ABSTRACTS

OF THE LECTURES HELD ON THE

FIRST JOINT CONGRESS OF THE HUNGARIAN SOCIETIES OF BIOCHEMISTRY, BIOPHYSICS AND PHYSIOLOGY

PÉCS, OCTOBER 12 TO 14, 1967

Supplementum

ad tomum 2

ACTAE BIOCHIMICAE ET BIOPHYSICAE

et

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ACTAE PHYSIOLOGICAE

Academiae Scientiarum Hungaricae

AKADÉMIAI KIADÓ, BUDAPEST 1967

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EXCITATION AND BIOCYBERNETICS

E. Ernst

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When speaking, e.g., about bioelectricity one should realize that it has a concrete meaning only in case of dealing with electric phenomena connected e.g. with nervous excitation or some functions of other organs. In speaking about biocybernetics it should unequivocally be determined what the meaning of cybernetics and its place among the sciences is. Wiener agreed with my proposal

$$\text{cybernetics} = \text{information theory} + \text{automatism.}$$

Dealing with excitation and biocybernetics we start from the well known phenomenon of pulling back one's hand from a painful physical impact. From this complex automatism only the information transport in the afferent and efferent nerve-channels will be dealt with in this lecture.

1. The *sensory nerve-channel* is known to display a series of impulses directly continuing e.g. the receptor potential of a Pacinian body impressed physically. As a result of this *frequency modulation* the frequency of the impulses is the higher the greater the amplitude of the receptor potential is. [The question of this frequency modulation will be dealt with in a later lecture; neither will the question now be discussed whether a d.c. impulse or another frequency will appear (in the nerve cell) after detection of demodulation of the frequent impulses (of the nerve fibre)]. In the sensory nerve channel the information is always lead as a series of impulses of a certain frequency.

The sensory nerve, however, must not be looked upon as a passive channel, because it is known to be able also to modulate the generator potential into frequent impulses. Furthermore, the Pacinian corpuscle-nerve preparation treated with procain produces only a receptor potential without these frequent impulses of the nerve. That means that procain, containing a strongly basic N atom and a benzene ring with six π electrons, probably performes a certain reaction with some compound of the sensory nerve. This inference is corroborated by the fact that the effect of thiocain is six times greater than that of procain, the O atom of the carbonyl group in the latter having been replaced by an S atom. On the other hand, the 4th p electron in the M electron shell of the S atom easily becomes a $d - \pi$ electron after jumping from orbit p to d. In short: *the sensory nerve seems to be not a simple channel, but also an actively modulating device.*

2. The *motor nerve* is known to *propagate* frequent impulses, but the property of frequency modulation can be considered as something which has been hypothesized rather than experimentally proved.

To investigate this question we started from the results obtained by drying e.g. striated muscles, as described in the literature. The so-called Läwen–Trendelenburg preparation of the frog (Rana esculenta) was perfused with

Ringer's solution and the plexus ischiadicus exposed to drying. After a certain time the m. gastrocnemius produced tetanic contraction and a series of electric impulses, upon stimulation of the drying plexus with a single electric shock of 0.1 msec. Against these results (and many others described in the literature) the so-called fundamental frequency of the muscle itself can be adduced as an objection.

Therefore, in another series of experiments the preparation was not perfused, but its lower leg was protected from drying by being covered with cotton-wool wetted with Ringer's solution. The drying plexus was stimulated as before and *frequent electric impulses were lead off from the nervus ischiadicus* fitted on a pair of electrodes inside the thigh of the frog. This indicates that the drying nerves of the plexus ischiadicus produced frequency modulation when stimulated with a short electric shock of 0.1 msec duration.

This statement can also be contradicted as follows: the ischiadic nerve is a mixed nerve containing besides motor, also sensory fibres. Hence frequent impulses can be conducted in these sensory fibres coming from the nerve endings in the spindels and/or the tendons while the muscle performs a tetanic contraction (may it be caused by anything).

To eliminate this possibility the fact that excitation and contraction can be experimentally separated was resorted to. The gastrocnemius, e.g., when perfused with hypertonic solution and stimulated, shows normal action current without any sign of mechanical activity. Correspondingly a new experimental result is shown demonstrating a series of electric impulses lead off from the nervus ischiadicus and simultaneously a single contraction of the gastrocnemius. Hence this series of impulses does not belong to the afferent fibres but, coming from the drying plexus ischiadicus, is conducted by the efferent fibres.

To sum up: *the motor nerve fibre has the property of frequency modulation*, hence neither this can be considered exclusively as a passive channel it plays an active role in the process of information.

Elaborating this topic further one comes to wide fields of investigation: the mechanism of the action of veratrine (many benzene rings of which contain a large number of π electrons), or the mechanism of the transmission of the excitation from the receptor and to the effector, respectively, etc. These and other similar topics surpass the problem of excitation, and are thus referred to the teams working in electronbiology and biocybernetics in our Biophysical Institute.

SOME QUESTIONS CONCERNING THE REGULATION OF CELLULAR PROCESSES

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The general properties of the genetic code — Survey of the different codons — The question of the stability of the code — Experimental investigations on the universal nature of the code — Data showing also reversed degeneracy in a small amount (one codon may code for different amino acids with different probability) — The statistical nature of the code — The questions of

the evolution of the code — Formulation of the problem of cell differentiation on the basis of self-reproducing automatons.

The cellular regulation system of Jacob and Monod — Induction and repression of enzyme synthesis — The example of Pitot and Heidelberger showing how the functioning of the regulation system will change under the effect of a carcinogen (also after the ceasing of the effect of carcinogen). — Possible activation of the duplication of the tumor virus DNA molecules under the effect of carcinogens or radiations. — The changed functioning of the regulation system after the ceasing of the primer effect.

ON SOME PROPERTIES OF CEREBRAL INFORMATION STORAGE

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On the basis of own experimental results and literary data the "two-process" theory of memory storage proposed by Hebb seems to be a suitable starting hypothesis. According to this theory, the initial form of memory acquisition, the so called temporary (short term) memory is not identical — concerning its mechanism — with the permanent (long term) memory storage.

The mechanism of temporary memory has been investigated by observing the storage of conditional evoked potentials. The topographic and temporal sequence of the stored bioelectric trace as well as its interference with electroconvulsive shock have been studied. The suggestion is proposed that short term memory depends upon changes in neuronal reverberating circuits: it seems to be essentially a membrane-process manifested in alterations of synaptic and neuronal bioelectrical activity.

