Union Schweizerischer Gesellschaften für Experimentelle Biologie

Berichte der 7. Jahresversammlung

Union des Sociétés Suisses de Biologie Expérimentale

Comptes rendus de la 7e Réunion annuelle

Union of Swiss Societies for Experimental Biology

Abstracts of the 7th Annual Meeting

Bern 11./12. April 1975

Unterstützt durch die Schweizerische Naturforschende Gesellschaft
über die Schweizerische Kommission für Experimentelle Biologie

PHYSIOLOGIE - PHYSIOLOGY

A comparative Study of the Effects of Norepinephrine and Vasopressin on Na^+ Transport and O_2 Consumption in Frog Skin

W. Arczynska, L. Girardier and R. C. de Sousa
Département de Physiologie, Ecole de Médecine, CH–1211 Genève 4

It is known that both vasopressin and norepinephrine (NE) increase Na^+ transport in frog skin, but only vasopressin has been tested for its simultaneous effect on O_2 consumption. In the present study, using skin from Rana ridibunda, transport of Na^+ was measured by the short-circuit current technique and O_2 consumption by an oxygen cathode. Both parameters were determined simultaneously on each piece of skin during 4 successive experimental periods: (1) no hormone, (2) after addition of hormone a, (3) after removal of hormone a, (4) after addition of hormone b. Hormones a and b were either NE ($4.10^{-7}M$) or vasopressin (20 mU/ml). In 16 experiments both hormones induced similar increments in Na^+ transport, $\triangle J_{Na}$ being $+280 \pm 37$ pmoles sec^{-1} cm^{-2} for vasopressin and $+262 \pm 33$ pmoles sec^{-1} cm^{-2} for NE, (mean $\pm$ SEM, $p > 0.4$). In contrast the effects on O_2 consumption were different, $\triangle J_{O_2}$ being $+21.1 \pm 4.4$ for vasopressin and $+4.6 \pm 2.1$ pmoles sec^{-1} cm^{-2} for NE ($p < 0.0025$). In conclusion it was found that, although both vasopressin and NE each increase Na^+ transport to the same extent, only vasopressin augments significantly the O_2 consumption at the same time. This suggests that the two hormones exert different effects on cell metabolism.

Supported by SNSF, grants 3.721.72, 1.300.73, 3.029.73 and Commission de recherche de l'Université de Lausanne

Increased Oxygen Uptake Induced by KCl in Brown Adipose Tissue Slices

Y.-A. Barde, A. Chinet and L. Girardier
Département de Physiologie, Ecole de Médecine, CH–1211 Genève 4

Beside its metabolic effects on brown adipocytes, the most important of which is an increase in respiration leading to heat production, noradrenaline is also known to depolarize these cells. In this study, an attempt has been made to reproduce hormonal action by a depolarization induced by increasing the KCl concentration in the medium. Brown adipose tissue slices (5–12 mg wet weight) were incubated for several hours and their oxygen uptake measured. An increase in KCl concentration did, in fact, mimic the hormonal action, i.e. 40 to 50 mM produced a 4 to 6-fold increase in respiration. However, samples taken from reserpine-treated animals failed to show any increase in respiration in response to KCl, their response to noradrenaline being the same as that of the untreated animals. Furthermore, L- but not D-propranolol at $10^{-7}M$ blocked more than three quarters of the KCl response, while moderate responses were potentiated by desipramine. Finally, chemical denervation by 6-OH dopamine almost completely prevented the KCl-induced increase in respiration. It has therefore been concluded that the KCl effect is entirely due to a release of noradrenaline by the still functional nerve endings of the tissue slices.

Ultrastructure of Spezialized Nodes of Ranvier in the Neurogenic Electric Organ of the Knife Fish Sternarchus

M. V. L. Bennett, K. Akert, C. Sandri and H. Moor
Albert Einstein College of Medicine, Department of Anatomy, New York 10461, USA, and Brain Research Institute, CH–8029 Zürich

Bennett (1966, 1971) demonstrated that electric organs in Sternarchidae are composed of spinal electrocyte axons which transform action potentials into diphasic external signals. Waxman et al. (1972) have described two types of serially arranged Ranvier nodes along these axons which may be responsible for this special property. One is classical, the other contrasts sharply with common nodal morphology. Freeze-etching studies of the latter confirm a vast surface elaboration of the nodal axolemma. It consists of a multitude of arborizing processes which are packed into an intertwining system of branches and twigs. The membrane faces of these processes are characterized by relatively particle-free B-faces and particle-rich A-faces. In contrast, the normal node displays a smooth axolemma whose B-face may be often, but not always, studded with membrane particles. These findings are consistent with Bennett's hypothesis that the typical nodes actively generate spikes whereas the modified nodes act as a series capacity.

The Decrease in Voltage of the T Wave at High Altitude

P. Berg, P. Laciga and E. A. Koller
Physiologisches Institut der Universität, CH–8001 Zürich

One of the characteristic electrocardiographic changes in man following acute exposure to high altitude (low-pressure chamber) is, as already shown, the decrease in amplitude of the T wave, predominantly in the left precordial leads. The present study was carried out on 33 healthy volunteers at 6,000 m altitude (P_{IO_2} 72.9 mm Hg). The results show that at this altitude the lower the P_{AO_2}, the higher the heart rate and the greater the decrease in amplitude of the T wave. In order to study the relative influence of the heart rate on the hypoxia-induced decrease in T voltage, the volunteers were grouped according to their P_{AO_2}. In all the groups with similar P_{AO_2} and different heart rate, the decrease in T amplitude is a function of the heart rate. In the groups of volunteers with similar heart rate and different P_{AO_2}, however, there is a significant correlation between the decrease in T amplitude and the P_{AO_2}. It is concluded that at high altitude the increase in heart rate is one of the factors contributing to the hypoxia-induced decrease in amplitude of the T wave.

NF = Schweiz. Nationalfonds zur Förderung der wissenschaftlichen Forschung
FNRS = Fonds National Suisse de la Recherche Scientfique
SNSF = Swiss National Science Foundation

Effect of Cholecystectomy Combined with Sphincterotomy on Bile Salt Synthesis and Pool Size in the Dog

K. von Bergmann, H. R. Schultheiss, G. Paumgartner and R. Preisig
Department of Clinical Pharmacology, University of Berne, CH–3012 Berne

Cholecystectomy combined with sphincterotomy is a frequently performed operation eliminating the mechanisms by which bile is intermittently released into the intestine. The consequences on bile salt (BS) metabolism, however, are as yet ill defined. Therefore, pool (BS-P) and rate of synthesis (BS-S) of BS were measured by biliary drainage (wash-out technique) and the frequency of enterohepatic circulations (EHC) calculated in each of 3 unanesthetized dogs equipped with a Thomas duodenal cannula, and repeated at monthly intervals following cholecystectomy and after additional sphincterotomy. In the intact dog, an average BS-S of 1.6 mmol/day was associated with a BS-P of 4.9 mmol and 3 EHC. Following cholecystectomy, the number of EHC increased to 12; however, in spite of an augmented BS-S (2.3 mmol/day), BS-P fell to 41% (to 2.0 mmol). Sphincterotomy resulted in a further decrease of BS-P to 1.5 mmol. These results suggest that following cholecystectomy the normal dog liver is unable to compensate for the increased BS loss, presumably induced by more frequent EHC. An additional sphincterotomy aggravates this loss and leads to a further fall in BS-P.

Role of TSH, and of Thyroid Cyclic AMP and Iodine on Goiter Development in Rats

C. Berthier and T. Lemarchand
Division de Biochimie Clinique, Département de Médecine, Hôpital Cantonal, CH–1011 Lausanne

Goiter induced by Propylthiouracil (PTU) or Low-Iodine Diet (LID) was studied in 240 normal male rats in regard to thyroid cyclic AMP (cAMP) and iodine, plasma and pituitary TSH contents, for 2, 3, 4, 7, 14, and 30 days. In the PTU group, parallel increases in thyroid weight, plasma TSH (from 0.42 ± 0,03 to 1.28 ± 0.09 µg/ml) and thyroid cAMP (from 1.79 ± 0.06 to 2.58 ± 0.32 p*M*/mg thyroid) were observed during the first week. At 14 days cAMP returned to control values, whereas TSH showed a slight decrease followed by a regular rise up to the end of treatment. Plasma T_3, pituitary TSH content and thyroid iodine decreased rapidly from the beginning and remained low. In the LID group, thyroid weight increased slowly, whereas plasma and pituitary TSH levels were not modified. Thyroid cAMP was enhanced on the fourth day (from 1.73 ± 0.07 to 2.26 ± 0.18 p*M*/mg thyroid) and returned to control values on the 14th day, at which time thyroid iodine was reduced to half its initial value. Although plasma TSH, cAMP and goiter growth correlate well throughout the first week in PTU-treated rats, no correlation exists between these parameters and the subsequent PTU goiter development or its induction by LID. Only the low thyroid iodine content seems to be responsible for the growth of the gland by enhancing TSH or/and cAMP actions on their own thyroid cell receptors.

Supported by SNSF, grant 3.112.73

The Microvibrations of the Body (MV), a Possible Index for Emotional Tension

M. Bircher, B. Nigg and E. A. Koller
Physiologisches Institut der Universität, 8001 Zürich, und Laboratorium für Biomechanik der ETH, CH–8006 Zürich

The MV were measured in 85 medical students immediately prior to taking a practical and an oral exam and later during the ensuing vacation (control). The force-time function as well as the rectified integrated force-time function (impulse) in the three dimensions was measured by means of a piezoelectric force-platform on which the subject was asked to stand at ease. The values obtained were then evaluated in relation to body weight. The high pre-examination values obtained in all subjects fell significantly in the control of the candidates who passed their exams, but remained high in those who failed. Habitual smokers and the candidates who resorted to tranquillizers before taking the exams had higher than average MV values, not only in the pre-examination tests but also in the control. In all subjects the MV results in the vertical direction were closely correlated with cardiac output, the mean values in all three tests being significantly higher in males than in females. It is concluded that MV measurement appears to be a reliable method for estimating emotional tension.

Thermal Stimuli at Onset of Sweating: Comparison of the Follicular and Luteal Phases of the Menstrual Cycle

J.-Ph. Bonjour, H. J. Welti and E. Jéquier
Division de Physiologie clinique, Institut de Physiologie et Département de gynécologie et obstétrique, Université de Lausanne, CH–1011 Lausanne

Thermal sweating (E) was studied in eight women exposed to 37 °C in a gradient layer direct calorimeter. The thermoregulatory response was found to be elicited when a characteristic mean body temperature ($\overline{T}_b$ set) was reached. Neither tympanic, nor vaginal, nor mean skin temperatures were by themselves a specific stimulus of E. $\overline{T}_b$ set in the luteal phase was observed to be higher than that in the follicular phase of the cycle. There was no correlation between the increment of $\overline{T}_b$ set and the rise of the urinary excretion rate of pregnanediol. The characteristics of the sweating response were that of a proportional control with a similar gain in the two phases of the menstrual cycle. Resting metabolic rate did not change significantly in the two phases. It is concluded that (1) Thermal sweating is initiated by changes in mean body temperature ($\overline{T}_b$) above $\overline{T}_b$ set and is directly proportional to variations of $\overline{T}_b$. (2) The rise of the post ovulatory basal body temperature is accompanied by a shift in $\overline{T}_b$ set which is not correlated with the urinary excretion of the main metabolite of progesterone.

In vitro Studies of Renin Secretion by Rats Kidney Slices

A. Capponi et M. B. Vallotton
Laboratoire de Physiopathologie clinique, Département de Médecine, Université de Genève, CH–1211 Genève 4

Rat kidney slices are incubated in Krebs-Ringer-bicarbonate buffer, pH 7.4 at 37 °C, with oxygenation. After 30 min preincubation the slices are incubated with fresh

medium for 90 min. 50 µl aliquots, taken at different times, are incubated for renin determination at 37 °C with excess rat renin substrate and anti-angiotensin I antiserum. The angiotensin I (AI) generated is measured by radioimmunoassay. Results are expressed in ng AI/mg fresh tissue/min. The results indicate an active release of renin which is linear during 90 min and temperature dependent. At 37°, 24° and 4 °C AI generation is 1.3 0.8 and 0.5 ng mg^{-1} min^{-1} respectively. Adrenalectomy of 300-g rats 8 days before nephrectomy followed by diuretics administration and salt-free diet increases the release to 2.6 ng mg^{-1} min^{-1}; if younger 200-g rats are used, the release is already as high when untreated and does not increase after adrenalectomy. Significant ($p < 0.05$) inhibition of renin release from 1.5 to 1.04 ng mg^{-1} min^{-1} occurs with epinephrine or norepinephrine ($10^{-5}M$) in the medium. In presence of dibenyline ($10^{-5}M$) inhibition no longer occurs with epinephrine (1.7 ng mg^{-1} min^{-1}) and a stimulation is observed with norepinephrine (1.9 ng mg^{-1} min^{-1}). With isoproterenol there is a dose-related stimulation of renin release from 10^{-8} to $10^{-6}M$ at which the stimulation is maximal.

Effect of Electrical Activity on the Energy-Rich Phosphates in Torpedo

M. Chmouliovsky-Moghissi
Département de Pharmacologie Ecole de Médecine
CH–1211 Genève 4

Previous experiments on small pieces of electric organ of Torpedo showed a decrease in ATP and CrP after long periods of activity [Chmouliovsky et al., J. Neurochem. *22* 73 (1974)]. A similar but smaller decrease of these compounds has now been found also after shorter periods of stimulation at 5 shocks/sec. Thus after 30, 90, 140 and 240 sec., the ATP fell to 79.6 ± 5.9, 97 ± 7.6, 84 ± 10 and 68.5 ± 11, and the CrP to 62.5, 64 ± 14.5, 53 ± 13.7 and 29.9 ± 10.9% of unstimulated controls (means of 4–5 experiments ± SE). Recovery to the resting level was slow, after 60 sec. recovery there was hardly any increase in the compounds. Thus, in contrast to isolated nerve [Chmouliovsky et al., J. Physiol. *202*, 90P, (1969)], where the fall in ATP is larger than that of CrP the electric organ appears to maintain a rather high ATP level, even during long periods of activity.

Supported by SNSF, grants No. 3.0890.73 and 3.3010.74.

The Modifications of Neuronal Activity after Infection with Pseudorabies Virus

M. Dolivo, E. Beretta, V. Bonifas and C. Foroglou
Institut de Physiologie de l'Université CH–1011 Lausanne

In rats infected with pseudorabies virus, the neurons of the superior sympathetic ganglion show a spontaneous activity characterized by periodic bursts of action potentials recorded on both the post- and presynaptic nerves [Dempsher et al., Am. J. Physiol. 182 (1955)]. We have repeated these experiments in order to find the origin of these bursts of action potentials and establish correlations of this abnormal activity with the metabolism and ultrastructure of infected ganglia. The results obtained until now show that the frequency of the spontaneous bursts of activity increases from the 30th hour after inoculation up to the 40th hour and then decreases until the 50th hour, following a very precise schedule. As the recorded bursts of activity can be blocked by curare and enhanced by eserine they are to be considered as 'cholinergic-like'. The ultrastructure shows virus in the post- and presynaptic fibers, in the neurons and in their satellite cells. Modified and newly formed membranes can be observed as well as synaptic lesions and viruses at various stage of their replication.

Supported by SNSF, grant 3.457.70.

Comparison of RHA and RLA Rats in Four Different Tests

H.-R. Dütsch and K. Bättig
Institut für Verhaltenswissenschaft, ETHZ
CH–8006 Zürich

In a behavioural comparison RHA rats were more active but defecated less frequently in an open-field test than RLA rats. They were also more active in an exploratory maze, learned more slowly the problems of the Hebb-Williams test of 'intelligence' and showed more rapid acquisition of active avoidance. Half of the 20 male rats tested for each strain were given these tests in the above order, while the other half were given the Hebb test before the exploratory maze in order to get some information with respect to possible transfer mechanisms. Activity in the exploratory maze was not altered through Hebb test experience with the RHA rats, but it was increased with the RLA rats. In neither of the two strains had experience in the exploratory maze influence on the problem-solving in the Hebb test. Correlation matrices between the parameters of the four tests for the RHA and the RLA rats showed strain differences especially in regard to the correlations between the parameters of the open-field and the shuttle box.

Respiratory Effects of Intermittent Electrical Bulbar Stimulation in the Rabbit

M. Fallert und K. Baum
2. Physiologisches Institut der Universität, D–65 Mainz, BRD

In anesthetized rabbits spirogram and diaphragmatic activity were examined during electrical stimulation of regions of the bulbar lateral reticular formation. The activity of respiratory bulbar neurons was recorded. One volley of some 150 m/sec duration at 100 pulses per sec, applied during inspiration, caused an immediate and transient inhibition of the diaphragmatic activity. After the end of the volley, the inspiration continued. The tidal volume, however, was increased above normal and the inspiration was prolonged. 'Inspiratory' neurons exhibited inhibition during the volley; the burst was lengthened and the discharge rate increased after the volley. The same was true for 'expiratory-inspiratory' phase-spanning units. The discharge of 'inspiratory-expiratory' phase-spanning neurons, however, was rather inhibited. When applied during expiration the volley caused an inspiratory twitch, correlated to a short post-stimulus firing of 'inspiratory' neurons. The burst of some phase-spanning units was shortened. The stereotaxic mapping of the respiratory neurons did not reveal a distinct grouping of inspiratory or phase-spanning units.

Amino Acid in Pigeon Tectum – An Autoradiographic and Iontophoretic Study

D. Felix, S. P. Hunt and U. Frangi
Brain Research Institute, CH–8029 Zurich

Autoradiographic studies have indicated, that certain neurones distributed throughout the optic tectum specifically take up GABA. Cell bodies as well as their pro-

cesses can be visualized, linear accumulations of silver grains passing between deep and superficial layers. There was no evidence for a selective uptake of glycine by tectal neurones, but it is possible that a glycinergic input may be supplied by an extratectal nucleus. The distribution of proline suggests an active site of uptake external to the radial dendrites of neurones in laminae 8–12. Microiontophoretic application of GABA, glycine and proline was tested on 134 tectal cells, using the same amount of current during equal test periods. GABA was the most potent amino acid (56%), followed by glycine (31%) and proline (13%), suggesting that GABA is of major significance as an inhibitory tectal transmitter. The highest percentage of GABA-neurones was found in layer 4 and 10–13. The cells inhibited by the other two amino acids did not appear to be grouped within any particular tectal layer.

Supported by SNSF, grants 3.822.72 and 3.823.72 and Slack-Gyr Foundation

Monovalent and Divalent Orthophosphate Uptake in Desheathed Rabbit Vagus Nerve

J. Ferrero, P. Jirounek, G. J. Jones, A. Salamin and R. W. Straub
Département de Pharmacologie, Ecole de Médecine, CH–1211 Genève 4

Previous experiments have shown that uptake of ^{32}P-orthophosphate in desheathed vagus is a slow, membrane-limited process. At pH 7.4, the rate of uptake depends on the extracellular phosphate with some saturation at high concentrations, but does not follow a simple Michaelis curve (Anner *et al.*, J. Physiol., in press). The uptake rates have now been measured at pH 8.4, where the orthophosphate is present mostly as the divalent ion. At this pH, for phosphate concentrations up to 2 m*M*, the rates fit closely a Michaelis curve (K_m, 0.5 m*M*; V_{max}, 20 µmole/kg wet wt. per min), suggesting that the divalent ion transport is by a saturable process. Experiments at pH 6.0 indicate that a fraction of the monovalent ion transport is by a similar process. At pH 7.4 phosphate uptake is largely dependent on the presence of extracellular sodium [Anner *et al.*, J. Physiol. *232*, 47P (1973)]. Replacement of sodium by choline also decreases the phosphate influx at pH 8.4 and 6.0. However, analysis of this effect is complicated by the finding that at low sodium, potassium also interferes with phosphate uptake.

Supported by SNSF, grant 3.0890.73

Local P_{O_2} and $\dot{Q}_{O_2}$ in Rat Superior Cervical Ganglia, at Rest and in Activity

C. Friedli
Institut de Physiologie de l'Université, CH–1011 Lausanne

P_{O_2} distribution was studied with an O_2-microelectrode in isolated ganglia perifused at 37 °C and 95% O_2. From the P_{O_2} gradients in the tissue and in the adjacent fluid layer [Whalen, Am. J. Physiol. *219*, 814, (1970)] an O_2 permeability of $2.29 \pm 0.12 \times 10^{-5}$ ml cm^{-1} min^{-1} atm^{-1} and an O_2 consumption at rest of $1.42 \pm 0.13 \times 10^{-2}$ ml cm^{-3} min^{-1} were calculated for similarly located central areas of 7 ganglia. With supramaximal stimulation of the preganglionic nerve, the early increase of the $\dot{Q}_{O_2}$ in each area (as measured by the local depletion rate of O_2) was linearly proportional to the frequency of stimulation F up to 15 Hz. In steady-state the corresponding increase of the $\dot{Q}_{O_2}$ was always smaller; it followed a saturation curve of the form $\triangle\dot{Q}_{O_2} = 1/(a/F + b)$. At half saturation, the calculated $\triangle\dot{Q}_{O_2}$ varied from 19% to 325% of the $\dot{Q}_{O_2}$ at rest, and the corresponding frequencies ranged between 2 and 15 Hz. Such variations might imply direct correlations with the local histological composition and the general metabolic state of the ganglia.

Supported by SNSF, grant 3.457.70

Control of Cardiac Action Potential Duration by $[Ca]_i$?

C. H. Fry, J. A. S. McGuigan and J. B. Bassingthwaighte
Physiologisches Institut der Universität, CH–3012 Bern, and Mayo Clinic, Rochester, Minn., 55901 USA

In a previous abstract (Experientia *30*, 680, 1974) evidence was presented that supported $P_{K\alpha}[Ca]_i$. This suggestion has now been further strengthened by the following observations. In low $[Ca]_o$, depolarizing current pulses applied at the beginning of the AP prolong it. Plots of AP duration in high and low $[Ca]_o$ at various frequencies cross each other; at low frequency the AP in high $[Ca]_o$ is longer than in low $[Ca]_o$, at high frequency the reverse is found. Voltage clamp experiments show that in 11 m*M* Na (to increase $[Ca]_i$) the outward current is increased when compared to Tyrode. In 70% $[Na]_o$ the outward current is not changed. The observed shortening of the AP in this $[Na]_o$ is partly due to the reduction in overshoot; this is supported by the fact that depolarizing current pulses prolong the AP. It is suggested that the level of $[Ca]_i$ sets the level of background current and provides a feedback mechanism for AP duration. In addition, at low frequencies with a presumably low $[Ca]_i$ and low potassium permeability, substitution experiments show that Cl current plays a relatively more important role in repolarization than at high frequencies.

C.H.F. is supported by The Royal Society

Effect of Cold-Induced Increase of $\dot{V}_{O_2}$ on the Growing Lung

P. Gehr, C. Hugonnaud, P. H. Burri and E. R. Weibel
Anatomisches Institut der Universität, CH–3000 Bern 9

Hypoxia and physical hyperactivity lead to a quantitative adaptation of the gas exchange apparatus during growth resulting in an increased morphometric pulmonary diffusion capacity (D_L). In order to test if cold exposure, which increases basic metabolism and hence O_2-consumption ($\dot{V}_{O_2}$) yields similar results, 2 groups of young rats were kept in climate chambers at temperatures of 11° and 24 °C respectively. The animals kept in cold showed a 87% higher mean oxygen consumption. After 3 weeks the animals were sacrificed and their lungs prepared for morphometry. Mean body weights were identical for both groups, but the test animals had a 14% larger lung volume. Morphometric analysis revealed that this was due to a 15% increase in alveolar volume. Capillary and tissue volumes, alveolar and capillary surface areas showed no significant differences. These findings indicate that the lungs of the test rats underwent a simple dilatation. Cold exposure increases $\dot{V}_{O_2}$ similarly to physical exercise. In animals exposed to cold, however, D_L was identical in experimental and control animals. It is concluded that cold exposure does not lead to a quantitative adaptation of the gas exchange apparatus.

Supported by SNSF, grant 3.682.71

Interhemispheric Integration in the Pigeon

M. Graf and H. Zeier
Swiss Federal Institute of Technology, Dept. of Behavioral Sciences, CH–8006 Zurich

Pigeons having their visual input selectively restricted by wearing goggles with a red and green filter, respectively, were trained on a successive pattern discrimination task. The stimuli (2 positive and 2 negative) all had a red and green component, which the animals had to interhemispherically compare with each other in order to perform the discrimination. During training, pecking on a S^+ was reinforced according to a 1 min variable interval schedule. A S^+ was presented for 5 min; a S^- terminated as soon as the bird abstained from pecking for 1 min. A session lasted until 60 reinforcements were achieved. The test session, run without any reinforcements, consisted of 12 2-min presentations of the 4 stimuli. On this test 4 out of 6 birds preferred the positive stimuli by about 66 to 88%. The other 2 remained at the 50% level. Tests run under training conditions, but with a white light instead of the stimuli, suggest that these birds probably used an easier but less effective strategy in learning the reinforcement schedule. Apparently, pigeons seem to be able to perform interhemispheric integration, although it is rather difficult for them.

Interactions of Oxytocin, Catecholamines, Calcitonin and Xanthines in Frog Skin

A. Grosso and R. C. de Sousa
Départements de Physiologie et de Médecine, Ecole de Médecine, CH–1211 Genève 4

Available evidence suggests that cAMP mediates the effects of oxytocin (OT) in frog skin. We tested further this hypothesis by taking OT as a reference substance, and examining its interaction with activators of adenylate cyclase (catecholamines, calcitonin) and with inhibitors of phosphodiesterase (theophylline and iso-butylmethylxanthine or IBM). Short-circuit current (SCC) was taken as a measure of net sodium flux. Oxytocin-induced increments in SCC were smaller ($p < 0.01$) in tissues exposed to catecholamines than in control tissues and, conversely, for catecholamines added after exposure to OT ($p < 0.01$). The same pattern of responses was found with OT before and after theophylline or IBM. Results with synthetic salmon calcitonin (Sandoz) were less clear, the hormone showing occasionally a natriferic effect competing with that of OT. In summary, work with several adenylate cyclase activators and phosphodiesterase inhibitors suggests that sodium transport is at a maximal rate when a critical level of intracellular cAMP has been reached, further stimulation of adenylate cyclase having no effect.

Supported by SNSF, grant 3.1300.73.

Histamine and Metiamide: Action on Hypothalamic and Cortical Neurones

H. L. Haas, U. M. Bucher, J.-C. Nussbaumer and P. Wolf
Department of Neurophysiology, Neurological Clinic and Department of Physiology, University of Basel, CH–4051 Basel

Biochemical and pharmacological studies suggest a transmitter role for histamine in the mammalian brain and an involvement in several hypothalamic functions e.g. ADH release. Microelectrophoretically applied histamine caused an excitation of many neurones in the hypothalamus of rat and cat including antidromically identified supraoptic neurosecretory cells in the cat. In the cortex histamine usually had a depressant action which was more pronounced in the rat than in the cat. Dual actions and excitations were observed infrequently. Metiamide, an antagonist of histamine at H_2-receptors, produced a depression or a dual effect, consisting of a short excitation followed by a longer lasting depression of firing, on 17 of 25 neurones. Excitant actions of histamine in the hypothalamus were not selectively antagonized by metiamide but on 6 of 9 cortical neurones tested the depressant action of histamine was blocked or reduced while the depressant action of GABA was unaffected. Betazol, an H_2-agonist also depressed cortical neurones but caused depressions and excitations in the hypothalamus.

Effects of Na^+ and Li^+ on the Excitation of Cultured Spinal Neurones by Amino Acid Transmitters

L. Hösli, P. F. Andrès and Elisabeth Hösli
Abt. für Neurophysiologie, Neurologische Universitätsklinik, CH–4051 Basel

There is strong evidence that glutamate and aspartate function as excitatory transmitters in the mammalian central nervous system. We have studied the action of these two amino acids on the membrane potential of human and rat spinal neurones in tissue culture. As has been observed on cat spinal motoneurones *in situ*, both glutamate and aspartate caused a depolarization of cultured spinal neurones when the amino acids were added to the bathing fluid at concentrations of 10^{-4} and $10^{-5}M$. In order to study ionic mechanisms associated with the amino acid induced depolarization we have altered the ionic composition of the extracellular fluid. Removing sodium ions reversibly abolished the depolarization produced by aspartate and glutamate, suggesting that the action of these amino acids is mainly dependent on an increase in sodium permeability. Replacement of sodium by lithium also reversibly reduced or abolished the glutamate depolarization which is in contrast to the effect of lithium on the action potential of various excitable membranes.

Initial Heat Production in Non-Myelinated Pike Olfactory Nerve

J. V. Howarth, R. D. Keynes, A. von Muralt and J. M. Ritchie
Physiological Institute, University of Berne, CH–3012 Berne

The pike olfactory nerve consists of extremely small non-myelinated fibres (average diameter, 0.25 μm). As a result it has an extremely large area of axonal membrane per gram nerve. Using this nerve, together with a faster thermopile than that used earlier with crab and rabbit nerve (Abbott, Hill and Howarth 1958, Proc. Roy. Soc. [B] *148*, 149–187; Howarth, Keynes and Ritchie 1968, J. Physiol. *194*, 745–793) we have shown that in pike nerve during the phase of depolarization one measures an evolution of heat (44 μcal/g), which is immediately reabsorbed as the fibre repolarizes. Because of overlap due to temporal and spatial dispersion, the true heat change

is probably about 4 times larger. The heat in the pike nerve is thus 3–5 times greater than in crab or rabbit nerve expressed per gram nerve, per unit area of membrane, however, it is of the same order. The positive heat seems to be derived from 2 sources: First, there is a dissipation of free energy stored in the membrane dielectric as the voltage changes. This voltage may be appreciably greater than the voltage measured between the axoplasm and the bulk of the external medium because of an asymmetrical distribution of fixed charges on the two sides of the membrane. Secondly, there is an evolution of heat corresponding with a decrease in entropy of the membrane dielectric with depolarization.

Effect of the CO_2/HCO_3^- System on the Membrane Potential of Frog Skeletal Muscle

F. Huguenin
Physiologisches Institut der Universität, CH–3012 Bern

The effect of the CO_2/HCO_3^- pair on the membrane potential of frog skeletal muscle in various bathing media has been studied. The introduction of CO_2 and HCO_3^- in various concentrations into Cl^--free Ringer (PCO_2 = 100 or 40 mm Hg; HCO_3^- = 5 or 25 m*M*/l) produced a rapid depolarization of 5 to 20 mV, reversible on withdrawal of the CO_2/HCO_3^- system. This response was primarily unrelated to the presence of Na^+; it was increased in solutions of decreased K^+ concentration. The observed facts can be explained if the cell membrane is assumed to exhibit a significant HCO_3^- permeability. Thus, the CO_2/HCO_3^- solutions established a transmembrane HCO_3^- ratio of such value as to cause a HCO_3^- efflux, i.e. an inward current tending to lower the membrane potential. Our observations seem directionally incompatible with a significant participation of H^+ ions themselves in the membrane diffusion potential. By a crude estimate, these effects would be accounted for by HCO_3^- effluxes of some 10^{-11} *M*/cm² sec, requiring a constant-field $P_{HCO_3^-}$ of about one order of magnitude less than P_{Cl}.

Die Bedeutung des intralaminaren Systems des Thalamus für das Kontrastsehen

R. W. Hunsperger und Daria Roman
Physiologisches Institut der Universität, CH–8001 Zürich

Elektrische Reizung im intralaminaren System des Thalamus der wachen Katze führt zu konjugierten Blickbewegungen nach der Gegenseite (Schlag und Schlag-Rey, Exp. Neurol. *33*, 498, 1971). Kombination der intralaminaren Reizung mit elektrischer Reizung im corpus gen. lat. bewirkt eine Verstärkung bzw. Verminderung der im visuellen Cortex ausgelösten spezifischen Potentiale, je nach dem zeitlichen Intervall der beiden Reize. Es stellt sich die Frage nach der sinnesphysiologischen Bedeutung dieser intralaminaren Effekte. Es wurde deshalb der Einfluss der künstlich herbeigeführten visuellen Zuwendung auf das durch Streifenkontraste ausgelöste Potential (Campbell, Maffei und Piccolino, J. Physiol. *229*, 719, 1973) untersucht. Als optischer Reiz dienten Streifenmuster, welche mit diffuser Belichtung gleicher Intensität mit einer Frequenz von 4 pro sec alternierten. Die Blickbewegungen wurden durch Mittelfrequenzdauerstrom erzeugt und die Kontrastpotentiale während der Endstellung der Augen über 32 sec lang gemittelt. Die Untersuchungen ergaben eine signifikante Verstärkung des Kontrastpotentials bei visueller Zuwendung (z. B. Tier *34:* 25,4 ± 2 mV gegenüber 19,4 ± 3 mV in den Kontrollen) und zeigen, dass das intralaminare System des Thalamus für die Erfassung optischer Reize von Bedeutung ist.

