockers were added to the assay without preincubation. The blockers showed a decrease of the excitated AN-activity. Propranolol (PR) inhibited AN 1.3× more than phentolamine. Even PR reduced the basal activities of both A. To conclude we suggest that NA probably takes part in regulation of neuronal resp. glial AN. Therefore adrenergic blockers as antagonists of NA possess influence on this mechanism too.

Fructose Metabolism and Glycogenolytic Effect of Glucagon in Perfused Rat Livers

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Livers from fed rats were perfused in a non-recirculating system with [U-^{14}C]-fructose (4 mM) and/or glucagon (3 nM) for 20 min. Net glucose, lactate and pyruvate production rates and fructose uptake were measured during the last 3 min of perfusion. The specific activities of glucose and fructose were determined in the perfusate leaving the liver after separation of glucose and fructose on Dowex I. Fructose enhanced net glucose production in the absence of glucagon but decreased it in the presence of glucagon, in spite of a slight stimulation of glucose formation from fructose. The decrease in net glucose production under these conditions was associated with a strong inhibition (65%) of glucose formation from glycogen, as calulated from the specific activity of glucose. From the balance of 6-C-units comparable values were obtained. The activity of phosphorylase *a* in homogenates of livers perfused with glucagon were the same in the presence and absence of fructose. It may be concluded, that the glucagon-induced conversion of phosphorylase *b* to *a* was unaffected. However phosphorylase *a* appeared to be inhibited, possibly by fructose-1-phosphate.

J-Chain in Murine IgA Myeloma Protein

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The MOPC-315 IgA myeloma protein from BALB/c mice comprises monomeric and oligomeric units and has an antibody-like affinity for DNP-groups. It was isolated by affinity chromatography and the purified protein was submitted to mild reductive cleavage (0.005M DTT; 0.015M iodoacetamide). Polyacrylamide gel electrophoresis (PAGE) showed a homogeneous band ($s_{20,w}$7 S). After stronger reductive cleavage of MOPC-315 protein (0.15M DTT; 0.45M iodoacetamide), a band migrating ahead of L-chains was observed; this material had M.Wt 26,000 Daltons (by Yphantis density equilibrium centrifugation). Components of varying molecular size were obtained when MOPC-315 protein was separated by sucrose density gradient ultracentrifugation. Reductive cleavage of these isolated components with graded concentrations of DTT should reveal the location of J-chain and perhaps also the structural linkage between this and other units.

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Neue Methode zur Isolierung der Granula aus reinen Pferde-Eosinophilen-Präparaten

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Eine gegenüber früher wesentlich raschere und schonendere Isolierung der Eosinophilen konnte erreicht werden durch spontane Sedimentation der eosinophilenhaltigen zentrifugierten Erythrocytenschicht, suspendiert in 6%iger Plasma-Polyvinylpyrrolidon-Mischung. Die in Suspension bleibenden Eosinophilen können abgesaugt, dadurch vollständig vom Erythrocytensediment abgetrennt und durch eine kurze Hämolyse (<1 min) von den noch verbleibenden Erythrocyten befreit werden. Die Freisetzung intakter Granula aus Pferde-Eosinophilen ist wegen ihrer Grösse schwierig. Von allen untersuchten Methoden ergab nur die Gas-Depressionsmethode gute und reproduzierbare Resultate, wobei eine nachfolgende Trennung der Zellstrukturen im Saccharose-Dichtegradienten (Zonalrotor) erlaubte, Präparate von Membranen, Granula, Kernen und löslichen Anteilen der Eosinophilen in relativ grossen Mengen zu erhalten. Diese Fraktionen wurden elektronenmikroskopisch auf ihre Reinheit überprüft (Dr. Hodel und Hauser, Sandoz) und für die Untersuchung des Fermentgehaltes und der Enzymverteilung in den Eosinophilen verwendet.

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Muscular Contraction in Crayfish: Regulatory Systems in Relation to Calcium

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The biochemical events and proteins implicated in the regulation of muscle contraction in crustaceans have not received much attention yet. From the physiological point of view, the regulatory systems of crustacean muscle appear less elaborate than those found in vertebrates. We have shown that the native tropomyosin of crayfish tail muscle, which induces the calcium sensitivity of desensitized actomyosin ATPase, is composed of 2 proteins, troponin and tropomyosin. These proteins have been purified and separated, their combined action is equivalent to that of native tropomyosin. Tropomyosin is a single chain of MW 40,000 while troponin is made of 3 components of

MW 54,000, 29,000 and 16,000. Crayfish sarcoplasmic reticulum functions as a calcium pump. Fragmented sarcoplasmic reticulum (FSR) has been obtained in the form of homogeneous vesicles probably made of a single glycoprotein combined with phospholipids. In the presence of ATP, FSR binds calcium and inhibits the superprecipitation of actomyosin. It appears that the biochemical regulation of muscle is essentially similar in crayfish and rabbit.

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Characterization of Human Clq with Respect to its Structure

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Clq was purified from fresh normal human serum according to three different methods: (1) DNA-precipitation (Agnello et al., Immunology *19*, 909, 1970), (2) low ionic strength (Yonemasu et al., J. Immunol. *106*, 304, 1971) and (3) a modified method of Müller-Eberhard.

Electron microscopy by negative staining with uranylformate revealed the three Clq preparations to consist of a central part surrounded by six peripheral parts. For biochemical analysis the different Clq preparations were radioiodinated using lactoperoxidase according to Heusser et al. (J. Immunol. *110*, 820, 1973). Dissociation and reduction of Clq revealed the same molecular ratio of the two noncovalently and the three covalently linked subunits in all preparations, demonstrating the smaller, radio-labelled subunits to be an integral part of the Clq molecule.

Adsorption of collagenase-digested Clq by ovalbumin-antiovalbumin complexes indicates its larger noncovalently bound subunit (presumably the peripheral part) to be involved in fixation to immune complexes.

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Temperature-Determined Functions in Octopine Dehydrogenase

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The body temperature of poikilotherms is a direct function of the environmental temperature, and, consequently, several biological processes may be affected by temperature changes. In the last few years, it has been shown that enzymatic systems of poikilotherms have interesting regulatory mechanisms which enable the animal to minimize the effect of temperature changes. We wish to report on the influence of temperature on the enzymatic functions of Octopine Dehydrogenase (ODH), a monomeric enzyme extracted from the molluscus *Pecten maximus L.* We found that the dissociation constant of NAD and NADH to ODH, as well as corresponding K_m, are practically temperature independent between 3° and 30 °C: the enzyme-coenzyme affinity is therefore not influenced by external temperature changes of the seaenvironment. We will discuss these findings in terms of thermodinamics and of the molecular mechanism which permits a temperature independent binding process.

Properties of Neurophysin from Neurosecretory Granules ('Native' Neurophysin?)

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The binding of tritiated lysine vasopressin (LVP) to the ('native'?) neurophysin isolated from bovine neurosecretory granules (NSG) was measured by equilibrium dialysis and analysed by Scatchard plots. Two populations of binding sites, with $K_{ass} = 2.3 \times 10^6$ and 2.4×10^5 l/mole and capacities $^1/_3$ and $^2/_3$ of the total, respectively, were found in addition to nonspecific ($K_{ass} < 10^4$) sites. The total high-affinity binding capacity is of the same order as the vasopressin content of the NSG. In disc gel electrophoresis and gel electrofocussing the NSG proteins showed a similar pattern as neurophysin acid-extracted from acetone-dried pituitaries but their stability was higher (binding unchanged on 7–24 h dialysis). Two components corresponding to neurophysins I and II were partially separated by electrofocussing (sucrose gradient, pH 4–6). Glc-ms analysis of hydrolysates indicated the presence of stearic and palmitic acids, supporting the suggestion [Mylroie, Koenig, J. Histochem. Cytochem. *19*, 738 (1971)] that the native neurophysins are lipoproteins.

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Sodium Transport in Isolated Vesicles of Brush Border Membrane

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The Na^+-permeability characteristics of brush border membrane were investigated in a system of isolated membrane vesicles from rat small intestine. These vesicles reversibly take up Na^+ from the medium into an osmotically active space, suggesting transport rather than binding. This uptake is little affected by temperature. The saturation kinetics and its independence from metabolic processes characterize it as a 'facilitated diffusion' type of transport. Interestingly, a proton gradient (vesicles > medium) can support a transient accumulation of Na^+ above the equilibrium concentration. This 'active' transport of Na^+ is abolished when the electrical potential across the brush border membrane is kept at zero by relatively high concentrations of permeable ions (e.g. KSCN in the presence of valinomycin). These findings suggest that the brush border membrane contains a 'carrier' or a 'channel' with a high conductance especially for Na (but not for K^+) and that this structure is not denatured by the isolation procedure for the purified membrane.

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Isolation and Respiration of Leucocyte Mitochondria

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Polymorphonuclear leucocytes and lymphocytes have been isolated from human blood using a modification of Böyum's technique (Scand. J. Clin. Lab. Invest. *21*, 1968, suppl. 97). Heparine (20 U/ml leucocytes) was used for

the destruction of the cells. Respiration of purified mitochondria was determined by the method of Barzu [Anal. Biochem. *21*, 344 (1967)], using oxyhemoglobin both as an oxygen donor and as a photometric indicator of the reaction (435.8 nm). Human lymphocyte mitochondria and guinea-pig macrophages both have an oxygen consumption of 50–100 nAtoms $\times$ $min^{-1} \times mg^{-1}$ protein, a respiratory control of 2, an acceptor control index of 4, and an ADP/O ratio for succinate of 1.87 (A case of lymphoblastosis: 300 nAtoms $\times min^{-1} \times mg^{-1}$ protein, ACI = 4.16, CR = 2.14, ADP/O = 1.83). These results provide a direct demonstration of oxidative phosphorylation in the leucocyte. Electron-micrographs of granulocyte mitochondria reveal an important granular contamination which explain the relatively low respiration (20–30 nAtoms $\times min^{-1} \times mg^{-1}$ protein) and the difficulty experienced in determining the respiratory control, acceptor control index and the ADP/O ratio. Whatever cell population, the oxygen consumption is always greater in the presence of FAD-dependent (succinate and α-glycero-phosphate) than in the presence of NAD-dependent substrates.

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Transplacental Control of Enzymes Involved in Epoxide Formation and Inactivation

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For several foreign compounds it has been demonstrated that they pass the placenta. Aromatic and olefinic compounds can be metabolized by microsomal monooxygenases via epoxide intermediates. Due to their electrophilic reactivity such epoxides can form covalent bonds with DNA, RNA and proteins thereby causing adverse (mutagenic, carcinogenic, cytotoxic) effects. The balance between epoxide forming and inactivating systems therefore appears to be of great importance. The present study delineates pre- and postnatal levels of monooxygenase(s) responsible for epoxide formation, and epoxide hydrase(s) which transform such epoxides to much less reactive dihydrodiols. The influence of several environmental chemicals and clinically used drugs on the levels of these enzymes was investigated. Benzo(a)pyrene, a product of incomplete combustion present in cigarette smoke and in polluted city air, transplacentally induce(s) monooxygenase(s) but not epoxide hydrase(s) thus potentiating possible adverse effects in the fetus. This differential induction indicates that the synthesis of the two enzyme systems is not under common control although both systems seem to be functionally and architecturally tightly coupled. This gives hope that a selective induction of the inactivating enzyme(s) might become feasable.

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Production of Specific Antisera to Mouse Thymus-Derived Cells with Purified Antigens from Mouse Thymocytes and Brain

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Mouse T-lymphocyte specific xenoantigen (MTLA) and θ-alloantigen have been isolated by Sephadex G-200 chromatography and preparative polyacrylamide gel electrophoresis of SDS solubilized mouse thymocyte membranes. The MTLA and θ antigenic activities, as tested by microcomplement fixation and inhibition of lymphocyto-toxicity are detected on the same protein having an apparent molecular weight of 35,000–40,000 daltons. The specific xenoantigenic activity was increased 120 fold compared to the activity of the original cell homogenate. The alloantigenic specific activity was 60 times higher on the purified material.

Similar results were obtained when the purification procedure was performed with butanol-extracted mouse brain proteins. Immunization of AKR mice with purified protein evoked anti-θ alloantiserum. By immunization of rabbits with 50–300 μg of purified protein, potent antisera were obtained which were specific for mouse T-cells and did not require absorption with mouse nonlymphoid tissue (liver, erythrocytes) or bone marrow cells.

The preparation of similar fractions from human thymocytes and brain is currently attempted since they could provide a suitable material to obtain specific antisera to human thymus-derived lymphocytes with minimal absorption procedures.

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Affinity Labeling of Sucrase-Isomaltase Complex from Rabbit Small Intestine

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Rabbit intestinal sucrase-isomaltase complex [Cogoli et al., Eur. J. Biochem. (1972) *30*, 7–14] is fully inactivated by a substrate-like epoxide, conduritol-B-epoxide [Legler, G., H.S.Z. physiol. Chem. (1966), *345*, 197–214], with pseudo-first-order kinetics. Substrates and competitive inhibitors protect both sucrase and isomaltase activities. Na^+ activates the reaction of conduritol epoxide with sucrase by about 15% at pH 6.0. Isomaltase is inactivated about ten times faster than sucrase. The pH dependence of the reaction as well as the hydroxylamine sensitivity of the covalent inhibitor-enzyme bond suggest the participation of carboxylic group(s) located in the active sites of sucrase and isomaltase.

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Fragilité des parois de levures en fonction de leur âge

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La fragilité des parois de levures *Saccharomyces cerevisiae* et *Schizosaccharomyces pombe* a été étudiée au cours de la cinétique de leur désintégration dans un broyeur à billes (selon Rehacek). Le degré de désintégration a été déterminé au moyen d'un calculateur de volume des particules (Coulter Counter). Cette méthode comparée aux méthodes conventionnelles (détermination de N total libéré, ensemencement sur plaques de Pétri et observation au microscope), permet une détermination précise du temps et du degré d'ouverture des cellules ainsi que des changements de volume de leurs particules au cours de la désintégration.

La répartition des catégories d'âge des cellules désintégrées a été observée au moyen de la technique de la microscopie fluorescente, coloration avec le Brightener CFW des cicatrices de parois indiquant l'âge des cellules (cicatrice

chez Saccharomyces et renflement chez Schizosaccharomyces).

Cette méthode a permis de trouver les différences essentielles de fragilité entre les parois de Saccharomyces et Schizosaccharomyces.

Les différentes fragilités des parois des diverses catégories d'âge de population de levures *Saccharomyces cerevisiae* trouvées au moyen de la combinaison des deux méthodes susmentionnées sont données non seulement par les propriétés structurales des parois mais aussi par la faible différence de leur composition chimique.