The problem of permanent memory storage is discussed on the basis of Hydén's molecular theory. Transfer experiments have been undertaken as suitable tests for intracellular storage. The initial aim has been the reproduction and control of the investigations on mammals which followed the well known experiments on flatworms. The learning and memory acquisition of rats could be facilitated by injecting brain extracts of trained animals provided some preliminary experimental conditions were kept. The optimal facilitation occurs using brain extracts of donor animals not being "over-conditioned" and in case of grown-up recipients not habituated previously to the conditional stimulus. Notwithstanding the intracellular molecular mechanism of the long term information storage, according to the experimental results the transfer-phenomenon seems to be rather of non-specific character.

On basis of our own data the possible ways of interrelations between temporary and permanent information storage are discussed.

ON THE CYBERNETICAL PROBLEMS OF BEHAVIORAL RESEARCH

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One of the necessary conditions of a cybernetical approach of behavior is its quantification. This requirement has partially been accomplished by experimental psychology in reducing complex behavioral actions to their basic and easily reproducible components. However, the elementary behavioral patterns (conditional reflexes) separated until now are still far from being ideal objects of a cybernetical approach because: *a)* conditioned reflexes (mainly motor events) do not allow of satisfactory quantification or only of their fractions, *b)* it became evident that a consistent behavioral theory can not be constructed on the basis of overt manifestations alone. It is now obvious that this task requires the recording and measuring of those peculiar internal (neural) "states" which have also been postulated by experimental psychology in the form of intervening variables or hypothetical constructs. A successful investigation of these factors has, however, only been commenced in the last decade, mainly with the introduction of the analytical methods of neurophysiology into behavioral studies. On the basis of this consideration the whole past of behavioral research can only be regarded as a preparatory first step towards the cybernetical approach.

The author gives a short account of the present status and some important electrophysiological discoveries of the field of motivation and conditioning. According to recent findings motivation (one of the principal intervening variables postulated by experimental psychology) proves to be an homeostatically regulated central event. The appearance of the notion of regulation in behavioral research has a twofold significance: *a)* it makes palpable and measurable those factors which were mainly responsible in the past for theoretical controversies in psychology, *b)* it represents a genuine cybernetical notion (control) and thus a spontaneous access to the cybernetical and neurophysiological viewpoints. Data concerning the neural mechanisms of regulation of motivation are presented and some principal questions of the basic mechanisms of regulation (feed-back and feed-forward) and the particular significance of inhibition in this context are discussed.

SIMULATION OF AN ASSUMED NERVOUS MECHANISM CONTROLLING CO-ORDINATED LIMB MOVEMENTS IN AMPHIBIA

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It is known that only the limb segments of the spinal cord are able to control co-ordinated limb movements. Experiments on the nature of these segments reveal the following: (1) Nervous activities determining the rhythm and movement pattern of the limb are inherent in the limb segments. (2) Thoracic

spinal cord segments, if they are transplanted in the place of limb segments at a very early embryonic age and if they develop more motoneurones than normally, are able to move a limb. The movement of the limb is the better, the larger is the number of motoneurones in the grafted thoracic segments. (3) The majority of limb muscles receive innervation from all three limb segments. Motoneurones supplying individual limb muscles are arranged in small groups in such a manner that muscles represented predominantly in these groups may act either as synergist or antagonist. (4) Model experiments with artificial neurones show that networks with recurrent cyclic inhibition generate rhythmic output in response to continuous regular or random input. The output patterns of these networks depend on *(a)* the time which elapses before an inhibited neurone starts firing; *(b)* the number of neurones composing the network; *(c)* the number of inhibitory connections. From these relationships simple equations can be derived, and the output patterns of networks composed of any number of neurones can be calculated. The function of such networks is exceedingly versatile and suggests an easy way to simulate the assumed nervous mechanisms controlling co-ordinated limb movements.

RECENT DATA ON BRAIN STEM LEARNING PROCESSES

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The authors reported recently (Hung. Physiol. Soc. and EEG Soc. Meetings, 1966) midbrain conditioning experiments. As a continuation of these studies the paper gives account on two series of experiments:

(1) The respiratory conditional reflex to acoustic stimulus in rats persists for several days after high decerebration. The differential inhibition elaborated before the surgical intervention persists likewise after transsection.

(2) Conditional evoked potentials can be recorded from the midbrain reticular formation in cats in acute experiment. This electrographic learned response persists after transsecting the brain stem on the level above the mesencephalon. The conditional potential can be extinguished and reestablished after repeated stimulus-pairings. The elaboration of a conditional response started in the decerebrated preparation is similarly succesful.

The role of midbrain structures in learning is discussed on the basis of the experimental data.

ELECTROGRAPHIC CHRONIC MEMORY TRACE IN THE CAT

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In previous papers (Acta Physiol. Hung. *26*. 1965; 6th Intern. Congr. EEG Vienna, 1965) the authors presented data on the conditioning of evoked potentials in acute experiment and reported some properties of this bioelectrical memory model.

In the present work on cats with chronically implanted electrodes it was demonstrated that:

(i) The association of an auditory stimulus (click) followed by electrical stimulation of the pad with a delay of 200 msec results in the appearance of two conditional evoked potentials on the auditory and somatosensory cortex: in the moment corresponding to the delivery of the CS and 200 msec later. in the moment corresponding to the previous cutaneous reinforcement. This latter conditional evoked response proves to be a chronic memory trace: it lasts for 8–10 days without reinforcement and it extinguishes showing amplitude-fluctuations. The extinction can be disinhibited.

(ii) The evoked response appearing to the conditional click-stimulus following the associations suffers some changes too: it seems to be likewise a lasting learned phenomenon.

(iii) A marked parrallelism between bioelectric and behavioral signs of learning is observable: the delayed evoked potential is accompanied by somatic motor response.

NEW DATA ON THE ELECTROGRAPHIC SIGNS OF HUMAN MEMORY TRACE

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In previous experiments (Acta Physiol. Hung. *30*. Suppl. 300, 1966) delayed conditonal evoked potentials were recorded in man following delayed association of acoustic (CS) and photic (US) stimuli, in the moment corresponding to the reinforcing photic stimulus. In the present series it has been found that:

(i) This human memory model persists for about 4 months without reinforcement.

(ii) Immediately prior to the conditional evoked response a negative wave is observable on the oscillogramme related perhaps to the learning process.

(iii) The bipolarly recorded bioelectric memory trace consists of three main wave-components.