Fluorescence Probe Signals from Excited Heart Muscle

A. Illanes and A. von Muralt
Physiological Institute, University of Berne, CH–3012 Berne

Certain fluorescent compounds act as probes for cell membrane potentials. (Cohen, L. B. et al. 1974, J. Membrane Biol. *19*, 1–36). The optical changes, which accompany the excitation of the atrial septum (frog heart), a thin tissue of transparent, excitable and contractile elements, were studied. Method: preparation incubated 20 min in frog Ringer + Merocyanine 50 µg/ml + ethanol less than 0.4%. Light source: 50 W halogen lamp on DC. Primary exciting light passes interference filters 493 or 544 nm. Fluorescent light passes 586 nm. Receptorphotodiode, registering KO + CAT 400. Results: we obtained fluorescent light signals, emitted by the preparation after electrical stimulation, with several components: (a) fluorescence from excitation, (b) fluorescence from contraction, (c) nonspecific light scattering. To identify these components we used the Ca-blocker D-600. With filters 493 and 586 almost no scattered light came through, but after staining with Merocyanine we observed the fluorescent signal (a).

Heat Losses of Newborn Infants of Different Body Size

P. Kajtàr, L. S. Prod'hom and E. Jéquier
Division de Physiologie clinique, Institut de Physiologie et Service de Pédiatrie, Université de Lausanne, CH–1011 Lausanne

(1) Small-sized infants (less than the tenth weight percentile), (2) appropriate for dates (tenth to ninetieth percentile) and (3) large for dates (above ninetieth percentile) were exposed to ambient temperatures (T_a) of 28 °C and 32 °C in a direct calorimeter. Total heat loss (R + C + E) expressed per unit body surface was similar in the 3 groups, but when expressed per kg of body weight, (R + C + E) was more elevated in group 1 than in groups 2 and 3. At 28 °C T_a, (R + C + E) was 4.35 ± 0.15 W/kg, 3.6 ± 0.1 W/kg, and 3.04 ± 0.09 W/kg in groups 1, 2 and 3 respectively. Metabolic rate was higher at 28 °C than at 32 °C; at 28 °C, group (1) had a lower heat production per kg body weight than the two other groups. Heat storage ($\dot{S}$) at 28 °C was negative in the 3 groups; at 32 °C, $\dot{S}$ was negative in group 1, but thermal equilibrium was reached in groups 2 and 3 ($\dot{S}$ = 0). Thermal body insulation between the 3 groups was not significantly different. In order to practically determine the 'dry heat losses' of a newborn infant, a 'cooling constant' of 5.7 W/m² × (T_{re} − T_a) was obtained (T_{re} = rectal temperature). Our results show that at low T_a the surface-body mass ratio of small-sized infants plays a more important role in their thermal balance than stimulation of heat production or thermal body insulation.

Supported by SNSF, grant 3.714.72

Effects of VMH-Lesions on Food-Competition in Paired Male Rats

H. P. Käsermann and R. A. Stamm
Institut für Verhaltenswissenschaft ETHZ, 8006 Zürich, and Zoologisches Institut der Universität Basel, CH–4000 Basel

Grossman (J. C. C. P. *78*, 274, 1972) suggested that in rats VHM-lesions moreover were followed by changes of the dominant-subordinate relationship (D-S). In the

present study D-S was determined in the homecage of paired male rats on the base of food possession and eating time after either offering of a piece to one of the two rats (a) or after releasing it between the two (b). Using method (b) a consistent D-S was found preoperatively in 28 of 32 pairs. In 11 pairs one rat was selected at random for bilateral VMH-lesions, which caused both a decrease of food possession and of capture of the released food piece ($p < 0.02$). Using method (a), in other 30 pairs the D-S could be established in 3 pairs if the same parameters were used and in 25 pairs if in addition efforts of a rat to prevent his partner from eating were also considered. In 9 pairs the dominant rat was lesioned and no difference compared with the behavior of the control group was seen. This indicates that rather the decrease of speed in capturing the piece than the D-S change is a consequence of VMH-lesions.

The Rat's Sleep in Oestrus

H. Kleinlogel
Wander Ltd., CH–3001 Berne

EEG-studies were carried out on 11 unrestrained, female Charles River rats with electrodes previously implanted in the brain, using the method of Sayers and Stille (Electroenceph. Clin. Neurophysiol. *27*, 87, 1969). During recovery and throughout the experiment the oestrous cycle of each rat was determined taking a vaginal smear. The cycle lasted 4 days. Monopolar tracings were recorded from the occipital cortex and the dorsal hippocampus, as well as the EMG from the neck muscles on every day of the cycle from 8 a.m. to 4 p.m. In order to avoid effects of adaptation, the tracings of the same rat were recorded again after at least 3 days. During oestrus, paradoxical sleep was augmented (dioestrus: 26 min; oestrus: 47 min), particularly because of the earlier beginning of that phase. Compared with the dioestrus data the rats were awake for shorter periods during oestrus (dioestrus: 188 min; oestrus: 151 min). There was very little difference between the sleep patterns of oestrus and prooestrus.

Microspectrofluorometry of Pyridine Nucleotides in Chick Embryo Cultured in Vitro

P. Kucera and Y. de Ribaupierre
Institut de Physiologie de l'Université, CH–1011 Lausanne

The redox state of pyridine nucleotides (PN) as measured by fluorescence is chosen to monitor globally the functional differentiation within the chick blastodisc. Embryos from the stage 3–4 HH are grown (up to 50 hours) in specially designed chambers allowing the continuous observation and measurement of fluorescence and/or absorption signals under controlled metabolic conditions. All the embryonic area is systematically examined by means of a scanning device built in our microspectrofluorometer. The on-line data processing is performed by a computer and the fluorescence signals corrected for the optical inhomogeneity of the specimen are obtained. The PN/PNH ratio displays are compared to the corresponding photographs in order to determine the regional and temporal variations of the embryonic tissue activity. This method should contribute to the establishment of physiological correlations with the early morphogenesis of the embryonic nervous system.

Supported by SNSF, grant 3.664.71.

Hypothalamic Representation of Defense Behavior in the Common Marmoset

H.-P. Lipp
Physiologisches Institut der Universität, CH–8001 Zürich

15 marmosets (*Callithrix jacchus*) with a total of 57 implanted electrodes were tested by means of electrical brain stimulation in order to elucidate whether hypothalamic representation of points eliciting defense behavior (flight, threat and attack) described for cat and oppossum also occur in primitive monkeys. Stimulation took place in familiar environment, the effects being observed by television. To disclose further effects depending on altered conditions, stimulation with the same electrical parameters was repeated under different environment and motivation conditions, particularly during flight behavior and situations in which spontaneous threat was likely to appear. The results show that all points yielding vocal threat and fighting lie under the fornix in the ventrolateral part of the N. ventromedialis, whereas points eliciting various types of flight behavior are less clearly distributed rostrad, dorsad and caudad to this zone. – It was concluded that in cat, oppossum and primitive monkeys the hypothalamic representation of defense behavior appears to be similar.

La^{3+}-stimulated Na Transport in Frog Skin: Independence from cAMP

J. Marguerat and R. C. de Sousa
Départements de Physiologie et de Médecine, Ecole de Médecine, CH–1211 Genève 4

La^{3+} added to the external surface of frog skin stimulates Na transport by a mechanism that appears to be independent of cAMP. To test further this hypothesis we compared the action of La^{3+} in the presence and in the absence of agents that modify intracellular cAMP. Short circuit current (SCC) was taken as a measure of net Na flux. In 10 paired experiments, La^{3+}-induced increase in SCC was 13.3 ± 1.83 and 13.8 ± 2.38 $\mu A\ cm^{-2}$, respectively before and after stimulation by oxytocin ($p > 0.7$). Similar results were seen with agents that either increase or decrease cAMP, strongly suggesting that the effect of La^{3+} is not mediated by changes in cAMP. We studied next the interaction between La^{3+} and propranolol (PR), an agent capable of displacing membrane Ca^{++}. PR also stimulated SCC by itself and diminished the subsequent effect of La^{3+} ($p < 0.005$). Conversely, previous esposure to La^{3+} diminished the response to PR ($p < 0.001$). We suggest that La^{3+} and PR compete for similar 'calcium sites' at the outer membrane of frog skin and that re moval of calcium from these sites by either agent results in an increased permeability to Na.

Supported by SNSF, grant 3.1300.73

Purified Myelin Fractions: Limitations in the Study of Pathological Material

J.-M. Matthieu
Service de Pédiatrie, Hôpital Cantonal, CH–1011 Lausanne

The method of Norton and Poduslo (J. Neurochem. *21*, 749, 1973) to purify myelin was used to prepare myelin from animals affected by demyelinating diseases or inborn

defects of myelin formation. Myelin isolated in these pathological conditions was analysed using electron microscopy and chemical markers. In hexachlorophene-intoxicated rats, a new fraction showing some of the characteristics of purified myelin was recovered on the top of the density gradient while classic myelin was isolated in reduced amounts at the 0.32–0.85 *M* sucrose interface. In Jimpy mice, a mutation with severe myelin deficit, no myelin could be demonstrated in the fraction obtained by density gradient centrifugation. This contrasted with *in situ* studies showing the presence of myelin in the brain of these animals. These results indicate that abnormal myelin can distribute abnormally on density gradient. Furthermore, the degree of contamination can increase and change the chemical composition. Therefore, conclusions from such results may have limited value and each case should be interpreted with great care.

Intrahypothalamic Nicotine, Induced Eating and Drinking and Plasma Glucose

Gisela Münster and K. Bättig
Institut für Verhaltenswissenschaft, Abt. Verhaltensbiologie, CH–8006 Zürich

It has been shown (Münster and Bättig, Psychopharmacol., in press), that single subcutaneous nicotine injection elevated the thresholds of hypothalamically elicited feeding and drinking in the rat. In the present experiments nicotine, injected into the site of stimulation in doses of 5 and 10 µg, raised the thresholds for eating and drinking and the related plasma glucose levels significantly about 15 min after injection. When contralaterally injected, the high doses of nicotine raised only the eating threshold and the related plasma glucose level. Pretreatment with subcutaneously injected 0.5 mg/kg mecamylamine reduced the nicotine-induced threshold increase and blocked the plasma glucose elevations. It is proposed, that nicotine influences the 'hypothalamic feeding and drinking center' by a direct central action.

Birefringence Measurements during Activity of Isolated Single Muscle Fibres of the Frog

S. M. Baylor and H. Oetliker
Physiologisches Institut der Universität, 3012 Bern and Schule für Biologie-Laboranten Wander, CH–3007 Bern

The authors reported that activation causes a three-component birefringence signal (Nature *253*, 97–101, 1975), whose second component might be related to Ca^{++} release from the SR. To test this idea further the following experiments were done: (1) increasing tonicity in steps up to 2.3 T reduced the second component by a factor of 5–10 and the twitch by a factor of 200–300. (2) stretching (up to 180% slack length) in various tonicities left the second component relatively unchanged, whereas later changes in light intensity were greatly reduced. These findings suggest that the second component arises from a step in EC-coupling preceeding interaction of myofilaments and is not related to the degree of overlap; whereas later changes in light intensity depend on the extent of overlap. The onset of the second component (easily seen in one sweep) is delayed as a linear function of distance from site of stimulation. At distances of over 9 mm the onset is obscured by movement artefacts. Propagation speeds of 2–2.5 m/s at 22° and 1 m/s at 7° were obtained. This method provides a simple way to measure speed of AP-propagation without using microelectrodes.

Supported by the Roche-Research-Foundation and by the US NIH (S.M.B.)

Preoptic Heating and Respiratory Rate during Sleep

P. L. Parmeggiani, C. Franzini and P. Lenzi
Istituto di Fisiologia umana, Università di Bologna, 40127 Bologna, Italia

In freely moving cats preoptic heating (0.75 MHz, 40–140 mW delivered by two electrode pairs; 0.5–2 °C) increases the respiratory rate, which is a non-linear function of preoptic temperature increments (recorded by a Yellow-Springs thermistor) during synchronized sleep (SS) and a linear function of the same increments during desynchronized sleep (DS). During SS respiratory rates fit two intersecting lines whose regression coefficients and related S.E.s are 26 ± 2.4/min °C^{-1} (b_1) below and 158 ± 9.7/min °C^{-1} (b_2) above the respiratory rate 40/min ($b_1 \neq b_2$, $P \ll 0.01$). During DS the regression coefficient (b_3) of respiratory rates is 34 ± 2.9/min °C^{-1} (b_1 and b_3 are not statistically different). The results show that a threshold for thermal polypnoea exists in SS, whereas it is lacking in DS, thus supporting the hypothesis of an alteration in homoiothermic regulation during the latter phase of sleep.

Effect of the Sugars Maltitol and Sorbose on Carbohydrate Metabolism in Man

Ph. Pittet, K. Acheson, K. Raman and E. Jéquier
Division de Physiologie clinique et Institut de Physiologie, Université de Lausanne, CH–1011 Lausanne

The metabolic rate (MR) of 5 subjects was measured continuously for 5.5 h after maltitol or sorbose ingestion (200 kcal). Blood samples were taken every 30 minutes and analyzed for glucose, insulin and FFA. Substrate oxidation rates were calculated from the non-protein RQ. Maltitol and sorbose caused an increase in MR of 6.4% and 6.9% respectively but no significant changes in the pattern of substrates oxidized. 200 kcal glucose caused an increase of 13.6% in MR and 70% in carbohydrate oxidation. Maltitol induced a weak increase in glycemia and insulinemia, and a short-term decrease in FFA. These changes are smaller in absolute values and shorter in duration than those obtained with glucose. Glycemia and insulinemia were not modified after sorbose ingestion. FFA slowly increased throughout the test. Our results suggest that maltitol and sorbose are incompletely absorbed and hence do not have the same metabolic effects as an equivalent dose of glucose.

Relations Between Ca Binding and Cation Movements in Human Red Cell Membranes

H. Porzig
Pharmakologisches Institut der Universität, CH–3010 Bern

Two separable effects of Ca on passive Na and K permeabilities could be demonstrated in human red-cell ghosts. (1) Increasing the Ca ion concentration during hemolysis from 10^{-8} to 10^{-5} *M* increased the yield of

ghosts which seal to Na and K after reversal of hemolysis at 21 °C (half maximal effect with $\sim 10^{-6}$ *M* Ca). (2) By increasing the intracellular ionized Ca in resealed ghosts from 10^{-8} to 10^{-6} *M* the K permeability is reversibly increased (half maximal effect with $\sim 2 \times 10^{-7}$ *M*). The binding of Ca to fragmented red cell membranes was measured using ^{45} Ca in an attempt to identify the permeability-controling binding sites. Using EGTA-buffered Ca solutions two binding sites were detected which had an apparent K_{diss} of $\sim 2 \times 10^{-6}$ and $\sim 2 \times 10^{-4}$ *M* and capacities of 1–2 and 100–200 n*M*/mg protein respectively. Ca binding to the high affinity site was partially abolished by 2 mM Mg, but was considerably enhanced by ATP. This binding site may be related to the Ca effect described above under (1) or to the Ca transport system. The Ca site which is involved in effect (2) probably has to low a capacity to be detected by the present method. No specific function could be assigned to the low affinity site.

Carrier-Mediated Transport of Bile Acids by the Liver

J. Reichen and G. Paumgartner
Department of Clinical Pharmacology, University of Berne, CH–3010 Berne

The hepatic uptake of bile acids (BA), an important aspect of BA metabolism and a major determinant of bile flow, has been shown to be a saturable phenomenon. To gain further evidence for carrier-mediated transport, competitive inhibition between free (C) and taurine-conjugated (TC) cholate was studied. The model of Goresky (Am. J. Physiol. *207*, 13, 1964) was applied to the perfused rat liver. ^{14}C-labeled C and TC (0.025–25 µmol) were injected rapidly into the portal vein. The two BA exhibited saturation kinetics, the apparent K_m being 88 nmol/g liver for TC and 124 for C. The corresponding V_{max} was 33 and 34 nmol/s · g liver, respectively. When C and TC were injected together, the K_m's were markedly and significantly increased, whereas the V_{max} remained unaffected, indicating competitive inhibition. The K_i was 28 nmol/g liver for C and 254 nmol/g liver for TC. Thus, C and TC appear to share a common pathway for the hepatic uptake the affinity to this carrier being greater for the more lipophilic free C than for the polar conjugated TC.

Effects of Testosterone on Basal and LH-RH Induced Secretion of Gonadotropins in the Normal Male Rat

M. Reymond and Th. Lemarchand-Béraud
Division de Biochimie Clinique, Département de Médecine, Hôpital Cantonal, CH–1011 Lausanne

LH and FSH secretions were studied in 50 day old male rats in vitro and in vivo. *In vitro*, after a 3 h incubation of pooled anterior pituitaries (AP), basal secretions of LH and FSH (measured by RI with NIAMD kits) were respectively 2192 ± 145 ng rLH-RPI/mg AP and 1031 ± 97 ng rFSH-RPI/mg AP. LH-RH induced a significant and log-dose related increase of LH and FSH secretion in the medium, whereas no changes in pituitary LH and FSH levels were measured. Testosterone (T), added in increasing concentrations in the medium, did not modify the LH and FSH basal secretions at any dose, but did affect in two ways the pituitary responsiveness to LH-RH: low levels of T (5 and 50 ng/incu) increased the LH and FSH release induced by 50 ng LH-RH ($p < 0.05$); high levels of T (5,000 and 50,000 ng/incu) inhibited the LH response ($p < 0.02$ and $p < 0.005$). *In vivo*, basal plasma LH and FSH were 84 ± 3 ng rLH-RPI/ml and 338 ± 18 ng rFSH-RPI/ml. Testosterone given s.c. as short pretreatment (30 min, 1, 2, 4, 8 h) had no effects on basal LH and FSH secretions, but significantly inhibited the LH and FSH responses to 100 ng LH-RH ($p < 0.005$). Earlier pretreatment with T did not present any effect either on basal gonadotropin secretion or on their response to LH-RH. This lack of T delayed action on gonadotropin secretion is due to the absence of TeBG in rat plasma resulting in a rapid disappearance of T in blood ($T^1/_2 = 7$ min and 32 min) as well as in the AP, hypothalamic area, testis, kidneys and liver. These results provide a confirmation that T can exert feedback actions directly at the pituitary level and may argue for an interaction of T at the pituitary binding site of LH-RH.

Supported by SNSF, grant 3.112.73

Hydrostatic Pressure and Inert Gas Pressure: Effects on Isolated Nervous Tissue

J.-F. Sauter
Institut de physiologie de l'Université, CH–1011 Lausanne

The CNS can be anesthetized by a mixture of N_2O and O_2 (80%: 20%, V/V) at barometric pressure, whereas the autonomic NS is sensitive to it only at higher pressure (more than 2 atm). Therefore the study of the narcotizing action of N_2 and the rare gases under pressure becomes more complex by the presence of two distinct factors: (1) the hydrostatic pressure (HP) and (2) the presence of foreign molecules. A device has been built which allows the electrical activity of the rat's isolated superior cervical ganglion (SCG) to be continuously monitored while applying either a HP (by compressing the incubation liquid) or by gas saturation under pressure. It could be shown that a HP of 9 atm improves the transsynaptic response of the SCG to a supramaximal stimulus at 6 Hz with respect to a control maintained at barometric pressure; on the other hand, helium or nitrogen at the same pressure suppress or even invert this evolution. The conductive properties of the preganglionic fibers remain unchanged in both experimental conditions. The effects described did not appear at 1 Hz.

Supported by SNSF, grant 3.457.70

Inhibition of Sodium and Sodium-Coupled Transport by Harmaline in the Intestine in vitro

F. V. Sepúlveda et J. W. L. Robinson
Département de Chirurgie Expérimentale, Hôpital Cantonal, CH–1011 Lausanne

The influxes of phenylalanine and β-methyl-glucoside across the brush-border membrane of the enterocyte are inhibited by the alkaloid, harmaline, but the Na^+-independent component of entry is unaffected by the drug. Furthermore the influx of sodium, together with its stimulation by sugars and amino-acids, is reduced by harmaline. These findings, together with the ubiquitous nature of harmaline inhibition of Na^+-coupled transport, led us to propose that harmaline interacts with the sodium-binding site of the brush-border membrane (BBA *373*, 527, 1974). The hypothesis is further supported by kinetic studies and experiments on transmural fluxes. The inhibition of the mediated component of sodium influx is

fully competitive, whereas the inhibition of non-electrolyte influx is not. Studies with flux chambers have revealed that harmaline is only effective when added to the mucosal face of the tissue, where it inhibits both sodium and non-electrolyte flux from the mucosa to the serosa, without influencing backflux. These results strongly indicate an action of the drug at the luminal face of the enterocyte.

Neuronal Spikes Interaction in the Medial Geniculate Body

A. Toros and F. de Ribaupierre
Institut de Physiologie de l'Université, CH–1011 Lausanne

Multi-unit activity is recorded in the medial geniculate body (MGB) of a nitrous-oxyde anaesthetized cat. Two spike trains are isolated through a window discriminator (Experientia *30*, 683, 1974) and processed on line by a minicomputer. When both of the simultaneously recorded cells are responding to acoustical stimulation, they exhibit the following properties: (1) Their tonal selectivity is generally in the same frequency range. (2) Their temporal response pattern and binaural properties can be similar or quite diverse. The two simultaneously recorded spike trains are tested for possible interaction by computation of a 'joint peristimulus-time scatter diagram', as proposed by Gerstein and Perkel (Science *164*, 828, 1969). Out of 480 cell airs tested, 70% show no sign of interaction. Only 30% display a possible direct influence of one unit on the other and/or a shared, but not acoustically driven, input. The low percentage of interacting unit pairs would indicate that there are very little feedback influences between closely located cells in the MGB.

Supported by SNSF, grant 3.239.69

Electrical Uncoupling in Mammalian Heart Muscle Induced by Cardiac Glycosides

R. Weingart
Physiologisches Institut der Universität, CH–3012 Bern

Exposure of heart muscle to ouabain results in an increase of $[Na]_i$ and a decrease of $[K]_i$, due to the inhibitory effect of the glycoside on the Na-K sensitive membrane ATP ase. If the Na-Ca exchange transport mechanism through the membrane indeed depends on $[Ca]_i/[Ca]_o = [Na]_i^2/[Na]_o^2$ as suggested by Reuter (Progr. Biophys. *26*, 1, 1973), a drug-induced increase in $[Ca]_i$ would be expected. This change in $[Ca]_i$ might be large enough to influence the coupling resistance between individual cardiac cells (see De Mello, The Physiologist *17*, 1974). To test this hypothesis, r_i/r_o (inside longitudinal resistance to outside longitudinal resistance) was measured in calf and cow ventricular preparations, using the method described by Weidmann (J. Physiol. *210*, 1047, 1970). A progressive increase of r_i/r_o was found on exposure to ouabain. The time taken for this to develop was concentration-dependent, as was the increase in r_i/r_o (ouabain dose: 5×10^{-7} to 2×10^{-6} *M*).

Thermoregulation of Obese Subjects Exposed to Cold (14,5 °C) Measured by Direct and Indirect Calorimetry

P. Weissbrodt and E. Jéquier
Physiologie clinique et Institut de Physiologie, Université de Lausanne, CH–1011 Lausanne

Energy balance of obese (Ob) and control (Co) subjects exposed to a cold environment (14.5 °C, 45% relative humidity) for 90 min was determined in order to investigate the relationship between heat debt, mean skin temperature (T_s), tympanic temperature (T_y) and stimulation of thermogenesis. Evaporative heat losses (E) were similar in the two groups; however radiative and convective heat losses were significantly higher in Co (+ 8.3%) throughout the test. Thermal insulation was greater in Ob than in Co by 19.8%. During the first 50 min metabolic rate (M) was similar in the two groups ($M_{Ob} = 46.6$ W/m², $M_{Co} = 50.3$ W/m²). After 90 min of cold exposure, Co had stimulated their metabolism 35.0% and Ob only 2.25%; this caused a significantly greater heat debt in Ob (− 19.7 W/m²) than in Co (− 35.3 W/m²). T_s was lower in Ob (26.5 °C) than in Co (27.4 °C) whereas T_y was not significantly different. We concluded that Ob tolerate a greater heat debt and a lower T_s than Co without stimulating thermogenesis, whereas a smaller heat debt and a higher T_s induce a thermogenic response by Co.

BIOCHEMIE – BIOCHIMIE – BIOCHEMISTRY

Mechanism of Inhibition of Clq Fixation to Immune Complexes (IC)

R. Allan, H. R. Knobel and H. Isliker
Institut de Biochimie, Université de Lausanne, CH–1011 Lausanne

Among the inhibitors of Clq fixation to IC, compounds with vitamin B_6 activity and collagen were found to be the most effective non-toxic agents. Pyridoxin, pyridoxal and pyridoxamine produced an inhibition of Clq fixation up to concentrations of 1–2 mg/ml, pyridoxal-5'-phosphate (P5P) up to 0.2 mg/ml. Using equilibrium dialysis and difference spectra, both Clq and IgG were shown to bind P5P through the formation of Schiff bases (60–90 P5P/Clq and 6–7 P5P/IgG). Combined with measurements of the Clq binding capacity, these data suggest that the inhibition of Clq fixation to IC is due to the modification of lysyl residues in Clq.

The first 78 N-terminal amino acids of Clq are similar to those present in collagen (K. B. M. Reid, Biochem. J. *141*, 189, 1974) and it was of interest to find bovine collagen to inhibit Clq fixation to IC up to concentrations of 0.2 mg/ml. Furthermore, antibodies to rat and guinea-pig collagen, as well as to a synthetic $(Pro\text{-}Gly\text{-}Pro)_n$ polymer cross-reacted with Clq. Antibodies to guinea-pig skin collagen were found to cross-react with the α_2-chain, but not with the α_1-chain of collagen.

Supported by SNSF, grant 3.061.73

The Mechanism of the Enhancement of Horse Liver Alcohol Dehydrogenase Activity with Thio-NAD as Coenzyme

A. Baici, O. Attanasi, R. Kuhn and P. L. Luisi
Technisch-Chemisches Laboratorium der Eidg. Techn. Hochschule, CH–8006 Zürich

When Thionicotinamide-adenine dinucleotide (thio-NAD) is substituted for NAD in the oxidation of ethanol, the turnover number of horse liver alcohol dehydrogenase (LADH) increases by a factor 4–6, depending on pH. Our studies in order to clarify the mechanism of such an enhancement incuded: (1) steady-state analysis with ethanol and benzyl alcohol as substrates, including product inhibition; (2) stopped-flow studies, also using deuterated alcohols; (3) fluorometric determination of the dissociation constants of the LADH thio-NAD and LADH thio-NADH complexes. Our studies show that: (1) the affinity of thio-NAD and thio-NADH to LADH is lower than that for NAD and NADH; (2) the isotope effect in the steady-state is 3.5–4 with thio-NAD, whereas with NAD only a secondary isotope effect is observed; (3) contrary to the NAD reaction, with thio-NAD the steady-state rate is attained with the stopped-flow without a preliminary rapid 'burst' of reactants. We believe that the differences between thio-NAD and NAD reactions are due to a change in the rate limiting step of the enzyme turnover. This change can be primarily ascribed to the lower affinity of thio-NADH for the enzyme. This decreased affinity is explainable in terms of the recent X-ray structure of the enzyme.

Studies on the Degree of Saturation of Gluconeogenic and Lipogenic Enzymes in vivo

S. Bas, J. Gerlach, P. Favarger and S. Rous
Département de Biochimie médicale, Ecole de médecine, CH–1211 Genève 4

Fed or 24-hours fasted mice received intravenous injection of 1- or 2-^{14}C acetate, 2-^{14}C or 3-^{14}C pyruvate, 1,5-^{14}C, 2,4-^{14}C citrate with or without an overload of unlabeled precursor and were killed at different times. The radioactivity of liver and carcass fatty acids and blood glucose were measured. The pyruvate carboxylase (PCX), phosphoenolpyruvate carboxykinase (PEPCK) and acetyl-CoA carboxylase (ACX) activity was also measured in the same experimental conditions. Results indicated that fasting increases gluconeogenesis from all the precursors except from 6-^{14}C citrate and 1- or 2-^{14}C acetate and increases PCX and PEPCK activities. In the contrary the activity of ACX decreases. The apparent dilution effect on incorporation of labeled precursors appears only when the animals were killed a very short time after the precursor administration and with a high overload. These results suggest that gluconeogenic and lipogenic enzymes were not saturated by their substrates in fed or fasted animals *in vivo*. Moreover the intramitochondrial acetyl CoA pool is in excess in relation to that of oxaloacetate in fasted animals. Our results are not in agreement with the generally admitted theory that ACX plays an important regulatory role in the process of fatty acid synthesis.

Supported by SNSF, grant 3.8970.72

Localization of Teichoic Acid in Bacillus subtilis W 23 Cell Walls

H. Bauer, D. R. Farr, H. Gerber and M. Horisberger
Société d'Assistance Technique pour Produits Nestlé SA, CH–1814 La Tour-de-Peilz

A rabbit antiserum against teichoic acid of *B. subtilis* W 23 was successfully obtained by immunization with cell wall and purified teichoic acid. *B. subtilis* cell walls were isolated and treated with trypsin and DNase as described previously (H. Bauer *et al.*, Arch. Microbiol. *97*, 17, 1974). Colloidal gold granules (average diameter 60 Å) coated with the antiserum were used as a marker for electron microscopy. When cell walls were incubated with the immunocolloid, it was specifically bound to the teichoic acid. No label was found when teichoic acid was added. The localization of teichoic acid at the surface of the cell wall of *B. subtilis* will be discussed in relation with the site of attachment of the bacteriophage SP 50.

Differences in Epoxide Forming and Inactivating Systems as a Possible Reason for Differences in Susceptibility to Carcinogenesis

P. Bentley and F. Oesch
Abt. Biochemische Pharmakologie der Universität, D-65 Mainz, BRD

Carcinogenic and mutagenic aromatic and olefinic compounds are presumed to owe these effects to their metabolic transformation to epoxides. Relative levels of enzymes involved in biosynthesis and further biotransformation of such epoxides may accordingly be of critical importance. Levels of microsomal monooxygenases (responsible for biosynthesis of epoxides) and epoxide hydratases (catalyzing their transformation to dihydrodiols) were therefore determined in rat organs with variable susceptibilities to carcinogenesis by polycyclic hydrocarbons and at different stages of their development. The results support the assumption of a critical role of epoxide hydratase in inactivating metabolically produced ultimate carcinogens.

Supported by SNSF, grant 3.877.72 and NIH, grant CA 12928

Cellular Distribution of Sarcoplasmic Calcium-Binding Proteins by Immunofluorescence

G. Benzonana, W. Wnuk, J. A. Cox and G. Gabbiani
Départements de Biochimie et de Pathologie de l'Université, CH–1211 Genève 4

All the calcium present in crayfish myogen is bound to 4 high affinity binding sites of a calcium-binding protein (CBP) of MW 44,000. CBP appears different from parvalbumin, its counterpart found in vertebrates. Antibodies against carp parvalbumin and crayfish CBP have been obtained and their specificity checked by immunodiffusion. The indirect immunofluorescence technique applied to cryostat sections of striated carp muscle shows parvalbumin diffusely in all the muscle cell cytoplasm; this wide distribution might be explained by the exceptionnally small size of parvalbumin (MW 11,400). In contrast, crayfish CBP is present exclusively along the sarcolemma and, similarly to glycolytic enzymes, on the I band, suggesting a possible functional relationship with the latter enzymes.

Supported by SNSF, grants 3.725.72 and 3.0330.73

Aggregation of Purified Glycophorin, the Major Human Red Cell Membrane Glycoprotein

D. Berthoud and Cl. Bron
Institut de Biochimie, Université de Lausanne, CH–1011 Lausanne

Gel electrophoresis of purified glycophorin in the presence of SDS shows at least three glycoproteins as revealed by Schiff reagent staining with apparent molecular weights of 96,000, 45,000 and 26,000 daltons. After elution and re-electrophoresis, each of the three proteins again yields three bands, which is suggestive of aggregation. The phenomenon is dependent on protein concentration; at low concentration, most of the protein migrates with the 46,000 daltons component, whereas at high concentration the mobility corresponds to 96,000 daltons. When tested with specific antibodies to glycophorin, blood groups A or MN, all types of antigenic activity can be detected in each of the three bands. On the basis of this evidence it appears that glycophorin has a strong tendancy to aggregate and thus can be found in monomeric and polymeric forms. Moreover, the specific anti-glycophorin and anti-blood group A sera react preferentially with the 46,000 daltons component while the anti-blood group MN serum shows a predilection for the large glycophorin aggregates.