Influence of Phosphodiesterase Inhibitors on Brain Protein Kinases in Vitro

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Partially purified ox brain soluble protein kinase (sPK) and free and membrane-bound protein kinase (mPK) of cat brain homogenate and its subcellular fractions, obtained by sucrose density gradient centrifugation, were investigated with and without cyclic AMP (cAMP). Stimulation of sPK was up to 10-fold, for homogenate PK 3-fold and less for the particulate fractions. Without histone, stimulation in synaptosomal fractions was not evident. We examined the effects of dihydroergotamine, dihydroergonine (DN), caffeine and papaverine, known phosphodiesterase (PE) inhibitors, on sPK and mPK and found, depending on drug and cAMP concentrations, deviations from the controls to a maximum of 10%. DN reduced the cAMP-stimulated synaptosomal PK to nearly the basal level. DN (10^{4}–M) showed strong inhibition of ox brain sPK (± cAMP), disappearing at 10^{-6}M. By phosphorylation mPK may cause changes in nerve transmission. PE and PK are found at high concentrations in synaptosomes (J. Biol. Chem. *246*, 134, 1971), thus drugs interfering with both enzymes are of special interest.

Presence of Peptidase in Bovine Pineal Gland

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During extraction and purification of neurophysin-like proteins and neurohormones from the bovine pineal glands, the yield of neurohormone measured as arginine-vasotocin (AVT) by radioimmunoassay (RIA) was found much lower (molar ratio of neurophysin to AVT: 22.5) than expected. The possibility for the presence of an enzyme was therefore searched by measuring AVT and neurophysin by RIA or by TLC followed by autoradiography of labelled AVP after incubation.

The crude pineal gland extract was found to contain an enzymatic activity that hydrolyzed synthetic AVT and 125-I-AVP but not pituitary neurophysins with the following caracteristics: its optimum is at pH 7.0–7.2, its activity is completely lost after 20 days at –20 °C, partially lost after dialysis against EDTA, or in presence of phenylisocyanate but unaffected by DFP, *p*-chloromercurobenzoate, iodination or iodoacetamide. The destruction of AVT is linearily related to the amount of crude pineal extract and 50% of 15 ng AVT is destroyed in 10 min.

Conclusion: pineal glands contain a proteolytic enzymatic activity able to attack the neurohormones AVT and AVP. Studies with inhibitors suggest that an amino-group of the enzyme is involved in the reaction.

Inhibitors of the Adhesiveness of Fimbriae Isolated from Entero-Pathogenic E. coli

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The entero-pathogenic strain of E. coli 0125:K70 is able to adhere to washed guinea pig erythrocytes and to cause their agglutination. Electron microscopy revealed this strain to be rich in fimbriae. The latter have been isolated according to Brinton (Trans. N.Y. Acad. Sci. 27,1003–1054, 1965); they are able to cause haemagglutination by themselves up to a dilution of 1 µg protein per ml. Bovine anti-E.coli lactoserum or serum have been shown in radial immunodiffusion studies to contain fimbrial antibodies and are able to inhibit haemagglutination caused by the whole bacteria or their fimbriae at a concentration as low as 1 mg protein per ml. D-mannose, α-methylmannoside and mannane are inhibitors at concentrations as low as 5 µg/ml. On the contrary, L-mannose, D-glucose or D-lactose have no inhibitory effect at concentrations as high as 50 mg/ml. The existence of inhibitors of the adhesiveness of the bacteria may be of practical importance in the prevention or treatment of chronic E. coli infections.

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Inhibition of Binding of Clq to Insoluble Antigen-Antibody Complexes

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The binding of Clq to soluble immune complexes in vivo may result in immune complex disease. Several groups of substances were tested for their ability to inhibit in vitro binding of 1 µg ^{125}I-labelled Clq [Heusser, J. Immunol. *110*, 820, (1973)] to 20 µg insoluble ovalbumin-antiovalbumin complexes. These included: 1. *Polyanions* of the carbohydrate and nucleotide type of which only those which precipitated Clq were inhibitors (e.g. polinosinic acid). 2. *Aliphatic amines* and diamines such as diaminopentane which caused a 50% inhibition at $1\times10^{-3}M$ concentration and hydroxylamine at $4\times10^{-2}M$. 3. *Aromatic amines* among which pyridoxal (vitamin B6) gave a 50% inhibition at a concentration of $2\times10^{-2}M$. 4. *Proteins* and peptides with molecular structures resembling IgG or Clq of which non-specific rabbit IgG and its fragments Fc, Pep III, and Pep V were inhibitory only when present at least at 100 times molar excess of immune complex. Bovine collagen, which resembles Clq in composition, decreased Clq binding to complexes by 50% when present at 200 times weight excess of Clq.

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A New Approach to the Isolation of RNA-Fragments from Specific Regions of the Bacteriophage Qβ Genome

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Various investigators have developed methods to obtain pure fragments from ^{32}P-labeled bacteriophage RNA by partial nuclease digestion followed by multistep poly-

acrylamide gel electrophoresis. By analysis of such frag ments large segments of the primary structure of R17- and MS2-RNA have been elucidated [e.g. Min Jou et al., Nature *237*, 82 (1972)]. However it is difficult to establish from which region of the RNA each of the many fragments produced by partial digestion originates. In order to detect all fragments derived from any particular region of interest in Qβ RNA, synchronized minus strand-directed synthesis by Qβ replicase was used to produce full-length plus strands labelled only in a restricted region with radioactive nucleotides. Such products and uniformly in vivo labelled RNA were subjected to a standardized partial digestion and the products were resolved electrophoretically side by side. Using this technique several fragments of Qβ RNA originating from the coat cistron region could be identified and isolated.

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Effect of a High-Fat Diet on Glycerol Utilization 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. When hemidiaphragms were incubated with 2 and 10 m*M* U-^{14}C-glycerol, incorporation into glyceride glycerol was increased 3–4 fold above the control value in diaphragms of fat-fed rats. In other experiments, glucose (5.5 m*M*) and glycerol (1 m*M*) incorporation into glyceride glycerol was measured in the presence of both substrates. Glycerol participates in reesterification up to 48% in tissues of fat-fed rats and up to 25% in control tissues. These results suggest that, even in the presence of glucose, glycerol can be significantly used by muscle for fatty acid esterification, a process which is further increased by high fat diet. This effect of fat diet can be explained by a stimulation of glycerokinase activity.

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Preparation and Properties of the Apo/Holo Hybrid of Aspartate Aminotransferase

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The cytoplasmic isoenzyme of aspartate aminotransferase was examined for possible subunit interactions by comparing the functional and structural properties of homomeric dimers with those of the corresponding apo/holo hybrids [one apo subunit and one subunit carrying the coenzyme, pyridoxal 5'-phosphate (PLP) or pyridoxamine 5'-phosphate (PMP)]. The apo/PLP hybrid was isolated from an apo enzyme preparation which had been restituted with PLP to 50 per cent of the original activity. Isoelectric focusing yielded 3 fractions: PLP/PLP, apo/PLP, apo/apo dimers in a ratio of 3:5:3. Both the apo/PLP and the apo/PMP hybrid do not differ from the respective homomeric forms with regard to active center activity, absorption and CD spectra of the coenzyme chromophore, and the rate of coenzyme binding. However, the thermal inactivation rate of the apo subunit of both the apo/PLP and apo/PMP is 5 or 3 times, respectively, slower than that of the apo/apo dimer, suggesting that the subunit unterface is involved in conformational changes occurring on binding of the coenzyme.

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Mechanism of Action of 2-C-Methylene-Myo-inositol Oxide in Schizosaccharomyces pombe

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2-C-methylene-*myo*-inositol oxide (MO) produces uninucleate, elongated cells in the susceptible strain, *Schizosaccharomyces pombe* CBS 1042. The mechanism of action of MO in this strain was studied. It took up 1.4 to 2.9% of the initially offered (^{14}C) MO from the medium during 44 h growth. At 30% inhibition of the culture, the surviving elongated cells took up 2.3 µg MO per mg dry cell mass. Resistant yeast strains, however, took up 6 to 8 fold less MO under identical conditions. The total uptaken MO by the susceptible strain was found to be distributed as follows: 72% in the 80% ethanol extract (Fract. I), 8% in the ethanol insoluble-water extract, and out of the remainder (20%), 11% was alkali hydrolysable whereas 9% was alkali insoluble. One of the metabolites from Fract. I was tentatively identified as guanine substituted at N-7 by MO. Further evidence is produced which suggests that MO acts by the alkylation of cellular DNA.

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Efficiency of calcium transport in rat liver mitochondria

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The recycling of endogenous calcium across the inner membrane of incubated mitochondria consists of a passive efflux of calcium from the matrix and an active re-uptake of this ion from the medium. After addition of ruthenium red which inhibits only the re-uptake, the concomitant decrease in the rate of state 4 respiration and the rate of calcium efflux from the matrix were measured. From these results the Ca/O ratio of the calcium recycling was calculated. When the calcium transport system was progressively inhibited with increasing concentrations of ruthenium red these Ca/O ratios became smaller, whereas the intramitochondrial ATP/ADP ratios increased. Moreover, in the presence of barely uncoupling concentrations of dinitrophenol the Ca/O ratios were larger than in the control, whereas in the presence of oligomycin they were smaller. This paradoxical behaviour of the efficiency of the calcium transport system will be discussed in the light of a thermodynamic theory [O. Kedem, S. R. Caplan (1965), Trans. Faraday Soc. *61*, 1897–1911]. The results suggest that the efficiency of the calcium transport system is very critically dependent on the mitochondrial energy charge.

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Topology of Carbamyl Phosphate Binding Sites in Aspartate Transcarbamylase

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In aspartate transcarbamylase, a regulatory enzyme from *E. coli*, pyridoxal phosphate mimics the binding properties of carbamyl phosphate, the first substrate in the ordered binding to the hexameric protein. The half-of-the-site saturation of carbamyl phosphate, and the unmasking of all 6 potential binding sites by succinate, an analogue of the second substrate, have been demonstrated [J. P. Rosenbusch and J. H. Griffin (1973) J. Biol. Chem. *248*, 5063]. Covalent linkage of pyridoxal phosphate by reduction with $NaBH_4$, followed by electrophoretic separation of the enzyme: pyridoxal phosphate complexes have yielded 4 species with either 0, 1, 2 or 3 moles of (^{32}P)-pyridoxal phosphate per mole of catalytic trimer. This result, in conjunction with the distribution and kinetics of appearance of the 4 species as a function of ligand concentration allows the conclusion that in the absence of succinate, the 3 pyridoxal phosphate molecules are bound randomly to both catalytic trimers, rather than asymmetrically to one of these subunits.

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CD-Titration of Enzyme-Coenzyme Complexes of Yeast Alcohol Dehydrogenase (YADH)

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We have shown previously that binding of NADH to YADH induces negative circular dichroism in the dihydronicotinamide chromophore of the bound coenzyme ($[\theta]_{333} = -25{,}000°$). Hence, measurement of the amplitude of the circular dichroism band provides a novel and convenient means to detect formation of the complex and to determine the binding parameters. Thus, circular dichroism (CD) titration of YADH with increasing concentration of NADH yields a stoichiometry of 4.0 moles/mole and for each binding site a dissociation constant, K_D, of $2.5 \times 10^{-5} M$ ($0.1 M$ phosphate, pH 7.5, 27°). The stoichiometry was also confirmed by equilibrium dialysis and is in agreement with the number of enzyme subunits. Unlike NADH NAD^+ does not induce circular dichroism above 300 nm but its interaction with YADH can be measured indirectly by its effectiveness to complete with NADH for the enzyme. The results indicate that all binding sites are equal and that coenzyme binding is non-cooperative.

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Compartmentation of Acetyl-CoA in Mitochondria of Rat Liver

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It has been proposed [Fritz (1968) FEBS Symp. *6*, 39; Müllhofer and Kuntzen (1972) Z. Physiol. Chem. *353*, 1461] that different pools of mitochondrial acetyl-CoA exist in the liver cell. To test this proposal we incubated rat liver mitochondria in the presence of 1–^{14}C-palmitate pyruvate, bicarbonate, ATP, phosphate and malonate and measured the ratio of the specific radioactivities of 3-hydroxy-butyrate: citrate. Without compartmentation this ratio would be maximally 2, however, under our conditions a value of 2.5–3.5 was observed. In further experiments with mitochondria, we tested the sensitivity of pyruvate carboxylase for acetyl-CoA produced from various precursors. It was found that acetyl-CoA produced either by pyruvate dehydrogenase, or by fatty acid oxidation from octanoate or palmitoylcarnitine, or from acetylcarnitine but not from leucine lead to a stimulation of pyruvate carboxylation. These results clearly indicate a compartmentation of mitochondrial acetyl-CoA in the liver cell. It cannot be decided from these results whether acetyl-CoA is compartmented within each mitochondria or whether different types of mitochondria with varying enzyme patterns and pool sizes are present in the liver cell.

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On the Subunit Structure of Particulate Aminopeptidase from Pig Kidney

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When solubilized by trypsin, particulate aminopeptidase from pig kidney yields 3 components upon denaturation. Replacing trypsin by Triton X-100 at pH 5 results in only 1 component, identical to the heaviest one of the 'trypsin enzyme'. Both enzymes are otherwise identical in every respect. Exposure of the 'triton enzyme' to trypsin yields the 3 components typical of the 'trypsin enzyme'.

After separation of the 3 components in $8 M$ urea followed by renaturation, only the heaviest one yields an active enzyme which seems identical to the 'triton enzyme', thus explaining the consistently higher yields of activity obtained for the 'triton enzyme' after reversible denaturation.

The results show that aminopeptidase is made up of 2 subunits of identical molecular weight. This fits with a content of 2 g atoms zinc per molecule shown previously. Trypsin introduces a nick into the molecule which becomes evident only under denaturing conditions.

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In vitro Interaction of Acetylcholine with Dopamine and Serotonin in Striatum

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The high affinity uptake mechanism that has been found for choline, analogous to those found for the biogenic amines, results in a rapid synthesis of acetylcholine which can be physiologically released on stimulation. Accordingly, striatal slices were prepared from nialamide-treated male rats and preincubated in Krebs-Ringer medium containing eserine ($10^{-4} M$) and choline chloride (10^{-3}–$10^{-6} M$) for 30′ at 37 °C. Further 10′ incubation was carried out after addition of ^{3}H-dopamine (DA) or ^{3}H-serotonin (5-HT) at 10^{-7} M and amine uptake measured. The remaining tissue was perfused sequentially with normal medium (10 fractions) and high K^+ (50 mM) medium containing choline (5 fractions). Choline at 10^{-3} M was found to significantly (Wilcoxon test for paired differences) inhibit DA uptake (67%) without affecting 5-HT uptake. There was no effect on efflux of either amine into the medium, but K^+-stimulated release was inhibited both for DA (63%, $p < 0{,}01$) and 5-HT (57%, $p < 0{,}05$) by 10^{-3} M choline.

Biosynthèse de mannolipides chez une levure, Schizosaccharomyces pombe

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Le GDP-mannose est généralement la source des restes mannosyles pour la biosynthèse des mannolipides. Chez *Schizosaccharomyces pombe*, la fraction microsomale est capable de transférer le reste mannosyle sur des accepteurs endogènes pour former quatre types de lipides. Par dégradation alcaline ménagée, on a pu mettre en évidence du mannosyl-diglycéride et une famille de lipides porteurs d'une chaine oligosaccharidique de plus de quatre unités glycosidiques. La fraction lipidique résistante à la déacylation alcaline a pu être résolue en deux lipides. Le premier est extrêmement acido-labile et ses caractéristiques correspondent à un mannosyl-1-phosphoryl-poly-isoprénol. La réaction de formation de ce lipide est réversible: on a pu régénérer de GDP-mannose radioactif en incubant la fraction enzymatique avec le lipide radioactif purifié et du GPD. La nature du second lipide est encore inconnue. Les enzymes de biosynthèse de tous ces lipides ont pu être solubilisés ainsi que les accepteurs au moyen de Triton X-100. Une précipitation acétonique a permis de séparer les accepteurs des enzymes.