EFFECT OF DIFFERENT RNA-EXTRACTS ON EXPERIMENTAL LEARNING

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As a continuation of earlier experiments on the molecular aspects of learning (Acta Physiol. Hung. *30*. 301. 1966) the aim of the present study was to characterize the brain RNA-fraction responsible for the enhancement of learning. The effect of ribosomal, soluble and labile brain RNA-fractions of rats performing an avoidance conditional response to acoustic stimulus on the learning of naive rats injected intraperitoneally by these extracts was investigated. According to the results the ribosomal RNA-fraction enhances the formation of the conditional reflex, whereas the two other fractions are less effective. On the basis of the results it is discussed whether the observed facilitation of learning is due to RNA-effect and whether this enhancing influence is a result of the donor animal's training or of some non specific factors.

TRAIN OF SPIKES ON MOTOR NERVE

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As it is known the sensory nerves can transform the generator potencial into frequent nerve impulses and the frequency of these impulses carries the information content of excitation. According to Frey's hypothesis the ability is ascribed also to motor nerves to transform the constant course of direct current into frequent impulses of excitation. In our experiments carried out on frog's sciatic-gastrocnemius preparation a comparison was made between the effect of supramaximal stimulation by direct current and by a rectangular impulse of 0.1 ms at the same voltage. The contraction of the muscle as well as the action current of the nerve and the muscle were recorded stimultaneously. Recording tetanus as a response of the muscle to d. c. stimulus, the oscillograms show the trains of action currents of both the nerve and the muscle. The appearence of the nerve action current having 5–10 waves — here it is not the question of α, β, . . . etc. waves — points to the fact that the motor nerves can really transform the constant course of the stimulus of direct current into frequent excitation.

BIOFREQUENCIES AND CYBERNETICS

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It is an essential property of the nerve fibers to tend to give a periodic electrical response to a constant stimulus (sometimes to a single stimulus as well). The most significant aspect of this is the transformation of the generator potential into a train of propagated pulses, another aspect is e.g. the damped-oscillation-like local response elicited by a subthreshold stimulus in certain cases. For the mechanism of the phenomenon it may be helpful to consider physical analogs with known mechanism. — Electrical, mechanical and electronic systems are reviewed, performing periodic response to an impulse or constant influence. The considered systems permit the understanding or interpretation of some details of the neural periodicity, including nonlinearity, self-control, frequency-modulation, the way and role of the threshold-change. The controlled supply of electrical energy seems to be a relevant factor. The question arises, which are the structural conditions as well as the molecular and electron processes which make these effects possible.

FORMATION AND TRAVELLING OF BIOLOGICAL SIGNALS — AN ELECTRONIC PROCESS

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This report — as a continuation of the other three lectures of our institute — deals with the problem how biological signals carrying information are formed. The hypothesis according to which electronic phenomena play an important part in biological excitation processes is supported not only by a number of former literary data, but also by our own experimental results. From among our experiments one series will be reported. This was carried out with frog hearts. These experiments showed that the beating of hearts formerly stopped, could be restarted again, when the hearts had been stained by eosin and illuminated with intensive visible light. The system of electrons in an eosin molecule is excited by the photons of light and — in our opinion — the formation of biological excitation could be restarted by accepting this energy, temporarily stored by the electron system of the dye molecules.

In accordance with Riehl's recent data proton-semiconductors are able to take part not only in travelling of information, but also in storing information, as it has been shown by their technical use.

ANALYSIS OF THE LOCOMOTION PATTERNS EVOKED BY ELECTRICAL STIMULATION OF THE HYPOTHALAMUS IN CAT

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The units, whose output is determined not only by their input but by the elapsed time as a parameter too, are often used in technical control systems. If we consider the effect of the hypothalamic stimulation as the input and if the state of the system is indicated by the direction of the elicited locomotion, it seems that in the hypothalamic regulation of the motivation probably similar units take part:

1. During a sustained (0.5–1 min) electrical stimulation the direction of the locomotion repeatedly changes. The direction reversal time and the time of return regularly varies depending on the parameters and the anatomical location of the stimulation as well.

2. If by the interruption of a short stimulation a rebound movement appears which is opposite in direction to the one which occurred during stimulation, then the direction of the locomotion induced by a second stimulation applied within a definite time (3–45 sec) will be also opposite. Such a "recent memory" for the direction of rebound movement could become, however, independent of the time parameter, if we switch off the stimulation on a predetermined point of the experimental cage (on a pedal) and so we establish a conditional pedal-approach or pedal-avoidance reaction.

A STUDY OF HYPOTHALAMO-NEOCORTICAL REGULATORY MECHANISMS BY EVOKED POTENTIAL AVERAGEING TECHNIQUE

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With freely moving cats the sensory evoked cortical potentials show definite changes in different states of awareness.

Recently the present authors established a correlation between the rewarding or punishing character of the behavioral reactions elicited by hypothalamic electrical stimulation and the concomitant hippocampal electrical manifestations.

In the present experiments the evoked potentials elicited by sensory stimuli and by electrical stimulation of the sensory pathways were studied during different motivational reactions. The potentials were averaged by a 128 channel digital analyzer.

Correlations were found between the characteristic behavioral reactions, the hippocampal electrical manifestations and the changes of the visual and auditory cortical evoked potentials.

The role of motivational mechanisms in the central control of sensory processes is being discussed.

ANALYSIS OF THE REGULATORY ACTION OF MIDLINE THALAMIC NUCLEI WITH ELECTRICAL, MOTOR AND VEGETATIVE INDICATORS

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At a previous meeting of the Hungarian Physiological Society a correlation between the neocortical and hippocampal electrical activities and behavioural manifestations elicited by electrical stimulation of the non-specific thalamic nuclei on freely moving cats was reported. In the present study attention was focused on the vegetative accompaniements of midline thalamic stimulation.

By applying different stimulation intensities to the same loci a strict covariation between the simultaneously recorded vegetative (Ecg, respiratory rate, pupillary size, blood pressure and the level of blood sugar) as well as cortical electrical and motor effects was found. In addition to that characteristic differences in the effects of different thalamic regions both in the direct- and the after-effects of stimulation were observed.

On the basis of present findings the integrative function of midline thalamic nuclei is discussed.

CYBERNETICAL PRINCIPLES IN REGULATING THE NEURONAL ACTIVITY OF THE RED NUCLEUS

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The distribution of electrical potentials along the cross-section of the midbrain after antidromic activation from the spinal cord through contralateral rubrospinal tract was investigated by microelectrode recording on the cats. The zone of the maximal activity was localized within a depth of 7.5–10 mm from the surface of the superior colliculus. The negative potentials were generated within a limited area about 2 mm across. This area corresponded to the magnocellular part of the red nucleus (RN).

The stimulation of the contralateral nucleus interpositus (IN) led to monosynaptic excitation of the neurones of RN. The monosynaptic excitation of the same neurones, but not of the same kind, had been shown by activation of the ipsilateral sensomotor cortex (SM). The possibility of the antidromic activation of some neurones of RN had also been found by the stimulation of the contralateral IN.