Supported by SNSF, grant 3.061.73

Antibodies and Glucosyltransferases

J. J. Burckhardt and B. Guggenheim
Abteilung für Orale Mikrobiologie und Allgemeine Immunologie, Zahnärztliches Institut der Universität Zürich, CH–8028 Zürich

Cariogenic streptococci of the species *mutans* synthesize water-insoluble predominantly $\alpha\,(1 \rightarrow 3)$ linked glucans by means of glucosyltransferases (GTF). These enable the bacterial colonization of tooth surfaces. The enzyme-antibody reaction of soluble and carrier-bound GTF (0.5 *M* eluate from hydroxylapatite), purified from the culture fluid of streptococcus mutans OMZ 176, was studied in serological and functional tests. Antisera reacted in two-dimensional immunoelectrophoresis with slow moving components of high GTF activity and with fast-migrating constituents having little or no enzyme activity. The passive hemagglutination test expressed mainly antibody titers to the latter components. Antibodies to GTF could therefore only be measured in a functional assay. They inhibited the soluble 0.5 *M* GTF to incorporate C^{14}-marked glucose from sucrose into the water-insoluble glucan. The degree of inhibition reached 75%. In contrast, control sera enhanced the same reaction up to 200%. Further studies with naturally cellbound GTF are necessary to predict an in-vivo efficacy of anti-GTF antibodies.

Purification partielle d'une substance 'ACTH like' extraite du placenta humain

N. Capolla et J.-P. Felber
Division de Biochimie Clinique, Département de Médecine, Hôpital Cantonal, CH–1011 Lausanne

Des techniques chromatographiques sur cellulose et Séphadex ont été employées afin d'isoler cette substance. Les poudres de chaque étape de purification ont été testées par essai biologique pour l'ACTH in vivo selon Lipscomb et Nelson (Endocr. *71*, 13, 1962). Les valeurs de la corticostérone obtenues avant toute purification donnent une moyenne de 0,04 µg / 100 ml de plasma par µg de protéines. Après nos essais de purification, cette moyenne est de 1,6 µg / 100 ml de plasma par µg de protéines. Les mêmes poudres ont été testées par essai radioimmunologique et ont donné des courbes de dilution présentant une réaction croisée partielle avec la courbe standard d'ACTH ($ACTH_{1-39}$ humaine synthétique CIBA). Après électrophorèse sur gel de polyacrylamide, l'essai radioimmunologique de chaque bande a montré qu'une seule de celles-ci donne une réaction croisée avec l'ACTH hypophysaire. Ces résultats préliminaires suggèrent que la substance extraite du placenta a une structure moléculaire semblable mais non identique à celle de l'ACTH hypophysaire.

Comparison of Plasma Membranes and Endoplasmic Reticulum Fractions Obtained from Whole White Adipose Tissue and Isolated Adipocytes

M. Chmelar and J.-P. Giacobino
Département de Biochimie médicale, Ecole de Médecine, CH–1211 Genève 4

The effect of the mode of preparation of white adipose tissue plasma membranes (PM) and microsomes upon some of their characteristics has been studied. 1. PM and microsomal fractions prepared from whole adipose tissue were compared with those prepared from isolated fat cells. The collagenase treatment was found to result in a decrease in specific activity of the PM enzymes Mg^{2+}-Na^{+}-K^{+}-ATPase and 5'-nucleotidase. The marker enzymes of endoplasmic reticulum (ER) fragments prepared from isolated fat cells had higher specific activities than those of ER fragments prepared from whole adipose tissue. This was due to the fact that the purification of whole adipose tissue but not of isolated fat cell crude microsomes by hypotonic treatment caused extensive solubilization of the ER marker enzymes, NADH oxidase and NADPH cytochrome c reductase. 2) PM fractions prepared from mitochondrial pellet were shown to have higher specific activities of Mg^{2+}-Na^{+}-K^{+}-ATPase than the PM originating in crude microsomes. The use of NADH oxidase and NADPH cytochrome c reductase as ER marker enzymes is discussed. It is apparent that in future adipose tissue membrane investigation, careful consideration should be given to the mode of preparation, the choice depending upon whether the study's purpose is to get membranes with high enzyme activities or with a high degree of purity.

Supported by SNSF, grants 3.8970.72 and 3.140.73

Specific Irreversible Inhibition of Enzymes Concomitant to Oxidation of Carbanionic Enzyme-Substrate Intermediates

Ph. Christen, Marianne Cogoli and M.-J. Healy
Biochemisches Institut der Universität, CH–8028 Zürich

The oxidation of carbanionic ES intermediates by extrinsic oxidants (Biochemistry *12*, 35, 1973) generates transiently reactive products capable of covalent modification of groups at the active site. Thus, Fru-1,6-P_2 aldolase (ALD) from rabbit muscle is inactivated concomitantly with the oxidation of its ES carbanion by $Fe(CN)_6^{3-}$. The inactivation is not due to the reaction

products hydroxypyruvaldehyde phosphate or $Fe(CN)_6^{4-}$ and is not reversed by extensive dialysis. Analogous inactivation is found with ES intermediates of ALD from yeast and of transaldolase and transketolase. The rate of inactivation obeys saturation kinetics with respect to substrate concentration and is independent of enzyme concentration suggesting that the inactivating agent is not released from the enzyme. Thus, addition of Fru-1,6-P_2 to a mixture of ALD and transaldolase with $Fe(CN)_6^{3-}$ initiates the exclusive inactivation of ALD, while Fru-6-P induces the exclusive inactivation of transaldolase. Hence, the combination of normal substrate with an electron acceptor constitutes a highly specific binary system for active-site-directed chemical modification of these enzymes.

Supported by SNSF, grant 3.1570.73

Solubilization and Characterization of Mouse P-815 Mastocytoma Membrane Antigens

K. J. Clemetson, M. Bertschmann and E. F. Lüscher
Theodor-Kocher-Institut, CH–3000 Bern 9

The P-815 mastocytoma can induce transferable cellular immunity in its syngeneic host (DBA/2 mouse) but so far humoral immunity has not been detected. By allogeneic or xenogeneic immunization followed by absorption of antisera in vivo in DBA/2, antisera have been obtained and shown to be specific for P-815, presumably directed against tumour specific/associated antigen(s) (TS antigens). Starting from a crude membrane preparation of P-815 we have established optimal conditions for the solubilization of H-2 and TS antigens using sodium deoxycholate (DOC) or DOC followed by papain treatment. This gives rise to two antigen types, one intact (DOC) and the other with a lipophilic region missing, which is more amenable to biochemical study. Using affinity-chromatography on *Lens culinaris* lectin bound to Sepharose 4B we have shown that whereas 90–95% of the H-2 antigens bind to the lectin and can be eluted subsequently, only 40–50% of the TS antigen binds. In a single step a very large degree of purification of both antigens is obtained.

Experimental Infection of New-Born Baby Rats with Enteropathogenic Strains of Escherichia coli

M. Darekar and D. Rivier
Institut de Biochimie, Université de Lausanne, CH–1011 Lausanne

Simulation of human gastroenteritis in laboratory animals under natural conditions has been a very difficult problem. The present evidence that a strain of E. coli is enteropathogenic is only based on its isolation from an epidemic. The suitability of new-born rats as a laboratory model producing experimental gastroenteritis was tested by challenging them orally with two enteropathogenic strains of E. coli isolated from an epidemic of gastroenteritis in children. The pathogenic strain produced an acute and progressive disease, with a generalized infection; whereas the saprophyte isolated from a healthy animal was only confined to colon. There were no deaths in controls given nutrient-broth. The above model also offers the possibility of testing colostrum from immunized animals and antibacterial drugs in vivo.

Supported by Nestlé-Alimentana S.A.

Inhibition of Horse Liver Alcoholdehydrogenase by 1,10-Phenanthroline and Hexafluoroisopropanol

A. Dubied und J.-P. von Wartburg
Medizinisch-chemisches Institut der Universität, CH–3000 Bern 9

The inhibition of horse liver alcohol dehydrogenase (isoenzyme AA) by 1,10-phenanthroline (OP) and hexafluoroisopropanol (FIP) was studied. With OP as inhibitor non-competitive inhibitions are found with constant NAD and variable ethanol (EtOH) concentrations and vice versa. In both cases the secondary plots reveal an intercept linear, slope parabolic pattern. EtOH concentrations leading to substrate inhibition abolish inhibition by OP. With FIP as inhibitor nonlinear primary plots best described by 2/1-functions (WRATTEN and CLELAND, Biochemistry *4*, 2442, 1965) are found. This partial inhibition shows that FIP does not form dead-end complexes and would indicate the functioning of a slower alternative pathway by formation of nonabortive E-NAD-EtOH-I complexes. Since FIP precludes the binding of EtOH and only one OP is known to bind per subunit, quaternary complexes or $E\text{-}OP_2$ complexes cannot be formed at the active site of a single subunit. These results are consistent with a half-of-the-sites reactivity of the dimeric enzyme, one subunit binding NAD and EtOH, the other binding the inhibitor.

Supported by SNSF, grant 3.8340.72 and USPHS, RO 1 AA00233-05

Norepinephrine-Metal Complexation and Binding to Membranes

J. Durand, J.-P. Giacobino, J. Deshusses and L. Girardier
Département de Physiologie, Ecole de Médecine, CH–1211 Genève 4

DL-Norepinephrine (NE $5 \times 10^{-7} M$) binding to brown adipose tissue (BAT) membranes was measured by Millipore filtration after 10 min incubation in the indicated buffer. It was observed however that even in the absence of biological material the concentration of NE as estimated by alumina assay decreased during the short incubation period in Krebs-Ringer bicarbonate buffer (KRBB). Oxidation could be ruled out by polarographic measurements. Since it is known that orthodiphenols complex readily with transition metals, NE-metal complexation was considered. The KRBB prepared from analytical grade chemicals contains contaminating metals in sufficient amounts to account for the disappearance of NE. In PO_4 buffer $5 \times 10^{-2} M$ an almost complete recovery of unmodified NE was observed. Addition of metals (Zn^{2+}, Cu^{2+}, Fe^{3+}, 10^{-6} M) to this buffer increased the amount of NE-bound to BAT membranes showing that NE-metal complexes bind more efficiently than free NE. Addition of glycine 10^{-3} M prevented the effect of added metals. Dopa, isoproterenol, ascorbic acid and dithiothreitol, which are competing with NE for the metals, prevented the disappearance of NE in the incubation medium. Addition of the same substances inhibited the binding not only in KRBB but also in PO_4-glycine buffer where no complexation of NE with metals occurs.

Supported by SNSF, grant 3.721.72

Isolation and Purification of two Extracellular Endo- and Exo-ß (1→3) Glucanases from the Myxomycete Physarum polycephalum

D. R. Farr, A. Schuler and M. Horisberger
Société d'Assistance Technique pour Produits Nestlé S.A., CH-1814 La Tour-de-Peilz

The plasmodia of the acellular slime mold *Physarum polycephalum* produce two extracellular glucanases, an endo-$\beta(1 \rightarrow 3)$ glucanase (MW 18,000) and an exo-$\beta(1 \rightarrow 3)$ glucanase (MW 14,000). The enzymes appear in the growth fluid after 6 days and reached a maximum after 12 days. These enzymes have been isolated and purified from the growth fluid. The glucanases had a pH optimum of 5.0, and the temperature optima were 40° for the endo-glucanase and 48° for the exo-glucanase. Yeast protoplasts were prepared using a mixture of endo- and exo-glucanases.

The Effect of Diphosphonates on Lysosomal Hydrolases in vitro

R. Felix, R. G. G. Russell and H. Fleisch
Pathophysiologisches Institut der Universität, CH-3012 Bern

Diphosphonates characterised by P-C-P bonds are known to inhibit bone resorption. This may be due to inhibition of dissolution of apatite crystals. However, since lysosomal hydrolases seem to be involved in bone resorption the possible direct effects of diphosphonates on these enzymes have been studied. Dichloromethylene diphosphonate (Cl_2MDP) was found to inhibit, in decreasing order, the following enzymes: acid phosphatase, acid pyrophosphatase, arylsulfatase A, deoxyribonuclease II and phosphoproteinphosphatase of rat liver lysosomes, but had no effect on β-glucuronidase, esterase and cathepsin D. This inhibition was competitive for acid phosphatase and arylsulfatase A. In contrast ethane-1-hydroxy-1,1-diphosphonate (EHDP) had little or no effect. Of several diphosphonates tested, only Cl_2MDP and undec-10-ene-1-hydroxy-1,1-diphosphonate (UHDP) inhibited acid phosphatase strongly. This correlates with the effect of these two compounds on bone resorption in vivo. Thus Cl_2MDP and UHDP may act in bone by inhibiting lysosomal hydrolases, in addition to any effect on apatite dissolution.

Identification of a New Onco-Foetal Antigen Extracted from Human Colon Carcinoma

R. Fritsché et J.-P. Mach
Institut de biochimie, Université de Lausanne, CH-1011 Lausanne

Rabbits were immunized with different fractions of saline extracts of human colon carcinomas obtained by gel filtration and ion-exchange chromatography. Three of the antisera, after absorption with normal plasma, red cells stroma and extracts of normal organs (intestin, liver, spleen, lung) gave a precipitin line of identity with extracts of several types of carcinoma (colon, lung, breast) and with extracts of different organs (colon, liver, lung, kidney) of 3–4 months old human foetuses. By the criterion of double diffusion the antigen does not appear to be present in any of the extracts of the normal adult tissues tested (colon, lung, breast, liver, spleen, kidney). The antigen was purified on an immunoabsorbant prepared by coupling IgG from specific rabbit antisera to Sepharose 4B. The antigen was eluted from Sephadex G-200 between the IgG and albumin peak, suggesting a molecular weight of about 100,000 daltons and had a β-mobility in standard immunoelectrophoresis. This antigen is immunologically distinct from previously identified human oncofoetal antigens: carcinoembryonic antigen, alpha-foetoprotein and ferritin.

Supported by SNSF, grant 3.061.73

Transport Studies in Yeast Plasma Membrane Vesicles

G.-F. Fuhrmann
Pharmakologisches Institut der Universität, CH-3010 Bern

Yeast plasma membrane vesicles can be isolated by mechanical disruption of the yeast cell wall (G. F. Fuhrmann, E. Wehrli and C. Boehm, BBA, *363*, 295, 1974). For the present transport study the previous method was modified in order to increase the yield of plasma membrane vesicles. The yeast cells were disrupted by a Cell Homogeniser MSK and subjected to differential centrifugation. Plasma membrane vesicles were separated from mitochondria by aggregation of the mitochondria at pH 4. As judged by biochemical and electronmicroscopical criteria a pure plasma membrane vesicle fraction was obtained. When the K^+ efflux from these vesicles was measured at zero K^+ outside and 0 °C an initial rapid loss of K^+ was followed by a slow efflux component. The slow component probably represents K^+ efflux from sealed vesicles. At pH 4, where the yield of tight vesicles was maximal, sealed vesicles accounted for about $^1/_3$ of the total vesicle volume. Divalent cations like Mg^{++}, Ca^{++} or UO_2^{++} added to the outside had no effect on K^+ permeability. As in intact yeast cells glucose permeability of the vesicles was inhibited by UO_2^{++}-ions and countertransport of sugars could be demonstrated.

Supported by SNSF, grants 3.770.72 and 3.3250.74

Syncatalytic Sulfhydryl Group Modification in Mitochondrial Aspartate Aminotransferase from Chicken and Pig Heart

H. Gehring and Ph. Christen
Biochemisches Institut der Universität, CH-8028 Zürich

One sulfhydryl group of the mitochondrial isoenzyme of aspartate aminotransferase from both chicken and pig heart exhibits syncatalytic reactivity changes similar to those found previously in the cytosolic isoenzyme from pig heart (J. Biol. Chem. *248*, 1751, 1973). The reactivity of the only titratable thiol group toward 5,5'-dithiobis (2-nitrobenzoate) is at a minimum ($k' = 20\ M^{-1}\ min^{-1}$) in the free pyridoxal and pyridoxamine enzyme and in the adsorption complexes; it is increased markedly when covalent enzyme-substrate intermediates are formed ($k' = 260\ M^{-1}\ min^{-1}$). Under all conditions 1 equivalent of 5-thio (2-nitrobenzoate) per subunit is incorporated. The enzymatic activity is not affected by the modification of the sulfhydryl group. This finding affirms and generalizes the earlier conclusion (cf. cit. above) that the syncatalytic reactivity changes are not due to a direct participation of this group in the active site but rather to conformational adaptations of the enzyme-coenzyme substrate compound occurring in the catalytic mechanism of aspartate aminotransferase.

Supported by SNSF, grant 3.1570.73

Mutagenicity of Benzo(a)pyrene: Metabolic Activation, Inactivation and Reactivation

H. R. Glatt and F. Oesch
Abt. Biochemische Pharmakologie der Universität, D–6500 Mainz, BRD

Benzo(a)pyrene is metabolized to species which are active in mutagenesis and in malignant transformation of cells. Synthetic derivatives of benzo(a)pyrene, representing known and potential metabolites, were therefore tested with *Salmonella typhimurium* strains designed for detection of different types of mutagens. From the compounds tested only the epoxide derivative, benzo(a)pyrene-4,5-oxide, was mutagenically active (as a frameshift mutagen) while the parent hydrocarbon as well as phenols, dihydrodiols and quinones were inactive. When the synthetic or enzymatically prepared mutagenically inactive metabolites (some of them derived from epoxides) were incubated with liver preparations and a NADPH generating system some of them were reactivated to mutagenic species. Metabolic activation of the parent hydrocarbon and of inactive metabolites was dramatically potentiated by epoxide hydratase inhibitors. It is concluded that benzo(a)pyrene is activated to epoxide(s) which are transformed to inactive metabolites, some of which can be reactivated by epoxidation at another site of the molecule.

Supported by SNSF, grant 3.877.72 and NIH, grant CA 12928. The authors thank Prof. B. Ames (Berkeley) for Salmonella mutants.

Preparation and Characterisation of Rabbit Antiserum Specific for Human T Lymphocytes

Nicole Gross and Cl. Bron
Institut de Biochimie, Université de Lausanne, CH–1011 Lausanne

Rabbit antiserum to a crude membrane extract of human thymocytes, after absorption with human erythrocytes, plasma and LIK B lymphoblasts, reacts with peripheral blood leukocytes (PBL), thymocytes and MOLT-4 cells (a non B cell line) when tested by indirect immunofluorescence. Pretreatment of PBL with this anti-T serum inhibitis the formation of spontaneous sheep red blood cell rosettes. An antiglycophorin serum, raised against the major red-cell membrane glycoprotein and reacting with PBL, induces no change in the percentage of rosette-forming cells. After anti-T serum and complement mediated lysis of PBL, 80–100% of the residual cells are membrane immunoglobulin positive as shown by direct immunofluorescence. A moderate increase in immunoglobulin-positive cells is caused by the same treatment with anti-glycophorin serum. These preliminary data suggest that the reagent is specific for human T lymphocytes and as such can be useful for the identification of T cells and isolation of specific antigens.

Supported by SNSF, grant 3.061.73

The Presence of Glycerokinase in Adipose Tissue and its Possible Role

S. Grote and P. Favarger
Département Biochimie médicale, Ecole de Médecine, CH–1211 Genève 4

Epididymal fat pads of fasted or fed male rats were incubated in vivo according to the method of Stein and Stein in 5 ml of a Krebs-Ringer phosphate-albumine buffer containing either 10 μCi of 1-^{14}C glycerol or U-^{14}C glucose (50 μmoles). The incorporation of both 1-^{14}C glycerol and U-^{14}C glucose into the glycerol and the fatty acids of adipose tissue triglycerides in the same animal was compared. The rate of penetration of both precursors into the tissues was measured. A significant incorporation of 1-^{14}C glycerol into triglyceride glycerol was observed but the value was only about 20% of that obtained when the precursor was U-^{14}C glucose. Fasting inhibited the incorporation of U-^{14}C glucose into glyceridic glycerol but had no effect on that of 1-^{14}C glycerol. Fatty acid synthesis was inhibited by both precursors. The fact that the intracellular pool of free glycerol in adipose tissue of fasted rats is higher than that in the controls should be considered in evaluating these results.

Supported by SNSF, grant 3.8970.72

Influence of Metabolic Inhibitors on the Rate of DTNB Reaction with Sulfhydryl Groups of Rat Liver Mitochondria

R. Guidoux and N. Haugaard
Institut de pharmacologie de l'Université, CH–1011 Lausanne

During incubation at 25 °C, mitochondrial –SH groups are continuously oxidized by the disulfide dithionitrobenzoic acid (DTNB) added to the mitochondrial suspension. The rate of the oxidation, measured as DTNB reduction, is influenced by the metabolic state of the mitochondria: it is inhibited by uncoupling agents as well as by antimycin A, with either glutamate or succinate. Rotenone does not depress the rate of the reaction. The inhibition seems to be linked to ATP-depletion by substances that activate mitochondrial ATPase reaction. In uncoupled mitochondria, ATP reactivates the rate of oxidation of mitochondrial –SH groups, in a reaction that is coupled to reduction of both DTNB bound to mitochondria as mixed disulfide and DTNB itself. The mitochondrial generation of some of the –SH groups that react with disulfides seems to require energy, presumably for the energydependent transhydrogenation of pyridine nucleotides that is prerequisite for reduction of mitochondrial glutathione.

Supported by the Swiss Academy of Medical Sciences and the Janggen-Pöhn Foundation

Immunochemistry by Scanning Electron Microscopy

M. Horisberger, J. Rosset and H. Bauer
Société d'Assistance Technique pour Produits Nestlé S.A., CH–1814 La Tour-de-Peilz

A general and rapid method has been developed which permits the visualization of cell surface receptor sites in the SEM. Colloidal gold of suitable diameter was prepared according to G. Frens (Nature *241*, 20, 1973). The gold granules were then coated with antibodies or Concanavalin A and stabilized against flocculation with polyethylene glycol. Colloidal gold (average diameter 500 Å) coated with specific anti-mannan antibody or with a complete antiserum was successfully used for marking *Candida utilis* cells. The localization of mannan at the surface of yeast cells of different species was also visualized using colloidal gold coated with Concanavalin A. As gold granules of different sizes can be prepared, the technique is applicable for double labelling.

Effect of Prostaglandin E_1 (PGE_1) on Gluconeogenesis and Esterification in Perfused Liver of Fasted Rats

Elisabeth Imesch and Simonne Rous
Département de Biochimie médicale, Ecole de Médecine, CH–1211 Genève 4

Using perfused livers of rats fasted for 48 hours, glucose production and incorporation of 2-^{14}C pyruvate (tracer dose) into perfusate glucose were studied. Both were found to be inhibited by PGE_1 (infused at a concentration of 0.5 µg/min) by about 60%. The incorporation of 1-^{14}C glycerol into perfusate glucose and into glycerol-glyceride part of liver glycerides were also studied, using the same test conditions. The former incorporation was significantly inhibited (56%) and the latter strongly stimulated (360%) by PGE_1 but the weight of the liver glycerides was unmodified. PGE_1 had no effect on glucose production in a perfusate overloaded with sodium pyruvate, nor on pyruvate carboxylase and phosphoenolpyruvate carboxykinase activity. This was in contrast with the results obtained in perfusions with a tracer dose of 2-^{14}C pyruvate. The results showed that PGE_1, at the concentration used, stimulated the incorporation of 1-^{14}C glycerol into glycerol-glyceride part of liver glycerides, and, when there was no overload of pyruvate present in the perfusion medium, inhibited gluconeogenesis at some point.

Supported by SNSF, grant 3.140.73

Kinetics of Oxidation of Deoxyhemoglobin A

E. E. Di Iorio and K. H. Winterhalter
Friedrich-Miescher-Institut, CH–4002 Basel

Autoxidation of oxyhemoglobin A has demonstrated the nonequivalence of α and β chains. With the present work we show that also in the deoxy-conformation the two chains behave differently. We therefore have studied the oxidation kinetics of deoxyhemoglobin by ferricyanide at pH 6 to 9 at different protein concentrations. At pH 9 and very low hemoglobin concentration (1.7×10^{-5} *M*) the reaction of oxidation is autocatalytic. By increasing the protein concentration to 10^{-4} *M* the progress curve becomes biphasic and the addition of 2,3-DPG causes the loss of biphasicity leaving a slight heterogeneity. If the protein concentration is increased to 2.5×10^{-3} *M* in the absence of 2,3-DPG, the curve is slightly heterogeneous and almost identical to the one obtained in the presence of 2,3-DPG at 10^{-4} *M* Hb concentration. We conclude that upon appearance of methemoglobin dimers are formed differing from the tetramers in their kinetic behaviour towards the oxidant. Their proportion is big enough to be detected only at low protein concentration and/or absence of 2,3-DPG. At high concentration their contribution is too small and only the difference between the chains is visible, producing a heterogeneous progress curve. Rapid chain separation after partial oxidation under identical conditions confirmed this concept.

Carbohydrate Analyses of Human IgA Myeloma Proteins Precipitating with Phaseolus vulgaris (PHA) Lectin

H. Jaquet, W. Wojnarowski, G. A. Spengler and G. I. Pardoe
Institut de Biochimie de l'Université, CH–1011 Lausanne; Institut für klin. Eiweissforschung, Tiefenauspital, CH–3000 Bern; WHO. IRTC, CH–1011 Lausanne

Lectins binding specific glycosyl groups can precipitate glycoproteins and indicate the presence of individual sugars in oligosaccharide components. The precipitation of carbohydrate-rich proteins was investigated in a series of human IgA myeloma sera using *Phaseolus vulgaris* (PHA) lectin. This lectin binds β-D-galactosyl groups in an oligosaccharide whose other components include N-acetyl-D-glucosamine and D-mannose. All myeloma proteins of the IgA2 subclass were precipitated by the lectin, whereas the majority of the IgA1 did not react. Among a few selected precipitating IgA1 proteins, only the polymeric, not the monomeric proteins precipitated. The presence of N-acetyl-D-galactosamine in the IgA1 proteins (absent from IgA2) suggested a second type of oligosaccharide. Fucose was present only in the IgA2 (precipitating) proteins; more neuraminyl residues were found in the fucose-free nonprecipitating IgA1 proteins. Lectins specific for individual sugars are potentially useful for clinical diagnostic screening.

Supported by SNSF, grant 3.061.73 and 3.1130.73

Kinetics of the Appearance of Estrogen Receptors in Chicken Liver

U. Joss
Friedrich-Miescher-Institut, CH–4002 Basel

Estradiol causes the appearance of estradiol receptors in the liver nucleus of immature chicken. Previous investigations in this system have involved subcutanous injection of high, unphysiological hormone doses not suitable for kinetic studies in the whole animal. We have established a methodology for i.v. administration of defined hormone pulses at concentrations close to the physiological range. This leads to the appearance of receptor within one minute and at levels comparable to those previously found. The characteristics of the receptor obtained with the two methods do not differ significantly. Cycloheximide inhibits more than 95 % of the aminoacid incorporation in the liver within less than 10 min after injection. The appearance of receptor, however, is only slightly inhibited 10 min after cycloheximide injection; only at longer times of exposure to cycloheximide, the appearance of receptor is reduced.

Insulin Removal by Perfused Livers of Lean and *ob/ob* Mice

C. Karakash and B. Jeanrenaud
Laboratoires de Recherches Médicales, CH–1211 Genève 4

Removal of insulin after a single passage through perfused livers of normal and obese-hyperglycemic (*ob/ob*) mice has been studied. Removal was measured at the steady state by the difference in immunoreactive insulin present in the perfusate entering and leaving the liver. In livers of both normal and *ob/ob* mice insulin removal decreased with rising concentrations of added hormone. At all concentrations tested, however, insulin removal was lesser in livers of *ob/ob* than in those of normal mice. When hyperinsulinemia of *ob/ob* mice was decreased by streptozotocin treatment and by fasting, or was neutralized by anti-insulin serum injection, removal of insulin in subsequently perfused livers was restored towards normal values. It is concluded that there is a defect in the hepatic insulin removal process of *ob/ob* mice that appears to be related to hyperinsulinemia as it is reversed by decreasing circulating insulin levels. This defect may contribute to the prevailing hyperinsulinemia of the *ob/ob* mice.

Supported by SNSF

Affinity Labeling of the Primary Bilirubin-Binding Site of Human Serum Albumin

C. C. Kuenzle, N. Gitzelmann-Cumarasamy and K. J. Wilson
Institut für Pharmakologie und Biochemie, Veterinärmedizinische Fakultät der Universität Zürich, CH–8057 Zürich

An affinity label for the high-affinity bilirubin-binding site of human serum albumin was prepared by reacting bilirubin with Woodward's reagent K. This converted both carboxyl groups of bilirubin into reactive enol esters. Coupling of this bilirubin derivative to albumin was achieved under nitrogen at 22° and pH 9.4 using imidazole as a catalyst. A yellow monomer of albumin was isolated by chromatography on Sephadex G-200. Dialysis in 8 *M* urea and 200 m*M* mercaptoethanol at pH 9.1 demonstrated that the yellow pigment was covalently attached to the protein. The specificity of the labeling reaction was strongly suggested by measurements of circular dichroism, fluorescence spectroscopy and competitive inhibition using regular bilirubin as well as by experiments showing that seven randomly chosen non-albumin proteins failed to give labeled monomeric products under identical reaction conditions. Preliminary evidence from degradative studies indicates that the high-affinity bilirubin binding site occupies a position in the carboxyl terminal two thirds of the albumin chain.

Variations of Monoamine Levels in 18 Rat Brain Regions in Relation to the Oestrus Cycle

W. Küng, E. Chappuis-Arndt and A. Wirz-Justice
Psychiatrische Universitätsklinik, CH–4025 Basel

From pharmacological investigations there is evidence that monoamines (MA) play an important role in the control mechanisms for gonadotrophic hormone release. We determined biochemically the concentrations of serotonin (5 HT), noradrenaline (NA) and dopamine (DA) in 18 brain regions at 10 a.m. and 3 p.m. of prooestrus (i.e. before and during the critical period) and of oestrus, and at 10 a.m. of metoestrus. The 5 HT concentration showed marked variations in several midbrain and diencephalic MA-containing cell groups, in the medio-basal hypothalamic regions, in the lateral preoptic area, in the septum and in the central area of the amygdala. In these regions, but not in cortex, hippocampus or in the nigro-striatal system, 5 HT falls in prooestrus from high values at 10 a.m. to low values at 3 p.m. The opposite occurs in oestrus (indicating that this is not a circadian variation). NA and DA showed only slight changes predominantly in hypothalamic regions, NA tending to higher values at 3 p.m. than at 10 a.m. in both prooestrus and oestrus. The results are interpreted as demonstrating removal of 5 HT inhibition during the critical period facilitating ovulation.

Properties and Partial Amino Acid Sequence of a Heme Protein from the Liver Parasite Dicrocoelium dendriticum

P. A. Kunz, P. E. Tuchschmid and K. J. Wilson
Biochemisches Institut und Kinderspital der Universität, CH–8032 Zürich

A heme-containing protein was isolated from *Dicrocoelium dendriticum* and was found to be a single peptide chain of a molecular weight of approximately 15,500 containing ferroprotoporphyrine IX which is not covalently bound. The monomeric heme protein reacts, as do other monomeric myoglobins and hemoglobins, to changes of ligands and to redox reactions. It has a very high affinity for oxygen. Isoelectric focusing separates two forms, having isoelectric points of 4.50 and 4.53, which do not differ in their amino-acid composition. The presently available partial amino-acid sequence of this protein suggests only limited homology with the sequences of other so-called primitive hemoglobins and myoglobins. In common with all other hemoglobins and myoglobins the 'invariant' residues Phe (CD1) and His (F8) (proximal His) are present. The distal His (E7) of the hemebinding region is absent as well as Tyr (H22) in the COOH-terminal region which is found in vertebrate hemo- and myoglobins only.

Influence of Ligands on the Oxidation of Hemoglobin

A. Mansouri
Friedrich-Miescher-Institut, CH–4002 Basel

Autoxidation of oxy-Hb A at pH 7.2 followed by rapid chain separation revealed that the oxidation rate of α chains is about 10 times higher than the one of β chains. This difference disappears at pH 9. It is present in case of isolated chains, but less pronounced. Analogous results are obtained by chemical oxidation ($K_3[Fe(CN)_6]$). Lowering the (O_2) pressure increased the rate of autoxidation, more so for β than α chains. 2, 3-DPG increases the rate of autoxidation for both chains, more for α than β. The situation is different when deoxy-Hb is oxidized by $K_3[Fe(CN)_6]$ under equilibrium condition. In this case the β chains are oxidized slightly faster than the α chains, regardless of pH, ionic strength or the presence of organic phosphates. This difference is not due to charge redistribution as is shown by timed mixing experiments. It is concluded that in the presence of oxygen the factors influencing the oxidation rate are mainly the chain structure and the affinity for the ligand, whereas in the absence of ligand the massive conformational change plays the most important role.