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NSILA-S, Epinephrine-Stimulated Lipolysis and cAMP Release in Rat Adipose Tissue

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The effects of nonsuppressible insulin-like activity (NSILA-S) and insulin on epinephrine-induced lipolysis and cyclic AMP release in rat adipose tissue have been compared: NSILA-S and insulin inhibit the rise of tissue free fatty acids (FFA) and FFA-release induced by epinephrine in a similar dose-dependent manner. Both compounds decrease epinephrine-stimulated glycerol release in the absence, but enhance it in the presence of glucose. Cyclic AMP release into the medium in the presence of $2.7 \times 10^{-6} M$ epinephrine and 0.7mM theophylline is reduced to less than 50% by 1 mU/ml of insulin and 0.6 mU/ml of NSILA-S, respectively. These results provide further evidence that NSILA-S and insulin act via the same molecular mechanism.

PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

Selective Stimulation by Dopamine of Adenylate Cyclase in Homogenates of Rabbit Retina

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Homogenates of rabbit retina were assayed for adenylate cyclase activity (ACA) in a medium containing Tris-HCl (pH 7.4), ATP, Mg^{2+}, NaCl, KCl, EGTA and theophylline, in optimal concentrations. The cAMP formed was then extracted and measured according the method of Brown. Control values were 130.5 ± 4.2 (S.E.M.) pmoles cAMP $\times$mg protein$^{-1} \times 2.5$ min.$^{-1}$. The rate of formation of cAMP was a function of time and amount of protein. Under our experimental conditions, dopamine was found to be the most potent activator of the enzyme: 10^{-6} to $10^{-3} M$ caused an increase in ACA up to 93% above control values with the peak effect at $10^{-4} M$ (253.9 ± 9.9 pmoles cAMP). This increase is much greater than any so far observed in mammalian brain or rat retina. Furthermore, the effect of $10^{-4} M$ dopamine was completely blocked by $5 \times 10^{-5} M$ haloperidol. Apomorphine, on the other hand, at $10^{-5} M$, caused a 49% increase in ACA (194.0 ± 15.1 pmoles cAMP). Noradrenaline ($10^{-5} M$) was found to stimulate ACA (48% above control values), whereas $10^{-5} M$ isoproterenol or phenylephrine had no effect. The results suggest that homogenates of rabbit retina can be very useful for studying dopamine stimulated ACA and possibly dopamine receptors.

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Effects of (-)-Δ^9-trans-Tetrahydro-Cannabinol (THC) on Venous Return in dogs

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Injection of THC (2.5 mg/kg i.v.) in pentobarbital anesthetized dogs with heart rate maintained constant decreased aortic blood pressure (B.P.), cardiac output (C.O.), peak (L.V.P.) and end diastolic left ventricular pressure (L.V.E.D.P.), as well as max. d L.V.P./dt. Conversely, the contractility index (max. d L.V.P./dt)/I.P. (isovolumic pressure) was not altered by the compound. The decrease in C.O. could be abolished by infusing saline-dextran in quantities sufficient to return L.V.E.D.P. to pre-THC level.

In intact dogs in which C.O. was maintained constant by a right heart bypass procedure THC decreased B.P. and venous return (V.R.) to the extracorporeal reservoir. This latter parameter was significantly less (70%) diminished in dogs with splanchnic arteries ligated. Conversely, no alteration in V.R. was produced by a THC in spinal dogs (infused with epinephrine).

These results indicate that the decrease in C.O. following THC is due to insufficient ventricular filling (decrease in V.R.) and not to insufficient ventricular emptying (decrease in myocardial contractility). In addition, the presence of splanchnic circulation and an active neurogenic tone to the vasculature are necessary for the full diminution of V.R. by THC.

Suppression of Conditioned Hyperthermic Response by l- and D-Oxprenolol in Rats

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In rats trained to avoid electric shocks (CS-light and platform presentation for 10 sec) an increase in rectal temperature could be observed at the end of each training-session. The hyperthermic response persists even after shocks are omitted and the conditioned avoidance response is established (A. Delini-Stula; Psychopharmacologia *20*, 153–158, 1971).

Repeated administration of *l*- and *d*-oxprenolol (2×50 mg/kg p.o./day over 14 days) to rats subjected to the conditioning procedure was found to prevent the development of conditioned hyperthermia. *d*-Oxprenolol was more effective in this respect than *l*-oxprenolol. The acquisition of conditioned avoidance behavior, which was studied in parallel, was not affected by these drugs. However, towards the end of the experiment (10th day) the number of failures resulting in increased shock frequency augmented slightly.

Observed effects could not be ascribed to the peripheral beta-blocking action of these drugs. The attenuation of fear in a stressful situation is suggested as a possible mode of action.

Correlation between Inflammatory Symptoms and the Release of Lysosomal Enzymes or Prostaglandins in an Acute Inflammation

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There is evidence that symptoms of acute inflammation are mediated by release of lysosomal content and/or by generation of prostaglandins (PG's) at the site of inflammation. Also, it has been proposed that Aspirin-like drugs exert their anti-inflammatory action by inhibiting either of these processes. Direct evidence, however, from in vivo experiments confirming these hypotheses are almost completely lacking. We have elicited an acute inflammation in chicken by injecting urate crystals into intertarsal joints. Nociception and temperature rise from the inflamed joints were assessed and joint fluids were investigated for appearance of leukocytes, lysosomal enzymes and, in addition, PGE_2 and $PGF_{2\alpha}$ using a radio-immuno assay. Then the effect of sodium salicylate was investigated. A correlation was found between the time course of the inflammatory symptoms and the amount of PG's found in the joint fluid but no such correlation with the time-course of granulocyte invasion and lysosomal enzyme release. Moreover, the inflammatory symptoms and the appearance of PG's were inhibited by sodium salicylate but not invasion of granulocytes and release of lysosomal enzymes. From this result, it is concluded that granulocytes and their lysosomal enzymes are not important mediators in this acute inflammation whilst PG's might be.

Effect of High Potassium and dbcAMP on Sympathetic Ganglia in Organ Culture

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In the peripheral sympathetic nervous system a prolonged increase in the activity of the preganglionic cholinergic nerves leads to a selective induction of tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) in the terminal adrenergic neuron. Since it had been suggested that depolarization as such might trigger TH and DBH induction and that cAMP might act as second messenger we studied the effect of 54 m*M* potassium and 5 m*M* dbcAMP on mouse sympathetic ganglia in organ culture. After 48 h in the presence of 54 m*M* potassium the TH level of the ganglia amounted to $189 \pm 13\%$ and that of DBH to $223 \pm 16\%$ of culture controls. The corresponding values for 5 m*M* dbcAMP were $202 \pm 14\%$ and $136 \pm 13\%$ respectively. However, the cycloheximide-sensitive increase was not confined to TH and DBH but involved DOPA decarboxylase and monoamine oxidase to a similar extent, enzymes which are never increased in vivo under experimental conditions leading to trans-synaptic TH and DBH induction. These findings together with the observation that high potassium does not change the cAMP level in sympathetic ganglia in organ culture make it doubtful whether an increase in TH in the presence of high dbcAMP can be taken as evidence that cAMP acts as second messenger in trans-synaptic induction of TH and DBH.

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Effect of TEA on Membrane Potential and Contraction of Vascular Smooth Muscle

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In strips of rabbit main pulmonary artery (RMPA), TEA-chloride (10 m*M*) reduced the membrane potential of the vascular smooth muscle cells from –60 mV to –46 mV, as measured with intracellular microelectrodes and sometimes caused a slight contraction. The drug decreased the threshold of the contractile response to noradrenaline (NA) by a factor of 30 and increased the maximum of the NA-induced contraction. Independent of the presence of TEA, NA (10^{-8}–10^{-6} *M*) depolarized the membrane of the vascular smooth muscle cells by virtually the same amount, resulting in maximal reductions of the membrane potential to –46 mV (without TEA) and –35 mV (with TEA), respectively. The residual contraction to NA in Ca-free solution was not altered by TEA. Consequently, the NA-induced release of Ca from cellular storage sites seems to be unaffected by the drug. In the RMPA, K^+-induced contraction depends only on extracellular Ca. The threshold of this contraction was shifted by TEA to lower K^+ concentrations. It is concluded that for a given depolarization, TEA increases the Ca permeability of the plasma membrane.

Progress in Isolation of Acetylcholinesterase [EC 3.1.1.7] by Affinity Chromatography

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The term 'Affinity Chromatography' is often used in a wide sense. In the most general sense it may be used for more or less specifically bound proteins to a column, and elution by a gradient of increasing ionic strength. For the isolation of acetylcholinesterase it can be defined in a specific way: 1. Binding of a specific enzyme-inhibitor to a solid matrix support. 2. Application of a crude protein mixture. 3. Elution of all nonspecifically bound proteins

with buffers of increasing ionic strength, and 4. Elution of the specific enzyme with the free specific inhibitor (same type as bound inhibitor). Affinity separation is only obtained when the 'spacer' has a considerable length. The spacer synthesis is laborious, time-consuming, and very expensive. With pre-synthesis of an inhibitor of sufficient length: 1-(N,N,N-Trimethylammonium)-10-decylamine bromide hydrobromide, and the use of a commercially available diamine (Spermine), first applied by J. Schmidt and M. A. Raftery, Biochem. *12*, 852 (1973) for separation of cholinergic receptor proteins, the synthesis could be simplified in any of the above mentioned ways with no loss in overall yield.

Disposition by Intact Rat Uterus of Oxytocin Analogues Modified in the Cystine Moiety

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[6,1-Cystathionine]oxytocin ['1-carba-oxytocin, *1*; cf. Jošt et al., Coll. Czechoslov. Chem. Commun. *38*, 1073 (1973)] and [1,6-α,α'-diaminosuberic acid]oxytocin (*2*) have been synthesised. The rate of relaxation of the K^+-depolarised rat uterus immersed in oil [Kalsner, Nickerson, Can. J. Physiol. *46*, 719 (1968); Furrer, Rudinger, Experientia *28*, 742 (1972)] after contraction with 1,2,desamino-oxytocin (*3*), its '1-carba' and 'dicarba' analogues (*4* and *5*), and of [1-hydroxy-2-mercaptopropionic acid]-oxytocin *6* [Hope, Wälti, Biochem. J. *125*, 909 (1971)] has been measured. The overall rate decreases in the order oxytocin $>$ *1* $\sim$ *6* $>$ *2* $>$ *3* $\sim$ *4* $\sim$ *5*. This structural dependence and the clearly multiphasic character of the relaxation curves indicated that one or more processes in addition to enzymic inactivation are involved in the disposition of the hormones.

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Effect of L-Dopa on Endogenous Noradrenaline in Rat Brain

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In the brain of rats, i.p. administration of L-dopa enhanced the disappearance of endogenous noradrenaline (NA) induced by FLA 63, an inhibitor of dopamine-β-hydroxylase. This effect was dose-dependent. In addition, 50 mg/kg L-dopa i.p. significantly increased the cerebral levels of 3-methoxy-4-hydroxy-phenylethylene glycol-sulfate, the main metabolite of brain NA. These findings indicate that L-dopa caused a release of NA, probably due to displacement of the amine by the newly formed dopamine. However, L-dopa administered i.p. alone or in combination with benserazid, an inhibitor of extracerebral decarboxylase, did not modify the content of endogenous cerebral NA.

It is concluded that L-dopa increases the turnover of brain NA whereby the exogenous amino acid is the source of the synthesized amine.

[2-*o*-Iodotyrosine]oxytocin Inhibits the Response of the Rat Uterus to Oxytocin

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[Tyr(I)^2]-Oxytocin has been prepared by iodination of oxytocin [Thompson, Freychat, Roth, Endocrinology *91*, 1199 (1972)] and by total synthesis using the S-Acm protecting group. The purified and chemically characterised analogue has no uterotonic activity but inhibits the response of the isolated rat uterus to oxytocin in media with or without Mg, with pA_2 ~7.2. Radioiodine-labelled oxytocin should therefore be suitable for studying binding to uterine receptors. The analogue has slight antidiuretic activity but no rat pressor activity in doses up to 250 μg/kg. It inhibits the pressor response to pituitary extract. [2-*o*-Methyltyrosine]oxytocin has similar properties, showing that the antagonism is due to the steric effect of the *o*-substituent and not to the change in pK_a of the phenolic hydroxyl caused by the iodine.

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Lack of Correlation between Changes in cAMP and Trans-Synaptic Enzyme Induction

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Treatment of neonatal rats with nerve growth factor antiserum or 6-hydroxydopamine producing an extensive (61–85%) specific destruction of the adrenergic neurons in the rat superior cervical ganglion led to a decrease in the ganglionic cAMP content by 16 to 28% only. This indicates that only a relatively small portion of the total cAMP is localized in the adrenergic neurons. However, administration of 0.4 mg/kg of isoproterenol produced a 12-fold increase in cAMP exclusively in this neuronal pool. Single or repeated injections of isoproterenol did not evoke a TH induction in the superior cervical ganglion. These results, together with observations that experimental conditions leading to a trans-synaptic induction of TH in sympathetic ganglia (swimming stress, treatment with reserpine) did not change cAMP levels do not support the assumption that cAMP plays a key role in trans-synaptic TH induction.
Dissociations between changes in cAMP and subsequent rise in TH also became apparent in the rat adrenal medulla.

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Drug-Induced Rotation in Rats after Unilateral Intracerebral Injection of 5,6-Dihydroxytryptamine (5,6-HT)

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Unilateral stereotaxic injection of 10 μg of 5,6-HT in the medial forebrain bundle (MFB) of rats produces, within 10 days, a marked unilateral decrease of the forebrain levels of serotonin (5-HT) and dopamine (DA) (Saner et

al. 1974), due to unilateral degeneration of serotonergic and dopaminergic non-terminal axons in the MFB (Lorez et al. 1974).

In these 5,6-HT-lesioned rats, apomorphine down to 0.1 mg/kg i.p. induced rotation towards the non-lesioned side, whereas *d*-methamphetamine down to 0.5 mg/kg i.p. evoked ipsilateral rotation. Haloperidol 1 mg/kg i.p. blocked the effect of apomorphine (1 mg/kg i.p.) and *d*-methamphetamine (3 mg/kg i.p.). LSD (100 and 200 µg/kg i.p.) elicited controlateral rotation. Injection of LSD for 5 consecutive days did not result in tolerance. Mescaline, up to 20 mg/kg i.p., proved inactive. Haloperidol 1 mg/kg i.p. was also able to block the rotation induced by LSD. It is tentatively concluded that LSD may exert an apomorphine-like direct stimulation of DA receptors.