The regulation of the neuronal activity of the RN was represented by several mechanisms: the inhibitory influence (disfacilitation) from the cerebellar cortex; two different excitatory influences from the RN and SM respectively and feed back mechanism from the RN to IN.

SIMULATION OF INSTRUMENTAL CONDITIONAL REFLEXES WITH COMPUTERS

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The brain structures as well as the combinations of irritations involved in our experiments with animals presume a learning matrice of at least 12 elements. Depending on the confirmation, the information content varies according to the learning characteristics and the generalization of irritation. Individual factors can be approached by simulation of the internal motive power. Reflex connections can be modelled as stochastic courses prevailing through series of random events. The input signals run through the periods of identification of irritations, determination of reactions and memorization; the latter one treats the returned information about the result of the reaction too.

The dog brain model reproduced the results of our experiment, namely: building up of the facilitating and hindering conditional reflexes, the role of internal motive power in the individual behaviour and in the changes taking place in consequence of extirpations; moreover it reproduced the further developments of the ruined conditional connections. This confirms our supposition on the central switching mechanism of the conditional reflexes and within it on the role of the forehead lobe: the external irritations, the reactions joining them, the information returned about their confirmation as well as the conditional connections on the basis of the internal motive power are all here integrated. The structure of the model makes it possible to model conditional connections as many as wanted. With this we gain possibility to approach complex behaviours.

ELECTRONICAL AND COMPUTER MODEL OF INSTRUMENTAL CONDITIONAL REFLEXES

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In the forehead lobe of the dogs facilitating and hindering structures were found which control instrumental conditional connections. Their functioning was approached with the model-analysis of the conditional connections.

Our electronic model contains switches for indication of irritations, reactions and confirmations, information-storing and logical elements, as well as lamps to indicate the working of these elements. If the irritation and the reaction are followed by confirmation, the information content of the storage device swings towards facility; in case of missing of confirmation, the information content swings towards hindrance. If the facilitating information content exceeds the hindering one, a temporary connection builds up in the apparatus between the given irritation and reaction. If the hindering information content

is greater than the other one, the connection between the irritation and reaction is hindered. In case of switching off the facilitating or hindering storage devices, the conditional connections built up by the model will be damaged in the very same manner as the conditional connections of the experimental animals when extirpating the analogue structures of their forehead lobe. The working of the nervous system structures taking over the tasks of the extirpated territory can be simulated by switching on the information storage devices switched off previously.

Modelling the dog's nervous system and the course of our experiment on a computer, all the essential factors of learning can be simulated. Therefore comparing the results of the computer and the experiment, our supposition on the working mechanism of the forehead lobe can be entirely checked.

RECORDING OF MUSCLE POTENTIALS FROM THE LIMB IN FREELY MOVING SALAMANDERS

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A pair of insulated silver wire, 50 μ in diameter inserted into individual limb muscles was used to record muscle potentials. The wires were fastened with sutures to the back, and with methyl-2-cyanoacrylate to the muscles. By this procedure the artifact caused by movements was so small that it could be filtered out with appropriately choosen resistors in parallel arrangement with the electrodes. The activity of sight muscles was recorded at the same time. From the length of active and resting periods the function of individual muscles was studied in a walking step. It was interesting to observe the extensive overlap in the activity of synergic and antagonistic muscle pairs, and that some of the muscles, depending on the actual position of the limb, could act either as synergist or antagonist.

FUNCTIONAL ADAPTATION OF THORACIC SPINAL CORD SEGMENTS GRAFTED INTO THE PLACE OF BRACHIAL SEGMENTS IN CHICKENS

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It is known from previous experiments that wing muscles, following replacement of the brachial segments by throracic segments of the spinal cord, gradually degenerate from the 12th day on of incubation, and disappear in spite of the presence of motor nerve fibers in the wing. If the transplantation is performed at the earliest possible embryonic age, i.e. at the closure of the medullary tube, nearly complete wing movements develop. A few hours delay in the operation results in a gradual loss of movements: first the shoulder,

then both the shoulder and elbow, and finally the whole wing remain motionless. Parallel to the increasing reduction of wing activity in the proximo-distal direction, the morphological differentiation of the grafted thoracic segments was gradually reduced in the cranio-caudal direction. Muscle potential recordings revealed relatively strong muscle activities even in apparently motionless wings. The findings suggest that the capacity of the spinal cord to throw the muscles into co-ordinated action is impaired in the case of decreasing functional adaptation.

EFFECTS OF GAMMA IRRADIATION (Co^{60}) APPLIED BEFORE SOWING ON PHOSPHORUS$_4$ UPTAKE AND PHOSPHATE INCORPORATION IN TOMATO PLANTS

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In the experiments carried out from the 3–4-leaf stage to inflorescence the uptake of phosphorus by tomato plants obtained from seeds, treated previously with gamma irradiation and the distribution of phosphorus in the different organs, and its appearence in the different phosphate fractions were examined on four occasions. The plants were grown until the time of the isotope treatment in a climate chamber, and were exposed to identical thermal, light, nutritional, etc. conditions. P^{32} was added in the form of a Na_2HPO_4 solution with a specific activity of 20–40 micro C/liter.

An interrelation may be demonstrated between the quantitative change of the radio-active phosphorus (imp./min./g) measured in the different organs and fractions on the one hand and the stimulating and inhibitory effects found in the growth and developemnt of the plants on the other.

A more active uptake of P^{32} was found to be accompanied by an increased rate of its incorporation into the different organic phosphate fractions. In the majority of cases, the RNS/DNS ratio diminished under the influence of the gamma ray treatment, except for the 500–750–1000 r values measured during the inflorescence.

THE INFLUENCE OF RADIOPROTECTIVE COMPOUNDS ON THE IONIZING RADIATION INDUCED DISTURBANCE OF IRON METABOLISM

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In recent years extensive studies were performed on a great number of mice to reveal the effects of lethal and sublethal doses of different types of ionizing radiations (as X-radiation, Co^{60} gamma radiation and mixed neutron-

gamma radiation from the atomic reactor) on the iron metabolism, particularly on the incorporation of a trace quantity of radioactive Fe^{59}, administered intravenously at different intervals after irradiation, into the newly formed erythrocytes and depot organs of animals. It has been established that in mice the disturbance of iron metabolism reflects rather sensitively and early the radiation injury of erythropoiesis. Less serious lesions were found in animals pretreated with radioprotective compounds, as compared to the untreated controls.