Dissimilar Effects of Biotin-Deficiency on Pyruvate Carboxylase and Acetyl CoA Carboxylase

Maria-Luisa Mayobre, P. Favarger and Simonne Rous
Département de Biochimie médicale, Ecole de Médecine, CH–1211 Genève 4

Biotin-deficient and control mice were injected intravenously with 3-^{14}C pyruvate and killed 5 min later. The radioactivity of carcass and adipose tissue fatty acids as well as that of glucose per ml of blood were mesaured. The total radioactivity of carcass fatty acids was found to be 822,000 dpm for control mice and 275,000 dpm for biotin-deficient mice. The glucose radioactivity was twice as high in the control mice as in the biotin-deficient mice. Measurements were also made of pyruvate carboxylase (PCX) and acetyl CoA carboxylase (ACX) activity with the following results: PCX activity in biotin-deficient mice decreased from 37.7 mμM to 11 mμM of $^{14}CO_3H^-$ fixed min^{-1} mg^{-1} of protein. ACX activity measured in presence of citrate was not affected by biotin-deficiency: 2 mμM of $^{14}CO_3H^-$ fixed min^{-1} mg^{-1} of protein compared with 1.8 mμM for controls. In the absence of citrate, the ACX activity was lower in biotin-deficient mice than in controls: 0.53 mμM compared with 1.1 mμM

of $^{14}CO_3H^-$ fixed min^{-1} mg^{-1} of protein. Biotin-deficiency was found to have a more pronounced inhibitory effect on fatty acid synthesis in adipose tissue than in the liver, and to inhibit PCX activity in the liver more than that of ACX. Citrate seemed capable of replacing biotin as a carrier of CO_2 to acetyl CoA.

Supperted by SNSF, grant 3.8970.72

Receptor-Binding Studies with Insulin and Nsila-S Using the Perfused Rat Heart

C. Meuli and E. R. Froesch
Med. Klinik, Stoffwechsellabor Kantonsspital, CH–8006 Zürich

NSILA-S (*n*onsuppressible *i*nsulin-*l*ike *a*ctivity) is a polypeptide extracted from human serum. It shares many metabolic effects with insulin but it has a different structure. Its activity is determined with the net gas exchange of the rat epididymal fat pad in comparison with insulin. (MW 7,500, purest preparation 375 mU/mg). On the isolated perfused rat heart insulin and NSILA-S are equally active on a molar basis with regard to glucose uptake. The dose-response curves of both peptides with respects to glucose uptake and lactate production are very similar. Both, insulin and NSILA-S accelerate the transport of 3-O-methyl glucose through the plasma membrane which can be considered as the classical and most important effect of insulin on its target tissues. Binding studies with I-125 labeled insulin and NSILA-S are indicative for two different binding sites for the two substances. For the insulin-binding site high affinity, specificity and saturability have been found. The saturability lies in the same range as the maximal stimulation of glucose uptake. Whereas NSILA-S shows some affinity for the insulin receptor, the reverse was not observed. It can be concluded that although insulin and NSILA-S show the same metabolic effects on the rat heart the two substances bind to different receptors.

Studies on the Differentiation of Red and White Skeletal Muscle in the Developing Rabbit

G. Müller, M. Ermini and E. Jenny
Institut für Pharmakologie und Biochemie der vet. med. Fakultät, CH–8057 Zürich

Biochemical studies revealed that 5 days before birth both myosin and myofibrillar ATPase specific activities are similarly low in red (M. soleus) and white (M. gastrocnemius) muscle. At birth white muscle exhibits a 50%, and 5–6 days later a more than 100% higher myosin ATPase activity than red muscle. Differentiation of the myofibrillar ATPase is retarded with respect to myosin, showing a clear distinction between red and white muscle 7–10 days, and full activity in both muscles 20–25 days after birth only. Histochemically both muscle types can be distinguished not earlier than 7 days after birth, using the reaction for succinate dehydrogenase, and 12–15 days after birth with the reaction for ATPase.

Molecular Parameters of Human Erythrocyte Acetylcholinesterase

P. Ott and U. Brodbeck
Medizinisch-chemisches Institut der Universität, CH–3000 Bern 9

Human erythrocyte acetylcholinesterase was solubilized by Triton X-100 and purified by affinity chromatography to a minimal specific activity of 3,800 IU/mg of protein. Polyacrylamide gradient gel electrophoresis of the detergent depleted enzyme showed seven corresponding bands of protein and enzyme activity. Carbohydrate stain showed six bands. SDS polyacrylamide gel electrophoresis gave one subunit only with corresponding stains for protein and carbohydrate. Gel filtration through Sepharose 4B could not be performed as the enzyme was adsorbed nearly quantitatively onto the column. However, when the column was preequilibrated with α-methyl-D-mannoside the enzyme eluted as broad peak in yields greater than 85%. Sucrose density gradient centrifugation carried out in presence of mannoside revealed six peaks of enzyme activity with *s* values of 7.3; 10.9; 12.9; 14.8; 16.8 and 18.1. Gel filtration of these individual enzyme forms gave stokes radii of 6.8, 10.1, 10.4, 10.7, 11.1 and 12.9 nm respectively. These data suggest, that the human enzyme exists in an even more complex state of aggregation than the eel enzyme.

Immunochemistry of Leishmania enriettii Surface Antigen

G. I. Pardoe, R. Hahn, H. Jaquet and W. Wojnarowski
WHO Immunology Research and Training Centre and Institut de Biochemie de l'Université, CH–1011 Lausanne

L. enriettii, a natural protozoal parasite of guinea-pigs is agglutinated by immune guinea-pig serum and can be cultured in vitro. It is Z-N-negative, Gram-negative, Giemsa-positive. After saline washing, it is Gram-positive, Giemsa- and Z-N-negative. Proteases disrupt the surface structure. It contains D-galactose, D-mannose, D-glucose, ribose, deoxy-ribose, N-acetyl-D-glucosamine and an unknown amino sugar. Storage carbohydrate, fucose, sialic acids, N-acetyl-D-galactosamine are absent. It is agglutinated by Con-A and mannose is the main sugar. An antigen, isolated by hypotonic saline extraction and differential centrifugation, migrated as a single PAS-positive band in PAGE/SDS and contained D-mannose, D-galactose, D-glucose and N-acetyl-D-glucosamine. It inhibited agglutination of both parasites and VCN-human Gr. O Rh^+ (T-positive) red cells by immune sera, indicating β-D-galactosyl immunodominance. An enhanced anti-T serum titre may be diagnostically significant in corresponding human infections.

Changes in Cyclic AMP-Dependent Beef Brain Protein Kinase with Age

K. Reichlmeier and P. Iwangoff
Sandoz Ltd., CH–4002 Basel

Basal and cAMP-stimulated (cAMP = 5×10^{-6} *M*) brain protein kinase (PK) activities were investigated according to Kuo and Greengard (J. Biol. Chem. *245*, 4067, 1970) in homogenate, synaptosomes and nuclei of 30 beef of three age groups (group A: 0.3 years; B: 1–1.5 years; C: 8 ± 1 years). Subcellular fractions were prepared by sucrose density gradient centrifugation. The results demonstrate an age-dependent decrease of the cAMP-stimulated PK values in comparison to basal activities which were set 100%, as there was no significant change with age. The cAMP-stimulated PK decline was found in all fractions studied. In synaptosomes a significant decrease (group A to C: $p < 0.01$) of the PK activity was observed as follows: group A to B: −12%, group A to C: −19%. Similar values were demonstrated in the nuclei fraction: A to B: −13%, A to C: −17%. In the homogenate a less pronounced effect was found (A to B:

−3.5%, A to C: −10.5%). Our results support existing hypotheses (Comfort, A. Mech. Age Dev. *3*, 1–31, 1974) postulating an increasing error rate for protein and enzyme synthesis with age.

Effects and Binding of Insulin-Dextran-Leucine Complex on Fat Cells

Y. Sakamoto and Y. Akanuma
Third Department of Medicine, Tokyo, and Laboratoires de Recherches médicales, CH–1211 Genève 4

The metabolic effect and the binding of insulin-dextran-leucine complex have been studied in rat isolated fat cells. The advantages of the use of such complex is that it does not penetrate the cell membrane and can be labelled with ^{14}C-leucine. Dextran (T70) was activated with cyanogen-bromide at pH 8.5–10.5 and was complexed to pork insulin and ^{14}C-leucine. The complex was purified by gel filtration on Sephadex G-75 column. It was found that 5–8 μg of insulin were bound to 1 mg of dextran. The labelled insulin complex had definite biological activity when measuring the oxidation of ^{14}C-gluccse to $^{14}CO_2$. The biological activity was, on a molar basis of insulin present in the complex, about 50% that of native insulin. Furthermore, the labelled insulin complex was found to bind to isolated fat cells in a way that was similar to that of labelled insulin. The labelled complex bound to found cells could be completely displaced by addition of an excess of unlabelled native insulin. The labelled insulin complex thus represent a interesting tool for the study of insulin-membrane interaction, and for its relationship with the biological effects of the hormone.

Supported by SNSF.

Importance of Intramuscular Lipid Concentration in Control of Glucose Oxidation by Rat Diaphragm in vitro

Ch. Schindler and J.-P. Felber
Division de Biochimie Clinique, Département de Médecine, Hôpital Cantonal, CH–1011 Lausanne

Immediately after weaning, groups of male Wistar rats were fed ad libitum during 4 weeks a high-fat carbohydrate-poor diet, or a control carbohydrate-rich diet. The glyceride content of diaphragm of fat-fed rats was 2–3 fold increased compared to control tissues. The oxidation in vitro of labelled glucose was reduced by the fat diet to 50% of the control value. An incubation with 4 m*M* 2-bromostearate, an inhibitor of fatty acid oxidation, leads up to a complete restoration of glucose oxidation in diaphragm of fat-fed rats. In an other experiment, we proceeded to a change of diet. Two groups of rats were fed either the high-fat diet or the high-carbohydrate diet during 28 days and were used as controls. A third and a fourth group received the carbohydrate diet during respectively 26 and 22 days and then the fat diet during respectively 2 and 6 days. All the rats were killed at the same time, and blood and diaphragm removed. After 2 days on high-fat diet, plasma FFA level and muscular glyceride concentration were already similar to those found in control fat-fed rats. Moreover 2 days on high-fat diet leads up to an impairement of in vitro glucose oxidation similar to that found in fat-fed controls. In conclusion, we propose that the increase of muscular glyceride concentration (following the elevation of plasma FFA) and a greater availability for oxidation of fatty acid proceeding from glyceride stored, are the most important factors in the control of glucose utilization by muscle in vitro and perhaps in vivo in certain cases of obesity and diabetes.

Supported by SNSF, grant No. 3.111.73

On the Mode of Action of Ethylenethiourea

J. P. Seiler
Swiss Federal Research Station, CH–8820 Wädenswil

Ethylenethiourea (ETU) is a decomposition product and a metabolite of the fungicidal ethylene-bis-dithiocarbamates. It has been described as a carcinogenic, teratogenic and mutagenic agent. In the *Salmonella typhimurium* mutagenicity test system the compound acts as a mutagen by causing base substitions. Due to the chemical and biological stability of ETU the direct alkylation of DNA bases as the mutagenic event can be excluded. Only an inhibitory or antimetabolite action on DNA biosynthesis can therefore explain the observed mutagenicity of ETU. Preliminary experiments with *Escherichia coli* demonstrated that ETU induces a condition of unbalanced growth, analogous to the action of hydroxyurea. This mode of action would explain the induction of the reported primary lesions in the rat, where low concentrations of ETU cause thyroid hyperplasia. Incorporation studies with various precursors of DNA, RNA and protein as well as kinetic studies demonstrated then that ETU inhibits preferentially DNA synthesis at concentrations which leave RNA and protein synthesis virtually unaffected.

Supported by SNSF, grant 3.7040.72

Energy Dissipation by Proton Recycling and the Efficiency of Oxidative Phosphorylation in Rat Liver Mitochondria

J. W. Stucki
Pharmakologisches Institut der Universität, CH–3010 Bern

Rat liver mitochondria were incubated in the presence of pyruvate-2-^{14}C, HCO_3, ATP and P_i. Different phosphate potentials in the medium were maintained at a steady state by addition of creatinekinase plus creatine as a phosphoroyl acceptor. Metabolic fluxes through individual reactions of mitochondrial pyruvate metabolism were calculated from the specific radioactivities of the formed products. The energy balance revealed that by adding increasing concentrations of creatine up to 20 m*M* the amount of ATP utilized for pyruvate carboxylation plus creatine phosphorylation rose but showed a local minimum at 13 m*M* creatine. When ATP utilization was maximal the overall P/O ratio was also maximal, whereas the energy dissipation was minimal. A positive correlation was observed between the amount of energy dissipated and the magnitude of the pH gradient across the inner membrane. From these results it is concluded that the recycling of protons across the inner membrane is an important energy dissipating process in mitochondria. Moreover, the efficiency of oxidative phosphorylation appears to be maximal when this dissipative process is minimal.

Supported by SNSF, grant 3.051.73

Ligand Binding to Aspartate Transcarbamylase and the Effect of the Donnan Equilibrium

P. Suter and J. P. Rosenbusch
Biozentrum der Universität, CH–4056 Basel

Ligand binding to catalytic and regulatory sites in the hexameric enzyme aspartate transcarbamylase from *E. coli* is characterized by positive and negative interactions. Previous studies indicated partial saturation with the substrate carbamyl phosphate in the absence of succinate, an unreactive aspartate analogue. In its presence, all 6 sites are available. Binding of succinate, in turn, exhibits positive cooperativity in the presence of carbamyl phosphate, but only 4 sites were detected. A binding study in 40 m*M* imidazole-acetate (pH = 7.0) at 22°, using a filter assay, has now yielded the following results. (1) The association of carbamyl phosphate with 6 sites of the enzyme in the presence of succinate shows positive cooperativity (Hill coefficient 1.7). (2) Succinate also exhibits partial saturation of the enzyme (2–3 sites) when carbamyl phosphate is absent. In its presence, the binding curve extrapolates to 6 sites. (3) Equilibrium dialysis experiments using ^{137}Cs as an indicator for the distribution of electrolytes show that the earlier finding of 4 sites was due to the Donnan effect.

Supported by SNSF, grant 3.0920.73

Properties of Cyclic AMP-Dependant Protein Kinase in Calf Ovaries

K. Talmadge, E. Bechtel, P. Huber, A. Salokangas and U. Eppenberger
Hormonlabor, Universitäts-Frauenklinik, CH–4000 Basel

A cyclic AMP-dependant protein kinase was purified from calf ovaries by ammonium sulfate fractionation and chromatography on DEAE-cellulose, Sephadex G-200 and hydroxylapatite columns. The holoenzyme has molecular weight of 230,000 estimated by Sephadex gelfiltration. Maximal rates or phosphorylation were observed between pH 6.5 and pH 8.0 with protamine sulfate as substrate. The apparent K_m value for protamine sulfate was 55 µg per ml and this value was affected little by cyclic AMP. The apparent K_m for ATP in the presence and absence of cyclic AMP were $5.5 \times 10^{-6} M$ and $6.2 \times 10^{-6} M$, respectively. Half maximal stimulation by cyclic AMP was observed at $3.0 \times 10^{-8} M$. Using other cyclic nucleotides at least 100-fold higher concentrations were required to obtain maximal activation of the protein kinase. The holoenzyme is converted on sucrose gradients to a single peak (catalytic unit) of a molecular weight of 40,000. The cyclic AMP-binding proteins had molecular weights of approximately 150,000 and 75,000 estimated by gel filtration.

Circular Dichroism (CD) of Enzyme-Coenzyme Complexes of Yeast Alcohol Dehydrogenase (YADH) below 300 nm

M. Vašák and J. H. R. Kägi
Biochemisches Institut der Universität, CH–8028 Zürich

We have shown previously that binding of NADH to YADH induces CD at the 340 nm transition of the coenzyme ($\Delta[\Theta] = -26{,}000°$). Measurements below 300 nm have now revealed that NADH or NAD^+ also generates a diffence CD-band at 280 nm ($\Delta[\Theta] = +4000°$). The same feature occurs in complexes with ADP-ribose but not with ADP or AMP suggesting its origin in an interaction of the second ribose moiety with an aromatic residue of YADH. Substantial CD-changes also occur at the 260 nm coenzyme transitions. Thus, binding of AMP, ADP or ADP ribose to YADH causes a 300% increase ($\Delta[\Theta] = -12{,}000°$) and binding of NAD^+ and NADH a 25% ($\Delta[\Theta] = +1{,}000°$) or 50% ($\Delta[\Theta] = +5{,}000°$) decrease of the amplitude of their ellipticity bands. The effect of the adenine-containing coenzyme fragments could indicate fixation of the N-ribosyl torsion angle in anti-conformation. The results with NAD^+ and NADH are dominated by contributions from changes of interbase coupling of electronic transitions and suggest that the coenzymes unfold on binding to YADH.

Supported by SNSF, grant 3.1070.73

Plasma Free Tryptophan, MAO Activity and Noradrenaline Uptake in Brain after TRH

A. Wirz-Justice and M. Lichtsteiner
Psychiatrische Universitätsklinik, CH–4025 Basel

Thyrotropin Releasing Hormone (TRH) may act as a short-term antidepressant by increasing noradrenaline turnover. Further possible mechanisms of TRH action were investigated in three groups of rats, male and ovariectomised female at 16 h, prooestrus female at 9 h, injected i.p. with TRH (0.4, 0.8 mg/kg) or saline. 1 h after TRH, plasma free and total tryptophan were unchanged in the first two groups. After stereotaxic microdissection of frozen brain, MAO activity was found to be unchanged in the cortex, lateral and medial preoptic region, lateral, dorso-medial and anterior hypothalamus, or pituitary. However in the ventromedial hypothalamus (including median eminence) TRH increased MAO activity in prooestrus females at 10 h (0.8 mg/kg TRH: 220 ± 45 nmoles isoquinoline formed from kynuramine/mg protein per hour; controls: 183 ± 50; N = 6) but decreased MAO activity in males at 17 h (0.4 mg/kg TRH: 196 ± 28; controls: 228 ± 31; N = 11). In males, in vitro ^{14}C-noradrenaline uptake in cortex, medulla, and anterior hypothalamus tended to increase with TRH, attained significance only in the ventro-medial hypothalamus (including median eminence) at $2 \times 10^{-4} M$ TRH (142 ± 59% control uptake; c.f. maprotiline (Ludiomil) 32 ± 7% control uptake; N = 10).

Cooperative Binding of Calcium by a Protein from Crayfish Muscle

W. Wnuk, L. Kohler, J. A. Cox and G. Benzonana
Département de Biochimie de l'Université, CH–1211 Genève 8

The crustacean myoplasmic calcium-binding protein (CBP) displays distinct physiochemical properties from its counterpart found in vertebrate myogen, parvalbumin. Stabilization of crayfish CBP by Mg^{++} is required for the complete and reversible removal of calcium. The binding of calcium to the apoprotein was measured at pH 7.4 (25 m*M* Tricine buffer, 80 m*M* KCl, 10 m*M* $MgCl_2$) by: 1. $^{45}Ca^{++}$-Chelex partition, 2. rate of dialysis, and 3. equilibrium dialysis. CBP has 4 high affinity sites ($K_d = 0.5 - 1.3 \times 10^{-6} M$) and 3–4 sites with lower affinity ($K_d = 0.4 - 3 \times 10^{-5} M$). The high affinity binding data yield a parabola-like Scatchard plot and a Hill plot with slope of 3.3 – 2.7 in the linear range. Thus the binding of calcium

is positively cooperative. In contrast, carp parvalbumin component 3 does not exhibit detectable cooperativity between its two high-affinity sites ($K = 1.8 - 3 \times 10^{-6} M$) as measured in the same conditions. Crayfish CBP is the first muscular protein for which pronounced positive cooperativity has been demonstrated.

Supported by SNSF, grant No. 3.725.72

Properties of Soluble High-affinity Ca^2S-ATPase from Human Erythrocyte Membranes

H. U. Wolf and W. Knipser
Institut für Biochemie der Universität, D–65 Mainz, BRD

Soluble high-affinity Ca^{2+}-ATPase (Adenosinetriphosphatephosphohydrolase, EC 3.6.1.3) is stable (half-life time ≈ 400 h) in a medium containing 100–200 m*M* K^+, 1 m*M* Mg^{2+}, 0.5 m*M* Ca^{2+}, 5 m*M* red. glutathione and 200 µg/ml of lecithine or sphingomyeline isolated from erythrocyte membranes. The yield of activity after solubilization under these conditions is 90% of the starting activity. The properties of the solubilized enzyme are not changed as compared to the membrane-bound enzyme: the Ca^{2+}-dissociation constant, the K_m-value and the shape of the v-pH-curve are nearly identical for both enzymes. Attemps for purification on a Sepharose 6B column yielded specific activities of 0.5–1 U/mg protein. Most of the activity appeared after an elution volume corresponding to MW ≈ 1×10^6. Gelelectrophoresis in 0.01% Triton X-100 yielded one slowly moving (v = 1 cm/6 h at pH 8.5) mayor band and one minor band after staining with Comassie Brillant blue. No PAS staining band could be detected.

High Resolution NMR Studies of the Molecular Dynamics of Proteins in Solution

K. Wüthrich und G. Wagner
Institut für Molekularbiologie und Biophysik der ETH, CH–8049 Zürich

In this communication we would like to illustrate with some experiments with the basic pancreatic trypsin inhibitor (BPTI) how the description of the three-dimensional molecular structure of proteins obtained from single crystal studies can be complemented by NMR measurements in solution. In the investigation of BPTI, which is a 'miniprotein' with molecular weight 6,000, ^{1}H-NMR gave direct evidence that the molecular conformations in single crystals and in aqueous solution are very similar. In D_2O solution, studies of the amide proton exchange with D of the solvent yields information on the kinetic stability of the hydrogen bonds of the α-helical and the β-sheet regions in BPTI, which revealed quite different dynamic properties for different segments of the polypeptide backbone in this molecule. Other experiments showed that the aromatic rings of Phe and Tyr in native BPTI rotate about the C^β–C^γ bond at a rate which is rapid on the NMR time scale. For two of the Phe residues, the free energy of activation for the rotational motion was found to be 14 and 17 kcal Mol^{-1}, respectively, at 27°. For all the four Tyr residues in BPTI, $\Delta G^\neq$ is smaller than 14 kcal Mol^{-1}.

Supported by SNSF, grant 3.1510.73

Presence of Intermediate Catalase Species in Heterozygotes for Swiss Type Acatalasemia; Evidence for Molecular Hybridization?

Sonja R. Wyss, B. Scherz and H. Aebi
Medizinisch-chemisches Institut der Universität CH–3000 Bern 9

Erythrocyte catalase of individuals heterozygous for an unstable enzyme mutant (Swiss-Type Acatalasemia) exerts properties that differ from those of the normal enzyme in respect to heat stability and electrophoretic mobility. There is no strict gene dosage inasmuch as heterozygotes exhibit more than 50% of normal activity (65–85%). Therefore, a clear-cut distinction between normals and heterozygotes is not possible at this level. The fact that the normal enzyme species is missing in the heterozygotes provides evidence that the classical scheme (i.e. presence of normal and mutant enzyme) is not applicable. Since catalase can be reversibly split into 2 or 4 identical subunits (dimer or monomer), the question arises how to explain this atypical behavior. The findings are compatible with the assumption that in heterozygotes normal and mutant monomer subunits are produced simultaneously and combined at random. Such hybrid molecules obviously exert properties intermediate to those of normal catalase or the unstable enzyme variant. This interpretation might also explain inter-individual variation of electrophoretic mobility and residual activity among heterozygotes of the same sibship. By means of model experiments (in vitro recombination of dimer subunits) the possibility of molecular hybridization is under investigation.

PHARMAKOLOGIE – PHARMACOLOGIE – PHARMACOLOGY

Blockade of the Presynaptic Alpha Receptor in Rat Cortex by Antidepressants

P. A. Baumann and L. Maître
Ciba-Geigy AG, CH–4000 Basle

Blockade of the presynaptic alpha receptor leads to an increase of ^{3}H-noradrenaline (NA) release by field stimulation from previously labelled cortical slices. Drugs which inhibit NA uptake in nerve endings (antidepressants, cocaine) also increase ^{3}H-NA release. This is due to the inhibition of the re-uptake of NA which is discharged in the synaptic cleft by nerve stimulation. It will be shown that the increase of ^{3}H-NA release by alphablockers can be distinguished from that caused by NA uptake inhibitors. After complete inhibition of NA re-uptake by a pure uptake inhibitor (for example, cocaine) antidepressants with only a NA uptake inhibiting action (such as desmethylimipramine and maprotiline) remain inactive in regard to release of ^{3}H-NA, while alpha-blockers retain completely their releasing action. We observed that imipramine, nortriptyline, amitriptyline and mianserin include a presynaptic alpha-receptor blocking component which increases in the above-mentioned order. With mianserin this component attains potency which is typical for known alpha-blockers such as phentolamine and phenoxybenzamine.

Binding of Lipophilic Drugs to Liver Microsomal Membranes as Differentiated from Specific Binding to Cytochrome P-450

M. H. Bickel, P. L. Gigon, C. di Francesco and R. Stegmann
Medizinisch-chemisches Institut der Universität, CH–3012 Bern

Many lipophilic drugs are taken up into hepatocytes and bound to their endoplasmic reticulum; they are also bound to the microsomal cytochrome P-450 and are substrates of P-450-dependent monooxygenases. Binding to P-450 (type I vis. difference spectrum) of a non-substrate, imipramine-N-oxide, could also be detected. On the other hand, equilibrium dialysis, sedimentation and UV-difference spectrophotometric experiments with substrates of P-450 disclosed total microsomal membrane binding of much higher binding capacities than estimated for P-450 (e.g., for imipramine 370 vs. < 2.5 nmol/mg microsomal protein). Furthermore, the following observations are pertinent. Comparable binding parameters were obtained with liver microsomes and with membranes containing less or no P-450 such as extrahepatic microsomes, mitochondrial or red-cell membranes, liposomes from the lipids of these membranes or from lecithin. With these binders identical ligand perturbation UV-spectra were obtained with phenothiazines, whereby the K_S-values represent total membrane binding. It is suggested that binding of lipophilic drugs to lipid constituents of membranes by hydrophobic interactions are quantitatively more important than binding to P-450.

Comparison of an in vivo and an in vitro Test for Evaluation of the Inhibitory Action of Drugs on the 5-Hydroxytryptamine Uptake in Rat Brain Neurons

W. P. Burkard
F. Hoffmann-La Roche & Co., CH–4002 Basel

Para-chloromethamphetamine (PCMA) 10 mg/kg i.p. reduces 5-hydroxytryptamine (5-HT) level to 56% after after 3 hours. Drugs which antagonize this PCMA-induced depletion are assumed to act by interfering with the common transfer mechanism for PCMA and 5-HT into 5-HT neurons and thus might give an estimate of the 5-HT reuptake inhibition in vivo. The uptake of ^{3}H-5-HT in vitro into a crude synaptosomal preparation from rat forebrain was measured both in the absence and in the presence of drugs. A significant positive correlation ($r = 0.88$; $p < 0.001$) was found between the potencies of 17 tricyclic antidepressants as antagonists of PCMA-induced depletion of brain 5-HT and as inhibitors of the synaptosomal uptake of 5-HT. The most potent drug was chlorimipramine, followed by imipramine and amitryptiline; iprindole, maprotiline, debenzepine being among the weakest compounds. A similar correlation existed between several drugs of different chemical structure, e.g. quipazine, tofenazine or tetrahydronaphtylamine. However, orphenadrine and amantadine markedly reduced the PCMA-induced depletion of 5-HT, but were weak inhibitors of its synaptosomal uptake in vitro, while cocaine was less active in vivo. Three antihistamines acted differently, tripelenamine and chlorpheniramine being active on both tests, while diphenhydramine was only active in the in-vivo test. The results suggest that both tests are indicative of an inhibitory effect of a drug on 5-HT uptake in brain neurons.

Plasma Glucose After Subcutaneous Injection of Nicotine in Rats

W. Classen, G. Münster and K. Bättig
Swiss Federal Institute of Technology, Dept. of Behavioral Sciences, CH–8006 Zurich

(a) After the sc injection of 0.2 mg/kg and 0.4 mg/kg nicotine in (Wistar-derived) female Roman High-Avoidance (RHA) and female Roman Low-Avoidance (RLA) rats, the RLA rats reacted with a significant rise in plasma glucose 5 and 15 min after the injection of the higher dose. The RHA rats showed no significant effect. (b) Chronic injections of 0.4 mg/kg nicotine, twice a day for three weeks, revealed a tolerance to the plasma glucose elevation in the RLA rats between the seventh and tenth day, which was still detectable after a 14-day no-treatment period. (c) In the RLA rats, pretreatment with a subcutaneous injection of 0.5 mg/kg mecamylamine or 3 and 9 mg/kg hexamethonium, 10 min before the injection of 0.4 mg/kg nicotine, completely abolished the plasma glucose elevation.

Effect of Carbamazepine (TEGRETOL®) on the Monosynaptic Reflex and Post-Tetanic Potentiation

H. Demiéville
Ciba-Geigy AG, CH–4002 Basle

The monosynaptic reflex (MR) and post-tetanic potentiation (PTP) were recorded in spinalized cats (C1) placed under artificial respiration. The lumbar spinal cord was exposed. A dorsal root and its corresponding ventral root were placed on stimulating and recording platinum electrodes respectively. Electric stimuli were monitored through a logic system allowing an automatic repetitive run of the stimulating sequences. The MR and PTP were recorded on a strip-chart recorder through a peak-detector. The MR were triggered every 10 sec and the PTP every 4 or 6 min. The time course of the response of the MR and the PTP to i.v. Carbamazepine (0.1 to 10.0 mg/kg) was multiphasic: first, both the monosynaptic (mass) potential and the degree of post-tetanic potentiation decreased; after 20 to 40 min. both indicators rose to above control level (up to 500% with the larger doses) and after 80 to 120 min returned to control. This response-pattern of PTP to Carbamazepine differs from the one produced by Diphenylhydantoin which only suppresses PTP. Evidently an exclusive inhibitory influence on PTP is not necessarily a prerequisite for anticonvulsant action.

Urinary Substances Protecting Juvenile Mice from Attack

A. K. Dixon
Wander AG (Sandoz Research Unit), CH–3001 Berne

Previous results have shown that adult female mice possess an aggression-inhibiting factor in their urine which prevents them from being attacked by adult males. Observations show that immature male and female mice, but not adult males, are also immune from attack. The mechanism affording this protection was investigated by analysing the behavioural responses of territory holding males towards male intruders which had been marked with urine from different donor mice. Urine from immature females, but not from immature males, was found to inhibit aggression. However urine from immature males did not share the aggression releasing properties normally

attributed to adult male urine. These findings indicate that the protection enjoyed by immature female mice is active, and like that of adult females is due to an aggression-inhibiting factor of urinary origin. In contrast, the protection associated with immature male mice is passive and probably involves other modalities besides olfaction.

Cholinesterases in Brain and Haemolymph of Ants

H. R. Frischknecht, W. H. Hopff und P. G. Waser
Pharmakologisches Institut der Universität, CH–8006 Zürich

Our aim was to detect the nature and quantitative composition of cholinesterases in brain tissue and haemolymph of *Formica pratensis* Retz. Enzyme activity was measured by Ellman's method (Biochem. Pharmacol. *7*, 88, 1961) with acetylthiocholine and and butyrylthiocholine respectively. For each test for acetylcholinesterase (ACHE) activity 1 brain (0.3 mg) proved to be sufficient. To estimate cholinesterase (CHE) activity, 10 brains per test had to be used. The brains were dissected stereomicroscopically and homogenized in a Kontes AA homogenizer. The haemolymph was cannulated by a capillary needle from the joints of the forelegs. The activity for ACHE in ant brains ($6.2 \times 10^{-2} \mu M$ units), was 2–4 timer higher than that found in the case of bees (Casida, Biochem. J. *60*, 487, 1955) and cockroaches (Smallman and Fisher, Canad. J. Biochem. *36*, 575, 1958). CHE activity was estimated to be $0.7 \times 10^{-2} \mu M$ units. The activity for ACHE in haemolymphe was estimated to be $5.5 \times 10^{-4} \mu M$ units, and for CHE $4 \times 10^{-5} \mu M$ units.

Supported by SNSF, grant 3.2110.73

Response of Cultured Cerebellar Purkinje Cells to GABA and GABA-Antagonists

B. Gähwiler and H. Stähelin
Sandoz AG, CH–4002 Basel

Cultures of newborn rat cerebellum were grown on glass coverslips in a plasma clot. Electrophysiological experiments were carried out after 2 to 3 weeks in vitro. Addition of GABA to the bathing medium inhibits the spontaneous electric activity of Purkinje cells. Low concentrations of GABA ($< 10^{-6}$ *M*) decrease the average spike frequency. The pattern of spontaneous activity changes from a Poisson-like distribution to a more regular firing pattern, thereby increasing the relative number of long intervals between consecutive action potentials. Higher concentrations of GABA (10^{-6} to 10^{-5} *M*) abolish the spontaneous activity. This effect is fast in onset and reversible. Concomitant addition of bicuculline or picrotoxin antagonizes the inhibition induced by GABA, while strychnine is ineffective in blocking this inhibition. Bicuculline ($10^{-11} - 10^{-5} M$) and picrotoxin ($10^{-8} - 10^{-4} M$) exert an excitatory effect on Purkinje cells. This suggests the existence of functional synapses in cultures of rat cerebellum in which endogenous GABA is used as transmitter. Both bicuculline and picrotoxin decrease the average firing rate of Purkinje cells when used at higher concentration.