Intracellular Uptake and Membrane Binding of Ca in Human Red Cell Ghosts

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The K permeability of resealed human red cell ghosts increases greatly when the intracellular Ca ion concentration (Ca_i) is raised from 10^{-7} to 10^{-6} *M*. This Ca-dependent permeability change was used as a measure of the inward movement of extracellular Ca. Ca-free ghosts were loaded with increasing concentrations of EGTA and incubated in NaCl media containing up to 5 m*M* Ca. Under these conditions the change in Ca_i resulting from uptake of Ca is determined by the intracellular Ca/EGTA concentration ratio and the apparent binding constant of the CaEGTA complex ($\sim 10^7$). The amount of Ca which had penetrated into the cell was calculated from the cellular EGTA concentration which was just insufficient to block the Ca-induced K loss. When the medium contained 5 m*M* Ca the cells took up a total of 0.8 m*M* Ca. The change in Ca_i was only 0.01 m*M*, the rest was probably bound to the membrane. The action of local anesthetics showed that Ca binding and Ca permeability behaved as independent variables: 1 m*M* tetracaine or propranolol decreased Ca binding by 45 and 20% respectively. The Ca membrane transfer was not changed by tetracaine but was increased nearly 5-fold by propranolol.

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5,6-Dihydroxytryptamine: Mode of Administration and Effect on Brain Monoamines

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After intraventricular injection in rats, 5,6-dihydroxytryptamine (5,6-DHT, 50 µg) produced a transient and rather selective depletion of cerebral 5-hydroxytryptamine (5-HT), and a more substantial and permanent reduction of this amine in the spinal cord. On the other hand, unilateral stereotaxic injections of 0.5–10 µg 5,6-DHT in the medial forebrain bundle and the nigrostriatal dopamine (DA) bundle of rats caused a marked dose dependent, and long lasting (> 170 days) decrease of both, 5-HT and DA with only a slight reduction of noradrenaline (NA) in the homolateral telediencephalon. Furthermore, direct application of 5,6-DHT in the dorsal NA bundle did not substantially affect the NA content. In addition, the uptake of 5-HT and DA into brain slices and synaptosomes of the telediencephalic regions homolateral to the stereotaxic injection was reduced. The results suggest that intracerebral in contrast to intraventricular administration of 5,6-DHT causes a destruction not only of 5-hydroxytryptaminergic but also of dopaminergic pathways in the brain, possibly as a result of anterograde degeneration.

Specificity of the Retrograde Axonal Transport of Nerve Growth Factor (NGF)

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NGF promotes growth and differentiation of the peripheral sympathetic nervous system. This protein is taken up into the adrenergic nerve terminals and reaches the cell body by rapid (2.5 mm/h) retrograde axonal transport (RAT) which is blocked by colchicine.

The specificity of RAT was investigated by injecting ^{125}I-labelled NGF and a series of other ^{125}I-labelled proteins with differing (6,000–500,000) molecular weights and isoelectric points (4.5–9.8) into the mouse anterior eye chamber. The preferential accumulation of radioactivity in the superior cervical ganglion of the injected side was taken as a measure for RAT. Of all the proteins NGF and tetanus toxin were the only ones which exhibited a statistically significant ($P < 0.001$) difference (3-fold for NGF, 2-fold for tetanus toxin) between injected and non-injected side. Moreover, small chemical changes of the NGF molecule resulted in a marked reduction of RAT. It is noteworthy that the RAT of NGF is confined to the adrenergic neurons whereas that of tetanus toxin occurs in all the neurons studied, so far. Thus, RAT of tetanus toxin seems to depend on general properties of nerve terminals whereas that of NGF on those specific for adrenergic neurons.

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Does Maprotiline (LUDIOMIL®) Influence Serotonin Uptake and Free Tryptophan Concentration in Human Plasma?

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The influence of maprotiline, a tetracyclic antidepressant, on plasma tryptophan binding, serotonin uptake and content of blood platelets has been studied in healthy volunteers. Plasma total and free tryptophan concentrations were measured after treatment with either maprotiline (100 mg/day) for four days or acetylsalicylate (2 g on the evening prior to and the morning of the experiment) as a known competitor of tryptophan binding to plasma albumin.

No alteration in plasma tryptophan binding was noted after maprotiline treatment in contrast to a marked increase in free tryptophan in the acetylsalicylate group. Neither maprotiline nor acetylsalicylate had an influence on the uptake or content of serotonin in blood platelets from these samples.

Dynamics of Depolarised Rat Uterine Muscle Upon Oxytocin Stimulation

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The behaviour of the K^+-depolarised rat uterus contracted with oxytocin and allowed to relax in hormone-free medium (wash-out) or after immersion in oil [Furrer and Rudinger, Experientia *28*, 742 (1972)] is qualitatively modeled by a 3-compartment model assuming transport, inactivation, and binding to nonreceptor sites and a hyperbolic dependence of response on hormone concentration in the receptor compartment. The poor *numerical* fit led us to record strictly timed series of stimulation-relaxation cycles. The observed time dependence of characteristics such as base-line tension and peak response necessitated introduction of time-dependent parameters into the model. Black-box identification by analogue computer afforded a transfer function and eigenvalues consistent with all experimental results. Detailed analysis of the transfer function revealed the existence of an additional compartment responsible for the 'fade' phenomena observed in several phases of the response-relaxation cycle.

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Etudes sur la Na-K-ATPase de l'organe électrique de la Torpille

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La Na-K-ATPase de l'organe électrique de la Torpille est localisée surtout dans les tubules membranaires de la face dorsale des électroplaques. Le tissu est homogénéisé, centrifugé; le culot est soumis à un choc osmotique puis recentrifugé. On obtient ainsi une fraction riche en tubules dont l'activité ATPasique est 45.6 µmoles Pi/mg protéines/h à 37°.

Dans cette fraction, l'ATPase est inhibée à 50% par $5 \times 10^{-7} M$ d'ouabaïne. L'ouabaïne tritée se lie à un composant de cette fraction dans deux conditions différentes: a) en présence de Mg et Pi la demi-saturation est de $10^{-7} M$; b) en présence d'ATP et Na, $3 \times 10^{-7} M$. C'est donc à des concentrations voisines que l'ouabaïne inhibe l'enzyme et se fixe sur la fraction. Après solubilisation partielle et filtration sur gel, l'affinité pour l'ouabaïne est retrouvée au même endroit que l'activité enzymatique.

1 g de tissu électrogène est capable d'hydrolyser 0,33 mmoles d'ATP par heure et de fixer environ 3 nmoles d'ouabaïne, ce qui représente $1,8 \times 10^{15}$ sites.

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

Hepatic Autophagy in Uncontrolled Diabetes Mellitus and its Relationships to Insulin and Glucagon

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Exogenous glucagon is known to increase hepatic lysosomes, but the relationships between endogenous glucagon and insulin levels and lysosomes have not been examined. To this purpose, glycogenosomes, dense bodies and autophagosomes were morphometrically quantitated in normal rats, in rats with mild streptozotocin-diabetes with normal hormone levels, and in rats with severe streptozotocin diabetes, with hyperglucagonemia, hypoinsulinemia and ketoacidosis. In the latter, volume density of lysosomes, especially autophagosomes, was significantly above the control value. Insulin treatment corrected the hyperglucagonemia, hypoinsulinemia and reduced the volume density of lysosomes significantly below both that of the diabetic rats and the non-diabetic controls. In mild diabetes, volume density of lysosomes was not different from the controls. A statistically significant correlation between all measurements of lysosomal volume density and plasma glucagon was observed. It is concluded that uncontrolled streptozotocin diabetes in rats is accompanied by hepatic autophagy which may be related to the increased plasma glucagon level and/or the decreased insulin, and which is corrected by insulin therapy.

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Isolation and Characterization of Xanthine Dehydrogenase (XDH) and XDH CRM from Drosophila

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Xanthine dehydrogenase (XDH) activity in *Drosophila* requires the expression of several gene loci. Files mutant at the ry^+ locus lack immunologically cross reacting material (CRM) while those mutant at the mal^+ locus, located on another chromosome, contain CRM. In an attempt to understand the action of the mal^+ locus, XDH and CRM have been isolated and compared.

1) Homogeneous preparations of XDH and CRM were obtained from wild type and mal^- files, respectively, using immunoadsorbent columns.
2) XDH and CRM have identical electrophoretic mobilities in polyacrylamide gels.
3) XDH and CRM show immunological 'identity' in Ouchterlony double diffusion tests.
4) XDH and CRM contain similar amounts of FAD, Fe, and Mo.

These results suggest that XDH and CRM are very similar proteins. Further comparisons between these molecules are in progress.

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Etude par le microscope électronique à balayage de la structure des capillaires pulmonaires au cours du gonflement du poumon

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Des poumons de rats sont gonflés à différents volumes sous pression positive ou négative; ils sont fixés par perfusion in situ, à thorax ouverts et sont examinés au microscope électronique et au microscope électronique à balayage (SEM). A volume de gonflement comparable, il n'y a pas de différence qualitative entre les poumons fixés par pression positive et par pression négative. Le lit capillaire des poumons dégonflés ou peu gonflés est très tortueux. Il forme un réseau anastomotique complexe séparant les espaces aériens restés ouverts. Les capillaires communiquent entre eux à travers de «diaphragmes». Ceux-ci correspondent à des plissements dans la paroi des alvéoles dégonflées, faisant protrusion dans la lumière vasculaire. Le lit capillaire des poumons totalement gonflés est mince et étiré entre les alvéoles surexpendues. Les «diaphragmes» vus sur les poumons dégonflés ainsi que les plissements ne sont pas retrouvés. Au cours du gonflement progressif des poumons, la disparition des diaphragmes dans une première phase puis l'écrasement du lit capillaire sont susceptibles d'expliquer la chute paradoxale initiale de la résistance vasculaire pulmonaire suivie de sa remontée lorsque le poumon est hypergonflé.

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Messenger RNA of Egg Yolk Proteins: Partial Purification, In Vitro Translation and Control by 17 β-Estradiol

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The estrogen-induced synthesis of the yolk phosphoprotein phosvitin in chicken liver is accompanied by a 100–160% increase in the rate of synthesis of poly[A] containing polysomal mRNA. We now show that in the membrane-bound polysomes of the liver of estrogenized immature chicks there is a large increase in mRNA coding for egg yolk proteins. Polysomes were isolated from the rough endoplasmic reticulum, and total RNA was extracted with Tris pH 9-sodium dodecyl sulfate-phenol. Total RNA was fractioned on a nitrocellulose column. A 32–39-fold enrichment of total mRNA was obtained by step elution with 0.5 *M* KCl at 6° and 19°, respectively, followed by the elution of mRNA with 0.1 *M* Tris, pH 7.5, 0.0025 *M* EDTA at 6°. The purified mRNA was analyzed by polyacrylamide gel elctrophoresis and its capacity for directing the synthesis of specific proteins was tested in a Krebs ascites cell-free system. 36% of the total protein synthesized in the presence of mRNA purified from the estrogenized chicks cross-reacted with anti-egg yolk antisera in contrast to 2.6% for the control. Estradiol did not influence the relative content of serum albumin mRNA.

Loss of Viability during Swelling of Cultured Cells Under Isotonic Condiditons

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Loss of cell viability due to cellular swelling was studied in cultures of a murine mastocytoma. In order to inhibit active cation transport across the cell membrane, sodium salts in the medium were replaced by corresponding potassium salts. This resulted in a rapid and pronounced increase in cell volume.

Numbers of morphologically 'intact' cells as seen by microscopic examination decreased more slowly than numbers of cells excluding trypanblue. On the other hand, the decrease in numbers of trypanblue negative cells was comparable to that of colony-forming cells and correlated well with the release of labeled proteins from cells prelabeled with L-leucine-^{14}C. After reincubation of cells in normal medium, no evidence for conversion of trypanblue positive into trypanblue negative cells was found, while the remaining trypanblue negative cells exhibited a delay in resumption of cell multiplication.

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Separation of the Strands of Polyoma DNA

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The ability to isolate separately the complementary strands of the double-stranded DNA of polyoma virus would be useful for the characterisation of polyoma-specific RNA molecules in virus-infected cells. We have synthesized in vitro from superhelical polyoma DNA with E. coli RNA polymerase, RNA which is complementary to only one of the strands of polyoma DNA. Denatured complete length polyoma DNA was annealed with a 10-fold excess of *in vitro* synthesized RNA. About half of the DNA formed DNA-RNA hybrid molecules while half remained single-stranded. DNA in double-stranded hybrids was separated from single-stranded DNA by chromatography on hydroxylapatite. After removal of RNA, neither DNA preparation formed double-stranded molecules when annealed by itself; double-stranded DNA was formed when the two were annealed together. Thus the two DNAs represent the complementary strands of polyoma DNA.

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The Regulation of λdv Plasmid Replication

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The λdv plasmid is a fragment of phage λ and consists, in essence, of only one operon, a block of genes used in replication and control (sequence *promotor cro tR ori O P*). λdv replicates in *Escherichia coli* without any known attachment to the bacterial chromosome; many plasmid copies are present per cell.

New λdv plasmids were generated from λ phages already mutant in genes suspected of being important in plasmid replication control. We find that plasmid replication requires the products of genes O and P. Transcription beginning at *'promotor'* is often terminated at *tR* by a host factor. When this factor is antagonized, λdv replication is stimulated. Since stimulation does not occur when *O* and *P* are supplied in trans, it seems that transcription per se near *ori* (replication origin) is also a prerequisite for replication. *Cro* is also indispensable; the role of *cro* product is probably the control of the rate of plasmid transcription such that the number of plasmid copies doubles once each cell generation.

Activation of Albumin Synthesis in Human Leucocytes by Somatic Cell Fusion

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A murine hepatoma cell line which secretes albumin was fused with peripheral human leucocytes. The isozyme analysis of the hybrid clones showed the presence of 25 mouse markers. Only two human autosomal and the x-linked markers were present in any hybrid clone, suggesting that most of the human chromosomes were lost. This was further confirmed by chromosome analysis using Q and C-Banding. All hybrid clones continued to secrete mouse albumin. Two clones produced in addition human albumin which was demonstrated by electroimmunodiffusion against non crossreacting monospecific antiserum. These results demonstrate the activation of a gene normally not expressed in leucocytes. The expression of the human albumin locus may reflect either the contribution of a mouse activator locus or the loss of a human repressor locus through chromosome segregation. The continued expression of the mouse albumin locus may reflect the absence of a cross reacting repressor in diploid human cells or its loss during chromosome reduction.

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The Complexity of the Genome of Avian Tumor Viruses

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The genome of avian tumor viruses consists of an RNA sedimenting at 60–70 S, indicating a MW of about 10^7. Denaturation of this RNA yields material sedimenting at 30–40 S, suggesting that the genome contains 3–4 subunits with a MW of $2.4–3.4 \times 10^6$ Daltons each [Duesberg and Vogt, J. Virol *12*, 594 (1973)]. To obtain an estimate of its sequence complexity, purified ^{32}P-labeled 60–70 S RNA of Rous sarcoma virus was completely digested with RNase T_1 and the oligonucleotides were resolved by two-dimensional polyacrylamide gel electrophoresis. The fingerprint displaying the large oligonucleotides showed a highly reproducible pattern only slightly more complicated than analogous fingerprints of Qβ RNA or 28 S ribosomal RNA (MW about 1.5×10^6). Analysis of the radioactive material recovered from 32 distinct spots revealed that 18 oligonucleotides are obtained pure and in almost equimolar yield. From the amount of input RNA and the yields of pure oligonucleotides (corrected for losses during the fingerprinting by use of marker oligonucleotides as internal controls) a sequence complexity of the genome equivalent to about 10^4 nucleotides (corresponding to a MW of about 3×10^6 Daltons) was calculated.