Further experiments were designed to determine quantitatively the effectiveness of the radioprotective compounds applied, namely AET (S,2-aminoethylisothiuronium) and Mexamin (5-methoxytryptamine) and their dose-reduction factors appearing in reducing the inhibition of Fe^{59} incorporation.

CONNECTION BETWEEN THE VITAMIN B_6-BALANCE AND THE MG METABOLISM

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Literary data show that upon administration of pyridoxine Mg uptake increases in the myocard, while the antimetabolite of pyridoxine, desoxypyridoxine decreases Mg uptake. Based on these data it was examined how Mg content of the blood serum and of the myocard changes in the experimental animals in the vitamin B_6-deficient stage.

70 albino rats were used. One group of the animals was kept for 8 weeks on semisynthetic vitamin B_6-deficient food labelled with ^{66}S. The control group was given pressed food preparation. The photometric method with titan-yellow was applied for Mg determination.

The results revealed, that under the effect of the vitamin B_6-deficient feeding the Mg level of the bloodserum compared to the average value of 2.2 maeq/l of the control group decreased to the value of 1.6 maeq/l. A similar change was found in the Mg content of the myocard. Compared to the value of 20.2 maeq/kg of the control group, vitamin B_6 deficient feeding effected a decrease to 15.8 maeq/kg.

The results corroborate the connection between the vitamin B_6 balance and the Mg metabolism.

THE ROLE OF VITAMIN D IN THE REGULATION OF THE SERUM CALCIUM LEVEL IN RATS

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The effect of fasting, calcium deprival and intravenous calcium loading has been investigated in normal, rachitic and vitamin D treated rats.

After fasting in normal and vitamin D treated rats serum Ca levels scarcely changed, whereas in rachitic rats a decrease of 40 per cent could be observed. According to tetracycline marked ossification tests the speed of hypocalcemia development is proportionate to the ossification process. Hypocalcemia may be prevented by administration of calcium through a stomach-tube. The effect of a calcium deficient diet equals that of fasting. For three hours after i.v. calcium injection calcium curves showed no significant changes in the different groups. When at the beginning of fasting i.v. Ca^{45} has been injected the specific activity of the serum calcium did not change in the first hour, while after 24 hours in rachitic rats it was significantly higher than in vitamin D treated rats.

Calcium balance investigations during fasting did not throw light on the hypocalcemia of rachitic rats. The results indicate that the development of hypocalcemia in vitamin D deficient animals is due to insufficient calcium mobilization from the bone.

THE ROLE OF TSH IN THE REGULATION OF THE IODOTYROSINE LEVEL OF THE PLASMA IN DOGS

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Even to-day it is disputed whether iodotyrosines are normal constituents of the plasma, and if they are demonstrated, where do they originate from? To determine plasma iodotyrosines the authors adopted a combined method based on gel-filtration (Sephadex G-25) and the use of an ionexchanger (Dowex 1 × 2). 24 hours after the administration of ^{131}I they could detect labeled iodotyrosines in low quantity in the dog's plasma drawn from the thyroidal, femoral vein and femoral artery. In acute experiments TSH — in a dose which increases the thyroxine secretion rate — did not change the ^{131}I-tyrosine level of the plasma. When TSH was applied in large doses (30 IU) the iodotyrosine level of the plasma increased. This was partly traced back to the enhanced output of iodotyrosines from the thyroids. It is assumed that after the extreme stimulation of the thyroids the iodotyrosines are set free in such a quantity that the dehalogenase enzyme system is incapable of deiodinating them all and so monoiodotyrosine and diiodotyrosine are secreted. Based on this observation they explain the relatively high iodotyrosine content of the plasma in thyrotoxic patients.

THE ROLE OF THE PITUITARY GLAND IN THE REGULATION OF THE GLYCOPROTEID LEVEL OF THE BLOOD

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The experiments were carried out on 45 male albino rats. In group I of the experimental animals (10 rats) muscle-necrosis was induced by ligature of a portion of the rectus abdominis muscle. The animals were sacrificed 72 hours after the operation. In experimental group II (10 rats) the animals were subjected to hypophysectomy, then three weeks later muscle necrosis was induced in the usual way. The animals were sacrificed 72 hours after the second operation. The difference between the animals of the experimental groups II and III was that those belonging to the latter were given — the week before their sacrifice — three times the extract of the anterior lobe of the pituitary (1 ml/100 g; Park Davis, dilution: 1/700). 15 rats served as control.

After sacrifice sialacid, hexosamine and hexose linked to albumin were determined in the blood serum and sialacid and hexosamine in the liver, while sialacid in the muscle was determined separately in the necrotic part, in the inflammatory barrier around the necrosis and in the intact part.

In both the groups I (necrosis) and II (necrosis + hypophysectomy) compared to the control animals, the glycoproteid level significantly increased in the blood serum, in the liver and in the muscle portion forming the inflammatory barrier.

THE ROLE OF CORTISONE IN THE REGULATION OF THE TISSUE PROTEIN METABOLISM

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It is well known that the body-weight of cortisone treated rats considerably decreases and their nitrogen excretion increases. The treatment is followed by reduced incorporation of labeled amino acids and their precursors into the muscle and into the lymphoid tissues. An opposite effect is caused by cortisone in the liver, where it increases protein synthesis and enzyme formation.

In their previous examinations the authors have shown that the absolute weight and nitrogen content of the parotid gland decreases in the cortisone-treated rats, while the weight and nitrogen content of the submandibular gland remains unchanged.

In the present experiments incorporation of S^{35} labeled methionine into the salivary gland of cortisone treated (daily 3 mg/100 g, for a weak) rats was examined. In an other group of the animals radioactive methionine was administered prior to cortisone treatment which lasted one week. The decrease of incorporated activity was examined in this group after the cortisone treatment which lasted one week.

According to the results cortisone decreases the incorporation of labeled methionine into the parotid, but does not change incorporation into the submandibular gland. In the parotid gland cortisone treatment accelerates the decrease of the previously incorporated activity, while this decrease is slightly slowed down by it in the submandibular gland. The results of the experiment show that the effect of cortisone on protein metabolism differs in the various organs.

THE EFFECT OF CORTICOTROPHINE ON LIPID METABOLISM IN RAT ADRENAL

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It has been shown that the stimulation of synthesis and secretion of steroid hormones by adrenal glands after treatment with ACTH is associated with a substancial decrease in lipids. An activation of the activity of lipase was found after the ACTH injection or ACTH addition to the incubation medium. The increase in lipase activity was specific for ACTH and was related to the dose of ACTH. A decrease in the content of free fatty acids (FFA) was found after the ACTH treatment in spite of the increased lipase activity. It was found, in further experiments, that ACTH stimulates the release of fatty acids by rat adrenal in vitro into the incubation medium containing 3 per cent bovine albumin. The release of FFA from rat adrenals was affected only with ACTH, with other pituitary hormones and adrenalin no significant effect was noted. All these hormone preparations had a stimulating effect on FFA release from adipose tissue.