Evaluation of Liver Function in Man by Breath Analysis

I. Gikalov, A. Küpfer and R. Preisig
Department of Clinical Pharmacology, University of Berne, CH–3012 Berne

Although in man partial hepatic functions may be assessed quantitatively, such tests are relatively cumbersome and annoying to the patient. To develop a simple procedure, breath analysis of $^{14}CO_2$ following p.o. application of 9 mg/kg ^{14}C-dimethylaminopyrine (DMA, 2 μCi) was performed in 17 healthy volunteers, in 13 patients with cryptogenic or alcoholic, and in 5 with primary biliary cirrhosis. Expired air was collected at regular intervals up to 12 hours. In the volunteers, peak activity (700 ± S.E. 140 dpm) was reached within 1 hour following DMA; the disappearance constant (K_B) of breath activity was 0.21 ± S.E. 0.04. In patients with cryptogenic or alcoholic cirrhosis, a lower peak activity (245 ± S.E. 100 dpm) was associated with a reduced K_B of 0.08 ± S.E. 0.04. As expected, patients with primary biliary cirrhosis exhibited practically normal metabolic function (K_B 0.18 ± S.E. 0.10). Since in all subjects K_B was significantly ($p < 0.001$) correlated with bromsulfalein disappearance rate and galactose elimination capacity, it is suggested that breath analysis of demethylation may be a useful, non-invasive liver function test and may contribute to a better understanding of drug metabolism in man.

Effects of Colchicine in Acute Inflammation

M. Glatt, P. Graf and K. Brune
Biozentrum der Universität, CH–4056 Basel

It is still unknown how colchicine exerts its anti-inflammatory action, e.g. in gouty arthritis. We have investigated into this problem using the urate arthritis elicited in chicken as a model (Agents and Actions *4*, 21–33, 1974). It could be shown that colchicine reduces the symptoms of inflammation in doses between 0.3 and 3.0 mg/kg. These doses inhibited histamine release, leukocyte immigration and the increase of vascular permeability otherwise seen in inflamed joints. However, colchicine led to increased concentrations of inflammatory prostaglandins (PG E_2 and $F_{2\alpha}$) in the fluid of inflamed joints. These results are completely different from those obtained with indomethacine or salicylates. With these nonsteroid anti-inflammatory drugs it is possible to inhibit prostaglandin release almost completely and to reduce histamine release and the increase in vascular permeability slightly whilst the invasion of leukocytes remains practically unchanged. From these results it is concluded that colchicine exerts its antiinflammatory action not through inhibition of prostaglandin synthesis. Instead in inflammation it appears to directly block the vascular and leukocytic response which is not influenced significantly by other nonsteroid anti-inflammatory drugs.

Effect of Intravenous Diazepam on Renal Function

J. P. Guignard, B. Filloux, J. Lavoie and A. Torrado
Division de Néphrologie, Service de Pédiatrie, Hôpital Cantonal Universitaire, CH–1011 Lausanne

Intravenous diazepam is often administered to patients undergoing accurate renal clinical tests, when bladder catheterization and repeat venous punctures are required.

The effect of iv diazepam on renal function was therefore investigated in children during renal clearance studies. Inulin and PAH clearances were measured by the constant infusion technique in 6 children weighing 25–42 kg. Urine was collected from a bladder catheter. Injection of 4 mg diazepam was followed by a fall in glomerular filtration rate (from 96 to 80 ml/min per 1.73 m^2) and effective renal plasma flow (from 461 to 341). This effect was significant only in the 15-min clearance period following drug administration. It was not accompanied by any change in systemic blood pressure, as measured by a sphygmomanometer technique. The effect of iv diazepam was also studied in 6 anesthetized rabbits undergoing isotonic saline diuresis. Urine was collected from two ureteral catheters. Administration of the drug (2 and 3 mg/kg) induced an immediate but very transient drop in blood pressure, as measured by a pressure transducer. A significant fall in GFR ($p < 0.02$) and urine flow rate ($p < 0.001$) was also noted in the 10-min clearance period following diazepam administration, without change in urine osmolality. It is concluded that iv diazepam depresses renal function and urine flow rate, independently from changes in ADH activity.

Supported by SNSF, grant 3.749.72

The Effects of Sodium Nitroprusside (SNP) on Vascular Smooth Muscle

G. Haeusler
F. Hoffmann-La Roche & Co., Ltd., CH–4002 Basel

In strips of rabbit main pulmonary artery (RMPA), the contractile responses to KCl and to noradrenaline were progressively inhibited by SNP in concentrations between 10^{-5}–10^{-3} *M*. SNP depressed the maximum of the dose-response curves for both KCl and noradrenaline. A competitive antagonism between SNP and Ca^{++} was found in strips of depolarized RMPA with a pA_{10} value of approximately 4. SNP caused an increase in membrane potential of the vascular smooth muscle cells of RMPA as measured with intracellular glass microelectrodes. This hyperpolarization was concentration-dependent in the range of 10^{-8} to 10^{-6} *M* and was prevented by ouabain or by exposure of the vascular smooth muscle to K^+-free solution. The depolarization produced by KCl or noradrenaline in the vascular smooth muscle cells remained virtually unaltered by SNP. It is concluded that a decrease in Ca^{++} permeability of the surface membrane of the vascular smooth muscle cells is the most likely explanation for the vasodilator action of SNP and that SNP-induced hyperpolarization is of minor importance for the relaxant effect.

Rise and Fall of the cAMP-Content in Human Blood Platelets after Stimulation of Their Adenyl-Cyclase with Prostaglandine E-1

U. Honegger, R. Trummer and P. Bally
Pharmakologisches Institut, Universität Bern, CH–3010 Bern

Prostaglandine E-1 (PGE^1, 5×10^{-7} *M*) rises cAMP-content, cAMP, of human blood platelets (in platelet-rich plasma) to a maximum after about 30 seconds. The decline of cAMP which then follows appears to be due to increased breakdown of cAMP by phosphodiesterase (PDE) rather than to inhibition of adenyl-cyclase, since the initial rate of cAMP-formation is the same whether PGE1 is added initially or whether an inhibitor of PDE (papaverine, saturat. concentration) is added subsequently (Abstract, Adv. cyclic nucl. Research, Vol. 5). Inverse relationship between cAMP and ATP was observed following the addition of papaverine plus PGE1 and appears to hold after PGE1-addition alone. This relationship was maintained only as long as the total ATP-pool was not severely depleted. It suggests a mechanism for the observed rise and fall of cAMP following the stimulation of ad. cyclase by PGE1 involving intracellular binding of cAMP. Calcium-depletion of the platelets prior to the addition of PGE1 leads to a decrease in the rise of cAMP but appears to leave its initial rate unaltered.

Supported by the SNSF and the Sandoz-Stiftung

Acetylcholinesterase Therapy in Organophosphate Poisoning?

W.H. Hopff und P.G. Waser
Pharmakologisches Institut der Universität, CH–8006 Zürich

Anticholinesterase organophosphates bind covalently to the esteratic site of cholinesterases. Thus the hydrolysis of acetylcholine (ACH) is no longer catalized, and ACH accumulates in the organism. Our aim was to show a beneficial effect of acetylcholinesterase (ACHE) applied i.v. in male white rats (SIV 50) 1 minute after sarin (SA) poisoning. In control rats excitation signs begin 1–3 minutes after SA, followed by death in 3–7 minutes. ACHE was prepared according to (Hopff et al., Symp. Cholinergic. Mech., 239 [P.G. Waser], Raven Press, NY. 1975); 1 ml of solution contained 52,000 μM units per 3 mg of protein. We proceeded as follows: after ether anaesthesia the rats were given $2 \times LD_{50}$ of SA i.p., followed by i.v. injection of 1.5 mg (6 rats) and 3 mg (6 rats) of ACHE. A matched control group of 12 rats remained untreated. A decrease in mortality rate was not observed and the symptoms of SA poisoning were not alleviated. Conclusion: Since ACHE cannot escape from blood vessels, it can only catalyze the hydrolysis of accumulated intravasal ACH. The increased ACH content in synapses, which is responsible for the acute death cannot be influenced.

Supported by SNSF, grant 3.2110.73 and by GRD, Abt. für Sanität, EMD

Effect of Caffeine and Chlordiazepoxide on the Motor Activity of the Chronic Thalamic Rat

M. Jost, A.A. Borbély, J.P. Huston and P.G. Waser
Institute of Pharmacology, University of Zürich, CH–8006 Zürich

Chronic thalamic rats were prepared by ablating the cortical and limbic telencephalic structures as well as major parts of the striatum. Thus the thalamus and hypothalamus were the highest brain structures left intact (Borbély et al., The Pharmacology of Thermoregulation, Karger 1973; Huston and Borbély, Physiol. Behav. *12*, 433, 1974). Experiments were conducted within 1 week after the operation. Motor activity was monitored by an ultrasonic technique. Low doses of caffeine (10 mg/kg i.p.) caused a weak motor stimulation, whereas higher doses (20 and 40 mg/kg) had a depressing effect. *d*-Amphetamine (3.7 mg/kg) caused a marked and prolonged stimulation of motor activity. Chlordiazepoxide HCl (5, 10 and 20 mg/kg) caused a dose-dependent depression of motor behavior. Thus, in contrast to amphetamine, the marked stimulatory

action of caffeine seen in the intact rat is contingent on the presence of telencephalic structures. The presence of meso-diencephalic structures is sufficient to account for the motor depression caused by chlordiazepoxide.

Supported by SNSF, grant 3.2120.73

Problems in Oxime Therapy

U.B. Junod, W.H. Hopff und P.G. Waser
Pharmakologisches Institut der Universität, CH–8006 Zurich

Severe organophosphorus poisoning is still an unsolved problem in therapy and generally oximes are recommended to reactivate the inhibited cholinesterases. Our aim was to show a beneficial effect against sarin (SA) and soman (SO) poisoning. For each test 10 C.R. male mice (22–24 g) were pretreated with Obidoxime (O) and Atropine (A), alone and in combination, and those mice serving for controle with saline. 3 minutes later the animals were treated with 2 × LD_{50} of SA or SO. Experiments were conducted as follows: each mouse treated with drugs was followed by a mouse treated with saline. The SA and SO solutions were prepared every 10 minutes. Dosis were optimized as follows: O dosage constant, A dosage variable and vice versa. Neither O (48 mg/kg) nor A (32 mg/kg) alone showed any effect on the mortality rate. In combination O (48 mg/kg) and A (8 mg/kg) protected all mice against SA poisoning, however SO poisoning could not be influenced.

Supported by GRD, Abt. für Sanität, EMD

Effect of Nicotine and Ambient Temperature on Hypothalamically Elicited Eating

R. Keller and K. Bättig
Swiss Federal Institute of Technology, Dept. of Behavioral Sciences, CH–8006 Zürich

As Münster and Bättig (Psychopharm. in press) showed, the threshold of hypothalamically elicited eating has increased by a single subcutaneous nicotine injection. On the other hand the threshold can be decreased by low ambinet temperature and be increased by elevating the ambient temperature (Bättig and Weber, Physiol. Behav. 1973). In the present experiments male rats, implanted with electrodes into the lateral hypothalamus and trained for stimulus-bound eating, are injected subcutaneously with nicotine (0.1 and 0.3 mg/kg) and exposed to different ambient temperatures (9°, 24° and 34 °C). At 9 °C the threshold decreased, but with nicotine this decrease was attenuated, at 34 °C the increase of the threshold was slightly enhanced, but only with the higher dose of nicotine. The anorexic effect of nicotine appears to be stronger than the effect of the ambient temperature. However this effect could be an indirect one due to the blood-glucose increasing effect of nicotine or it's effect on body temperature.

Differences in the Response to Drugs of Limbic and Striatal Dopamine Neurons

H.H. Keller, G. Bartholini and A. Pletscher
F. Hoffmann-La Roche & Co. AG, CH–4002 Basel

The turnover of dopamine (DA) in rat limbic system (LS) and striatum (S) has been investigated by measuring the changes in tissue concentration of homovanillic acid (HVA). Neuroleptics (chlorpromazine and haloperidol) increased HVA more markedly in the S than in the LS. In contrast, oxotremorine, caused a more pronounced rise of the acid in the LS as compared to the S. Trihexyphenidyl antagonized the effect of chorpromazine in both regions but that of haloperidol only in the S. The anticholinergic drug was more potent in antagonizing oxotremorine in the S. It is concluded that cholinergic influences are involved in the regulation of the activity of DA neurons in both brain structures. The different response to drugs of the two regions is explained on the basis of the finding (Bartholini et al., p. 741; Lloyd et al., p. 777, in: E. Usdin and S. Snyder, Frontiers in Catecholamine Research, Pergamon Press 1974) that in the S dopaminergic and cholinergic neurons mutually regulate their activity whereas in the LS only the cholinergic influence on DA neurons seems to exist. However, differences in DA and acetylcholine receptor sensitivity to both neuroleptics and anticholinergics must be considered.

Guanylate Cyclase System in Nervous and Other Tissues

P.J. Magistretti and M. Schorderet
Département de Pharmacologie, Ecole de Médecine, CH–1211 Genève

Guanylate cyclase activity (GCA) was measured in rabbit parotid glands, retinas and cervical vagus nerves. Isolated tissues were collected in a cold medium containing 50 m*M* Tris-HCl (pH 7.4), 10 m*M* KCl, 10 m*M* NaCl and 2 m*M* EGTA, homogenized with 9 volumes of the same medium and centrifuged at 20,000 *g* for 15 min at 4 °C. Next, 100 µl of supernatant were added to 1.0 ml of a standardassay mixture containing 50 m*M* Tris-HCl (pH 7.4), 6 m*M* theophylline, 7.7 m*M* $MnCl_2$, 2 m*M* EGTA and 0.50 m*M* ^{3}H-GTP (approximately 1.5 × 10^6 cpm). Incubation was allowed to proceed for various periods of time up to 30 min. Then, 0.1 ml TCA 50% was added, the suspension centrifuged, the supernatants applied to columns of AGl-X 8 (formate) according to Murad et al. (PNAS *68*, 736, 1971) and the labelled cGMP was counted. Preliminary results showed that GCA was higher in cervical vagus than in other tissues which might suggest a possible rôle of cGMP in nervous function. Experiments were also made to measure labelled cGMP in slices of rabbit parotid glands using ^{3}H- or ^{14}C-guanine, H_3-guanosine, ^{3}H-hypoxanthine as precursors. However, the penetration of these compounds was small, and no labelled cGMP was found, in the absence and in the presence of cholinergic drugs.

Supported by the Fondation Sandoz

Effects of Dihydroergotamine on cAMP Content in Cultured Glial Cells

R. Maurer and A. Grieder
Sandoz AG, CH–4002 Basel

Different biochemical properties have been determined in cultured rat astrocytoma cells C 6 (Gilman and Nirenberg, Proc. Natl. Acad. Sci. *68*, 2165, 1971). Content in cAMP is strikingly elevated in these cells by norepinephrine (NE) and isoproterenol. This effect can be blocked by preincubation of cultures with β-, but not with α-blockers. We have found that preincubation of confluent cultures with dihydroergotamine (DHE) inhibits the stimulatory effect of NE on cAMP level (determined as described by Fisch et al., Experientia *28*, 630, 1972) in

a dose-dependent fashion. The lowest dose to produce a significant inhibitory effect was found to be 5×10^{-8} *M* of DHE. Incubating cells with DHE in concentrations from 2×10^{-6} to 7.5×10^{-5} *M* (without stimulation by NE) increases the intracellular concentration of cAMP up to sevenfold. The interpretation of the inhibiting effect of DHE on NE stimulation in C6 cell line cultures is difficult, since there are no indications for a β-blocking activity of DHE. The increase in cAMP by DHE is probably mediated via inhibition of cAMP phosphodiesterase.

Immunopharmacological Investigations on Tracheal Segments from Rats and Guinea-Pigs

Rita K.M. Müller and F.M. Dietrich
Ciba-Geigy AG, CH–4002 Basel

Anaphylactic contractions were elicited in vitro in single minute tracheal segments from nematode-infected rats exhibiting a reaginic type of immune response and from ovalbumin-sensitized guinea-pigs producing antibodies of the IgG class. Drugs with known anti-anaphylactic or anti-allergic properties were studied in both systems. Their capacity to relax tracheal smooth muscle from unimmunized animals was also tested. In addition, their effectiveness in inhibiting contractions due to histamine, 5-hydroxytryptamine or carbachol was assessed. Disodium cromoglycate and related compounds had a potent anti-allergic effect, distinguishable from relaxation and antagonism. Similarly, isoprenaline was anti-allergic but only in the rat system. On the other hand, the effect of theophylline, mepyramine and methysergide proved to be partly due to directly relaxant or antagonistic properties. As confirmed by tests with clinically established anti-asthmatic drugs, the isolated tracheal strip from reaginic rats can serve as an in-vitro model for certain components of the pathogenesis of human extrinsic asthma, permitting the evaluation and characterization of anti-allergic compounds.

Enhancement of the Pressor Effect of Val^5-angiotensin II Amide (at II) by Propranolol in Urethane-Anesthetized Rats

Lise Peters-Haefeli, E. J. Kirchertz and G. Peters
Institut de pharmacologie de l'Université,
CH–1011 Lausanne

In urethane-anesthetized rats 0.25–0.5 mg/kg D, L-propranolol i.v. increased basal mean blood pressure (BP) by 0–20 mm Hg and considerably enhanced pressor responses to single doses of 2–18 ng/kg at II. The pressor potency of at II increased approximately 2 fold, but the slope of the log dose-response regression also increased. Preinjection of 2.6 mg/kg i.v. pentolinium did not abolish these effects of propranolol (Regoli et al., Canad. J. Physiol. Pharmacol., *50*, 207–214, 1972.) The rise of BP induced by infusion of 9 ng/min at II was negatively correlated ($b = 0.24 \pm 0.04$) to basal BP: after 0.5 mg/kg propranolol pressor responses to infused at II fell above the fiducial limits of this regression. In pentobarbital-anesthetized rats propranolol enhanced pressor responses to at II, independently of its own depressor effect. The enhancement of pressor responses to at II after propranolol appeared to be due to suppression of vasodilator effects of adrenal adrenaline released by at II.

Supported by SNSF, grant 3.742.72

Cerebellar Purkinje Cells Discharge Rate after Diphenylhydantoin or Diazepam

L. Pieri and W. Haefely
F. Hoffmann-La Roche & Co. Ltd., CH–4002 Basle

The spontaneous firing rate of cerebellar Purkinje cells (PC) from the vermian lobules VI, VII or VIII was continuously recorded in unanaesthetized rats and cats, immobilized with tubocurarine, and in rats anaesthetized with urethane. Diphenylhydantoin in doses of 10 to 18 mg kg^{-1} i.v. did not alter PC activity. Diazepam (0.1 to 4 mg/kg^{-1} i.v.) consistently and markedly decreased the firing rate of PC, for a span of approximately 50 min with 0.1 mg kg^{-1}. Pentetrazol in subconvulsive i.v. doses dramatically increased the discharge rate of PC. Prior administration of diazepam greatly reduced this effect of pentetrazol. The results with diazepam and diphenyldantoin are at variance with those of R.M. Julien (Neuropharmacology *11*, 683–691, 1972). The effect of diazepam on PC discharge rate could explain the ataxia observed after high doses of the drug, and would suggest a potentiation of the GABA-ergic inhibition of PC on the basis of findings that diazepam enhances GABA-ergic pre- and post-synaptic inhibition (Haefely W. et al., Adv. Biochem. Psychopharmacol., in press).

The Effect of Diazepam on Inhibition in the Cuneate Nucleus of Decerebrate Cats

P. Polc and W. Haefely
F. Hoffmann-La Roche & Co. Ltd., CH–4002 Basle

Diazepam (D) (1 mg kg^{-1} i.v.) augmented the P wave, the dorsal column reflex and the increase in excitability of cuneate presynaptic terminals elicited by conditioning stimuli to the ipsilateral median nerve. The effect of diazepam on these 3 indices of presynaptic inhibition was antagonized in a surmountable manner by the GABA-receptor blockers, bicuculline (0.5 mg kg^{-1} i.v.). and picrotoxin (1 mg kg^{-1} i.v.). The resting excitability of cuneate primary afferents was not modified by D. Postsynaptic inhibition, assessed by a decrease after the conditioning volleys in the median nerve of the fast lemniscal response to cuneate stimulation, was also enhanced by D. Picrotoxin, but not bicuculline, antagonized this effect. Acute inhibition of GABA synthesis was achieved with thiosemicarbazide (TSC) or 3-mercaptopropionic acid (MPA). TSC (30 mg kg^{-1} i.v.) reduced presynaptic and postsynaptic inhibition (after 90 min), whereas MPA (20 mg kg^{-1} i.v.) depressed only presynaptic inhibition (after 30 min). The effects of TSC and MPA were only partially overcome by D. It is suggested that D enhances GABA-ergic transmission involved in presynaptic and postsynaptic inhibition in the cuneate nucleus, as previously demonstrated for presynaptic inhibition in the spinal cord (Polc et al., Arch. Pharmacol. *284*, 319, 1974).

Accumulation of cAMP in Bovine Superior Cervical Ganglia Induced by Depolarizing Media

Ph. Roch and P. Kalix
Institut de Pharmacologie, Ecole de Médecine,
CH–1211 Genève 4

The content of cAMP in superior cervical ganglia is known to increase with electrical stimulation and upon treatment with cholinomimetic agents (Kalix et al, J. Pharmacol. Exp. Therap. *188*, 676, 1974) or biogenic amines (Roch and Kalix, Neuropharmacology *14*, 21, 1975).

We have observed that similar increases can be obtained by exposing the ganglion to veratridine or ouabain and also by raising the potassium content of the incubation medium. Accumulation of cAMP occurs only when a phosphodiesterase inhibitor is present during stimulation. The cAMP increase elicited under these conditions is rapid and roughly proportional to the concentration of the depolarizing agent. The effect of veratridine on the cAMP level can be prevented by preincubation with tetrodotoxine or tetracaine. Antagonists of the muscarinic or nicotinic action of acetylcholine do not interfere with the increase in cAMP brought about by depolarizing media, whereas antiadrenergic substances have an inhibitory effect on the cAMP accumulation. The results show that a cAMP synthetizing system is present in bovine superior cervical ganglia that can be activated by depolarization of the tissue. An adrenergic mechanism seems to participate in this activation.

Supported by SNSF, grant 3.0890.73

The Effects of Diazepam and Bicuculline on the Strio-Nigral Evoked Potential

R. Schaffner and W. Haefely
F. Hoffmann-La Roche & Co. Ltd., CH–4002 Basel

Supramaximal electrical stimulation of the head of the caudate nucleus evoked a response in the substantia nigra in unanaesthetized curarized cats, assumed to represent GABA-mediated IPSP's (Yoshida and Precht, Brain Res. *32*, 225, 1971). Cumulative i.v. injections of bicuculline depressed the nigral response in a dose-dependent manner. Diazepam injected i.v. up to a dose of 3.0 mg kg^{-1} did not affect the evoked potential. However, the effect of bicuculline, tested 2 h after a single dose of 3.0 mg kg^{-1} i.v. diazepam, was markedly reduced. The dose-response curve for bicuculline was significantly shifted to the right. Inhibition of GABA-T by pretreatment with 10 mg kg^{-1} i.v. of aminooxy-acetic acid or hydroxylamine for 3 h respectively 2 h in order to increase the GABA level in the CNS did not change the bicuculline effect on the strio-nigral evoked potential. The effect of ethanol and phenobarbital on the evoked response and on the bicuculline-induced depression of the nigral response is compared with that of diazepam. Diazepam has been found to enhance transmission in various GABA-ergic pathways. The present results are compatible with such a mode of action.

Interference of Inhibitors of Dopamine-β-Hydroxylase with the Uptake of Aromatic Monoamines in vitro

Doris Schlichter, M. Da Prada and A. Pletscher
F. Hoffmann-La Roche & Co. AG, CH–4002 Basel

The ATP/Mg^{++}-dependent uptake of dopamine (DA) by isolated membranes of bovine adrenal chromaffin granules was totally blocked by some inhibitors (disulfiram, FLA 63; 10^{-4}–10^{-5} *M*) of DA-β-hydroxylase (DAβOH). However, 5×10^{-6} *M* disulfiram (DS) caused only partial inhibition of DA uptake, while DAβOH was totally inactivated. Other inhibitors of DAβOH: diethyldithiocarbaminate (DDC), fusaric acid (FA) in concentrations which induced a virtually complete blockade of the enzyme did not markedly interfere with the DA uptake. Therefore, the interference of DAβOH inhibitors with DA uptake is not causally connected with inhibition of DAOβH, but may be related to the activity of a Mg^{++}-dependent ATPase which was partially blocked by DS and FLA and not inhibited by DDC and FA. DS also inhibited the ATP/Mg^{++}-dependent accumulation of noradrenaline, 5-hydroxytryptamine (5-HT) and tryptamine. Reserpine, however, interferes only with the uptake of catecholamines (CA) and 5-HT but not with that of tryptamine. It is concluded that (a) the change of the CA metabolism in vivo caused by some inhibitors of DAβOH may be partly connected with their interference with granular uptake of these amines, and (b) DS inhibits both the reserpine-sensitive and resistant uptake of monoamines.

Intracellular cAMP: Hormone-Induced Changes in Frog Skin Epithelium

M. Schorderet, A. Grosso and R. C. de Sousa
Départements de Pharmacologie et de Physiologie, Ecole de Médecine, CH–1211 Genève 4

Effects of oxytocin and norepinephrine on sodium and water transport in frog skin appear to be mediated by cAMP. Direct evidence of such mechanism can be provided by measuring cAMP content of epithelial cells, both during basal conditions and after stimulation with several agents. The epithelium was separated from the dermis by means of a standard technique employing collagenase. The typical protocol consisted in the comparison of a control group where the skins were exposed to a low dose of theophylline (10^{-3} *M*) and an experimental group exposed to the same dose of theophylline and to a hormonal agent during 5 min. Significant increases in cAMP ($p < 0.05$) were obtained with oxytocin and with several catecholamines. Moreover, exposure to a high concentration (10^{-2} *M*) of theophylline or iso-butylmethylxanthine also resulted in cAMP values significantly higher ($p < 0.01$) than those measured in control tissues not exposed to the drugs. These results support the concept that effects of catecholamines and oxytocin on frog skin are mediated by cAMP.

Supported by SNSF, grant 3.1300.73

A New Device for Ultrafiltration and Concentration of Fluids e.g. Blood Plasma

U. Trechsel and H. Fleisch
Pathophysiologisches Institut der Universität, CH–3012 Bern

Various problems arise during ultrafiltration of plasma: (1) The deposition of protein on the filter diminishes the filtration rate and may change the molecular cut-off. (2) Since pH is dependent on the pCO_2, the latter has to be kept constant and in the physiological range. (3) The plasma volume should be as small as possible. (4) The device should permit the processing of several samples simultaneously. Commercial instruments available at present do not fulfil all these requirements. We have devised a new apparatus based on filtration under pressure with the following characteristics: (1) The deposition of protein on the filter is avoided by means of a movement of the fluid backwards and forwards over the filter surface. (2) The pCO_2 is maintained constant since the fluid is not in contact with a gas space. (3) The minimum plasma volume is 0.4 ml. (4) Six samples can be processed simultaneously. (5) A filtration pressure up to 5 atmospheres can be applied. (6) All commercially available filters can be used. The apparatus can also be used for concentrating fluids from a chosen initial volume to an end volume of 100 µl e.g. for use in chromatography.

A Dynamic Model of the Oxytocin Effect on the Depolarized Rat Uterus

O. Wanner and V. Pliska
Institut für Molekularbiologie und Biophysik ETH, CH–8049 Zürich

We have earlier analysed the dynamics of the K^+-depolarized rat uterus contracted with oxytocin and allowed to relax in hormone-free medium on an analogue computer by an identification technique and concluded that the dynamics were at least sixth order (Wanner & Pliška, Experientia *30*, 698, 1974). From this result we have devised various structures for the model and tested them by experiment. This iterative procedure has now resulted in a model consisting of the originally suggested 3 compartments (Pliška, Il Farmaco Ed. Sci. *23*, 623, 1968); a third-order process describing the time course of the hormone concentration in the organ bath (particularly accounting for the fact that neither hormone addition nor wash-out are truly stepwise processes); an additional compartment generating the so-called 'fade' phenomenon; and a strong nonlinearity associated with saturation of the hormone receptors. The structure of the model requires that the 'fade' generating compartment be situated *behind* this nonlinearity and suggests strongly that the 'fade' is generated by the contractile system of the muscle rather than at the receptor level, as assumed by Paton's 'rate' receptor theory (Proc. Roy. Soc. London, [*B*] *154*, 21, 1961). The model now contains the minimum number of elements necessary to describe completely the behaviour of the depolarized rat uterus, including the slow, time-dependent alterations in the properties of the muscle.

Supported by SNSF, grant 3.0780.73

Screening of Anthracycline Antibiotics for Cardiotoxicity in Rats

G. Zbinden
Laboratorium für Experimentelle und Toxikologische Pathologie, Institut für Pathologische Anatomie, Universität Zürich, CH–8003 Zürich

Daunomycin (D) and adriamycin (A), 2 useful antitumor drugs, may cause lethal cardiomyopathy in man at total doses exceeding 550 mg/m². In order to identify derivatives with a better therapeutic index a toxicological screening was developed. Rats were treated 5 times weekly i.p. ECGs monitored in unanesthetized animals showed QRS widening, often with appearance of a distinct S-wave trough. In later stages intraventricular block, ventricular extrasystoles, bradycardia, and heart failure occurred. These changes sometimes developed weeks after discontinuation of treatment. 7 antibiotics were tested and ranked in decreasing order of cardiotoxicity depending on the minimal cumulative dose necessary to induce ECG changes: A (68 mg/m²), D (80 mg/m²), NSC-149584 = 14-octanoate of A (110 mg/m²), NSC-164011 benzylhydrazone of D (148 mg/m²), NSC-143496 = N-carbamoyl of D (195 mg/m²), NSC-143114 = semicarbazine of D (490 mg/m²), NSC-118114 = N-acetyl of D (> 800 mg/m²).

Supported by SNSF; antibiotics were obtained from Drug Development Branch, NCI, Bethesda, Md.

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

Strand Origin of Polyoma Virus-Specific 'Giant' Nuclear RNA and 16 S and 19 S Messenger RNA

N. H. Acheson and P. Beard
ISREC, CH–1011 Lausanne

Nuclear polyoma-specific RNA synthesized late during productive infection of mouse cells consists of 'giant' molecules ($\geq$ 26 S) one to several times the size of one strand of polyoma DNA. Cytoplasmic late polyoma-specific messenger RNA lacks these giant molecules but consists of 2 classes with sedimentation coefficients of 16 S and 19 S. We have separately hybridized giant, 19 S and 16 S RNAs with labeled separated strands of polyoma DNA. Preliminary results indicate that the giant RNA is complementary to all of the sequences in the 'L' DNA strand, suggesting that these giant molecules represent transcripts of the entire L strand. 16 S mRNA is complementary to 60% of the L strand, while 19 S mRNA is complementary to the same L strand sequences as well as to 40% of the E strand. These results suggest that (1) there are two classes of 19 S mRNA, one complementary to the L strand of polyoma DNA and one complementary to the E strand; and (2) there are also two classes of 16 S mRNA, each one containing a fraction of the sequences present in the 19 S species complementary to the L strand.

Determination of the Fine Structure of a Morphological Variant of *E. coli* Phage T4 which Produces Giants

U. Aebi, R. Bijlenga, A. Steven and B. ten Heggeler
Biozentrum der Universität, CH–4056 Basel

When *E. coli* B^E bacteria are infected with a temperature-sensitive mutant in gene 24 of phage T4 and grown at intermediate temperatures, then they produce in addition to normal phage particles a small amount of so-called giants similar to those which have been described by Eiserling et al. (J. Virol. *12*, 1973) and Cummings et al. (J. Virol. *13*, 1974). They are infective, show an increased uv resistance, are about 10 times longer than normal phages and often have two tails. We have optically diffracted and filtered electron micrographs of negatively stained preparations and found that the lattice constant of the hexagonal surface net is a 125 Å – compared with a 112 Å in T4 polyheads – and has a unique pitch angle of 14°. The filtered giants show a rather complex capsomer morphology which is significantly different from that of polyheads (De Rosier et al., J. Mol. Biol. *65*, 469, 1972). However we were not yet able to structurally identify the different protein species which appear to be involved in the head architecture in stochiometric quantities as it can clearly be seen in the corresponding gel electrophoresis patterns.