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Possibility of Changing the Origin of Chromosome Replication in E. coli

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Temperature-sensitive mutations in the gene *dna*A affect the initiation of chromosome replication at 41° but not 30°. We have shown that they were not complemented by the presence of an R-factor in the plasmid state but that they could be suppressed as a result of the integration of R at a variety of sites in the chromosome. We postulated that in this 'integrative suppression' the entire chromosome becomes part of the episome (R) replicon and is replicated passively under R control. We have synchronized *dna*A strains suppressed by integration of R at different sites and studied the origin and directions of chromosome replication. We find that, in these strains, replication originates on the R replicon instead of on the normal origin. R-controlled replication is, like the normal one, bi-directional.

Secondary Monolayer Cell Culture from Rat Endocrine Pancreas

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Endocrine cell cultures are obtained from pancreas of newborn rats according to Lambert et al. Endocrinology *90*, 239 (1972). From these primary cultures, secondary cultures can be obtained provided that the clusters of endocrine cells are detached before 48 hours of culture and with a solution of trypsin not exceeding 0,125%. The trypsin cell suspension must be gently transferred into another dish and diluted by at least five volumes of medium-containing 10% calf serum. Monolayer cell cultures thus obtained usually contain larger clusters of endocrine cells and less fibroblasts than the primary cultures of equivalent age. This preparation is suitable for combining morphological and biochemical studies.

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Co-ordinated Stereological-Biochemical Model for the Rat Liver

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A method, based on a co-ordinated analytical model, has been developed which allows direct quantitative comparisons to be made between morphological and biochemical data derived from cellular fractions. For example, using Glucose-6-Posphatase as a marker enzyme for the endoplasmic reticulum (ER), the activity of this enzyme determined biochemically was compared with the surface of the ER obtained using combined stereological and cytochemical techniques. Recoveries, comparing the liver homogenate with the subsequent fractions, were 96% for the enzyme activity and 96% for the surface area of the ER. The following relative distributions of ER surface area and its marker enzyme, Glucose-6-Phosphatase, were observed in the fractions: Nuclear (13% Er surface, 14% enzyme activity), heavy mitochondrial (13%, 17%), light mitochondrial (5%, 7%), and microsomal (69%, 62%). In the above example, a direct comparison was made between the surface area of a cellular structure (ER) and the activity of an enzyme attached to it. The model, however, can also be used to compare the volume of a cellular organelle with the activity of an included marker enzyme.

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Estradiol-Induced Synthesis of Nucleoproteins in Chicken Liver

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Previously we demonstrated that injection of 17β-estradiol into immature chicks (5 mg/100 g body wt.) lead 12 hours later to the appearance of phosvitin in the serum. During the first 2 hours of this lag phase total polysomal mRNA increased over 160% while no increased rate of rRNA was observed. In our present study concerning the role of nucleoproteins in estradiol stimulated transcription, we show that 2 hours after estrogenization, the rate of incorporation of [^{3}H]leucine into nuclear protein in general was increased by 150% and in particular that the synthesis of three non-histone nucleoproteins was specifically enhanced during this time. They were characterized by means of SDS polyacrylamide gel electrophoresis using a double labeling technique. One of the nucleoproteins was a basic yet non-histone protein with a mol. wt. of 26,000, the others were acidic nucleoproteins with mol. wts. of 17,000 and 20,000 respectively and isoelectric points of 5.0, 5.35 and 6.2. Although the exact function of these nucleoproteins cannot be stated at this time, the evidence given above suggests a specific role during the early period of transcription.

The Ordering of Structural Proteins on the RNA-Tumorvirus Precursor Polypeptide Using Pactamycin

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The major structural proteins of RNA-tumorviruses are synthesized as a large precursor polypeptide of 76,000 MW. Using pactamycin, an inhibitor of initiation of protein synthesis, an attempt was made to determine the order of their synthesis. At various times after the addition of pactamycin to avian myeloblastosis virus (AMV) infected cells, proteins were labeled with ^{35}S-methionine for 10 min. Following a 2 h chase with unlabeled medium virus was collected from the supernatant and the amount of label in the virion proteins determined after electrophoresis. In addition the cells were lysed and the intracellular virus-specific proteins precipitated with antiserum against AMV proteins. The amount of label in these intracellular proteins was determined by electrophoresis of the immune precipitate. The results indicate that pactamycin preferentially reduces the incorporation of label into the 24,000 MW viral protein (VP24), while the labelling of the 11,000 MW protein (VP11) is relatively less affected. This was true for both intracellular viral proteins and proteins in assembled extracellular virus. We conclude that VP24 is N-terminal to VP11 on the precursor. No conclusion can be made concerning the other two major proteins since these do not incorporate enough label.

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Ultrastructural Localization of Paternal Genome in Early Mouse Embryos by Autoradiography

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Mouse zygotes after fertilization by tritiated thymidine-labeled sperm were processed for electron microscopy. Early mouse embryos were fixed in glutaraldehyde followed by osmium tetroxyde and embedded in Epon. Ultrathin sections were prepared for electron microscope autoradiography and preparations developed after 8–12 months. Initial results show the presence of radioactivity in about 10–20% of the nuclei examined. Practically all of these labeled nuclei show a non-homogeneous distribution of silver grains within the nucleus, thus confirming light microscopy results. Labeling is localized either in one region of the nucleus or sometimes in two more or less opposite nuclear regions. In some nuclei silver grains are observed as several spots. In some cells the label is associated with the nuclear periphery or is found over the nucleolar region. The labeled regions do not seem to be morphologically different from those which are not labeled.

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Estimation of the Number of Immunoglobulin Genes by (^{125}I) mRNA/DNA Hybridization

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The 14 S mRNA of immunoglobulin light chain is prepared from mouse myeloma tumor polysomes by oligo(dT)-cellulose chromatography, repeated sucrose gradient centrifugations and analysed on acrylamide gel electrophoresis in 98% formamide. The homogeneity of the 14 S mRNA is mesured from kinetic complexity data determined by hybridisation of the mRNA with enzymatically synthesized complementary DNA. 14 S mRNA labeled with (125 I) (spec. act.: 2×10^7 cpm/μg) is hybridised with a large excess of sonicated cellular DNA in 50% formamide. Conditions are chosen in which the size of the mRNA remains unchanged during the hybridisation. When hybridisation is measured (RNase) in function of Cot (concentration × time) it can be shown that about 80% of the hybridised RNA corresponds to near unique sequences in the DNA, and that 14% corresponds to sequences reiterated about 100 times.

The implications of these results on the number of genes existing for the variable (V) and the constant (C) part of immunoglobulin chains will be discussed.

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Attachement spécifique de l'ACTH à la G6PD (1.1.1.49) de cortex surrénalien bovin

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L'isolation conventionnelle de la glucose-6-phosphate déhydrogénase à partir du cortex surrénalien bovin (Int. J. Protein Res. *II*, 173, 1970) est grandement simplifiée par l'introduction de la chromatographie d'affinité sur

sépharose substituée par NADP. La déhydrogénase ainsi purifiée se lie spécifiquement in vitro au tétrakosipeptide synthétique [2-phénylalanine, 4-(4′, 5′-déhydro-4′5′-ditritio)-norvaline]-ACTH-(1–24). Ce dérivé hormonal à radioactivité spécifique élevée possède également des propriétés pharmacologiques très proches de celles de l'hormone à séquence naturelle (Liebig's Ann. Chem. *1973*, 1298). La constante d'association $K = 1.8 \cdot 10^6$ mesurée par équilibre de dialyse à pH 5.9 est en bonne concordance avec celle observée par mesure de la polarisation de la fluorescence avec le dérivé analogue [21-N-e-dansyllysine]-ACTH-1–24) (Helv. Chim. Acta *54*, 897, 1971).

Fingerprints of the Polypeptides of Polyoma Virions

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Polyoma virus particles were highly purified and then disrupted by heating in sodium dodecylsulfate and mercaptoethanol. The polypeptides were separated by electrophoresis in polyacrylamide gels in the presence of sodium dodecylsulfate.

Each polypeptide was eluted, lyophylized, oxydized and digested with trypsin. The resulting peptides were separated in two dimensions by electrophoresis and chromatography on thin layer cellulose sheets. The α-amino groups react with Fluram (Roche), which then shows visible fluorescence under ultraviolet irradiation. Approximately one nanomol of protein is sufficient for a good fingerprint.

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Site-Directed Mutagenesis

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Stepwise synthesis of Qβ RNA plus or minus strands [E. Bandle and C. Weissmann, Experientia *28*, 743 (1972)] allows the insertion of mutagenic base analogs at defined positions in the nucleotide sequence. When such a substitution is made near the 5′ terminus of minus strands and these are used as template for Qβ replicase, plus strands with a mutation in the 3′ extracistronic region can be obtained in good yield. A minus strand containing N^4-hydroxyCMP in place of CMP at position 15 from the 5′ end led to the formation of plus strands, 33% of which had a G→ A transition in position 16 from the 3′ end. The mutant RNA is more efficiently replicated by Qβ replicase than is the wild type and hence could be enriched by extensive in vitro propagation. A second extracistronic mutant with a A → G transition in position 40 from the 3′ end has been generated by a similar approach, using as template a minus strand in which UMP was replaced by hydroxyCMP in position 39 from the 5′ end. This general approach to mutagenesis can be extended to the 5′ extracistronic region of Qβ RNA as well as to the intercistronic regions, in particular to the ribosome binding sites.

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RNase-Sensitive DNA Synthesis in Antigen-Stimulated Mouse Lymphocytes

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Mouse spleen lymphocytes are cultured in the presence of bacterial lipolysaccharide (LPS) which induces the differentiation of a significant % of the cells into immunoglobulin-producing cells. A DNA synthetic activity is found in a cytoplasmic high-speed pellet prepared from the LPS-stimulated cells. This pellet can be fractionated on a glycerol gradient, where the DNA synthetic activity bands as a discrete peak. It is an endogenous activity (no added template is necessary), it is abolished by preincubation with RNase, it is dependant on the presence of the 4 triphosphates and it is almost absent in non-stimulated cells. The product of the reaction has been characterised as DNA by several criteria and its buoyant density in CsCl is similar to that of mouse nuclear DNA. 85% of the product hybrides to nuclear DNA. Attempts to hybridise this cell-free DNA with various cellular RNAs have thus far not provided information as to its nature.

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Evidencing Cytoplasmic DNA in Ultrastructural Cytochemistry

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Osmium-Ammine, a newly synthesized complex of Osmium-polyammine (Cogliati and Gautier, C. r. Acad. Sci. [D] *276*, 3041, 1973; Gautier et al., *Electron Microscopy and Cytochemistry* 1974, 271) can be successfully used in a Feulgen-type reaction to localize DNA in thin sections from tissues fixed with either aldehydes, aldehyde-OsO_4, OsO_4 or $KMnO_4$, and embedded in plastics.

Preliminary experiments show that the cytoplasmic DNA may be clearly demonstrated with this technique, e.g., in mitochondria and toxycysts (but not in trichocysts) from some ciliates, as well as in the cytoplasm of gametophytic *Allomyces* (Ojha and Turian, Molec. Gen. Genetics *112*, 49, 1971; Khandjian et al., in progress). The ultrastructural appearance of these cytoplasmic DNAs is compared to that of bacterial and viral DNAs obtained in similar conditions.

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The Okazaki Fragments of Mouse P-815 Cells

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Mouse P-815 cells from spinner cultures were incubated with ^{3}H-thymidine for 15 to 240 sec at 37 °C or 25 °C. DNA was extracted by a modified Hirt procedure (J. Mol. Biol. (1967) *26*, 365–369) and molecular weights of single stranded ^{3}H-DNA were measured by sedimentation analysis in alkaline sucrose gradients or electrophoretically in agarose gels. After pulses of 2 to 8 min at 37 °C, most of the radioactivity was found in molecules of 25 to 60 S, reflecting the replicon size distribution (3×10^4 to 3×10^5 base pairs). After a 30 sec pulse at 25 °C, however, only short DNA strands of 50 to 200 nucleotides were labelled,

suggesting discontinuous strand elongation via Okazaki fragments both at the 5′ end and at the 3′ end. In neutral CsCl or Cs_2SO_4 gradients Okazaki fragments had the same buoyant density as bulk DNA and no evidence for covalently attached ribonucleotides was obtained.

Addition of Polyadenylate Residues to the 3′ End of Qβ RNA Does not Reduce its Infectivity

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Most eukaryotic messenger RNAs have polyadenylic acid sequences at their 3′ end, while this type of modification appears to be absent from messengers of prokaryotes and their viruses. An enzyme capable of adding poly A to oligo A primers has been purified from calf thymus cytoplasm [Tsiapalis et al., Biophys. Biochim. Res. Commun. *50*, 737 (1973)]. We have tested the ability of this enzyme to utilize Qβ RNA as a primer and have determined the properties of Qβ RNA carrying a polyadenylate extension. Using [α-^{32}P] ATP and [^{3}H] Qβ RNA as substrates and displaying the product on a sucrose gradient it was possible to show that AMP residues can be transferred to high molecular weight Qβ RNA. In two separate experiments products carrying an average of 7 and 70 AMP residues respectively per chain were generated. The RNAs were then purified by hybridization to a poly U-Sephadex column and elution by formamide. In a control experiment it was shown that unmodified Qβ RNA passes through such a column quantitatively. The poly A-Qβ RNAs, both with the short and long A sequences, were found to be fully infectious when tested in the spheroplast assay of Guthrie and Sinsheimer.

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Application of the Fluorescent Antibody Technique to the Study of Muscle Cells in Culture

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The fluorescent antibody technique was used to detect selectively the M and B forms of creatine kinase (CPK) in cultured cells derived from embryonic chicken skeletal muscle. Presumptive myoblasts (in medium containing 5-bromodeoxyuridine) could be distinguished from myoblasts (prevented from fusing by the addition of EGTA) in that, while both contained B-CPK, only the myoblasts contained the M form characteristic of differentiated muscle. Myotubes (in cultures allowed to develop normally) contained large amounts of M- and B-CPK; the few fibroblast-like nonmyogenic cells had only B-CPK. These results, as well as those obtained with other techniques (electrophoresis, histochemistry) for the same and other marker enzymes, indicate that terminal muscle differentiation can proceed, to some extent at least, in the absence of myoblast fusion.