MECHANISM OF FFA-MOBILIZING EFFECT OF BACTERIAL ENDOTOXINS

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Previous experiments have demonstrated a rise of serum FFA-level in rabbits after a single intravenous injection of S. typhi endotoxin. The maximum was reached after 120 minutes. The present studies were aimed to clarify the mechanism of this reaction. Serum FFA was determined by the method of Mosinger.

Transsection of the spinal cord abolished elevation. Sham-operation had no effect, the same was true after the administration of an epinephrine-liberation blocking drug (Sanotensin) (2-octahydro-1-azocinyl-aethyl-guanidine-sulphate) and the β receptor blocking agent "Aderlin" [1-(naphtyl)-2-isopropylamino-aethanol-hydrochloride]. On the other hand α-receptor blocking

drugs such as yohimbin and dibenamin inhibited the FFA mobilizing effect of the endotoxin. These experiments confirm the role of the nervous system in the FFA-mobilizing effect of bacterial endotoxins.

INVESTIGATIONS OF SERUM BETA-LIPOPROTEINS IN VARIOUS DISORDERS OF LIPID METABOLISM

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Authors examined the extraction of human serum with various solvents of different dielectric constants. The extracted lipids were fractionated and determined by quantitative thin-layer chromatography. They established that more apolar solvents (hexane, benzene, ether) elute the loosely bound lipid content of the beta-lipoproteins (chylomicrons) only. Disorders of the lipid metabolism of the organism (hyperlipaemia) result in a characteristic change in the lipid structure both quantitatively and qualitatively. Molecules of ionic affinity towards lipoprotein structure (phosphatids, polyanions) loosen the structural binding of lipids. Formation of abnormal beta-lipoproteins is present in the cases of obstruction jaundice. This can be demonstrated by ether extraction of serum. The losening of neutral lipids suggests the possible function of the physiological heparinoids. It may be assumed that this is a predisponating factor of the lipid deposition on the sclerotic wall of blood vessels.

METABOLIC CHANGES IN THE MYOCARD IN EXPERIMENTAL CARDIAL LESIONS

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The authors started from the observation, that by myosine, prepared from the myocard, in addition to adenosinetriphosphate (ATP) also monophosphates (cytosine-, uridine-, guanosine-5-monophosphates) are considerably dissociated. It was established that the above mentioned monophosphates, in larger doses than ATP, are able to normalize the function of the frog's heart, that had been made hypodynamic by chinine.

By cardipathogenic diet (S_{65}) myocardiac lesion was induced in rats, in which the uridine and ATP-dissociating capacity of the heart was examined. The ATP content of this organ in rest and after increased charge was also studied. It was established that the ATP-content and ATP-ase activity changed neither in the initial stage (5th week) of the infarctoid changes nor in their developed stage (9th week). After charge, in the 5th week of diet, the ATP-content significantly decreased, ATP-ase activity showed a slight

and U-5-MP dissociation a significant increase. It may be assumed that in cardiopathy capacity of ATP synthesis in the myocard is inferior to the normal one.

FACTORS CONTROLLING GLYCOLYSIS IN THE ISCHEMIC MYOCARDIUM

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Arrest of the coronary flow causes in the myocardium within a few seconds an activation of the phosphorylase system and an outburst of glycolysis (Biochem. Z. *342*. 171. 1965). In the present study the level of all glycolytic intermediates was measured in the left ventricle of dogs and rabbits before and at various periods during the first 120 seconds following circulatory arrest. Graphic arrangement of the results according to Gosh and Chance (BBRC *16*. 174. 1964) revealed that the shift to anaerobic metabolism did not proceed monotonically, but in an oscillatory fashion. Crossover points in the glycolytic chain identifiable as metabolic control sites could be located at the phosphofructokinase, glyceraldehyde dehydrogenase and, with less certainty, at the pyruvate kinase steps. In ischemic rabbit myocardium an additional control site was found at the enolase step. The frequency of the damped oscillations was 2.3 min^{-1}; the damping factor was about 2. Part of the increase in phosphorylase *a* (and phosphofructokinase) activity can be attributed to the release of endogenous cardiac catecholamines following the onset of ischemia. Among the non-humoral factors likely to play a role in the regulation of the rate of glycogenolysis and glycolysis in the acutely ischemic myocardium, the level of cellular orthophosphate appears to be of prime importance. Lactate accumulation in the ischemic ventricles was indeed closely correlated to the rise in orthophosphate in the tissue.

INFLUENCE OF GLUTAMATE-DEHYDROGENASE INHIBITORS ON THE ELECTROPHORETIC BEHAVIOUR OF THE ENZYME

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Rat liver, kidney and heart tissues were subjected to agar gel electrophoresis. Glutamate dehydrogenase activity was demonstrated by means of ultraviolet optical test. Two fractions were obtained: a fast fraction, which could be seen already at the start line and a slow one which could hardly be demonstrated and separates slower from other fractions. For the analysis of the aforementioned two fractions the following glutamate dehydrogenase (GDH) inhibitors were used: 1. sodium dodecyl sulphate, 2. diethylstilboestrol

and 3. o-phenantrolin. Sodium dodecyl sulphate, as a known enzyme inhibitor — dissociates proteins to subunits. In the experiments it was found that this inhibitor in low concentrations inhibits the appearance of the fast fractions completely, while the inhibitory effect on the slow one is less pronounced. Application of sodium dodecyl sulphate in higher concentrations results in a total disappearance of both fractions. Diethylstilboestrol and o-phenantrolin — being specific GDH inhibitors — suppress of the fast fraction but have no effect on the slow one. Increasing the applied concentrations, the result has not changed.

GDH seems to be identical with the fast fraction, while the slow one gives only a nonspecific GDH reaction.

ACTIVITY AND LOCALIZATION CHANGES OF LYSOSOMAL ENZYMES IN HUMAN PLACENTAL TISSUE

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One of the earliest manifestations of cell damage is the decrease in enzyme activities bound to lysosomes and a simultaneous increase in the activity of free enzymes in the cytoplasm. Our investigations showed that in the term placenta the activity and localization of lysosomal enzymes — in contrast to the early placenta — were similar to the changes which were observed during cell injuries.