Glomerular Lesions Induced in Rats by Prolonged Immunization with Peroxydase

A.-Cl. Bataillard and M. Campiche
Institut d'anatomie pathologique, Hôpital cantonal, CH–1011 Lausanne

Adult rats were immunized by 3 subutaneous injections of 5 mg of horseradish peroxydase (HRP) in incomplete Freund's adjuvant at intervals of 2 weeks, then received 2 intravenous injections of 0.75 mg of HRP per week. Low-grade proteinuria developed after 20 to 30 i.v. injections; hematuria was exceptional. Kidney biopsies were performed in animals with proteinuria. Tissues were processed for immunofluorescence and light and electron microscopy, and HRP was demonstrated cytochemically. HRP was occasionally found in vacuoles of mesangial cells. Glomerular lesions were observed after 65 i.v. injections. The mesangium was slightly enlarged and hypercellular, and contained granules positive for IgG. Ultrastructurally, the foot-processes were partly fused and the lamina densa showed irregularities in the mesangial areas. Dense granular deposits were observed in the mesangial matrix in contact with the lamina densa. HRP was localized in these deposits.

Supported by SNSF, grants 3.786.72 and 3.1800.73. We are grateful to Dr. V. Mirkovitch for performing the kidney biopsies.

Polyoma Virus-Specific RNA in Productively Infected and Transformed Cells

P. Beard and N. H. Acheson
Swiss Institute for Experimental Cancer Research, CH–1011 Lausanne

Using the separated strands of highly radioactive polyoma DNA as hybridisation probes we have investigated the nature of virus-specific RNA in the nuclei and cytoplasm of productively infected mouse cells and two lines of transformed hamster cells. Late in the infectious cycle, cytoplasmic viral RNA is complementary to 40% of one polyoma DNA strand (the E-strand) and 60% of the other strand (the L-strand). In cytoplasmic RNA isolated early in the cycle before DNA replication, only the E-strand transcript is seen. In marked contrast the nuclei of infected cells, both early and late in infection, contain RNA complementary to most or all of the viral L-strand together with lesser amounts of E-strand transcript. Therefore in certain regions of the polyoma genome both DNA strands are transcribed throughout the lytic cycle. Transformed cell nuclear RNA hybridsies to 60% of the E-strand and cytoplasmic RNA to 40% of the same strand. Neither cytoplasmic nor nuclear RNA from the two transformed cell lines tested contain sequences complementary to the viral L-strand.

Drosophila cells: Colony Formation and Cloning in Agarose Medium

H. P. Bernhard and W. J. Gehring
Biozentrum der Universität, CH–4056 Basel

Established cell lines of *Drosophila melanogaster* generally have a low plating efficiency and require higher inocula than transformed mammalian cells. Four established cell lines tested do not form agar colonies if plated at densities below 10^6 cells per ml. We have developed a cloning technique in soft agar using X-irradiated or Mitomycin-C treated feeder cells. Clones could be obtained from each cell-line at low densities (100–1,000 cells/ml) and with high plating efficiency, and subsequently readapted to liquid culture. A complete chromosome analysis was performed on the stem lines and on six clonal sublines. All stem lines were aneuploid and showed a broad distribution of chromosome numbers, whereas the clonal sublines displayed characteristic and substantially homogeneous karyotypes.

Membrane Changes During Muscle Cell Differentiation Detected with Plant Lectins

B. Betschart and M. M. Burger
Biozentrum der Universität, CH–4056 Basle

Cultured muscle cells from chick pectoral muscles were examined for agglutination by lectins from wheat germ, soybean, lentil and Concanavalin A. Specific hapten inhibitable agglutination with WGA and Con A increased prior to or with the onset of the fusable state. An increased agglutinability was found even under conditions where fusion could not occur (EGTA), suggesting that fusion by itself cannot be the cause for the increased agglutinability. Binding studies with radioactive WGA showed that the number of receptor sites per mg protein did not increase during development. ESR experiments are in progress to detect a possible change in membrane fluidity, which might explain the increased agglutinability. Fusion can also be inhibited by ara-C, a DNA synthesis inhibitor. DNA synthesis seems to be a prerequisite for the change in agglutinability and probably also for fusion.

Supported by SNSF, grant 3.1330.73

Early Transcriptional Events in Liver of Estrogen-Stimulated Immature Chicks

Françoise Bieri and Christa Dierks-Ventling
Friedrich-Miescher-Institut, CH–4002 Basel

In liver of immature chicks treated with a single injection of 17β-estradiol we have previously established time-dependent changes of polysomal RNA synthesis (Jost et al. J. Biol. Chem. *248*, 5262, 1973) and of nuclear protein and nuclear RNA synthesis [C. Dierks-Ventling et al. Europ. J. Biochem. *50*, 34 (1974)]. In correlation with these findings we now present data pertaining to the DNA-dependent RNA polymerase activities in isolated liver nuclei. The nucleoplasmic polymerase II (mRNA) activity was distinguished from that of the nucleolar polymerase I (rRNA) by the addition of α-amanitine. The following kinetics were obtained: Polymerase I activity rose to a maximum at $1^1/_2$ hours (+30% above the controls), then returned to control levels. In contrast polymerase II activity rose to a maximum only at 2–3 hours (+25% above the controls) and returned to control levels. At 6 hours after estrogen treatment, both polymerase activities increased again and reached a plateau (+100% above the controls) at 18 hours. This plateau remained constant for 48 hours. Cycloheximide given before or together with estrogen inhibited completely the activation of polymerase I. Cycloheximide given one hour after estrogen treatment did not produce unequivocal results with regard to the activity of polymerase II. We concluded that the activation of polymerase I at least is dependent on protein synthesis.

Nucleotide Sequences of Qβ RNA: a Progress Report

M. A. Billeter, J. E. Dahlberg, C. Escarmis, C. de Giorgi, P. Goldfarb, H. M. Goodman, J. Hindley, L. King, A. Luder, F. Meyer, P. Sastry, G. Vögeli, H. Webev and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich; Institut de Biologie Moleculaire, Université de Genève; Dept. of Biochemistry, University of Bristol

The nucleotide sequence of Qβ RNA is being analyzed using a variety of approaches. So far, the 5′ terminal region (229 nucleotides), the 3′ terminal region (161 nucleotides), the intercistronic and adjacent regions (112 and 143 nucleotides, respectively), segments derived from the coat cistron (154 nucleotides), A_2 cistron (68) and (probably) replicase cistron (21, 60 and 164 nucleotides), and 3 sites of unknown origin (total 91 nucleotides, including a sequence determined by Argetsinger-Steitz) have been elucidated. Furthermore 21 large oligonucleotides have been sequenced and their approximate locations within the Qβ genome have been determined. All known sequences, adding up to 1,498 nucleotides (about 33% of the genome), as well as their location in the genome and their biological function, as far as they are known, will be presented.

Supported by SNSF and the Jane Coffin Childs Fund

Molecular Structure of the Histone Polygenes

M. Birnstiel, E. Southern, J. Telford, M. Wilson, W. Schaffner and K. Gross
Institut für Molekularbiologie II der Universität, CH–8057 Zürich

Highly-labelled 9 S histone messenger RNAs has been isolated from cleaving sea urchins. Gel electrophoresis separates these RNAs into at least four major messenger sub-species, three of which have been obtained in high purity by re-electrophoresis. The genes coding for the different mRNAs are repeated several hundred times in the sea urchin genome. Chromosomal DNA containing these reiterated genes has been enriched 130 fold by repeated centrifugation in actinomycin-CsCl and Hg^{2+}-Cs_2SO_4 density gradients. Digestion of the histone DNA of *Psammechinus* with the endonuclease EcoRI (gifts of Drs Murray and Yuan) yields a prominent 4.0×10^6 dalton DNA fragment. When individually tested, all three histone mRNA subfractions hybridize exclusively to this DNA fragment, as does unfractionated 9 S mRNA. These results demonstrate that genes coding for different histone proteins are linked together within a relatively short DNA segment that is repeated manyfold in chromosomal DNA. Preparative amounts of the EcoRI fragment have been isolated for integration into λ-receptor phages.

Supported by SNSF, grant 3.8630.72SR and the Helen Hay Whitney Foundation

Studies on In Vitro Processing of Avian Myeloblastosis Virus Precursor-Polypeptide

R. Braschler and Heidi Diggelmann
Swiss Institute for Experimental Cancer Research, CH–1011 Lausanne

Chick embryo fibroblasts infected with avian myeloblastosis virus (AMV) synthesize a viral precursor-polypeptide that is subsequently cleaved to form virion structural proteins (group-specific antigens) (Eisenmann, Vogt, PNAS *70*, 1973). We have attempted to find conditions under which this cleavage can be studied in vitro. The ^{35}S-methionine-labelled precursor-polypeptide was isolated from infected cell lysates by immunoprecipitation with an antiserum against total disrupted AMV, and purified by SDS-polyacrylamide gel electrophoresis (PAGE). The purified precursor-polypeptide, still immunoprecipitable, was incubated with AMV-infected and uninfected cell lysates, with purified virus and with different subviral fractions under different conditions. No specific nor unspecific cleavage could be detected. However the precursor-polypeptide can be completely digested by added trypsin. In crude lysates of infected cells obtained by sonication, specific cleavage of endogenous precursor-polypeptide can partially occur. Treatments that destroy membrane structures (NP-40, chloroform) block this in-vitro cleavage. We therefore isolated the precursor-polypeptide in native membrane-bound form and are trying to cleave it as in the case of the purified precursor-polypeptide. In addition the interaction of cellular and viral proteins with the cellular membrane was investigated.

Freeze Etching of Myogenic Cells under Varied Culture Conditions

M. Chiquet, H. M. Eppenberger, H. Moor and D. C. Turner
Institut für Zellbiologie, ETH, CH–8049 Zürich

The freeze-etching technique was used to study the surface morphology of myogenic cells under culture conditions which either allowed fusion into myotubes or prevented formation of multinucleated cells (addition of 1.66 m*M* EGTA). Cells were grown directly on the gold specimen carriers used for subsequent freeze etching and thus at no stage detached from the substratum. Damage to the delicate filopodial structures extending from the cell bodies either towards other cells or towards the substratum could therefore be avoided. Myogenic cells grown in EGTA medium are typically bipolar but neither filopodia nor close cell-to-cell contacts are observed. Vermiform elevations on the surface of such cells may represent incomplete or degenerate filopodia that are unable to protrude properly in calcium-deficient medium. Four hours after replacement of EGTA medium with standard medium protrusion of filopodia, frequent intercellular filopodial contacts, as well as extensive cell fusion were observed. It is tentatively concluded that EGTA blocks the proper functioning of the filopodia thus preventing cell contacts leading to fusion.

Supported by SNSF, grant 3.8640.72, and the Muscular Dystrophy Association of America

Isolation of the Genes Coding for $tRNA_f^{met}$ from *Xenopus laevis*

S.G. Clarkson
Institut für Molekularbiologie II der Universität, CH–8057 Zürich

DNA containing the reiterated genes for $tRNA_f^{met}$ has been partially purified from *Xenopus laevis* by centrifugation in actinomycin-CsCl and Ag^+-Cs_2SO_4 gradients. Complete purification has been achieved by digestion of this DNA with the restriction endonuclease EcoRI, and by separation of the resulting fragments by agarose slab gel electrophoresis. DNA complementary to $tRNA_f^{met}$ has been found solely within one discrete fragment, whose

molecular weight has been estimated to be 2.0×10^6 from its electrophoretic mobility. Partial digestion products whose molecular weights form an arithmetic progression up to the pentamer of the 2.0×10^6 dalton fragment have been resolved, thus confirming that the fragment is tandemly repeated. The results demonstrate the existence of at least one gene for $tRNA_f^{met}$ within a 3,000 base pair repeating structure that shows little if any length heterogeneity. Whether each repeat contains more than one gene for this or another tRNA species is under investigation, but the results already preclude the possibilities that it contains genes for $tRNA_m^{met}$, $tRNA^{Val}$, 5 S RNA or rRNA.

Supported by SNSF, grant 3.8630.72 SR.

A Tentative Physical Map of the Genome of an Avian Tumor Virus

J.M. Coffin and M.A. Billeter
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich

Recently it has been established that the nucleotide sequence complexity of the avian tumor virus genome (65 S) RNA is about 10^4 nucleotides, suggesting that each of the 36 S subunits obtained from it by heat-denaturation has the same or very similar nucleotide sequences (Billeter et al., PNAS *71*, 3560, 1974). To decide whether all subunits contain these sequences in an identical or in a permuted order, ^{32}P-labeled 65 S RNA of the Prague strain of Rous sarcoma virus (subgroup B) was denatured and fractionated into 5 size classes ranging from intact 36-S subunits to 10-S fragments. The poly (A)-containing fragments representing the original 3' end were isolated by chromatography on poly U-Sephadex and characterized by complete digestion with RNase T_1 followed by two-dimensional gel electrophoresis. Only full-length RNA contained all large T_1 oligonucleotides of unfractionated 65 S RNA whereas in shorter fragments successively more oligonucleotides were missing. We conclude that at least the vast majority of 36 S RNA subunits have a non-permuted structure. A physical map of 36 S RNA with the approximate location of 28 T_1 oligonucleotides has been constructed. It is of particular interest that this map locates the 3 oligonucleotides which are lacking in non-transforming variants nearest to the 3' end of the RNA.

Supported by SNSF and the Jane Coffin Childs Fund.

Intracellular Events During in vitro Stimulation of Lymphocytes with Mitogens

A. Cogoli, G.E. Matthyssens and D. Givol
Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel

Lymphocytes from mouse spleen were stimulated in vitro with Con A, Periodate or LPS. The newly synthesized proteins were labeled with pulses of ^{35}S-methionine or ^{14}C-lysine and arginine at different times after stimulation. Soluble cytoplasmic content (cytosol) was obtained by freezing and thawing, and nuclei were obtained by treatment of the cells with 0.25% NP-40. The nuclear proteins were solubilized by treatment of the nuclei with 1% SDS and DNAse, occasional cores were removed after centrifugation: 100% of the radioactivity was found in the supernatant. Nuclear and cytoplasmic proteins were analyzed by SDS electrophoresis on slab gels and autoradiography of the dried gels. Labeled histones were found only in nuclei. The synthesis of all histone classes is specifically enhanced after stimulation. Several new proteins appear in the cytoplasm of stimulated cells. Free nuclei from normal cells preferentially absorb one protein (mol. size about 50,000) if incubated with the cytosol of Con-A stimulated cells.

Purification of Globin RNA from Friend Cells Induced by Dimethyl-Sulfoxide

P.J. Curtis and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich

Friend cells can be induced by 1.5% dimethylsulfoxide to produce substantial quantities of hemoglobin [Friend et al., PNAS *68*, 378 (1971)] and so provide a system in which globin RNA synthesis and processing can be studied. The procedure for the isolation of RNA-DNA hybrids of Coffin et al. (J. Mol. Biol. *86*, 373, 1974) has been adapted for the purification of RNA molecules containing globin sequences. Essentially, globin cDNA elongated with dCMP residues was hybridized to radioactive RNA. The RNA-DNA hybrid was specifically bound to poly I-Sephadex by virtue of its poly dC tail. Using this method (with some further elaborations) hybridization of labeled RNA from uninduced and induced Friend cells yielded 0.01% and 0.15% labeled RNA in the hybrid fraction, respectively. However hybridization analysis of the RNA purified from induced cells indicated only 5–10% purity in regard to globin mRNA. This low purity was due to attachment of ribosomal RNA to the globin mRNA moiety of the RNA-DNA hybrid. The contamination by labeled ribosomal RNA was eliminated by addition of excess degraded yeast RNA. Labeled RNA from induced Friend cells purified by this modified method consisted of more than 70% globin specific sequences.

Supported by SNSF and the Jane Coffin Childs Fund

Demonstration of A-Cells and Glucagon in the Canine Stomach

C. Rufener, D. Baetens, L. Munoz, C. Srikant, R. Dobbs, R. Unger and L. Orci
Institut d'Histologie et d'Embryologie, Ecole de Médecine, CH–1211 Genève 4 et VAH and U. of TX SW Medical School, Dallas, Texas (USA)

Hyperglucagonemia has recently been demonstrated in depancreatized dogs and is believed to play a mediating role in their hyperglycemia. These studies were designed to identify the source of this extrapancreatic glucagon and to characterize its cells of origin. Extracts of canine gut were immunoassayed with 30 K (highly specific for pancreatic glucagon) and 78 J (highly cross-reactive with GLI). Fundus contained ~ 200 ng of glucagon/g of tissue but no GLI. Post-pyloric gut had < 2 ng of glucagon/g. EM studies of the fundus revealed cells ultrastructurally indistinguishable from pancreatic A-cells. Those cells were virtually absent elsewhere where glucagon was either low or undetectable. Incubation of fundus with peroxidase-conjugated specific anti-glucagon serum revealed an abundance of positive cells in fundus but very few elsewhere. Immunofluorescent staining with the same antibody gave similar results. We conclude that the canine fundus contains: (1) glucagon immunologically indistinguishable from pancreatic glucagon; (2) A-cells with granules ultrastructurally indistinguishable from pancreatic A-cells; and (3) cells which are immunohistochemically positive for pancreatic glucagon.

Supported by SNSF, grants 3.8080.72, 3.0310.73 and N.I.H. 02700-15

Synthesis and in vitro Replication of an Extracistronic Mutant of Qβ RNA

E. Domingo, R.A. Flavell and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich

The method of site-directed mutagenesis (Flavell et al., J. Mol. Biol. *89*, 255, 1974) has been used to produce a further defined mutation in the 3'-extracistronic region of the Qβ genome. N^4-hydroxyCMP was introduced in place of UMP at position 39 from the 5'-end of Qβ RNA minus strands. When substituted minus strands were used as template for Qβ replicase to direct a single round of plus strand synthesis 20% of the product contained an A→G transition at position 40 from the 3'-end, as evidenced by the appearance of a new large T_1-oligonucleotide that was isolated by bidimensional polyacrylamide gel electrophoresis and characterized by nearest neighbor analysis. When the reaction initiated by substituted minus-strands was allowed to proceed for several rounds the proportion of mutant RNA decreased, suggesting that under the in-vitro conditions used the mutant RNA was replicated less efficiently than wild-type Qβ RNA. The biological activity of the mutant RNA is under investigation.

Supported by SNSF and the Jane Coffin Childs Fund

Intercellular Junctions between Pituicytes. A Freeze-Fracture Study

J.J. Dreifuss, C. Sandri, K. Akert and H. Moor
Département de Physiologie, Ecole de Médecine, CH–1211 Genève 4
Hirnforschungsinstitut und ETH, CH–8049 Zürich

Neuroglial cells (pituicytes) constitue the great majority of the nucleated elements present in the neurohypophysis, but little is known of their function. Using freeze-fracture, we observed intercellular junctions between pituicytes, but neither between these and neurosecretory axons, nor between axons themselves in the rat neural lobe. Two types of junctions occur. Gap junctions appear as macular aggregates of membrane-intercalated particles with a ~ 10 nm center to center spacing (face A) or a complementary array of small depressions (face B); they interconnect the thin, intertwining pituicyte processes which cover the axon endings. Complex junctions are characterized by a combination of gap and tight junctions, one or more patches of particle aggregates typical of a gap junction appearing between the ridges of a tight junction. Complex junctions occupy larger areas than gap junctions and were seen only between pituicyte cell bodies. These observations provide evidence in favour of electrical and metabolic coupling between pituicytes in the mammalian neurohypophysis.

Supported by SNSF, grants 3.133.69, 3.134.69, 3.712.72

La thiamine et ses esters interviennent-ils dans le métabolisme de l'actéylcholine?

L. Eder et Y. Dunant
Département de Pharmacologie, Ecole de Médecine, CH–1211 Genève 4

Le tissu nerveux exige pour son activité la présence de thiamine. Dans l'organe électrique de la Torpille, l'acétate externe est utilisé comme précurseur de l'acétylcholine (Israël et Tuček, J. Neurochem. *22*, 487, 1974). D'autre part, au cours de la stimulation, le taux d'ACh présente des oscillations périodiques soutenues (Dunant et al., Nature, Lond. 212, 485, 1974). Afin de déterminer si la thiamine et ses esters phosphoriques jouent un rôle dans le transport et le recyclage de l'acétate. leurs taux respectifs sont mesurés dans l'organe électrique au repos et pendant l'activité. Le fractionnement subcellulaire est utilisé pour localiser l'activité des enzymes impliquées dans le métabolisme des thiamines-phosphates (thiamine pyrophosphatase, triphosphatase, phosphotransférases).

Characterization of Fragments of Bacteriophage Qβ RNA and Sequence Determination of a Fragment of the Coat Cistron

Cristina Escarmis, P. Sastry, M.A. Billeter and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich

31 large oligonucleotides produced by complete digestion of [^{32}P] Qβ RNA by RNase T_1 were isolated by two-dimensional polyacrylamide gel electrophoresis and sequenced to a large extent. Their location within the genome was established by preparing short-term Qβ replicase products on a minus strand template, isolating the resulting Qβ plus-strands at various stages of completion and determining which large T_1 oligonucleotides were present within each product of defined length. Now we used these oligonucleotides as markers to characterize partial RNase T_1 degradation products of [^{32}P] Qβ RNA which can be reproducibly fractionated into 49 bands by polyacrylamide gel electrophoresis. The material from each of these bands (which still consisted of a number of RNA fragments) was characterized by fingerprinting with respect to its content of the marker oligonucleotides. Two of these bands, yielding a high amount of two oligonucleotides characteristic for the coat cistron, were further purified by a modified two-dimensional polyacrylamide gel electrophoresis using formamide at pH 3.5 in the first dimension. Two pure fragments could thereby be isolated. The sequence of the shorter of these fragments, corresponding to the coat aminoacid sequence No. 16 to 66 has been almost completely determined and will be presented.

Supported by SNSF and the Jane Coffin Childs Fund

The Entire Sequence of L Chain mRNA Corresponds to Single Copy Genes

M-G. Farace, M.-F. Aellen, P. Vassalli and B. Mach
Department of Pathology, CH–1211 Geneva 4

We have carried out experiments aimed at estimating the number of immunoglobulin genes in mouse plasmocytoma DNA. L chain mRNA, isolated by procedures previously described from myeloma MOPC 41 and 104, and chemically labeled with ^{125}I (5×10^7 cpm/μg) was hybridised under conditions of DNA excess. The Cot curve showed doubletransition kinetics, with one component corresponding to about 300 gene copies and the other to 1 copy. Since data with cDNA had shown that there is one gene for the C region, the reiterated component, also observed by other authors, had been interpreted as being either the V region or a non-translated 5'-segment of L chain mRNA. We have further investigated the question of the reiterated RNA by two approaches: (1) Further purification of L chain mRNA as a single band on formamide gels results in a Cot curve with a single transition

corresponding to 1–2 genes, with the disappearance of the reiterated component. (2) Hydroxyapatite chromatography of the hybrides obtained with purified RNA shows that L chain mRNA (MOPC 41) cannot have a reiterated sequence longer than about 20 nucleotides. The data indicate that, contrary to previous reports, the entire sequence of L chain mRNA corresponds to unique genes and that the observed reiterated RNA component results from contaminating species. These have been identified on the side fractions of the acrylamide gel.

Supported by SNSF, grant 3.1310.73

Demonstration of Carbohydrate Residues on the Outer Surface of Rabbit Leukocyte Granules

M. E. Feigenson, H. P. Schnebli and M. Baggiolini
Wander Ltd, CH–3001 Berne and
Friedrich Miescher Institute, CH–4002 Basel

In polymorphonuclear leukocytes (PMNs), fusion of azurophil and specific granules with phagocytic vacuoles is a selective process which governs the release of bactericidal and digestive proteins, and which may depend upon mutual recognition of the merging membranes. Characterization of the granule membranes is, therefore, a logical approach to the study of this process. Using the plant lectin ricin, conjugated to ferritin (RF), we have demonstrated the presence of carbohydrate residues on the surface of intact granules. Granules prepared by density gradient fractionation and incubated with RF are processed for microscopy under conditions which insure random sampling. The electron-dense RF is bound to the outside of azurophil and specific granules but not to mitochondria. When RF is pre-incubated with the hapten α-lactose, the binding is essentially abolished. Morphometrical analyses show no difference in the RF binding density of either type of granule. This demonstration of exposed carbohydrate moieties on the granule surface suggests the presence of membrane glycoproteins, which may be involved in the recognition process preceding fusion.

Comparative Peptide Analysis of Structural Proteins from Simian Virus 40 and Some Temperature Sensitive Mutants

G. Fey and B. Hirt
Institut Suisse de Recherches Experimentales sur le Cancer, CH–1011 Lausanne

Tryptic peptides from structural proteins of SV 40 wild type (labelled with ^{14}C-arginine) and temperature-sensitive mutants of three late complementation groups (labelled with ^{3}H-arginine) were compared by double label ion exchange chromatography on chromobeads P. The aim is to establish a correlation between structural proteins and complementation groups, i.e. to map the structural proteins on the genome, since the location of the complementation groups is known with respect to the restriction enzyme map of the SV 40 genome. Results: (a) We find one peptide of altered mobility in the major capsid protein VP 1 (46,000 dal) for each ts B 201 and ts C 219, indicating that both complementation groups B and C correspond to VP 1. (b) The two minor capsid proteins of the wild type: VP 2 (*c.* 43,000 dal) and VP 3 (30,000 dal) have identical arginine peptide patterns. (c) We find two additional peptides in VP 3 for ts D 238 and one additional peptide in VP 1 for ts D 101 but no peptides with altered migration in both cases. The pattern of VP 1 from ts D 238 is indistinguishable from wild type.

DNA Replication in Brij-58 Treated Mouse P-815 Cells

J. R. Gautschi
Pathologisches Institut, Universität Bern, CH–3010 Bern

DNA replication in mammalian cells involves the formation of at least two replication intermediates: Okazaki fragments (50–200 nucleotides) and replicon-sized molecules ($\sim 10^5$ nucleotides). To study mechanisms controlling initiation and elongation of intermediates, an in vitro system was developed. Cultured cells were treated with the detergent Brij-58, washed and incubated with the eight ribo- and deoxyribonucleoside triphosphates, creatine phosphate, creatine phosphokinase, $MgCl_2$, $MnCl_2$, KCl, EGTA, cadaverine, dextran, sucrose, dithiothreitol and HEPES. Characteristics of the system are: (1) During the preparation no detectable strand breaks are formed in preexisting DNA ($Mn > 2 \times 10^8$ daltons). (2) ^{3}H-TTP is incorporated into DNA at a constant rate during 20–30 min at 30 °C. (3) Okazaki fragments and replicon-sized molecules are labeled after a 5 min pulse at 30 °C. (4) After a 30 sec pulse at 25 °C more than 60% of incorporated radioactivity are found in Okazaki fragments. (5) In pulse-chase experiments, Okazaki fragments are converted to replicon-sized molecules. Thus, characteristics of DNA chain elongation in vitro are similar to those in intact cells.

Supported by SNSF, grant 3.923.72

Evidence for a Viral Maturation Complex in Parvovirus Infected Cells

M. Gautschi and G. Siegl
Institut für Hygiene und medizinische Mikrobiologie, CH–3000 Bern

By lysis of nuclei from parvovirus Lu III-infected Hela-cells at low ionic strength (1 m*M* NaCl) a nucleoprotein complex is released sedimenting with > 200 S in sucrose gradients containing 0.35 *M* NaCl. This complex is infective. The protein component is largely composed of the two virus-specific structural polypeptides present in a similar relative amount as found in infective virions. About 90% of the DNA is accessible to digestion by DNase I. This treatment releases viral particles, empty capsids (60 S) and capsomeres (< 20 S). RNase has no effect on the stability of the complex. During density equilibrium centrifugation in 3.5 *M* CsCl the complex is dissociated into a main component banding at 1.46 g/ml, DNA, and four particle species containing a variable amount of nucleic acid. Since reconstruction experiments provided no evidence for the complex being artificially generated during the isolation procedure, it may be assumed that it plays an important role in parvovirus maturation.

Growth Inhibition of Organ Cultured Embryonic Bones by Metal Chlorides

H. Gerber, M. Bürge, J. Cordey, W. Ziegler and S. M. Perren
Labor für experimentelle Chirurgie, CH–7270 Davos-Platz

Modern implant alloys, although exhibiting good corrosion resistance, release metal ions into the tissue. There is an interest in sensitive and reproducible standard tests of tissue toxicity. In vitro experiments were conducted

which enabled the examination of the effect of metal chlorides on growing bones. 18-days-old pairs of embryonic rat femora were cultured according to Fell an Weiss (J. Exp. Med. *121*, 551, 1965). The right and the left femur from each animal were randomly assigned to the experimental or control group. Parameters examined were: wet weight, dry weight and histology. In the first experiment solutions of 10^{-3} were used. $CrCl_3$, $FeCl_3$, and $TiCl_3$ exhibited a low, and $CoCl_2$ and $NiCl_2$ a marked inhibition of growth. In the second experiment with II logarithmic concentrations of $CoCl_2$ and $NiCl_2$ between 10^{-5} *M* and $3{,}16 \times 10^{-3}$ *M* the scatter of the values was low and we found a good correlation between the applied concentration of metal ions and the resulting growth inhibition. The described standardized tissue toxicity test method is of interest for further studies on the effect of soluble metal salts.

Supercoiling of SV40 DNA and Chromatin Structure

J. E. Germond, B. Hirt, P. Oudet, M. Gross-Bellard and P. Chambon
Institut Suisse de Recherche Expérimentale sur le Cancer, CH–1011 Lausanne, et Institut de Chimie Biologique, Faculté de Médecine, F–67085 Strasbourg Cedex

Supercoiled DNA molecules from simian virus 40 were converted by untwistase to relaxed, covalently closed circles. The relaxed DNA was associated in vitro with those four histones, which are present in the virus particles ($F2A_1$, $F2A_2$, F2B and F3). In electron micrographs, the resulting complexes appear twisted, with globular structures (nucleosomes) along the DNA. Incubation with untwistase removes the twists from the complexes. Extraction of the DNA from untwisted complexes yields supercoiled molecules. The number of superhelical turns per molecule corresponds to the number of nucleosomes per DNA molecule in the histone-DNA complexes. The in vitro associated complexes look similar to those extracted from virus particles or from infected cells. The nucleosomes formed in vitro resemble closely those in chromatin. Each nucleosome contains about 200 base pairs of DNA. The tortional deformation of the DNA in the nucleosome is equivalent to the unwinding of the double-helix by one turn.

Supported by SNSF, DGRST, INSERM and FRMF

Trans-Synaptic Enzyme Induction in the Rat Adrenal Medulla in Organ Culture

R. Goodman, U. Otten and H. Thoenen
Biozentrum der Universität, CH–4056 Basel

As a prerequisite for studying in vitro the complex processes involved in trans-synaptic enzyme induction, organ culture conditions for the rat adrenal medulla representative for the in vivo situation were developed. The processes of trans-synaptic enzyme induction initiated in vivo by injecting 5 mg/kg of reserpine 2 h prior to the removal of the adrenal medulla, continued in this culture system and the final levels of tyrosine hydroxylase (TH) were comparable to those seen in vivo. Transection of the splanchnic fibers supplying the adrenal medulla or administration of actinomycin D prior to reserpine administration abolished the rise in TH activity both in vivo and in culture. The finding that 0.29 m*M* corticosterone added to the culture medium inhibited the increase in TH activity initiated by reserpine supports the hypothesis that glucocorticoids act as modulatory agents in trans-synaptic enzyme induction. That the morphology of adrenal medullae in culture and in vivo are indistinguishable is further evidence that this culture system is representative for the in vivo situation.

Supported by SNSF, grant 3.563.71

Thermoadaptation of Enzymes in Thermophilic and Mesophilic Cultures of *Bacillus Caldotenax*

H.-U. Haberstich and H. Zuber
Institut für Molekularbiologie und Biophysik der ETH, CH–8049 Zürich

The extremely thermophilic *Bacillus caldotenax* could be adapted to both, thermophilic and mesophilic conditions. With some enzymes of the glycolytic pathway, it could be shown that 70°-cells produced more thermostable enzyme-forms than 37°-cells. 37°-precultures of B. caldotenax were further cultivated at 5° intervals between 30° and 70°. It was observed that cells cultured in the temperature range between 30°–50° produced thermolabile enzyme variants, while cultures between 60°–70° synthesized thermostable variants. At 55° both types were probably formed. Mesophilic cultures, further cultivated above 50° showed a pronounced lag-period, expressing extensive metabolic changes. In the lag-period, thermolabile enzymes were no longer present as early as 20 min after increasing the temperature (70°), and synthesis of thermostable enzymes started about 1 h before the beginning of thermophilic growth. Similar results were obtained with B. caldotenax precultivated at 70° and cultivated between 30 °C and 70 °C.

The Rifampicin-RNA Polymerase Complex: Influence of Substances which Interact with the Enzyme

J. Handschin and W. Wehrli
Ciba-Geigy AG, CH–4002 Basel

The binding and dissociation kinetics between rifampicin and DNA-dependent RNA polymerase of E. coli change during enzyme purification. The complex with an enzyme purified by $(NH_4)_2SO_4$-fractionation is formed and dissociated about ten times more slowly than that with an enzyme further purified by DEAE-cellulose chromatography. The RNA present in the cruder enzyme is responsible for this difference. Nucleic acids such as rRNA, tRNA and DNA when added to the purified RNA polymerase have a similar effect, which is enhanced by the addition of Mg^{2+}. Since the K_{eq} remains almost constant, nucleic acids probably do not alter the rifampicin-binding site, but might interfere sterically with the enzyme, inhibiting both release of bound rifampicin as well as binding of free antibiotic. Studies with ribonucleoside triphosphates showed that only purine nucleotides, and only as their Mg^{2+}-salts, stabilize the enzyme-antibiotic complex. This raises some interesting questions as to the action of rifampicin on RNA chain initiation.