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Cytochalasin B: Action on Domes Formed in Kidney and Mammary Gland Cell Cultures

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Dense monolayer cultures of mammary gland epithelium from pregnant mice organize into 3-dimensional structures, called 'domes' by McGrath et al. and supposed to be analogous to acini [J. Virol. *9* (1972) 367]. Here, domes are reported to arise likewise in primary cell cultures derived from the kidneys of 10-days-old mice; they possibly correspond to Bowman's capsules. Both mammary gland and kidney cells were cultivated in the hormonal medium designed by Lasfargues and Moore [in Vitro *7* (1971) 21]. 10 μg/ml of Cytochalasin B (CB; Phomin, Sandoz) cause the domes of both cell types to collapse mostly within 5 to 15 minutes, the action being reversible: the domes are partially or completely restored within one to several hours after CB has been washed out. Collapse and restoration can be repeated several times. In view of the known action of CB, microfilaments are likely to produce dome formation in vitro. Furthermore, one would expect many other epithelia exhibiting CB-sensitive morphogenetic processes to build up domes in monolayer cultures under appropriate conditions [cf. Wessells et al., Science *171* (1971) 135].

Changes in the dCTP and dTTP Pools of P-815 Cells Following Exposure to VP 16-213

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The pyrimidine deoxynucleoside triphosphate pools, dTTP and dCTP, were altered in nonsynchronized P-815 mastocytoma cells after treatment with VP 16–213 (4′-demethyl-epipodophyllotoxin-ethylidene-β-D-glucoside) in vitro. The amount of dTTP per 10^6 cells increased from 110 pmoles to about 160 pmoles within 1 h of treatment with 1 or 10 μg/ml VP 16–213, while the dCTP pool decreased from 25 to about 7 pmoles per 10^6 cells. Maximum alterations were present after 1 h of treatment and prolonged exposure to the cytostatic drug had no further effect on pool sizes. The changes in pool sizes were independent of drug concentration. Earlier studies with VP 16–213 concerning DNA, RNA and protein synthesis had shown a continuation of the synthesis of these macromolecules, although the mitotic index was drastically decreased within 45 to 60 min treatment with the drug. In conclusion we assume that fluctuations of the dTTP and dCTP pools caused by VP 16–213 do not influence the macromolecular synthesis. It seems that alterations of pool sizes may occur as a consequence of changes in enzymatic activities of the pyrimidine nucleotide metabolism. They probably have no direct correlation with the inhibitory action on mitosis.

Glycoprotein Metabolism during Morphological Differentiation in Neuroblastoma Cells

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Conditioned medium by glial cells induces process formation in neuroblastoma cells grown in tissue culture. The morphologically differentiated cells show a twofold increase in the incorporation of radioactive glucosamine. The incorporation of fucose remains unchanged.

Glycoproteins from normal and differentiated neuroblastoma cells were labelled with ^{3}H- or ^{14}C-glucosamine and fractionated on SDS-polyacrylamide gels. Glycopeptides were enzymatically removed from the surface of such cells and fractionated on Sephadex G-50. By these two methods glycoproteins of normal and morphologically differentiated cells were found to be identical.

Morphologically differentiated cells take up glucosamine from the medium faster than normal cells. This increased uptake can account for the difference in incorporation. The rate of uptake is also enhanced for deoxyglucose, galactosamine and leucine, but not for fucose, thymidine and uridine.

Effect of Cholesterol and Other Lipids on Cell Surfaces of Transformed and Normal Cells

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The growth of serum requiring tissue culture cells is substantially impaired in the absence of fatty acids and biotin. The cessation of growth can be reversed by the exogenous addition either of fatty acids or of biotin. Stearate, oleate, palmitate and elaidate are among the fatty acids that support appreciable growth of the normal 3 T 3 fibroblast cell line, whereas linoleate and myristate fail to do so. In the presence of biotin, linoleate further stimulates growth compared to cultures containing biotin alone. Similar results are obtained with the transformed SV 101 3 T 3 cell line, except that palmitate does not support growth. Analysis of the cellular lipids by TLC and GLC shows that the exogenously added fatty acid is incorporated into the phosphatide fraction.

In an attempt to explore the role of membrane fluidity in cell surface events, the lectin agglutinability of 3 T 3 and SV 101 3 T 3 cells grown on various fatty acids was measured. The presence of elaidate decreases the agglutinability at room temperature. These and other data suggest to us that the lectin receptors for conA and for WGA are separated in different lipid environments.

A similar conclusion has been reached from data that will be presented on the involvement of membrane cholesterol in agglutination phenomena of transformed cells.

Thiostrepton Binding Protein of *E. coli* Ribosomes

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The antibiotic thiostrepton inhibits protein synthesis in *E. coli* by binding tightly to the 50 S subunit. This binding inhibits the interaction of the ribosome with both EF-Tu and EF-G. We report here on the use of three separate methods to determine the specific binding site of thiostrepton on the 50 S subunit, utilizing a preparation of high specific activity [^{35}S]thiostrepton to quantitate this binding. The methods were: 1. Correlation of the loss of thiostrepton binding ability with selective removal of ribosomal proteins. In this procedure 50 S subunits were treated with either NH_4Cl-ethanol or LiCl, with subsequent identification of the proteins in both the split fraction and on the depleted cores determined by 2-D gel electrophoresis; 2. reconstitution of these depleted cores with selected groups of ribosomal proteins to restore their thiostrepton binding ability; 3. treatment of reconstituted cores with specific ribosomal protein antibodies to determine which one(s) caused inhibition of thiostrepton binding. The results of these experiments indicate protein L_{11} is the thiostrepton binding site on *E. coli* 50 S subunits.

Qualitative and Quantitative Studies of the DNA-Nuclear Membrane Complex Isolated from Eukaryotic Cells

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Procedures without detergent treatment for isolation and purification of nuclei from two lines of cells in culture have been developed. The degree of purity of the nuclei has been checked by electron microscopy. The nuclear membrane was labelled with choline and the DNA with thymidine. The DNA-nuclear membrane complex has been isolated by 2 gentle techniques which avoid excessive fragmentation of the membrane. After a first isolation, the complex contains 1–10% of the nuclear DNA. Treatment of the complex with DNAase I shows that DNA is still accessible to the enzyme. In certain cases, repeated washes in different solutions of high ionic strength allow the remaining DNA to be completely detached. Non-ionic detergents disaggregate the membrane and liberate in this way the DNA previously bound. CsCl gradient profiles show that the fraction of DNA from this complex which is the most intimely bound to the membrane contains about 2 times more AT-rich sequences than total DNA. Experiments on synchronized cells show that the DNA of the complex is not preferentially replicated during late S-phase.

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Myo-Fibroblasts in the Cirrhotic Liver

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Hepatic cirrhosis was produced in rats by repeated weekly gastric intubation of CCl_4 during a period from 4 to 9 months. Tissue samples from animals with typical lesions were studied by means of electronmicroscopy and immunofluorescence using sera containing specific anti-actin antibody. Contractility of small strips of cirrhotic tissue was tested in a specially designed micro-bath. In the fibrous areas of the affected livers, many typical myofibroblasts were present as demonstrated by electronmicroscopy. Immunofluorescence studies revealed the presence of actin in such cells. There was also evidence of contractile activity in strips of cirrhotic livers when compared to strips of normal livers, when Serotonin or Epinephrine were added into micro-bath. Addition to the microbath of Prostaglandin Flα and Fl, Phenergan and Vasopressin did not produce any significant contraction. All strips tested were relaxed by Papaverine. In analogy to the previous works on the contraction of granulation tissue, it is probable that the presence of contractile cells such as myo-fibroblasts may be a factor playing a role in the retraction of the cirrhotic liver and in the associated hemodynamic alterations.

The Morphogenesis of the Tail of Phage λ

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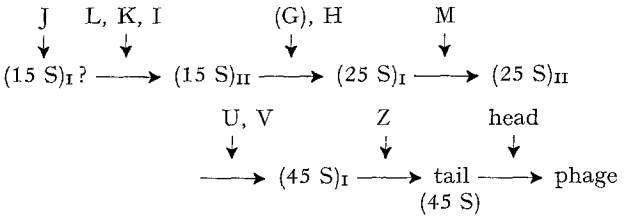
By measuring serum blocking activity and in vitro complementation activities in sucrose gradients of defective lysates, we found various precursors of λ tail which indicate the following pathway:



The major protein (pV) in tails can be dissociated into disks (~ 10 S) or smaller units (~ 2.5 S) under extreme conditions in vitro. The disks formed polytails efficiently under physiological conditions but the smaller units did not. But the latter had in vitro complementation activity with V^- lysate. V^+, U^+ and Z^+ activities were detected in the dialyzed extracts of SDS gel electrophoresis of tail in the fractions corresponding to MW of 31,000, 14,000 and 20,000 daltons respectively.

Although it is known that H^- produces no visible tail-related structure, the double mutant U^-H^- produced polytails of several microns in length without a tail fiber and a basal part. The same double mutant had V^+ activity which sedimented at about 2.5 S but did not complement purified 25 S precursor in V^-.

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Persistance of Segregation between Paternally and Maternally Derived Chromatin During Cleavage Divisions of Mouse Eggs

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Mouse zygotes with only the male parental half of the genome specifically labeled by tritiated thymidine have been studied at various stages of cleavage. Previously described techniques have been used with improvements.

The observation of a 'gonomeric' segregation between paternally and maternally derived chromatin was substantiated by observing over 4,000 interphase nuclei with light microscope autoradiography. The density of labeling in the paternal component of an egg chromatin is related to the amount of label present in the fertilizing sperm. A definite localization of paternally derived chromatin can be followed up to the 32 cell stage. Further observation is limited by dilution of initial radioactivity, at each cleavage mitosis.

This is the first observation in a mammal of a process well known in several species ranging from invertebrates to higher fishes.

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The Regulation of the Synthesis of Bacteriophage T4 Gene 32 Protein

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The synthesis of gene 32 product (P 32) has been followed by gel electrophoresis of infected cell lysates. In wild-type infection its synthesis starts soon after infection and begins to diminish about the time late gene expression commences. The absence of functional P 32 results in a marked increase in the amount of the non-functional P32 synthesized. For example, infections of T4 mutants which contain a non-sense mutation in gene 32 produce the non-sense fragment at greater than 10-fold the maximum rate of synthesis of the gene product observed in wild-type infection. This increased synthesis of the non-functional product is recessive, since mixed infections (wild-type, gene 32 mutant) fail to markedly overproduce the non-sense fragment.

In addition, evidence will be presented that because of particular biochemical properties of P 32 the nature and amount of DNA replicated in an infected cell also has an effect on the expression of gene 32. These results are compatible with a model for the regulation of expression of gene 32 in which the synthesis of P 32 is either directly or indirectly blocked by its own function.

An Ultracytochemical Investigation of Rat 'Specific Heart Granules' with Regard to the Presence of Catecholamines

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The 'specific heart granules' (SHG) of right atrial muscle cells were investigated in order to determine whether they are able to store or to accumulate biogenic amines. For this purpose, tissues were fixed for electron microscopy using a modified chromaffine reaction, rendered more specific and sensitive for amine localization. The SHG were investigated in reserpinized and untreated rats for the presence of endogenous amines, and in 5-hydroxydopamine-incubated or noradrenaline-perfused heart tissues for the presence of exogenous amines.

There were neither indications for the storage of endogenous nor for the accumulation of exogenous amines. Thus, the electron density of SHG is preferentially induced by uranium staining, to some extent by osmium fixation, but not by chromium treatment. Moreover, the content of SHG could be enzymatically digested with pronase on ultrathin sections.

Therefore, it is concluded, the SHG are unable to store appreciable amounts of endogenous or exogenous biogenic amines. Their content consists of an uranophilic, pronase sensitive, probably proteinaceous, material.

Cytochemical Demonstration of a Transport ATPase in Mammalian Cornea

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Biochemical and physiological findings suggest a cation dependent, ouabain sensitive ATPase as playing a predominant role in regulating the state of hydration of the

cornea. Localizations obtained with Gomori-type lead procedures are equivocal with regard to transport ATPase. Using the Ernst method (J. Histochem. Cytochem. *20*, 23, 1972), the first cytochemical procedure to be readily responsive to ions and ouabain, for in situ localization of transport ATPase in corneas of rats, rabbits and mice, the major sites of activity are the intercellular spaces of the endothelium. This localization was not found with the so-called ATPase methode of Wachstein and Meisel (Am. J. Clin. Path. *27*, 13, 1957). These observations lend support to the conclusions derived from the physiological data which increasingly emphasize the endothelium's importance in dehydrating the cornea via a transport ATPase.

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D,L-*p*-Chloro-N-Methylamphetamine (P) Induced Accumulation of 5-HT in Nonterminal Axons

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P is known to produce a longlasting depletion of 5-HT in the brain. After standard (35 mg/kg ip) P treatment of rats, we observed with the Falck fluorescence-histochemical method an indolamine (IA) depletion in terminal axons. However, in nonterminal axons of pros-mes-metencephalon, e.g. the medial forebrain bundle (MFB) an IA accumulation was present. Moderate yellow formaldehyde induced fluorescence (FIF) occurred in nonterminal axons 1–6 days after P. This FIF was abolished by reserpine (10 mg/kg ip 16 h prior to sacrifice), but intensified by nialamide (300 mg/kg ip 6 h) and by reserpine + nialamide in P pretreated (64 h) rats. Biochemically, 48 h after P, striatal 5-HT was reduced to 2.2 µg/g protein from 6.1 µg/g in controls ($p < 0.01$) whereas the 5-HT in dissected MFB was unchanged. Histofluorescence analysis of similar MFB sections also revealed an accumulation of IA FIF in nonterminal axons, but a decrease in terminal axons. The data indicate that the accumulated FIF represents 5-HT. Thus P treatment may be used for mapping 5-HT pathways.

Quantitative Characterization of Microsomal Membranes by Freeze-Etching

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A stereological method for freeze-etching has been developed which allows different membrane types to be identified in rat liver microsomal fractions. First, the densities of 'protein particles' found on identifiable membrane fracture faces of intact tissue replicas were determined. These fracture faces were chosen to correspond to those displayed by the concave fracture faces of the microsomal vesicles. The collection of particle density data from microsomal vesicles was restricted to a constant projection area on concave polar faces displaying no cast shadow. Both theoretical and experimental analyses demonstrate that these faces account for 12–15% of all vesicles exposed and that the procedure provides a reliable estimate of relative vesicle surface area. The mean particle densities in intact tissue were 1,350/µ² for plasma membrane (PM), 2,940/µ² for endoplasmic reticulum (ER), and 4,250/µ² for mitochondrial membranes (MiM). The composition of the microsomal fraction was 17% PM, 65% ER, and 16% MiM, which agrees with biochemical determinations.

A Bacteriophage T4 Mutant Affecting the Expression of Many Early Genes

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A bacteriophage T4 temperature sensitive mutation has been isolated which affects the expression of many early T4 induced proteins. All of the proteins affected by this mutation are initially synthesized in reduced amounts. Some of the proteins affected have been identified with specific T4 genes. The proteins specified by gene 43 (the T 4 induced DNA polymerase), gene 32 (a protein able to bind to single stranded DNA), gene 45 and the *r*II B gene are among those affected by this mutation. Various kinds of evidence suggests that in cells infected with phage carrying this mutation, *ts*Gl, transcription of some genes is reduced.