At the end of the pregnancy the bound activity of acid phosphatase, acid ribonuclease, β-glucuronidase, cathepsin D and cathepsin B was considerably decreased in the placental tissue. On the other hand the free activities of the same enzymes showed an increase in the cytoplasm. Consequently the ratio of bound to free activities was also decreased. No changes were found in the localization of leucinaminopeptidase and alkaline phosphatase which are of no lysosomal origin.

Apart from the obvious presence of lysosomal injury the disturbance in the process of recomposition of new lysosomes could be also observed. This was well illustrated by the fact that total activities of free and bound enzymes were conspicuously lower in the term than in the young placental tissue. All these were confirmed also by electronmicroscopic observations.

A shift in balance between desintegration and recomposition of lysosomes indicated a significant modification in the regulation of the lysosomal system in the term placenta. All these phenomena are supposedly connected to the biological aging process going on in the placental tissue.

ACTIVITY AND LOCALIZATION CHANGES OF LYSOSOMAL ENZYMES IN EXPERIMENTAL KIDNEY INJURY

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The desintegration and recomposition of tissue lysosomes are in balance under physiological conditions. In the course of cell injury this balance is upset: not only an increased desintegration of lysosomes can be observed but the recomposition of new lysosomes decreases or stops completely. These changes can be studied by determining enzyme activities bound to lysosomes and those released from lysosomes into the cytoplasm.

In our experiments the changes in the activity and localization of lysosomal enzymes of kidney homogenates were studied during both CCl_4 and sublimate poisoning in rats.

It has been stated that the rate of lysosomal injury i. e. the release of enzymes from lysosomes into cytoplasm and the possibility and grade of recomposition of new lysosomes are dependent on the dosis of the substances used for damaging the cells. 0.5 mg/100 g sublimate caused a reversible damage: the largest quantity of lysosomal enzymes leaked into the cytoplasm could be observed during the first 24 hours following poisoning. Later on recomposition of lysosomes prevailed. This process was especially intensive after 72 hours.

1.0 mg/100 g sublimate caused an irreversible effect: the desintegration of lysosomes lasted, without any recomposition, till the death of the animals occurred.

The disturbances of the regulation of the lysosomal enzyme system is presumably one of the main factors in the development of both functional and morphological alterations during kidney injury.

ON THE SIGNIFICANCE OF CONFORMATIONAL CHANGES IN THE FUNCTION OF TRANSPORT ADENOSIN TRIPHOSPHATASE

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Incubation of a $(Na^+ + K^+)$-activated ATPase preparation with 20 μg trypsin/mg protein at 25° C caused an approximately 50 per cent decrease in ATP-ase activity during 10 min. A similar loss in ATPase activity was observed after 10 min. incubation with 200 μg subtilisin A/mg protein or after 20 min incubation with 300 μg chymotrypsin/mg protein. On the other hand, the simultaneously measured proteolysis was five times higher in the presence of subtilisin A or chymotrypsin than in the presence of trypsin.

The loss in ATPase activity was partly counteracted by Mg^{++}, Na^+ or K^+. The protective effect of cations could be modified by ATP: the effect of Mg^{++} was completely abolished by ATP; the protection by K^+ — in the presence

of Mg^{++} — was considerably lowered by ATP, whereas the action of Na^{+} — under similar conditions — was not influenced at all.

Conclusions: 1. Trypsin — in contrast to chymotrypsin and subtilisin A — acts presumably in the region of the active centre of ATPase. 2. Cations and ATP exert their action by changing the conformation of ATPase. 3. In the presence of Mg^{++} + ATP + Na^{+} the conformation of the enzyme system is not identical with that in the presence of Mg^{++} + ATP + K^{+}. 4. Presumably the changes in conformation of ATPase constitute the basis of the active ion transport across the cell membrane. 5. Arginine or lisine may have an important functional role in the active centre of (Na^{+} + K^{+})-activated ATPase system.

CATION AND ANION ACCUMULATION IN ISOLATED MITOCHONDRIA

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Isolated mitochondria accumulate Ca^{++} ions from the incubation medium. Limited amount of Ca^{++} is accumulated in the absence of penetrating anions: Ca^{++} is exchanged for H^{+}. For translocation of larger amounts of Ca^{++} some penetrating anion i.e. phosphate, arsenate or acetate is required. Parahydroxymercuribenzoate (PMB) inhibits Ca^{++} uptake in the presence of phosphate but not if acetate is the anion.

In an acetate medium mitochondria swell parallel with Ca^{++} uptake. If phosphate is present Ca^{++} uptake is not accompanied by swelling, irrespective of the presence or absence of acetate. In PMB-treated mitochondria phosphate is not inhibitory on swelling accompanying calcium acetate accumulation.

Some antibiotics as gramicidin or valinomycin induce K^{+} uptake in mitochondria. K^{+} uptake is partially dependent on the presence of penetrating anions. After PMB-treatment of mitochondria phosphate-dependent swelling is inhibited while acetate-dependent swelling is unaffected.

It is concluded from the experimental data that PMB in the concentration employed blocks the entry of phosphate into mitochondria. The phosphate penetration accompanying active cation uptake is probably dependent on some SH-group.

THE EFFECT OF ANTIBIOTICS ON THE ION EXCHANGE IN BACTERIA

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The effect of streptomycin and chloramphenicol was investigated on the ^{42}K-exchange in sensitive bacterial cells. It has been stated that owing to the effect of streptomycin the ^{42}K efflux of the sensitive bacteria has accelerated

compared to the control. The permeability constants have been calculated and it has been found that the ^{42}K ion permeability of the sensitive E. coli B. cells treated with streptomycin has increased by 61.3 per cent. Streptomycin causes damage to the wall of the sensitive cells thus changing the permeability barrier.

The ^{42}K exchange of bacteria treated with chloramphenicol slowed down, the calculated permeability constant amounted to 49 per cent of the value obtained in the case of control cells. We suggest that chloramphenicol decreases ion transport by excluding the energy source of transport, the chemical energy of the intermediate products arising from ATP breakdown.

The common effects of streptomycin and chloramphenicol on ^{42}K exchange in bacteria have also been investigated. It has been found that in the case of equal concentration the two antibiotics do not alter the exchange of ions compared to the control. Our results point to the existence of a competitive antagonism between chloramphenicol and streptomycin.

THE TRANSPORT MECHANISM OF Cl^{36} THROUGH THE BRAIN-CEREBRO-SPINAL FLUID-EYE-BARRIER

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It is a well known fact that the concentration of Na and Cl ions is higher in the CSF and the aequeous humor, than in the blood plasma. These ions can move from the blood to electropotentially higher, body fluids only by active transport. We examined this mechanism with the aid of Cl^{36}.