Effect of Insulin and Non-suppressible Insulin-like Activity (NSILA) on Activity of Ornithine Decarboxylase

G. Haselbacher and R. E. Humbel
Biochemisches Institut der Universität, CH–8032 Zürich

Ornithine decarboxylase (ODC) is the rate limiting enzyme in polyamine biosynthesis. High tissue levels of polyamines have been associated with rapid growth. The

activity of ODC in non-dividing tissue is very low, but can be greatly increased by various growth stimulants. We compared activities of ODC after addition of some growth promoting substances to synchronized chick embryo fibroblast cultures. Insulin (200 mU/ml) and NSILA (200 μU/ml) stimulate ODC approximately sixfold, 10% fetal calf serum about eighteenfold. Nerve growth factor does not increase ODC activity above control levels. The extent of ODC activation achieved with serum, NSILA or insulin corresponds to the respective increase in cell number obtained with these growth stimulants. A sharp increase in ODC activity occurs between 2.5 and 5 hours after addition of the growth factors, with a peak at 4 to 4.5 hours. The potential to activate ODC decreases progressively as cells leave G_1 phase and pass through S phase of the cell cycle.

GABA-Uptake in Primary Cultures of Neonatal Rat Cortex

Kathleen L. Hauser
Ciba-Geigy AG, CH–4002 Basel

Monolayer cultures of cells from newborn rat cerebral cortex accumulate γ-aminobutyric acid (GABA) from the incubation medium. Uptake of GABA is dependent on the temperature and time of incubation and requires sodium ions in the medium. Kinetic studies at 25 °C show that both high affinity and low affinity uptake mechanisms are present. The K_m for the high affinity uptake (2.5×10^{-6} *M*) is similar to the value published for cortex slices from newborn rats (5×10^{-6} *M*; G. A. R. Johnston and L. P. Davies, J. Neurochem. *22*, 101–105, 1974). GABA uptake can be inhibited by 2,4-diaminobutyric acid and β-alanine, as well as by phenothiazines and other neurotransmitter uptake inhibitors. At present, it has not been possible to determine whether GABA is taken up into neurons or glial cells, or both. Various cell types are seen in these cultures, including cells with long processes and typical neuronal morphology. Radioautographs of cultures incubated with ^{3}H-GABA show many of these cells to be heavily labeled, on a background of lightly labeled cells without processes.

Translation in vitro of Polyoma Virus Specific (16 and 19 S) m-RNA

K. von der Helm and N. Acheson
ISREC, CH–1011 Lausanne

Late polyoma-specific messenger RNA consists of two size classes, with sedimentation coefficients of 16 S and 19 S. Hybridization studies (Acheson and Beard, these abstracts) have shown that both 16 S and 19 S RNAs are complementary to the same part (60%) of the L strand of polyoma DNA, while 19 S RNA also contains sequences complementary to 40% of the E. strand. To further characterize these RNAs, we have begun to translate them into proteins in a cell-free system of wheat germ. Cytoplasmic RNA from polyoma-infected mouse cells was passed over an oligo-dT cellulose column, poly A-containing RNA (85% of the total polyomaspecific RNA) was eluted and sedimented twice consecutively on a sucrose gradient. The 16 S RNA stimulated the synthesis of a polypeptide with the same electrophoretic mobility as the major capsid protein of polyoma virus, in addition to numerous other polypeptides. The 19 S RNA also stimulated the synthesis of a polypeptide of the same mobility, but to a much lower level.

Distribution of Glutamate Decarboxylase and Choline Acetyltransferase in the Pigeon Optic Tectum

H. Henke and F. Fonnum
Brain Research Institute, CH–8029 Zurich and Norwegian Def. Res. Establ., Kjeller (Norway)

The optic tectum was cut into 20-μ sections parallel to the surface. The sections were freeze-dried and used for microdissection; sections stained for acetylcholinesterase were used as controls. It was possible to prepare each of the 15 laminar layers of the optic tectum separately. The activity of glutamate decarboxylase (GAD, L-glutamate 1-carboxy-lyase, EC 4.1.1.15) and choline acetyltransferase (acetyl-CoA-choline O-acetyltransferase, EC 2.3.1.6) was measured in each layer. GAD is an enzyme typical for GABA-containing nerve terminals, choline acetyltransferase is a marker enzyme for cholinergic nerve endings. GAD activity was localized mostly in layers 1–7, peaking in layer 4 and 5. Choline acetyltransferase activity overlapped with the acetylcholinesterase staining pattern. Less than one third of the activity was found in layers 1–7 but the highest activity was localized in layers 3 and 5. Neither GAD nor choline acetyltransferase activity was found in the optic nerve.

Supported by European Training Program and SNSF, grants 3.124.73 and 3.823.72

A New RNA-Dependent RNA Polymerase Activity in Influenza Virions

M. Horisberger
Ciba-Geigy AG, CH–4002 Basel

A new RNA polymerase activity has been detected in influenza virions. Its assay requires the presence of ribosomes, Mg^{2+} and salt at low concentration (30 m*M* KCl). There is no activity in the absence of ribosomes. Ribosomes washed with 1 *M* KCl are still active. The effect of ribosomes can be partly mimicked by Mn^{2+} and salt at high concentration (200 m*M* KCl) (usual assay conditions described in the literature). Histones, protamine or RNA primers cannot substitute for ribosomes. The function of ribosomes could be to displace an inhibitory protein or to change the structure of the ribonucleoprotein by binding to the RNA. The study of the product (base composition, sedimentation properties, hybridization) indicates that the new RNA polymerase activity of the virion is probably responsible for the primary transcription of the viral genome during infection.

DNA Polymerases in Brain Neuronal Nuclei: Increased Activity During Learning

U. Hübscher, A. Knüsel, Rita Heidelberger and C. C. Kuenzle
Institut für Pharmakologie und Biochemie, Veterinärmedizinische Fakultät der Universität Zürich, CH–8057 Zürich

Neuronal nuclei were isolated from the cerebral cortices of trained and control rats and DNA polymerase activities determined using various templates. The training apparatus consisted of a large cage designed to provide a maximum of social and intellectual stimulation, while at the same time requiring true learning for survival. Thus, in order to get access to food and water, animals were

forced to balance on ropes, select and open appropriate gates and traverse a maze placed in front of the food dispenser. Twenty-five rats were maintained in this 'superenriched environment' for 10 days (postnatal days 21 to 30). DNA polymerase activities were observed with all templates tested (native and denaturated DNA, poly d(A-T), poly(rA) · $(dT)_{10}$. Most important, however, was the finding that DNA polymerase activities were significantly greater in trained as compared to control rats when poly d(A-T) was used as a template. No such effect was evident with the other templates. The significance of this finding with respect to learning processes remains to be elucidated.

Evidence for a Glycinergic Nucleus in the Midbrain of the Pigeon

S. P. Hunt, H. Künzle and M. Cuénod
Brain Research Institue, CH–8029 Zurich

Neurons within the central nervous system can be classified according to their abilities to take up relevant transmitter substances or their precursors (Hökfelt, T. and Ljungdahl, A., Exp. Brain Res. *14*, 354–362, 1972). Using this approach and injecting tritiated glycine into the optic tectum of the pigeon we have found cell bodies distant from the tectum, within the nucleus isthmi, pars parvocellularis (IPC) were heavily labelled. IPC neurons were further found to selectively take up glycine and from histochemical and retrograde marking techniques, to receive an acetylcholinesterase-positive projection from layer 11 of the tectum. IPC projects back to the tectum notably to layer 5 (Karten, H. J. pers. comm.) a region also receiving a heavy projection from the retina. Removal of the retina and the subsequent analysis of IPC projections with autoradiographic techniques suggested that there had been a loss of structure of the terminal arborisation within lamina 5 accompanied by an increase in the intensity of the projection within the retinally deprived lamina 5.

Supported by SNSF, grants 3.124.73 and 3.823.72

The Reiteration Frequency of Mammalian Histone Coding Sequences

E. Jacob and M. C. Wilson
Institut für Molekularbiologie II der Universität CH–8057 Zürich

Histone mRNA has been isolated from two mammalian cell lines, HeLa and mouse L 929 cells. These 8–10 S RNA fractions lack poly (A), require continued DNA synthesis for their appearance in the cytoplasm, cross hybridize to purified sea urchin (*Psammechinus miliaris*) histone DNA, and may be translated in vitro into histone protein. To estimate the reiteration frequency of the mammalian histone coding sequences, probes of unfractionated histone message and electrophoretically distinct histone mRNA subspecies, or histone messenger sequences further selected by hybridization to sea urchin DNA were hybridized with their homologous DNAs. Comparison with kinetic standards of known reiteration (human ribosomal RNA, poly (A) containing mRNA and E. coli cRNA) has indicated that the histone genes are represented 20–50-fold in the human and murine haploid genomes.

Supported by SNSF, grant 3.8630.72 SR

Release of Phosphoglucomutase from *E. coli* by Osmotic Shock or Spheroplast Formation

G. R. Jacobson and J. P. Rosenbusch
Biozentrum der Universität, CH–4056 Basel

Phosphoglucomutase catalyzes reversibly the conversion of glucose-1-P to glucose-6-P. In *E. coli* this enzyme has a role in both the glycolytic and cell wall biosynthetic pathways. In monitoring the release of enzymes from *E. coli* after osmotic shock, we have observed that 60–70% of the total phosphoglucomutase activity is reproducibly released when the plasmolyzed cells are shocked by ice-cold water, while less than 10% of either of the intracellular enzymes β-galactosidase or glucose-6-P dehydrogenase is liberated. More phosphoglucomutase activity is released from spheroplasts (up to 40% of the total) than can be accounted for by the low level of general cell lysis, while less than 6% of the total activity could be found in the envelope fraction from cells lysed by agitation with glass beads. The identity of the activity we measured with that of the purified *E. coli* enzyme was established by its requirement for Mg^{+2}, its stimulation by mercaptan and its sensitivity to *p*-mercuribenzoate. These results suggest that phosphoglucomutase in *E. coli* is localized at or near the surface of the cell.

Supported by SNSF, grant 3.0920.73

Metabolic Differences in *Bacillus stearothermophilus* Grown at 55 °C and 37 °C

L. Jung, R. Jost, E. Stoll and H. Zuber
Institut für Molekularbiologie und Biophysik der ETH, CH–8049 Zürich

Bacillus stearothermophilus was adapted to grow at 55 °C and 37 °C in a complex medium with almost equivalent yields in cell mass. In both temperature ranges the maximum specific growth rates μ_{max} were identical. Cellular extracts of this bacterium showed remarkable differences in the activity levels of several enzymes depending on the respective growth temperature. High activities of alcohol-, lactate- and glyceraldehyde-3-phosphate-dehydrogenase were found in 55°-cultures and the respiratory quotient exceeded 1.0. Enzymes of the TCC-cycle and the respiratory chain were of low activity. Pyruvate, lactate, and ethanol were formed. Succinate- and isocitrate-dehydrogenase activity was high in 37°-cultures and the respiratory quotient was below 1.0. No alcoholdehydrogenase was detected in cells from mesophilic cultures. Lactate and pyruvate were readily metabolized as second carbon sources. Under anaerobic conditions at 55 °C enzyme activities, metabolites and μ_{max} were the same as under aerobic conditions whereas at 37 °C no growth was possible when oxygen was excluded.

Radioactively Labeled Spermatozoa and the Cytology of Fertilization in Mice

T. P. Keneklis and N. Odartchenko
Institut suisse de recherches expérimentales sur le lancer CH–1011 Lausanne

Fertilization in mice is known, on morphological grounds, to belong to the classical *Ascaris* type: two distinct parental groups of chromosomes form without

fusion of pronuclei and thus no true zygote nucleus. The association of paternal and maternal genomes characteristic of biparental heredity has been studied autoradiographically following fertilization of mouse ova by spermatozoa which were previously labeled with tritiated thymidine in their DNA, or with tritiated arginine in their arginine-rich histores. Eggs collected at very early stages during or shortly after fertilization reveal a sharp separation between post-pronuclear parental chromosome groups at initial stages of chromatin condensation. Later, when observed on the metaphase plate of the first cleavage mitosis, chromosomes originating from each parent are not randomly intermixed as classically expected. This is expressed in interphase chromatin later in cleavage as a gonomeric separation between paternally and maternally derived chromatin. Only labeled paternal DNA at the exclusion of paternal arginine-rich histones appears to participate in cellular events beyond swelling of the sperm head shortly after penetration into the egg, as judged by an early loss of all tritiated arginine-labeled material.

A Simple Method for the Preparation of Cell Cultures for Ultramorphological Investigations

H. Kuhn
F. Hoffmann-La Roche & Co., AG, CH–4002 Basel

The preparation of cell cultures for ultramorphological investigations is often difficult because of the tight adhesion of the culture layer to the supporting medium, and also the delicate nature of the cell layer. A simple method is described which allows the preparation of cell cultures for electron microscopy in plastic Falcon® dishes. The cultures are fixed, then dehydrated in ethanol (up to 100%) in situ. Immediately before the change to propylene oxide, 3–5 mm squares are cut in the culture layer, and, after adding the propylene oxide, the dishes are carefully shaken. Because of the effect of propylene oxide in dissolving the plastic material, the culture squares separate from the Falcon® dishes. They are immediately decanted into a glass vessel, washed with propylene oxide and embedded in Epon. Using this method, the culture layer is completely surrounded by Epon, so that the difficulties usually encountered in preparing ultrathin sections do not arise. Areas of interest in the culture layer may be selected by carefully cutting the squares upon microscopic examination. Moreover, with this method it is not necessary to have confluent layers further it is also possible to prepare growing explants for electron microscopy.

Tuberal DA Neurons and Tanycytes: Response to Electrical Stimulation and Nicotine

W. Lichtensteiger and J.G. Richards
Pharmakologisches Institut der Universität, CH–8006 Zürich, und F. Hoffmann-La Roche & Co., AG. CH–4002 Basel

Microfluorimetric observations on the effect of brain stimulation on the tuberoinfundibular dopamine (DA) neurons indicate that different patterns of anterior pituitary secretion can accompany similar reponses of this DA system. Such a modulatory action might be explained, i.a., by an effect on non-neuronal elements at the neurohaemal contact zone of the median eminence. This possibility was studied on oestrogen-progesterone-pretreated or untreated ovariectomized rats. The animals were subjected to treatments known to elicit fast bilateral reactions in the tuberal DA neurons, i.e., medial preoptic or medial amygdaloid stimulation (15 min) or injection of nicotine (1mg/kg, 20 min). The fluorescence intensity of tuberal DA neurons was then measured on one side, whereas on the other side the proportion of the neurohaemal contact zone covered by tanycytes was determined by electron microscopic examination of ultrathin sections of the remaining half of the median eminence. Although differences between steroid-injected and untreated rats were observed, the overall changes were consistent: the response of the DA neurons, an increase in mean fluorescence intensity, was correlated with the proportion of capillary surface covered by tanycyte processes. This suggests that the tuberal DA neurons may modulate anterior pituitary function partly through an action on these elements.

Microscopic Observation of Liberation of Fluorescent Compounds from 5HT Organelles of Live Blood Platelets

H. P. Lorez, M. Da Prada, W. Boguth, H. R. Baumgartner and A. Pletscher
F. Hoffmann-La Roche & Co. Ltd, CH–4002 Basel

Subcellular distribution studies with blood platelets of rabbits, rats and guinea pigs exposed to mepacrine, acridine orange or daunomycin in vitro and in vivo showed that these fluorescent compounds markedly accumulated in the 5-hydroxytryptamine (5HT) storage organelles. Fluorescence microscopy of live platelets loaded with the compounds revealed fluorescent granular elements of about 400 nm in diameter similar in size and appearance to 5 HT organelles isolated from these platelets. In rabbits the average number of the fluorescent granules per platelet (approx. 17) was of the same order as that of the 5 HT organelles previously estimated by electron microscopy. On irradiation for 10 or more seconds with violet-blue light, the loaded platelets started to emit subsequent flashes. These were characterized by a marked increase for a few seconds of the fluorescence of the whole platelets including their pseudopods followed by fading. According to microfluorimetric measurements, 10–25 flashes could be emitted by a single rabbit platelet. Each flash was probably due to the liberation of the fluorescent compound from one 5 HT organelle.

Growth Control and the Mitotic Cell Surface Change

R. J. Mannino, Jr., and M. M. Burger
Biozentrum der Universität, CH–4056 Basel

A non-agglutinating lectin preparation, Succinyl-Con A, inhibits the growth of untransformed 3T3 mouse fibroblasts in a concentration dependent, non-toxic, reversible manner. Succinyl-Con A inhibited cells accumulate in the G_1 phase of the cell cycle and by several parameters appear identical to a density-inhibited monolayer. Growth inhibition requires specific binding of Succinyl-Con A to the cells and not to a necessary factor in the growth medium. The final density reached by a population of cells in the presence of Succinyl-Con A is independent of the initial (i.e. plating) density; this suggests that both cell-cell interactions and Succinyl-Con A-cell interactions are necessary for the termination of growth. Succinyl-Con A inhibits growth only through an interaction with cells in the mitotic and/or early G_1 phase of the cell cycle.

Supported by SNSF, grant 3.1330.73

Antigen-Binding Capacity of Cytotoxic T Lymphocytes (CTL)

A. Matter and D. Snary
F. Hoffmann-La Roche & Co. AG, CH-4002 Basel, and NIMR, Mill Hill, London (England)

B cell antigen-binding measures specific and clonally expressed surface receptors whose function is inhibited by anti immunoglobulins. T cell antigen binding is not clear: In contrast to B cells the receptors have a lower affinity or are less numerous, they require 37° to function and their structure is still under debate. In contrast to these precursor cells the differentiated CTL show a specific and mechanically robust binding as a prerequisite for the death of the target cell. CTL react with serologically defined (SD) antigens of the targets. Membrane fragments with demonstrably well-preserved SD antigens inhibit at high concentration the function of CTL nonspecifically whereas at low doses there is a slight but specific enhancement. EM autoradiography of CTL incubated under appropriate conditions with surface labelled (^{125}I) intact target cells shows a high percentage of CTL attached to target cells. There is no transfer of label inside the CTL. The number of CTL that bind under identical conditions iodinated membrane fragments is lower; quite often label is found inside CTL within small vacuoles and lysosomes. These discrepancies are unexplained so far.

Qβ RNA Elongated with a 3′ Terminal Oligo C Tract

P. Mekler and M.A. Billeter
Institut für Molekularbiologie I, Universität Zürich, CH-8049 Zürich

To study the requirements for recognition of Qβ RNA by Qβ replicase we have prepared Qβ RNA with a 3′ terminal oligo C tail. By incubating Qβ replicase with Qβ RNA and ^{32}P-GTP as the only substrate, poly G was synthesized (presumably by slippage). This upon addition of non-radioactive ATP, was elongated to the sequence pppGpGpG (pG) $_n$pApGpGpApGpApGpApGpGp G_{OH} at which point CTP is required for further elongation. After removal of substrates all 4 unlabeled triphosphates were added to complete the minus strands. Analysis of the RNase T_1 digestion products revealed that the modified minus strand contained about 10 G residues more than normal minus strands ($n \approx 10$ as compared to $n = o$). After hybridization with poly C up to 15% of this RNA bound to poly I-Sephadex. Plus strands synthesized on these minus strands were analyzed by T_1 fingerprinting: almost no normal 3′ terminal oligonucleotide was detected but instead a new oligonucleotide appeared, containing about 9 residues more than the normal 3′ terminal oligonucleotide. If the modified minus strands were used for extensive replication only about 10% of the plus strands produced at the end of the cycles contained a modified 3′ end, suggesting that the modification allows less efficient replication.

Supported by SNSF and the Jane Coffin Childs Fund

Separation of Repetitive and Non Repetitive sequences of HeLa Cells HN-RNA and their Relationship with Poly-A Attached Messenger

Marialuisa Melli
Institut für Molekularbiologie II der Universität, CH-8057 Zürich

It has been shown that the reiterated high molecular weight fraction of hetero-geneous nuclear RNA of HeLa cells hybridizes to clusters of repeated sequences in the DNA. This finding suggests that the reiterated sequences of Hn-RNA might be also clustered in a structure which is somewhat in contrast with some of the current hypothesis. To distinguish between clustering and fine interspersion of repeated sequences, the repetitive and not repetitive sequences of the Hn-RNA were separated with the technique of RNA-DNA habridization. It was found that Hn-RNA in which the molecular weight after hybridization was ranging from $0.15\text{-}3 \times 10^6$ Daltons, hybridizes to approx. 50%, under conditions which allow the hybridization of reiterated RNA. The hybridized and not hybridized fractions of the RNA were collected and hybridized again in excess DNA. The results were as follows: the fraction of RNA eluted from the hybrid shows a kinetic which covers several orders of magnitude and high enrichment for reiterated sequences. This hybrid is also very sensitive to RNAse digestion. The hybridization of the sequences which did not anneal in the first reaction is instead very uniform, with a kinetic consistent with the presence of essentially non repetitive sequences. The hybrid shows low RNAase sensitivity. The existence of separate sequences. containing essentially reiterated sequences is consistent with clustering of repetitive sequences.

Supported by SNSF, grant nº 3.8630.72 SR

The Binding Sites of Qβ Replicase on Qβ RNA

F. Meyer, H. Weber, H.J. Vollenweider and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, and Institut für Zellbiologie ETH, CH-8049 Zürich

Earlier work (Weber et al., Experientia *30*, 711, 1974) had shown that, depending on reaction conditions, Qβ replicase is able to bind specifically to either or both of two regions on Qβ RNA. One site has been identified by sequence determination with the beginning of the coat cistron. It is probably responsible for the translational repressor action of Qβ replicase (Weber et al., Nature New Biol. 237, 166, 1972). The second site, which is thought to be essential for the recognition of Qβ RNA as template for its replicase, has been characterized by three non-overlapping RNA segments originating from a region situated between 2,100 and 2,700 nucleotides from the 5′ end. We now report the complete nucleotide sequences of these three fragments which have chain lengths of 164, 60 and 21 nucleotides. The fragments do not overlap with the known sequences at the ribosomal binding site of the replicase cistron which are located nearby. Electron microscopic visualization of Qβ replicase-RNA complexes (see also H. J. Vollenweider et al., these abstracts, 1975) also suggests a two-site interaction between Qβ replicase and RNA.

Supported by SNSF and the Jane Coffin Childs Fund

Association with Ribosomal Subunits and with Viral Protein of in Vitro Synthesized Semliki Forest Virus (SFV) RNA

M. R. Michel
Sloan-Kettering Institute, New York, N.Y., USA., and Swiss Serum and Vaccine Institute, CH–3001 Berne

Using an extract from infected cells which catalyzes the in vitro synthesis of all species of SFV RNAs, it is found that none of the newly synthesized virus-specific RNAs are free, but all are associated with structures of varying size, separable from each other by differential centrifugation. We have concentrated on the structures to which the in vitro synthesized 26 S SFV RNA is associated. This RNA is the major viral messenger RNA in SFV-infected cells. The 26 S RNA synthesized in vitro is found associated with nucleoproteins in ribonucleoproteins (RNPs) sedimenting between 40 and 60 S. These RNPs, after fixation with glutaraldehyde, have heterogeneous densities in CsCl ranging from 1.37 to 1.50 g/ml. In the particles at a density of 1.37 g/ml the 26 S RNA is associated with viral nucleocapsid protein. This RNP may be the precursor to the viral nucleocapsid. In the RNPs with density greater than 1.37 g/ml the in vitro synthesized 26 S RNA is bound to ribosomal subunits. These RNPs (> than 1.37 g/ml) are thought to be intermediates in the proces of formation of polyribosomes.

DNA Synthesis Rate vs. Thymidine Incorporation in Synchronous Mammalian Cells

G.G. Miller and R. Schindler
Pathologisches Institut der Universität, CH–3010 Bern

The incorporation of labeled thymidine (TdR) in concentrations ranging from 5×10^{-8} to 10^{-5} *M* was compared with the true rate of DNA synthesis in synchronous murine mastocytoma cell cultures. The DNA synthesis rate was measured as moles TdR incorporated per 10^6 cells per h in cultures where endogenous TMP production was blocked by amethopterin, and was compared with incorporation of ^{3}H-TdR plus TdR in the absence of the inhibitor. At all TdR concentrations studied, maxima and minima of ^{3}H-TdR incorporation occurred at the same time as those of DNA synthesis rate. Using 5×10^{-8} TdR, the difference between maximum and minimum incorporation was, however, smaller than that of DNA synthesis rate and that at higher precursor concentrations. CsCl gradient centrifugation analysis indicated that newly synthesized DNA of cells in early S period had a lower adenine-thymine content than that of cells in late S period.

Supported by SNSF, grant 3.923.72

In vivo Assembly of Tight Junctions

R. Montesano, D. Friend, A. Perrelet and L. Orci
Institut d'Histologie et d'Embryologie, Ecole de Médecine, CH–1211 Genève 4

Bile canaliculi of 14-day embryonic rat liver have some fully-developed tight junctions (TJs). To study the intramembranous events during their formation, we freeze-fractured glutaraldehyde-fixed livers of embryos removed at various intervals. The following observations are presented in the sequence in which we envisage them. Particles aggregate in otherwise particle-deficient areas of plasma membrane near the luminal surface. Lines of contiguous particles branch peripherally from the edges of the irregular arrays, frequently forming a network joining other particle-islands. Even at this stage complementary B-fracture faces contain narrow furrows rather than rows of pits, distinguished the linear aggregates on the A-face as forming tight rather than gap junctions. Short segments of the linear arrays become beaded rows which merge with smooth ridges, now identifiable as parts of discontinuous TJs. With the continuing confluence of beaded and smooth ridge segments, mature TJs become fully appreciable. As noted previously, the mature TJ contains gap junctions within its domain. Our observations favor the interpretation that the *de novo* assembly of TJs consists of the 'fusion' of separate particles into beaded ridges which in turn become confluent and transformed into continuous smooth ones.

Supported by SNSF, grants 3.8080.72 and 3.0311.73

In Vitro-Induction of Endogenous C-Type Viruses in Lymphoid Cells

Ch. Moroni and G. Schumann
Friedrich-Miescher-Institut, CH–4002 Basel und Ciba-Geigy AG, CH–4002 Basel

Lipopolysaccharide induces C-type viruses in short-term spleen cultures of mice. Concanavalin A is also inductive, but only in combination with 5-bromo-2'-deoxyuridine (BrdU). In contrast, phytohemagglutinin is not inductive, with or without BrdU. Induced viruses band at 1.16 g/cm^3, contain reverse transcriptase and can be found as budding particles on the surface of induced cells. In vitro induction of virus depends on: 1. cell type, 2. mitogen specificity, 3. genotype.

Differential Chromatin Fluorescence and its Relationship to the Cell Cycle

H. Müller and Gertrude C. Moser
Abteilung Genetik, Universitäts-Kinderklinik Basel, CH–4005 Basel, and Dept. of Genetics, Albert Einstein College of Medicine, New York, N.Y. 10461 (USA)

Interphase nuclei stained with quinacrine dihydrochloride show different fluorescent patterns according to their position in the cell cycle. In general, a decrease in fluorescent intensity occurs during the G1 phase and an increase begins in the DNA synthesis period. This observation primarily based on the analysis of human embryonic fibroblasts and synchronized HeLa cells (S_3) provides further evidence for conformational changes of the chromatin that span the entire interphase. The fluorescent characteristics were used as cytological parameters to study the behavior of constitutive heterochromatin in relation to the cell cycle and to analyze mammalian temperature sensitive cells (BHK 21, ts AF8 and others) which are assumed to carry mutations in the cell cycle regulating mechanisms.

Quantitative Determination of BB-Creatine Kinase in Crude Extracts Containing all Three Isoenzymes

J. C. Perriard, E. R. Perriard und H. M. Eppenberger
Institut für Zellbiologie, ETH, CH–8049 Zürich

Purification of rabbit antibodies against chick MM creatine kinase (MM-CPK) was achieved by immunoadsorption of antisera on MM-CPK immobilized on Se-

pharose 4B. Highly purified antibodies were eluted with acidic buffers or with high ionic strength and subsequently covalently coupled to Sepharose 4B beads. This material retained its immune reactivity and could adsorb more than 1 mg MM-CPK/ml packed adsorbent. All the BB-CPK activity of an isoenzyme mixture was observed in the flowthrough of such an immunoadsorption column, while the MM and MB species of the enzyme were quantitatively retained. This single step procedure provided an accurate method for determining BB-CPK activity in crude extracts from chick muscle and cultures of myogenic cells. Bound isoenzymes could be eluted in highly purified form from the column with high ionic strength at neutral pH and regained up to 80% of their activity after exhaustive dialysis against low ionic strength buffer. Similar methods are being developed for the determination of MM-CPK with anti BB-CPK immunoadsorption columns.

Supported by SNSF, grant 3.8640.72 and the Muscular Dystrophy Association of America

SEM Investigations on Physiological Cell Death in the Chick Embryo Heart

T. Pexieder
Institut d'Histologie et d'Embryologie de l'Université, CH–1011 Lausanne

The physiological cell death in bulbar cushions of normal embryonic hearts is characterized by the presence of numerous prenecrotic cells with cytosegresomes. Later on many macrophages appear. Even if TEM investigations have shown a exocytosis of cytosegresomes into the intercellular spaces and a digestion of ingested materials in phagosomes, their further fate was not known. Chick embryonic hearts between the fourth and sixth day of incubation were investigated by a S4-10 Stereoscan scanning electron microscope after a glutaraldehyde microperfusion, microdissection, osmium tetroxyde postfixation, graded ethanols dehydration and critical point drying from Freon 13. The friction surfaces of bulbar cushions were found to be covered by a continuous layer of flat squamous-like endocardial cells with some microvilli. On the other hand on cushions' borders, in the 'dead water' zones, exocytosis of cytosegresomes into the bulbar lumen could be observed. In these regions the endocardial coverage was frequently not continuous and there were, especially at the cell limits, numerous dehiscences communicating with the cardiac jelly. In some cases the macrophages were seen entering the blood stream in the neighbourhood of such holes.

Supported by SNSF, grant 3.8440.72

A New Cleavage Assay for Restriction Endonucleases

J. Reiser and R. Yuan
Biozentrum der Universität, CH–4056 Basel

Four different assays have recently been used for restriction enzymes. Both sucrose gradient and gel electrophoresis assays do not give quantitative values whereas transfection is very time consuming. The standard filter-binding assay is based on the formation of an enzyme-DNA complex which can then be trapped on nitrocellulose filters. However, the restriction endonucleases from *E. coli* (P1) and *E. coli* (15) do not show such binding, and a new cleavage assay that was quick and quantitative had to be developed. The basis for the assay is the observation of Saucier and Wang (Biochem. *12*, 2755, 1973) that circular λ DNA binds to nitrocellulose filters under specified conditions, whereas linear λ DNA will pass through. For the cleavage assay linear λ DNA is circularized to form hydrogen-bonded circles (Hershey circles). The circles are then exposed to the enzyme in a complete reaction mixture; the reaction is stopped by SDS and filtered rapidly through nitrocellulose filters without any subsequent washing. The filters are dried and counted in a liquid scintillation counter. The assay is specific (as checked with modified DNA and dependence on cofactors), linear and quantitative. It has been used successfully in the purification of the restriction endonucleases from *E. coli* (P1) and *E. coli* (15).

Supported by SNSF, grant 3.2530.74

Supra-Ependymal Serotonergic Nerve Fibres in Rat Brain: Selective Demonstration, Distribution and Origin

J. G. Richards, H. P. Lorez and L. Pieri
F. Hoffmann-La Roche & Co. Ltd., CH–4002 Basel

Recent investigations by fine structural cytochemistry and fluorescence histochemistry have identified serotonergic nerve fibres on the ependymal surface of cerebral ventricles in the rat. The selective ultrastructural demonstration of serotonin in these fibres was achieved by fixation with a modified chromaffin reaction: the highly electron dense cores of small (50 nm) and large (100 nm) vesicles present in varicose regions of these nerves disappeared after reserpine and para-chlorophenylalanine but persisted after α-methyl-para-tyrosine. In correlation, a formaldehyde-induced yellow fluorescence, specific for indolealkylamines, was observed supra-ependymally which reacted similarly to the above-mentioned drugs and was additionally intensified after nialamide and reserpine + nialamide. Serotonergic nerve fibres were observed throughout the lateral ventricles and interventricular foramina, in parts of the third ventricle (e.g. nucleus medialis habenulae), throughout the aqueduct and the floor of the fourth ventricle. Investigations of the effect of intracerebral injection of 5,6-dihydroxytryptamine and of electrolytic lesions placed in the posterior hypothalamus, suggest that, in the prosencephalon, the supra-ependymal nerve fibres originate from uncrossed axons of the medial forebrain bundles.