Presence of Native 40 S Ribosomal Subunits on Microsomal Membranes of a Murine Plasmocytoma

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P3 K myeloma cells were labelled in culture with ^{3}H-uridine (from 30 min to 24 hours). Free and membrane-bound ribosomes were separated quantitatively and obtained completely free of reciprocal contamination by one-step centrifugation using a new technique of sucrose density gradient sedimentation. Sucrose gradient analyses of the material released from the microsomes by detergents revealed polysomes and small amounts of 60 S and 40 S native ribosomal subunits. Kinetics of appearance on the microsomal membranes of the newly synthetized native 40 S subunits were identical to those of the 40 S subunits free in the cytoplasm. The native 40 S, but not the native 60 S subunits could be released from the microsomal membranes by mild RNase treatment. Pulse labelling for 3 min with ^{35}S-methionine showed the presence of ^{35}S Met-tRNA on the membrane bound 40 S native subunits, with a specific radioactivity 5 to 6 folds greater than on the free 40 S subunits. It thus appears that protein synthesis is initiated on the membranes of the endoplasmic reticulum by the attachment of free native 40 S subunits to mRNA bound to the membranes, followed by the formation of an initiation complex; this may subsequently bind to 60 S subunits already present on the membranes.

Comparison of the Physico-Chemical Properties of Nuclear and Cytoplasmic-Non-Mitochondrial DNA from Duck Erythroblasts

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Non-dividing duck erythroblasts were labeled with either ^{32}P or ^{3}H-TdR in vitro. DNA was extracted from isolated nuclei with $CHCl_3$-isoamyl alcohol and purified

on Cs_2SO_4-Urea density gradients. On SDS-sucrose gradients the component smaller than 16 S, had 15–20 × higher specific activity of labeling than the faster component. After pelleting mitochondria, the cytoplasmic supernatant was successively centrifuged to pellet polyribosomes and free mRNPs. These were centrifuged on SDS-sucrose gradients and were found to contain DNA sedimenting slower than 16 S. These DNAs were further purified on Cs_2-SO_4-Urea density gradients. The specific activity of total ^{32}P labeled DNA derived from polyribosomes and free-mRNPs was about 20 × that of total nuclear DNA. Thermal melting of the DNA was monitored by measurements of either hyperchormicity or S_1 endonuclease sensitivity. Tm for erythroblast nuclear DNA, duck embryo nuclear DNA, and DNAs derived from polyribosomes and free mRNP was the same. Differentiated melting data revealed that nuclear- and polyribosome-derived DNAs contained identical components in identical proportion. Both of these fractions reassociated in a similar with their $C_0t^1/_2$ to 10^3. Their 5'-mononucleotide composition was similar. We conclude that the labeled nuclear and non-mitochondrial cytoplasmic DNA are very similar; they probably represent metabolic DNA. The bulk of (unlabeled) nuclear DNA differs in some respects from the labeled DNA. However the analysis of this newly synthesized DNA is complicated by inevitable contamination from the unlabeled nuclear DNA.

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Effects of Conformation and Structure of Collagen on Platelet Aggregation

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The loss of aggregating activity upon denaturation of tropocollagen (triple helix conformation) to gelatin (random coil conformation) indicates that a rigid stereostructure is a *necessary* requirement for collagen to promote platelet aggregation. It is, however, *not a sufficient* requirement as we found monodisperse tropocollagen to be completely inactive. It is proposed that platelet aggregating activity resides in a multimer of tropocollagen, either an intermediate structure (e.g. nucleus) and/or the end product (fibril) formed in the process of tropocollagen-fibril conversion.

Further studies established that the quarter-staggered packing arrangement of tropocollagen as present in native-type fibrils is not a necessary condition for the ability to induce platelet aggregation. In addition to native fibrils long spacing segments (SLS), long spacing fibrils (FLS) and electron optically amorphous fibrils of collagen were capable of inducing platelet aggregation.

A Freeze-Etching Study of the Epithelium of the Toad Urinary Bladder

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Toad urinary bladder has been used intensively as a model for the study of water and ion movements across epithelia. Available data suggest two possible pathways for such movements, one through epithelial cells (transcellular), the other along extracellular spaces (paracellular). The transcellular permeability is ultimately controlled by the plasma membrane at the luminal pole of the epithelial cell whereas the permeability of the paracellular pathway could be modulated by the tight junction. We applied to the urinary bladder of the toad the technique of freeze-etching, which gives a clear insight into membrane structure and membrane differentiation. The luminal membrane of epithelial cells has an unusual appearance since the membrane-associated particles in its external leaflet (B-face) are more numerous (1,500/μ^2) and larger (160 Å) than in its inner leaflet (A-face) (800/μ^2, 100 Å). The B and A faces of the lateral membranes have respectively 300 and 1,700 particles/μ^2 measuring 120 Å. The tight junction between epithelial cells is composed of several strands forming a complex network. The implications of such membrane structures on water and ion transport are currently being studied.

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Studies on the Synthesis of Rous Sarcoma Virus RNA in vivo

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A new procedure which permits the specific isolation of a labeled RNA by hybridization to its complementary DNA has been used to study the in vivo synthesis of avian tumor virus RNA. Purified DNA complementary to avian myeloblastosis virus RNA is extended at its 3' terminus with 40–60 dCMP residues, using terminal deoxynucleotidyl transferase. The elongated DNA is annealed with the labeled nucleic acid preparation and the mixture is passed through a column of Sephadex to which poly (I) has been covalently bound. The poly(I) retains the specific RNA-DNA hybrids by virtue of their poly(dC) extension. After washing with RNase to degrade non-hybridized RNA, the RNA retained on the column is eluted with formamide and its radioactivity is determined. The proportion of virus-specific RNA labeled with [^3H] uridine in RSV-infected chicken cells during a 2-h period was found to be 0.6 to 0.9% as compared to 0.05% in uninfected cells. Actinomycin D specifically inhibits RSV RNA but not Newcastle Disease virus RNA synthesis in doubly infected cells. This observation provides strong evidence that tumor virus RNA is synthesized on a DNA template.

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Gap Junctions in Mesangial and Lacis Cells

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The existence of mesangial cells in the mammalian kidney glomerulus is now well established. Situated between the basement membrane and the endothelium, these are stellate cells embedded in the intercellular matrix. In a study of the rat kidney with freeze-etching, we found that mesangial as well as lacis cells have plasma membrane differentiations characteristic of gap junctions. Accordingly, conventional thin-section electron microscopy show in both cell types frequent zones of plasma membrane apposition which correspond to gap junctions. Gap junctions occur not only between different cells, but also between processes of the same cell. These findings correlate with the concept that mesangial and lacis cells are modified

smooth muscle cells. Gap junctions in mesangium could represent therefore, as in muscle, sites of electrical coupling suggesting a coordinated contractile function.

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Localization of Amine Storage Sites in the Adrenergic Cell Body by Fine Structural Cytochemistry

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The superior cervical ganglion of the rat was examined by electron microscopy after fixation with an improved (more sensitive and specific) cytochemical technique for the ultrastructural localization of biogenic amines using a modified chromaffine reaction. Several cells contained many small dense core (50 nm, SDC) vesicles and occasionally large dense core (100 nm, LDC) vesicles together with a tubular reticulum (TR) with a highly electron dense content. The SDC and LDC vesicles and TR were characteristically distributed within the neuron: (i) isolated SDC and LDC vesicles dispersed throughout the cytoplasm of the perikaryon and dendrites, (ii) groups of SDC vesicles and TR at the cell periphery, in dendritic processes and non-terminal axons, and (iii) SDC and LDC vesicles and TR occasionally associated with the Golgi apparatus. The chromaffine cells contained predominantly large (100 to 300 nm) electron dense granules. Electron dense reaction product could be observed in non-osmicated tissues from control animals but not in those treated with reserpine.

These findings provide strong evidence that the electron dense material present in the different organelles of the ganglion cells represents a biogenic amine, probably noradrenaline.

Immunocytochemical Localization of Chromomembrin B

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Antiserum to bovine *chromomembrin B*, a membrane protein of the chromaffin granule, (Winkler H., Phil. Trans. Roy. Soc. Lond. [B] *261*, 293, 1971) was produced in rabbits and IgG fraction conjugated to peroxidase. Rat adrenal glands were fixed with a periodate-lysine-paraformaldehyde solution (McLean I. W. and Nakane P. K., J. Cell Biol. *59*, 209a, 1973) and rinsed overnight in phosphate-buffered saline (PBS) containing 12% sucrose. Frozen sections were mounted on albumin slides and incubated for immunohistochemistry using the direct peroxidase-labeled antibody method (Nakane, P. K. and Pierce G. B., J. Cell Biol. *33*, 307, 1967). At light microscopic level, peroxidase activity was localized only in the medulla of the adrenal gland. At electron microscopic level reaction product was identified in nuclear envelope, rough endoplasmic reticulum, Golgi complex, membrane of secretory granules and plasma membrane of the chromaffin cells. No reaction product was found in control sections reacted with a peroxidase-labeled anti-human gonadotropin serum instead of the labeled anti-bovine chromomembrin B.

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In-vitro Synthesis of Rous Sarcoma Virus RNA

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The synthesis of Rous sarcoma virus (RSV)-specific RNA has been examined using an in vitro system for RNA synthesis and a novel hybridization assay for radioactive virus-specific RNA (Coffin et al., J. Mol. Biol., in press). Nuclei from infected and uninfected cells were incubated with ^{32}P-labeled ribonucleoside triphosphates, $MgCl_2$ and $(NH_4)_2SO_4$. ^{32}P-nucleotides were incorporated into both total and viral RNA with similar kinetics for up to 25 min at 37 °C. Approximately 0.5% of the RNA synthesized by nuclei from RSV-infected chicken cells was scored as virus-specific, compared to 0.03% of the RNA from uninfected chicken cell nuclei and less than 0.01% of the RNA from monkey kidney cell nuclei. Preincubation of isolated nuclei with either DNase or actinomycin D completely suppressed both total and virus-specific RNA synthesis. α-Amanitin, a potent inhibitor of eukaryotic RNA polymerase II, completely inhibited RSV-specific RNA synthesis, while reducing total RNA synthesis by only 50%. We conclude that tumor virus-specific RNA is synthesized on a DNA template, most probably by the host-specified RNA polymerase II.

Supported by SNSF, International Union against Cancer, Jane Coffin Childs Fund

Studies on the Synthesis of '6 S RNA' by Qβ replicase

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Qβ replicase incubated with substrates, in the absence of Qβ RNA, synthesizes so-called 6 S RNA after a lag of 5–20 min. This synthesis is suppressed by Qβ RNA and selectively retarded by high ionic strength (0.2 *M* KCl). Analysis by gel electrophoresis and fingerprinting shows that 6 S RNA is heterogenous. Its origin is unclear. It cannot be hybridized to Qβ RNA. Traces of 6 S RNA originating in the infected cell could be present as impurity in the enzyme preparation and initiate autocatalytic synthesis; alternatively, replicase could spontaneously synthesize a variety of more or less random sequences, some of which are selectively replicated. Three ^{32}P-labeled 6 S RNA preparations were synthesized in separate but identical incubations; their T_1 fingerprints were indistinguishable, making it unlikely that the RNA arose by 'spontaneous generation'. On the other hand no purification procedures yielded Qβ replicase devoid of 6 S RNA-synthesizing activity. One main species of double-stranded 6 S RNA was purified and the complementary strands were separated by gel electrophoresis. Nucleotide sequence analysis is in progress; it is clear that this RNA is completely different from the one sequenced by Spiegelman and his colleagues.

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Inhibition of Barium Stimulated Release of Rat Growth Hormone by Linear Somatostatin*

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In the presence of $BaCl_2$ (4.6 m*M*), the release of growth hormone from rat hemipituitaries incubated in sulphate-free Krebs-Ringer bicarbonate buffered medium was

shown by radioimmunoassay to be increased from 1.25 ±0.25 to 7.10 ± 1.12 μg/mg wet wt/h. The stimulated rate of release was maintained during three successive 30 min. incubations in the presence of $BaCl_2$, and was not decreased by reduction in Ca^+ concentration. Linear somatostatin (2–2,000 ng/ml) did not affect basal growth hormone release but inhibited release in the presence of $BaCl_2$, half maximal inhibition being observed at about 10 ng/ml. Observation of hemipituitaries with freeze-etching and conventional thin-section electron microscopy suggests that $BaCl_2$ stimulates a secretory pathway which involves exocytosis. Since this stimulation is not sensitive to Ca^{2+} depletion, it is suggested that Ba^{2+} can replace Ca^{2+} in the control of release, and that somatostatin interferes with the handling of Ba^{2+} or Ca^{2+} by the pituitary.

*Given by Dr. R. Guillemin

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Oxidation of α-Ketoglutarate by Mitochondria from Neurospora Crassa

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In *Neurospora*, the inception of the glyoxylate shunt triggered by growing the mold on acetate requires a regulation of the Krebs cycle. A control point was sought by measuring the oxidative properties of tightly coupled mitochondria isolated from cultures grown on sucrose and/or acetate. Isocitrate is the only Krebs cycle intermediate whose rate of oxidation increases readily upon derepression by acetate.

The oxidation of α-ketoglutarate (α-KG) depends on both growth conditions and cofactor supplementation: (1) oxygen uptake is consistently low in the absence of ADP; (2) respiratory control (averaging 3.0) is affected neither by CoA nor by TPP, but raised by NAD up to 7.0; (3) in the presence of NAD, mitochondria isolated from derepressed cultures oxidize α-KG much more efficiently than those obtained from sucrosegrown cultures; however, CoA and TPP cancel this difference.

The results show that the α-KG oxidation step regulates the Krebs cycle by the interplay of the cofactor levels, depending themselves on the growth conditions.

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Somatic Cell Genetics Applied to Species Hybrids of Drosophila

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In crosses of *D. melanogaster* (*mel*) with *D. simulans* (*sim*) all offspring without a *simulans* X-chromosome die as larvae. A search for imaginal disk cells in such lethal larvae or embryos was unsuccessful. The question arose whether imaginal disk cells with a lethal chromosome constitution will always express this lethality.

Viable female hybrids, heterozygous for the X-linked recessive mutations *y* = *yellow* and *f* = *forked* (*mel, y/sim, f*) were X-rayed with 1,000 R at different times of development. This treatment should induce somatic crossing over (mitotic recombination) and should produce twin clones of genetically marked cells, one of the clones being homozygous *mel, y/mel, y* (and therefore lethal), the twin partner clone being *sim, f/sim, f*). Such twin clones were in fact found on the abdomen in frequencies comparable to non-lethal controls. They suggest that the lethal effect may be overcome in somatic mosaics.

The fact that somatic crossing over can be induced in these hybrids is in itself interesting.

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Phage T4 Head-Gene Proteins: Purification, Characterization and in vitro cleavage

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Four proteins are cleaved during T4-head maturation. We have purified proteins P22, P24, P23 and cleaved P23 ($P23^6$) from lysates of phage mutants. All have large numbers of acidic residues: P22 is 29 mole % Asx plus Glx. P23, $P23^6$ and P24 have similar compositions: 20–23% Asx plus Glx and 4–5% Pro.

Mutations blocking capsid formation prevent cleavage in vivo. However, we find that lysates of most head-gene mutants contain a specific protease which cleaves purified P22, P23 and IP-III. The in vitro cleavage products migrate exactly on SDS polyacrylamide gel as those produced in vivo. This cleavage activity is present in late lysates of all head-gene mutants except 21^-. P21 dependent protease is not present in uninfected bacteria or $T4^+$ lysates. Most activity sediments with the membrane-cell debris.