Effects of Aldosterone on Poly(A)-rich RNA in Toad Bladder Epithelium

B. C. Rossier, P. A. Wilce and I. S. Edelman
University of California, San Francisco (USA), and Institut de Pharmacologie de l'Université, CH–1011 Lausanne

Previous studies revealed that aldosterone increased the incorporation of ^{3}H-uridine into a 'mRNA' like fraction. This effect was mineralocorticoid: it was not elicited by cortisol and was antagonized by spironolactone. In the present study, paired hemibladders of the toad (*Bufo marinus*) were incubated in vitro with or without aldosterone (7×10^{-8} *M*) for a period of 14 hours. Na^+ transport was monitored simultaneously by the short-circuit

current (SCC) technique of Ussing. Control hemibladders were continuously labelled with ^{14}C-uridine and aldosterone treated hemibladders with ^{3}H-uridine of same specific activity. Epithelial cells from both incubations were mixed and cytoplasmic RNA extracted in one pool by a phenol-sodium dodecyl sulfate technique. Poly(A)-rich RNA was separated by oligodeoxythymidylate cellulose column chromatography and analyzed on 5–20% sucrose density gradients. ^{3}H/^{14}C dpm ratio was determined in each of the 27 fractions collected. Aldosterone increased SCC 2.26 times control values ($p < 0.006$) and induced a major peak of the ^{3}H/^{14}C dpm ratio profile (peak ratio = 2.2 vs baseline ratio = 0.8 – 1.0). These results indicate that aldosterone induced the synthesis or (and) increased the stability of a few classes of Poly(A)-rich RNA.

Supported by USPHS HL-06285, US Nat. Kidney Found. and SNSF, grant 3.2630-74

Complexity of Cytoplasmic RNA Measured by Hybridization of Poly-Adenylated RNA to Complementary DNA

G. U. Ryffel and B. J. McCarthy
Zoologisches Institut, Universität Bern, CH–3012 Bern

The kinetics of hybridization of polyadenylated RNA from mouse L-cells with complementary DNA synthesized with reverse transcriptase revealed three frequency classes of polyadenylated RNA: 5%, 45% and 50% of the total polyadenylated RNA represented about 3, 300 and 7,600 different RNA sequences of 6×10^5 Daltons, respectively. The complementary DNA synthesized with L-cell polyadenylated RNA as template hybridized efficiently with RNA from different mouse tissues, indicating that most species of L-cell RNA in the high- and middle-frequency class are present in all mouse tissues. Kinetics of hybridization of complementary DNA synthesized with cytoplasmic polyadenylated brain RNA as template suggested a higher complexity for brain RNA. 35% of this brain cDNA failed to hybridize with L-cell RNA. This complementary DNA fraction, isolated by hydroxylapatite chromatography, represented approximately 11,000 different RNA sequences specific for the brain.

Site-Directed Mutagenesis: Effect of an Extracistronic Mutation in Phage Qβ RNA

D. L. Sabo, E. Bandle and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich

The mutagenic analog N^4-hydroxyCMP was inserted into position 15 of the Qβ minus strand by stepwise enzymatic synthesis and plus strands were prepared using such minus strands as template. 55% of the plus strands had a G → A transition in the expected extracistronic position (cf. J. Mol. Biol. *89*, 255, 1974). Spheroplasts were infected with a 1:1 mixture of wild type and mutant RNA. All resulting clones examined were wild type, suggesting that the mutation was lethal, even though the mutant RNA was a more efficient template for Qβ replicase in vitro than wild type. To prove that lethality is due to the extracistronic mutation it is necessary to revert the mutated site and show recovery of infectivity. Mutant RNA was separated from wild type by using the plus-strand mixture to direct stepwise synthesis of the minus strand. The chain termination analog, 3′-desoxy-3′-amino-AMP was incorporated into position 16 of the wild type product; on further elongation only mutant minus strands were completed and these were used to prepare mutant plus strands. Specific reversion was accomplished by again incorporating N^4-hydroxyCMP into position 15 of the minus strand. The plus strands made on this template were about 30% wild type with regard to the extracistronic mutation; the infectivity is under investigation.

Supported by SNSF and the American Cancer Society

Characterization of Polyoma m-RNAs

C. Salomon and H. Türler
Département de Biologie Moléculaire, Université de Genève, CH–1211 Genève 4

The virus-specific RNAs extracted at various times after infection from the cytoplasm of polyoma virus-infected mouse kidney cells were characterized by sedimentation in sucrose gradients and by hybridization to polyoma DNA fragments obtained by the restriction enzyme of *Hemophilus parainfluenzae II* (in collaboration with Prof. B. Allet). Three RNA preparations were characterized (i) 'early RNA' synthesized from 11–14 h p.i., (ii) 'late RNA' synthesized from 27–30 h p.i. and (iii) 'late FdU-RNA' synthesized from 27–30 h p.i. under conditions where cellular and viral DNA replication was inhibited by 5-fluorodeoxyuridine. Early 19-S RNA hybridizes to the early region, preferentially to fragment 2. Small amounts of late RNA may also be present. 'Late RNA' consists of more than one size class (16 S and 19 S) and hybridizes to the late region, preferentially to fragment 1. Small amounts of early 19-S RNA are also present. Late FdU-RNA con-sists of a mixture of all kinds of polyoma-specific RNA found early and late in infection.

Supported by SNSF, grant 3.0790.73

Polyoma- and SV40-Specific Messenger RNAs

C. Salomon and R. Weil
Département de Biologie Moléculaire, Université de Genève, CH–1211 Genève 4

Earlier results from this laboratory led to the conclusion that polyoma- and SV40-specific 'early 19-S messenger RNAs' are the contiguous transcript of the early region of the viral DNAs and that they carry most of the genetic information for induction of lytic and abortive infection and for initiation and maintenance of the 'transformed phenotype'. Judged from their mol. wt. (7×10^5 Daltons) 19-S mRNAs contain about 40% of the genetic information of the virus and thus should be able to specify a polypeptide (s) with a mol. wt. of about 7×10^4 Daltons. To reconcile the paradox of the small amount of genetic information with the broad spectrum of biological effects we suggested, as working hypothesis, that early 19-S mRNAs direct synthesis of a novel type of virus-specific regulatory protein(s) ('pleiotropic effectors') which, among other, trigger cell proliferation. Recently Grässmann et al. obtained the first direct experimental evidence in support of this hypothesis. We will report on attempts to isolate intact 'early' and 'late' polyoma- and SV40-specific mRNA molecules and, in collaboration with P.-F. Spahr, to translate them in vitro.

Supported by SNSF, grant 3.0790.73

Enzymes of Thymidine Nucleotide Metabolism as Related to DNA Synthesis Rate

J. C. Schaer, U. Maurer and R. Schindler
Pathologisches Institut der Universität, CH-3010 Bern

In cultures of a murine mastocytoma, the average rate of DNA synthesis, as calculated from ^{3}H-thymidine incorporation into DNA in the presence of amethopterin, was found to be equivalent to 0.5×10^{-15} mole thymidine/cell $\times$ h. Comparative activities of enzymes involved in thymidine nucleotide metabolism were determined in cell extracts, using respective substrates at a concentration of 4×10^{-5} *M*. Lowest activities were obtained for TMP synthetase (0.53×10^{-15} mole/cell $\times$ h) and for DNA polymerase (0.23×10^{-15} mole/cell $\times$ h), whereas activities of thymidine kinase, TMP kinase and TDP kinase were significantly higher. TMP synthetase and DNA polymerase may, therefore, be considered as rate-limiting enzymes for DNA synthesis. In synchronous cultures, however, variations with time of these two enzyme activities were not correlated with those of DNA synthesis rate, indicating that in the model system used, none of the enzymes studied has a rate-limiting function with respect to DNA synthesis.

Supported by SNSF, grant 3.923.72

Sequence Determination of a 6 sRNA (WSI) Arising in Non-Templated Qβ Replicase Reactions

W. Schaffner and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, CH-8049 Zürich

In the absence of added template Qβ replicase synthesizes a mixture of different short RNAs with a sedimentation coefficient around 6 s. The main species was isolated and the complementary strands separated by electrophoresis in 20% polyacrylamide gels. The complete nucleotide sequence of both strands was determined. The plus and minus strands, which comprise 90 nucleotides each, can be folded into extensive but different secondary structures. The 3′ ends, where replication starts, apparently remain single-stranded. Two subspecies, differing only in a few nucleotides from the main component have been found as well. A sequence comparison between the 6 s RNA main species and the internal replicase-binding sites of Qβ RNA showed no similarities. Possible homologies with the first 100 nucleotides of Qβ RNA were noted. The most striking homology to the Qβ strands is the 3′ terminal sequence ..$CCCA_{OH}$ which seems to be a necessary but not sufficient requirement for replication by Qβ replicase. Except for the terminal C's no homology was observed with 2 other 6 s RNAs isolated and sequenced by Spiegelman. The origin of 6 s RNA as well as the mode of recognition by Qβ replicase is still unclear.

Supported by SNSF and the Jane Coffin Childs Fund

Changes in Size and Secondary Structure of the Ribosomal Transcription Unit During Vertebrate Evolution

U. Schibler, T. Wyler and O. Hagenbüchle
Zoologisches Institut der Universität, CH-3012 Bern

Ribosomal RNA (rRNA) and precursor ribosomal RNA (pre-rRNA) from at least one representative of each vertebrate class have been analyzed by electron microscopic secondary structure mapping and length measurements. All these pre-rRNAs have a common design. The 28S rRNA is located at or near the presumed 5′-end and is separated from the 18S rRNA region by the internal spacer region. In addition there is an external spacer region at the 3′-end of all pre-rRNA molecules. In cold-blooded vertebrates the precursor (Mol. wt. = $2.6–2.8 \times 10^6$) contains two short spacer regions; in birds the precursor (Mol. wt. = $3.7–3.9 \times 10^6$) bears a long internal spacer and a short external spacer region, and mammalian pre-rRNA (Mol. wt. = $4.2–4.7 \times 10^6$) has two long spacer regions. Both electron microscopic secondary structure mapping and analysis of single-strand specific S_1 nuclease-resistant fragments indicate a higher proportion of secondary structure loops in the pre-rRNA and rRNA of mammals when compared to lower vertebrates.

Supported by SNSF, grant 3.872.72

Membrane Fluidity is not Required for Concanavalin A Mediated Cell Agglutination

H. P. Schnebli
Friedrich-Miescher-Institut, CH-4002 Basel

In order to understand the factors that govern lectin mediated agglutination reactions we have analysed the kinetics of erythrocyte agglutination by Con A. At low concentrations of Con A, binding of the lectin appears to be the rate limiting step. At higher concentrations of the lectin the rate of agglutination becomes concentration-independent, indicating that the aggregation reaction is rate-determining. Lowering of the temperature to 0 °C (which 'freezes' the cell membrane), reduces the rate but not the extent of agglutination. Similarly, the Con A mediated agglutination of Ehrlich ascites carcinoma cells, trypsinized baby hamster kidney cells (BHK 21), L 1210 cells (mouse leukemia, ascites form) and murine thymic lymphocytes is not, or only slightly affected by lowering of the temperature to 0 °C. Metabolic poisons (iodoacetamide, azide, fluoride and dinitrophenol) as well as cytochalasin B and colcemid do not affect any of the agglutination reactions. It is concluded that metabolic activity and receptor mobility within the membrane are not required for lectin mediated cell agglutination. Agglutination appears to be governed primarily by the rate and extent of binding of lectin to the cell surface, the cell surface charge and the shear forces in the suspension.

Autoradiographic Tracing of Peripheral Projections of Spinal Cord Motoneurons by Retrograde Axonal Transport of ^{125}I-Tetanus Toxin

M.E. Schwab and H. Thoenen
Biozentrum der Universität, CH-4056 Basel

^{125}I-labelled, highly purified tetanus toxin injected into various regions of the forepaw of adult rats has been shown to be taken up with high selectivity and transported retrogradely to the spinal cord (Stöckel et al., this issue). Light microscopic autoradiography of the spinal cord segments C_6 and C_7 revealed heavily labelled cells (motoneurons only) and fibers. The location of the label with regards to different motor cell groups is highly dependent on the topographical site of injection suggesting a somatotopic representation for different muscles. With this system, a method is given for retrograde tracing of the efferent projections of spinal cord motoneurons. This may be of special importance in species where the chromatolytic reaction to nerve section is not reliable. e.g. in many rodents. A further advantage is the possible com-

bination of light microscopic autoradiography with various other histological stainings and the possibility to extend the retrograde identification of individual cells to the electron microscopic level.

Supported by the Sandoz Foundation

In vitro Transcription and Translation of SV40 DNA

P. R. Sharma and R. Weil
Department of Molecular Biology,
University of Geneva, CH–1211 Genève 4

Simian Virus 40 (SV 40) induces subcutaneous tumors, leukemias and lymphomas in hamsters and is able to 'transform' tissue-culture cells derived from several species. The 'transformed' cells contain T-antigen. The 'early' region of SV 40 genome carries the information necessary for the synthesis of virus-specific T-antigen. Using *E. coli* RNA-polymerase, we transcribed in vitro the SV 40 DNA under conditions leading only to the transscription of the 'early' strand. The c-RNA thus obtained was translated in vitro using the 'wheat-germ' system (in collaboration with Prof. P.-F. Spahr). The properties of the polypeptides obtained are being studied and compared with virus-specific polypeptides synthesized in vivo.

Supported by SNSF, grant 3.0970.73

Effects of Sialidase and DMSO on Erythroid Colony Growth in Culture

F. Sieber
Friedrich-Miescher-Institut, CH–4002 Basel, and
Institut für Zellbiologie, ETH, CH–8049 Zürich

When mouse bone marrow cells are grown in a viscous culture medium, two different classes of erythropoietin (epo)-responsive red cell progenitors can be detected by their ability to form colonies: One consists of 'erythroid colony forming units' (CFU-E) and gives rise to small erythroid clusters after 2 days in culture, and a second consists of 'erythroid burst forming units' (BFU-E) and gives rise to so-called 'bursts', which reach macroscopic dimensions after 10 days. Both classes exhibit an enhanced response to suboptimal doses of human urinary epo desialated either by neuraminidase or by acid hydrolysis. Inclusion of sialidase in the culture medium containing saturating amounts of epo increases the plating efficiency of BFU-E, but not of CFU-E. Addition of DMSO to the culture medium enhances the response of CFU-E to suboptimal doses of native epo and suppresses the growth of bursts and granulocytic colonies.

The Purification and Properties of the DNA Dependent RNA Polymerases of *P. polycephalum*

S. S. Smith, J. Stalder, V. M. Vogt and R. Braun
Institut für allgemeine Mikrobiologie der Universität,
CH–3013 Bern

Using nuclei isolated from *P. polycephalum* microplasmodia grown in 12 l fermenter cultures, we have found that the two known RNA polymerases activities can be seperated reproducibly on DEAE-Sephadex if a batchwise adsorption and step elution precedes column chromatography. The activities thus seperated are absolutely dependent on added DNA, and are more active on single stranded templates than they are on more intact templates. Both activities are stabilized by glycerol, and show an apparent activation on dilution, while a variety of protein preparations, including BSA in high concentration, inhibit polymerase I.

Presence of Globin mRNA Sequences in the Polyadenylated and Non-Poly-Adenylated Fraction of Pre-mRNA from Duck Erythroblast Nuclei

G. Spohr, V. Manzari and G. Dettori
Institut Suisse de Recherches Expérimentales
sur le Cancer, CH–1011 Lausanne

The pattern of nuclear pre-mRNA and the kinetics of its synthesis and decay allows the characterization of three metabolically distinct size fractions: (1) nascent pre-mRNA (2) intermediate size pre-mRNA and (3) small pre-mRNA (Spohr, Imaizumi, Scherrer, Proc. Nat. Acad. Sci. USA *71*, 5009, 1974). In duck erythroblasts the nascent pre-mRNA fraction contains a precursor to globin mRNA (Imaizumi, Diggelmann, Scherrer, Proc. Nat. Acad. Sci. USA *70*, 1122, 1973). The amount of globin mRNA sequences present in the non-polyadenylated and polyadenylated fractions of the pre-mRNA was determined. In duck erythroblasts the majority of the globin mRNA sequences are found in the non-polyadenylated fraction on molecules of different sizes. The distribution of globin mRNA sequences in the non-polyadenylated fraction shows a predominance in the low molecular weight region (2×10^{-5}–4×10^{-5} MW) as for total nuclear globin pre-mRNA. These results suggest that, at least in the case of duck globin mRNA formation, the processing of the pre-mRNA precedes polyadenylation.

Comparison Between the Retrograde Axonal Transport of Nerve Growth Factor (NGF) and Tetanus Toxin in Rats

K. Stöckel and H. Thoenen
Biozentrum der Universität, CH–4056 Basel

NGF, a protein which is essential for the embryonic development of adrenergic neurons and the maintenance of their function in adulthood, is taken up by adrenergic nerve terminals with high selectivity and is transported retrogradely to the cell body by a colchicine sensitive mechanism. Although in sensory neurons the responsiveness is confined to a short time of embryonic life the high selectivity of retrograde transport of NGF persists throughout the whole life as in adrenergic neurons. No retrograde transport of NGF could be demonstrated in motor neurons. In contrast to NGF, tetanus toxin is transported retrogradely in all the neurons tested (adrenergic, sensory and motor neurons) and is blocked by simultaneous administration of gangliosides and previous administration of neuraminidase. It is concluded that the uptake and retrograde transport of tetanus toxin seems to depend on membrane structures which are common to all the nerve terminals, and which contain as a common denominator sialic acid. In contrast the uptake and retrograde transport of NGF seems to depend on more specific structures restricted to sensory and adrenergic neurons.

Supported by SNSF, grant 3.653.71

Site-Directed Generation of Nucleotide Substitutions in the Coat Cistron Ribosome Binding Site of Qβ RNA

T. Taniguchi, M.A. Billeter and C. Weissmann
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich

The mutagenic nucleotide analog N^4-hydroxyCMP was introduced into the region of the minus strand complementary to the coat cistron ribosome binding site. Following the procedure of Kolakofsky et al. (J. Mol. Biol. *76*, 271, 1973) a ribosome was bound to Qβ RNA at the coat cistron and the complex was used as template for Qβ replicase. Synthesis of the minus strand proceeded from the 5' terminus up to the position corresponding to the sixteenth nucleotide of the coat cistron. After removal of the ribosome by EDTA, the nascent chain was elongated by stepwise synthesis (Flavell et al., J. Mol. Biol. *89*, 255, 1974) and the nucleotide analog was inserted into the positions complementary to the third and fourth nucleotides of the coat cistron. The purified minus strands were used as template to synthesize radioactive plus strands. Analysis showed that 4 RNA species were present: (1) Wild type, (2) G→A-substituted in position 3 of the coat cistron, changing the initiator AUG into AUA, (3) G→A-substituted in position 4, changing the alanine codon GCA into ACA, and (4) G→A-substituted in both positions 3 and 4. Preliminary ribosome-binding studies show that the AUG→AUA modification greatly reduces ribosome binding, while the GCA→ACA change does not.

Supported by SNSF and the Jane Coffin Childs Fund

Sulphatierte Proteoglycans in normalen und in transformierten Zellen

L. Tarcsay und H.-P. Schnebli
Friedrich-Miescher-Institut, CH–4032 Basel

Sulphatierte proteoglycans (SPG) bilden bei einer Reihe von in vitro kultivierten Zellinien einen integrierten Bestandteil der äusseren Membran. Auf Grund ihrer variablen Struktur und ihrer Fähigkeit, mit Proteinen Komplexe zu bilden, könnten sie eine wichtige Funktion in spezifischen interzellulären Kontakten und somit auch in Wachstumsregulationsprozessen ausüben. Wir führten in vitro vergleichende Untersuchungen durch über die Synthese, Sekretion und Degradation von SPG in 3T3- und SV 40-transformierten 3T3 Fibroblasten, sowie in NRK (Normal Rat Kidney Cells), RSV-transformierten NRK und ts-339 (temperatursensitives RSV) – transformierten NRK. Unsere Resultate sind wie folgt: 1. Der Turnover der SPG wird durch die Transformation nicht verändert. 2. An der Membran der transformierten Zellen erfolgt während der Sekretion ein im Vergleich zu Normalzellen erhöhte Degradierung der SPG zu Oligosacchariden. 3. Das Phänomen ist assoziiert mit dem transformierten Phänotyp: die Degradierungsrate bei der permissiven Temperatur ist mit derjenigen in den RSV-transformierten NRK, bei der nichtpermissiven Temperatur jedoch mit der Degradationsrate in den NRK identisch. 4. Das Phänomen ist dichte- und zyklusunabhängig.

A Procedure for Cell Separation Based on Density, Volume and Shape Differences

J. Thummel, C.L. Voûte and M. Brenner
Institut für Org. Chemie der Universität, CH–4056 Basel und Lab. für Exptl. Nephrol., Kantonsspital, CH–4051 Basel

In the course of our work on aldosterone stimulation of isolated epithelial cells (frog skin) it became desirable to enrich carbonicanhydrase (CA)-active cells present in collagenase digests of the epithelium. Density-gradient centrifugation proved quite effective. Enrichment could be doubled, however, when centrifugation was combined with a method which differentiates with respect to an additional parameter, viz. frictional resistance to sedimentation, as expressed by Stocke's law. Differences in sedimentation velocities are such as to produce cell separation during transport through a multistage sedimentation machine. A simple apparatus has been constructed with 30 4-ml stages and adjustable sedimentation path (3–30 mm). Best total enrichment was achieved by first passing the crude cell suspension through this machine and then subjecting the CA-rich fractions of the resulting binomial distribution to a density gradient centrifugation. As a side result, these combined procedures yield information on the density and on the radius of spheric cells.

Supported by Fritz Hoffmann-La Roche Stiftung, nº 124

Structure and Replication of Ribosomal Genes in *Physarum*

V. M. Vogt, H. Moser and R. Braun
Institut für allg. Mikrobiologie der Universität, CH–3013 Bern

The DNA coding for ribosomal RNA in *Physarum* is known to form a dense satellite band in CsCl centrifugation and to replicate in the G-2 phase of the cell cycle. In contrast to main-band DNA, which replicates in S phase, the replication of rDNA appears to be unscheduled from one cell cycle to the next. That is, replicating rDNA sequences pulse labeled with ^{3}H-TdR during any time in G-2 will have equal probabilities of replicating again in any time interval (except the first two hours) in the subsequent cell cycle. At least two thirds of purified rDNA can be visualized in the electron microscope as linear molecules of 38×10^6 Daltons. The remaining molecules are smaller and are not clustered in size classes. We have found less than 1% circular molecules. The size of rDNA probably is not due to endonucleolytic cleavage, since the sedimentation of rDNA from nuclei lysed on top of sucrose gradients is the same as that of purified rDNA. We have tried to elucidate the structure of rDNA with restriction nuclease mapping, denaturation mapping, and heteroduplex analysis in the electron microscope.

An Electron Microscopic Method for Studying Complexes of Single Stranded Nucleic Acid with Proteins

H. J. Vollenweider and Th. Koller
Institut für Zellbiologie, ETH, CH–8049 Zürich

We have developed a protein-free nucleic acid preparation method, especially for studying complexes of single-stranded nucleic acids with proteins. The basic procedure

is very similar to the classical protein monolayer spreading techniques. The carrier protein, however, is replaced by dimethylbenzylalkylammoniumchloride (BAC). We show that both the hypophase method and the microdiffusion or droplet method can be used with BAC to visualize single-stranded and double-stranded unfolded molecules. BAC does not lead to any apparent thickening of the nucleic acid strands. Partially denatured DNA shows a loosened structure with a foamy appearance in the 'unmelted' regions, which opens up locally into melted loops of different size. That specifically bound protein can also be visualized will be shown for Qβ-replicase bound to Qβ-RNA. The binding position of this enzyme can be mapped within specific regions of the Qβ-RNA (see also abstract by F. Meyer, H. Weber, H. J. Vollenweider and C. Weissmann).

Supported by SNSF.

Binding of Qβ Coat Protein to the Replicase Cistron Initiation Site of Qβ RNA

H. Weber
Institut für Molekularbiologie I, Universität Zürich, CH–8049 Zürich

Genetic and biochemical evidence suggests that in the replication of coliphage Qβ, the coat protein acts as a translational repressor for the synthesis of the replicase protein. We have been able to show that, like in the case of R17 (Bernardi and Spahr, PNAS *69*, 3033, 1972), this repression is the result of a physical association of coat protein with the beginning of the replicase cistron. Codialysis of [^{32}P] Qβ RNA and coat protein (30–50-fold molar excess) from 9.4 *M* urea into buffer solution converts up to 40% of the input RNA to a complex recoverable by filtration through nitrocellulose filters. If the complex, before filtration, is treated with ribonuclease T_1 in the presence of excess unlabeled Qβ RNA, the amount of nitrocellulose-bound [^{32}P] RNA is reduced to about 1% of input. From this material, three main fragments of a chain length of 88, 71 and 27 nucleotides were isolated by 20% polyacrylamide gel electrophoresis. Fingerprinting and further sequence analysis established that all three fragments consist of sequences extending from the intercistronic region to the beginning of the replicase cistron.

Supported by SNSF and the Jane Coffin Childs Fund.

Clonal Analysis of Early Development in *Drosophila melanogaster*

E. Wieschaus and W. Gehring
Biozentrum der Universität, CH–4056 Basel

Using gynandermorph mapping, the precursors of the second leg and wing disks were shown to lie close to each other in the embryo. In a parallel study, progeny of single cells were genetically marked using X-ray induced somatic recombination. Most of the clones induced at the blastoderm stage (3 h) were restricted to single disks. However, clones were found extending from the wing into the second leg, and thus the blastoderm cells which gave rise to these clones had not yet been determined to form specific disks. No such overlapping clones were induced by irradiations later in development (at 7 h or 10 h). Clones extending from the eye into the antenna could be induced at all times up to 10 hours. No clones were found extending between different legs of across segment borders.

Poly (A) Synthesis in T2L-Infected *E. coli*: A Combination of Poly-Nucleotide Phosphorylase and ATPase

W. Wunderli, R. Hütter, M. Staehelin and W. Wehrli
Ciba-Geigy AG, CH–4002 Basel

In extracts of T2L phage-infected *E. coli*, the formation of poly (A) from both ADP and ATP was observed. In addition, cleavage of ATP to ADP was found. Purification of these activities led to the isolation of polynucleotide phosphorylase and an ATPase. The polynucleotide phosphorylase had the same properties as the enzyme from uninfected cells except for the molecular weight (290,000 Daltons). The ATPase had a molecular weight of 165,000 Daltons and its properties were different from any known ATPase. It was specific for ATP and produced ADP at a high rate (turnover number: 2.5×10^4 min^{-1} at 37 °C). This rate is about 40× higher than the one measured for polynucleotide phosphorylase. The optimal assay conditions for ATPase were very similar to those for polynucleotide phosphorylase. Therefore, traces of ATPase present in polynucleotide phosphorylase were sufficient to produce poly (A) from ATP very efficiently and so could simulate the presence of a poly (A) polymerase.

Optische Filtration von Elektronenmikrographien beschatteter biologischer Objekte

M. Wurtz, T. Kistler, T. Hohn and E. Kellenberger
Biozentrum der Universität, CH–4056 Basel

Die optische Filtration (Klug et al. 1966, Nature, Lond. *212*, 29; Aebi et al. 1974, J. Supramol. Struct. *1*, 498) von negativ gefärbten Objekten mit periodischer Struktur erwies, dass signifikante biologische Information nur bis 25–30 Å erzielt wird, trotz 2–3 Å instr. Auflösung. Die Gründe dafür sind: strahlbedingte chemische Destruktion biol. Materials, topographische Deformationen bei der Präparation (Oberflächenspannung) (Anderson 1952, Revue d'Optique, 576) und thermaler Kollaps (Anderson 1954, Trans. N.Y. Acad. Sci. *16*, 242) und Eigenstruktur des Farbstoffes. Auf gefriergetrockneten periodischen biologischen Objekten, die nachher beschattet wurden, konnten wir Filtrationen herstellen, die zeigen: 1. signifikante Details bis zu 30 Å, 2. die Körner des Beschattungsmaterials (WO_2) werden statistisch abgelagert, so dass nach Ausmittlung, d.h. im filtrierten Bild, eine echte dreidimensionale Wirkung vorliegt. Die Methode gestattet: a) beide Seiten einer einschichtigen Struktur (T-layer von bac. brevis) unabhängig darzustellen, b) bei abgeflachten, tubulären Strukturen, deren obere und untere Schicht kristallographisch in Register liegen, diese getrennt und korrekt abzubilden.

Growth Control in a Clonal *Drosophila* Cell Line by Juvenile Hormone and Ecdysone

Ch. Wyss
Institut für Zellbiologie, ETH, CH–8049 Zürich

The established *Drosophila melanogaster* cell line Kc was cloned in semi-solid agar medium. The effects of a juvenile-hormone analogue, ethyldichlorofarnesoate (EDCF), and α- and β-ecdysone on the in vitro growth of one of these hypotetraploid clones, Kc C 7, were investigated. Increasing concentrations of EDCF alone in-

hibited proliferation, whereas the two ecdysones alone each showed a growth stimulating activity at low concentrations and strong growth inhibition at higher concentrations. Effective doses for α-ecdysone were two orders of magnitude higher than for β-ecdysone. The bimodal response to the ecdysones was also consistently observed in cultures with varying concentrations of EDCF. There are, however, concentration ranges where EDCF and α- or β-ecdysone in combination not only cancel each others inhibitory effect but even cause a net stimulation of cell proliferation. For all three hormones significant effects were observed at concentrations comparable to those reported from assay systems using whole animals. The morphological changes induced by these hormone treatments are also reported.

Supported by SNSF, grant 3.8640.72

Search for a Precursor of Histone Messenger in the Nuclear RNA of HeLa Cells

H. Wyssling and Marialuisa Melli
Institut für Molekularbiologie II der Universität, CH–8057 Zürich

It has been shown that histone messenger sequences from HeLa cells can cross-hybridize to some extent to sea urchin histone DNA. If the HeLa histone mRNA is the only major messenger species cross-hybridizing with sea urchin DNA, it should be possible to use this technique to detect histone-mRNA sequences in a complex RNA population. We found that, under certain conditions, it is possible to cross-hybridize total sea urchin DNA to fractions of a formamide gradient of HeLa cytoplasmic RNA, and to find the majority of the hybridization in the position corresponding to histone mRNA. When the same conditions were used to hybridize nuclear RNA from synchronized HeLa cells to sea urchin DNA, the pattern of hybridization across the denaturing gradient was similar to the one found in the cytoplasm. Nuclear RNA extracted from cells in which DNA synthesis was inhibited with cytosine arabinoside did not show any clear peak of hybridization, confirming the specificity of the reaction. Provided that cytosine arabinoside ultimately inhibits transcription rather processing of nuclear RNA in synchronized cells, it may be tentatively concluded that our present techniques do not reveal the existence of a high molecular weight nuclear precursor to cytoplasmic histone mRNA.

Supported by SNSF, grant 3.8630.72SR

PRAEMIA

Prize 'Biochemical Analysis'

The prize of DM 10000.– is donated from Boehringer, Mannheim, and is awarded every 2 years at the conference 'Biochemical Analytic' in Munich, Germany, for outstanding work in the field of biochemical instrumentation and analysis. The donation will take place during the 1976 conference between the 9th and 13th of April. One or several papers concerning one theme, either published or accepted for publication between October 1st 1973 and September 30th 1975, may be sent in triplicate before November 15th 1975 to: Prof. Dr. Ivar Trautschold, secretary of the prize 'Biochemical Analysis', Medizinische Hochschule Hannover, Karl-Wiechert-Allee 9, D–3000 Hannover-Kleefeld, Germany.

Ruzicka-Preis 1975

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. Kandidaten dürfen in dem Jahre, in dem sie den Preis erhalten, das 45. Lebensjahr nicht überschritten haben. Kandidatenvorschläge können bis spätestens 28. Juli 1975 dem Präsidenten des Schweizerischen Schulrates, ETH Zürich, Rämistrasse 101, 8006 Zürich, unterbreitet werden.

CONGRESSUS

Denmark
The 2nd International Symposium on Vascular Neuroeffector Mechanisms

in Odense, 29 July – 1 August 1975

Topics: Morphology, development and differentiation of blood vessels; mechanisms of neural control of vascular tone; ionic and metabolic control of vascular muscle; pathophysiology of vascular disease and clinical aspects. Further information by Dr. O. A. Nedergaard, Institute of Pharmacology, University of Odense, Niels Bohrs Alle, DK–5000 Odense, Denmark.

Spain
EUCHEM Conference on Enzymatic and Homogeneous Catalysis

in Santander, 13–18 July 1975

Further information by: Dr. A. O. Ballestros, Departamento de Catalisis, C.S.I.C., Serrano 119, Madrid-6, Spain.