P21 dependent protease is inhibited by non-polar compounds: organic acid esters, some butanol isomers, $CHCl_3$, CCl_4, but also pyridine and 2-chlorethanol. TPCK, DFP, and PMSF do not inhibit. $CHCl_3$ inhibition is reversible: P23 cleavage is more sensitive to it than P22 cleavage.

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Colchicine-Induced Autophagocytosis in Liver

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Colchicine, an inhibitor of microtubular function, was injected i.v., into normal mice (0.05 mg/100 g body weight) and their livers examined 4 to 24 hours later. The treatment resulted in a marked accumulation of autophagic vacuoles in hepatocytes. Four hours following the injection, clustering of autophagic vacuoles and dense bodies was observed in hepatocytes. Between 8 and 11 hours following the treatment, the number of such bodies further increased. Their lysosomal nature was indicated by localization of acid phosphatase activity in some of them. Total acid phosphatase activity was 20.6 ± 0.9 U/g liver (8 h) and 22.8 ± 1.0 (11 h) in controls, and 17.9 ± 0.5 (8 h) and 20.1 ± 1.1 (11 h) in treated animals. Microtubules were observed in control livers but were virtually absent in the treated mice at 4 hours and up to 11 hours after treatment. Fifteen hours after colchicine administration, there was a marked diminution of lysosomal bodies at a time when some microtubules were again visualized. After 24 hours, the morphology of livers from treated mice was similar to that of controls. This system can be used to investigate the mechanisms involved in the formation and disposal of autophagic vacuoles.

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Synthesis and Processing of Nuclear RNA Precursor to Messenger RNA in Duck Erythroblasts

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As reported at last year's USGB meeting [Experientia (1973) *29*, 774, 779] sequences identical to globin mRNA are found in duck erythroblasts covalently linked in nascent, intermediate size and small nuclear pre-mRNA. These three classes of pre-mRNA have half lives of appr. 30 min, 3 hours and 15 hours, respectively. The molecular weights of the RNA in these three classes, measured by exponential polyacrylamide gel electrophoresis under completely denaturing conditons (99% formamide, 45 °C) are: 2,5–5 $\times 10^6$, 0,9–2,5 $\times 10^6$, and $< 0{,}9 \times 10^6$ respectively).

A mathematic analysis of the kinetic data obtained by comparing the RNA in these three classes after various time periods of labeling and chase shows that the nascent pre-mRNA of 20–30 min half life behaves as would be expected for a precursor to the mRNA which appears in the cytoplasm with a lag of 20–30 min.

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Tumor Invasion into the Diaphragm: a Scanning Electron Microscopic Analysis

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Penetration of an ascitic reticulum cell sarcoma (HaTu 25) of the golden hamster into the diaphragm was analysed by scanning electron microscopy. 5×10^7 tumor cells were implanted intraperitoneally, and on 6 consecutive days, specimens of the diaphragm were fixed, dehydrated by critical point drying, and coated with carbon-gold. The presence of the tumor inoculum altered the pattern of the serosa: mesothelial cells, single and in groups, contracted and assumed a hemispherical shape, thereby exposing the submesotheilal connective tissue/basement membrane layer. Tumor cells preferentially adhered to these denuded areas. Penetration into the deeper layers was characterized by the advance of single cells and loose aggregates of cells. On day 5, single tumor cells were recognizable in widened intercellular spaces of the serosa on the pleural side of the diaphragm, and on day 6, groups of tumor cells were found within conspicuous defects of the pleural mesothelium and submesothelial tissues. In most instances, penetrating tumor cells were found to have maintained their spherical shape and loose arrangement. This spacious mode of infiltration is facilitated by the porous lymphatic system of the diaphragm, and it may be further promoted by lytic factors produced by the tumor cells.

Transcription of the Globin Gene in Avian Erythroblastosis Virus-Transformed Cells not Producing Hemoglobin

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Erythroblasts infected with the Avian Erythroblastosis Virus (AEV) could be kept in culture for several months (while normal erythroblasts die rapidly); they fail to synthesize hemoglobin and, unlike the Friend Virus transformed cells to differentiate into red cells on stimulation with DMSO. – RNA from the virus transformed non-inducible cells growing in tissue culture was analysed using hybridization with specific cDNA to globin mRNA as the probe. Preliminary data indicate that the percentage of globin-specific sequences in the total RNA is as low as 0.001. This figure when compared with the figures we obtained (Imaizumi et al.) on the RNA population normal duck erythroblasts is comparable in amount to that in the nascent pre-mRNA class. Hence this differentiating cell may be blocked at a stage where the globin gene is transcribed but the pre-mRNA not processed to translatable mRNA. Experiments are now being performed to understand the level within the 'Cascade' of regulation at which the leucemigenic RNA virus interferes with mRNA formation in the target cells, leading to continous growth in vitro and as a corollary, the arrest in terminal differentiation.

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Ribosomal RNA Genes in Germ Line and Somatic Cells of Ascaris lumbricoides

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DNA from spermatids and 10–12-day-old larvae of *Ascaris lumbricoides* was isolated and used as a source for germ line and somatic DNA. Saturation hybridization experiments with in vitro labeled ribosomal RNA from *Ascaris* larvae to germ line and somatic DNA revealed that 0.2% of each DNA is complementary to 18 S and 28 S rRNA. This value is equivalent to approximately 345 and 250 ribosomal genes per haploid spermatid and larval genome, respectively. Polyacrylamid gel electrophoresis showed that the 18 S and 28 S ribosomal RNA have molecular weights of 0.76×10^6 for the smaller and 1.42×10^6 for the larger unit. About 40% of the 18 S and 28 S rDNA base sequences of *Ascaris* and *Xenopus* are homologous.

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Integration of Polyoma DNA into Mouse DNA during a Lytic Infection

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The investigations on a possible integration of polyoma DNA into the chromosomal DNA of permissive (mouse) cells were continued with the same approach described earlier [Experimentia *28*, 752 (1972)]. Using a different extraction procedure, it has been possible to separate completely the mouse DNA from the free polyoma DNA. Thus it was found, that no integration takes place before 10 h p.i. This indicates that integration is not required for the transcription of early viral RNA which starts around 7 h p.i. Association of polyoma DNA with mouse DNA was observed in small amounts (1–2 genomes per cell) at 10 h p.i. and thereafter in increasing amounts both in the presence and in the absence of FdU. It coincides therefore with the appearance of T-antigen and the activation of the DNA-synthesizing system of the host cell. The amount of viral DNA found to be associated with host DNA at 18 h p.i. was largely decreased, when cycloheximide (20 µg/ml) was added from 10 to 18 h p.i. Indications for a linear integration of polyoma DNA into mouse DNA

were obtained by subjecting the isolated mouse DNA to alkaline CsCl density gradient centrifugations.

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Fractionation of Chromatin in 2-Phase Aqueous Polymer Systems

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Deoxyribonucleoproteins (DNPs) prepared by shearing chromatin of mouse cells may be fractionated in 2-phase aqueous Dextranpolyethylene glycol mixtures. A partial separation of DNPs with different nonhistone protein/DNA ratios may be obtained in a single-step partition. Separation of DNP into 40–60 fractions of different nonhistone protein/DNA ratio has been obtained using countercurrent distribution in the same system. DNP fractions which bear nascent RNA may be separated from the major fraction of DNP inactive in transcription.

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Synthesis of Structural Proteins of Avian RNA Tumor Viruses

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The major structural proteins of avian oncornaviruses are synthesized as a single large precursor polypeptide of molecular weight 76,000 (Pr 76). We have elucidated the scheme of specific proteolytic cleavage of this polypeptide by pulse-labeling infected cells with S^{35}-methionine, purifying intracellular viral-specific polypeptides by immune precipitation and dodecyl sulfate gel electrophoresis, and then comparing the tryptic fingerprints of these polypeptides with those of H^3-methionine virion proteins on ion exchange chromatography. The met-labeled tryptic peptides of each of the major methionine containing virion proteins (VP11, VP19 and VP24) are contained in Pr76. These virion proteins do not have any met-labeled tryptic peptides in common. From analysis of the several other intracelullar viral specific polypeptides in pulse-labeled cells, we propose the following cleavage scheme (where numerals denote kilodaltons molecular weight and Pr means precursor and VP, virion protein): (1) Pr 76 → Pr12+Pr66. (2) Pr12 → VP11. (3) Pr66 → Pr55. (4) Pr55 → Pr31 + VP24. (5) Pr31 → VP19.

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In vitro Translation of Rous Sarcoma virus RNA

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RNA from Rous sarcoma virus was translated in vitro in a cell-free protein synthesizing system from mouse Ascites Krebs II cells. A part of the in vitro synthesized proteins could be precipitated by antiserum against the group specific (gs) antigens of Rous sarcoma virus. Analysis by SDS-gelelectrophoresis shows that the in vitro synthesized proteins have a higher MW than in vivo produced viral gs-antigen proteins. Analysis of tryptic digests of the in vitro proteins indicates similarity to tryptic digests from viral in vivo produced gs-proteins. It is concluded that at least part of the RNA from RSV is translated in vitro into a high molecular weight precursor of gs-proteins. This in vitro precursor appears to be similar to a precursor to the gs-antigen proteins observed in vivo in RSV-infected chick cells [Vogt and Eisenman, PNAS *70*, 1734 (1973)].

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Interactions between Qβ Replicase and Qβ RNA

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The interaction between Qβ replicase and Qβ RNA was analyzed by subjecting binding complexes to limited ribonuclease T_1 digestion and retaining protein-bound RNA fragments on nitrocellulose filters. Under near-physiological ionic conditions (10 m*M* Mg^{2+}, 0.15 *M* NaCl), replicase interacts mainly with two regions of the Qβ genome. The first region overlaps with the ribosomal initiation site for the coat cistron and is responsible for translational repression by replicase [H. Weber et al., Nature New Biol. *236*, 166 (1972)]. Interaction at this site is independent of Mg^{2+} ions but requires monovalent cations and incubation at 37 °C. The interaction at the second site requires Mg^{2+} and takes place at 0° in the absence of salt. This site is located between the 2,100th and the 2,700th nucleotide from the 5′ end, but does not overlap with the ribosomal initiation site of the replicase cistron which is located in the same area. In contrast to the first site, the second site may be essential for the recognition of Qβ RNA by Qβ replicase [M. Schwyzer et al., Experientia *28*, 750 (1972)].

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Effects of Starvation on DNA Synthesis in Polytene Chromosomes of Drosophila

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We have shown that imaginal disks do not reach the competence for metamorphosis if cultured in starved adult females. This block appears to be due to an absence of cell divisions. The tool of starved hosts was now further explored by examining whether DNA-synthesis is possible in polytene chromosomes when salivary glands are cultured in starved flies.

The DNA-content of nuclei was measured cytophotometrically with a modified Feulgen technique using the fluorescent dye BAO. Salivary glands of early 3rd instar larvae were cultured in adult hosts whereby age of hosts and length of culture were varied. Fluorescence being directly proportional to DNA-content, our results indicate that in starved hosts the nuclei may finish an already initiated round of replication, but then any further DNA-synthesis is blocked. After prolonged culture the newly synthesized DNA is degraded. The block is reversible as shown by a steep increase in DNA-content after transferring the starved hosts onto complete yeast food.

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Purification and Properties of Mouse Interferon

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Interferon from Mouse E. Ascites cells was purified to a specific activity of 1.5×10^9 NIH ref. units per mg protein. After neuraminidase treatment, the highly purified Interferon banded as a single activity peak at pH 8.8 on electrofocusing, whereas untreated interferon showed several peaks between pH 5.4 and 7.2. On SDS-poly-acrylamide there were 2 components, interferon with a MW 23,000d (60%) and a major contaminant with a MW 65,000d (40%). Ultracentrifugation showed one single activity peak with a MW of 20,000d.

In this interferon preparation we detected a specific endonuclease activity which was associated with interferon throughout the purification. The activity peaks of interferon and endonuclease were not separated on sucrose gradients under the conditions used. This endonuclease degrades in our assay system mRNA of L-cells, EMC virus and reovirus, but not mouse tRNA Val and E. coli $tRNA_2^{Gln}$, poly-U and dsreoRNA (W. D. Graziadei III et al, 1973).

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Reiteration Frequency of Mammalian Histone Genes

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Mammalian histone messenger RNA has been isolated from mouse and human (HeLa) cell lines and investigated employing DNA-RNA hybridization techniques. Criteria of early appearance in the cytoplasm, in the case of mouse, and preferential synthesis during early S phase of HeLa, in addition to cytosine arabinoside sensitivity have allowed the characterization and isolation of a RNA population of 150,000–200,000 daltons molecular weight of a high specific radioactivity. Hybridization experiments in vast DNA excess to the homologous DNA have suggested a reiteration frequency of less than forty for each histone gene per haploid genome; in human over ten copies are indicated. Moreover, we have observed hybridization of the mammalian RNA to purified histone DNA of sea urchin indicating a 5–10% homology.

Conversion of Cell Type in Lens Regeneration in Adult Newts

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Although a number of descriptive evidence is available for conversion of iris epithelial cells into lens cells, experimental evidence for the conversion so far obtained does not exclude the possibility of participation of cells from the iris stroma in lens formation. Since the iris stroma contains a large number of cell types whose state of differentiation is mostly not well characterized, the above situation creates ambiguity in interpreting the alleged cell type conversion. The reported fact that the newt dorsal iris epithelium cultured in the presence of retina of frog larvae produces lens tissue supported strongly conversion of epithelial cells into lens cells. The further works have been done to assess the extent of contamination of the sample of dorsal iris epithelium by non-epithelial cells, to study the ability of dorsal iris stroma to produce lens tissue in vitro in the presence of frog retina, and to test the authenticity of lens tissue produced by iris epithelium in vitro by specific immunofluorescence. The results exclude participation of cells from the stroma in production of lens tissue and demonstrate that the iris epithelial cells of adult newts, which are fully differentiated and completely withdrawn from cell cycle become dedifferentiated and converted into lens cells.

PRAEMIA

RUZICKA-Preis 1974

Aus dem Fonds für den Ruzicka-Preis wird alljährlich einem jungen Forscher für eine hervorragende veröffentlichte Arbeit auf dem Gebiete der allgemeinen Chemie, die entweder in der Schweiz oder von Schweizern im Ausland ausgeführt wurde, ein Preis erteilt. Kandidatenvorschläge können bis spätestens 29. Juli 1974 dem Präsidenten des Schweizerischen Schulrates, ETH Zürich, Rämistrasse 101, 8006 Zürich, unterbreitet werden.

CONGRESSUS

Austria

1st International Congress on Human Ecology

in Vienna 15–19 September 1975

With regard to the extent of the problems involved in 'Relation between Man and his Environment', the Executive Committee is opening the Congress with a preparatory comprehensive discussion between the different groups of specialist. This written exchange of deas between members of the different discussion groups should serve to orientate the particularly interesting points in human ecology. The appropriate instructions should be requested from:
Dr. Helmuth Knötig, Secretary of the Board of the 1st International Congress of Human Ecology, Karlsplatz 13, A-1040 Wien (Austria).