We determined in "in vitro" experiments the active transport of Cl^{36} in the epithelium of plexuses chorioideus and ciliaris of horses. We found a difference between the activities of the plexuses of the 3rd and 4th ventricles.

Apart from the above-mentioned biological membranes we also examined the accumulation of Cl^{36} ions in the cornea, lens, retina, the grey and white matter of the brain.

We also examined the effects of a few substrates, which play a role in the transport mechanism of Cl^{36}, such as glucose, lactose, galactose, and pyruvic acid; and such enzyme inhibitors as dinitrophenol, cyanide, ouabaine and Diamox.

CHANGES IN THE IONIC COMPARTMENTS IN SLICES OF CAT CEREBRAL CORTEX BROUGHT ABOUT BY THE EFFECT OF OMEGA-AMINO ACIDS

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0.35–0.50 mm thick slices from the cat cerebral cortex were excised by the method of McIlwain (in Practical Neurochemistry, Churchill Ltd. London, 1962. 109 p.). Slices were incubated in saline solutions of different ionic

compositions, all having physological tonicity, in the presence of glucose and under fluent oxigenation. During incubation the slices swelled and took up Na as a function of time. In the presence of 5 mM K, swelling was of smaller degree and the uptake of Na was more pronounced than in the presence of 27 mM K, or, if Ca was absent from the medium. 110 mM K increased the swelling further and caused an uptake of K, while probably because of the activation of the Na pump the Na content decreased. The effect of gamma-aminobutyric acid in different concentrations on the above-mentioned phenomena was investigated.

BIOLOGICAL TRANSPORT OF BROMIDE ION

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Characteristics of the biological transport of bromide ion have been examined in the dog and rat, as the role of bromide ions has become to be of increasing interest because of the extracellular volume determinations (activation analysis) and the investigation of the effect of bromide containing cytostatics.

Concentration changes of the bromide ion ($NaBr^{82}$) between the corpuscular elements and the plasma of the blood, kidney- and liver-clearance and the changes in time of the virtual bromide space after intravenous and peroral treatment with $NaBr^{82}$ have been examined in the dog.

After intravenous and peroral administration of Br^{82} into rats a two directional transport of bromide ion has been observed in the gastro-intestinal tract.

EXPERIMENTS ON ENTERAL ABSORPTION OF CALCIUM

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Regarding calcium absorption from the duodenum and the proximal jejunum the authors carried out *in vivo* experiments with Ca^{45} isotopes on white rats weighing 80–120 g. The method applied was the so-called "*in vivo* loop technique".

It has been established, that in studies on Ca transport, the Ca^{45} content of the intestinal wall has also to be considered. It has also been found that under the conditions applied the measure of Ca penetration from the blood into the intestinal sac is irrelevant.

On examining the dependence of the absorption from the quantity of the administered Ca, it has been established, that in the case of Ca doses of 100–500 μg considerably more Ca is absorbed from the duodenum, than from the jejunum.

A calcium-deficient diet lasting two weeks resulted in a considerable increase in Ca absorption in both the duodenum and the jejunum. In animals kept on normal diet the treatment with large doses of Cortison (250–500 μg pro die pro animal) for two weeks resulted in a slight, not significant decrease in Ca absorption. By the same treatment the increase of Ca transport due to the Ca deficient diet was inhibited in the jejunum and hardly influenced in the duodenum.

STUDIES ON THE INTESTINAL ABSORPTION OF DRUGS IN THE RAT

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The absorption of benzimidazole across the intestinal epithelium was investigated in experiments *in vivo* (perfusion of the small intestine according to Schanker et al.) and *in vitro* (using the everted small intestinal sac according to Wilson and Wiseman).

The following two mechanisms seem to be involved in the passage of benzimidazole across the intestinal epithelium: 1. at low concentrations the presence of an active transport mechanism may be assumed (characterized by saturation phenomenon, depression of transport with inhibitors of energy-yielding reactions, competitive inhibition with similar compounds and movement against a concentration gradient); 2. at higher concentrations — at which the active transport becomes saturated — passive diffusion may prevail.

These data suggest that benzimidazole and its derivatives may be transferred by transport mechanisms of physiological importance involved in the absorption of food constituents.

ELECTRON MICROSCOPIC DEMONSTRATION OF MEMBRANE BOUND ADENOSINE TRIPHOSPHATASE ACTIVITY IN HEART MUSCLE CELLS

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By the use of the lead phosphate technique a Mg^{++}, Na^{+} and K^{+} activated, ouabain-sensitive ATPase has been localized on the plasma membrane of the sarcolemma in electron migrographs of heart muscle cells of several animals.

A second, Mg^{++} activated, Na^{+} and K^{+}-independent ATPase activity has been demonstrated in the various regions of the intercalated disks. Ouabain did not noticeably reduce the enzyme activity in these intracellular membranes.

EFFECT OF CELLULINE ON THE ACTIVITY OF THE SODIUM CARRIER SYSTEM IN THE CAT AURICLE

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In previous works performed with one form of Knoll's celluline ("celluline-A" it has been shown that this substance specifically enhances the activity of the sodium carrier system of the frog's ventricle. Recent experiments with intracellular techniques proved that celluline-A significantly increases the rate of depolarization and the overshoot of action potential in the cat auricle bathed in Tyrode solution, but the effect is inferior to that observed in the frog heart. However, if unfavourable conditions are brought about for the sodium carrier system by increasing the extracellular potassium concentration, the celluline effect becomes conspicuous in the mammalian heart too. Similarly to previous observations on the frog heart, epinephrine and isoproterenol do not affect the rate of depolarization in the mammalian heart either. It is concluded that celluline plays a specific role in the facilitated sodium diffusion of the cell membrane in the mammalian heart too, but in contrast to the frog's ventricle, the cells of the cat auricle are nearly optimally supplied with celluline under physiological conditions.

CHANGES OF RESTING POTENTIAL AND ACTION POTENTIAL ON THE VENTRICLE MUSCLE OF FROG HEART FOLLOWING THE DOSING OF DIFFERENT CHEMICAL MEDIATORS

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It has been established by means of microelectrode investigations carried out on intact and injured ventricle muscle of frog heart that small doses of adrenalin, noradrenalin and acetylcholine bring about changes in the resting potential and in the action potential opposite to the classical effects of these drugs. In certain cases the same was experienced immediately after the administration of the classical doses of the above mentioned drugs. This phenomenon was observed with contact electrodes as well. It is supposed that in the case of small doses, opposite to the effect of the administered drugs, an "adaptation phenomenon" prevails.

Recent investigations have revealed the role of protein bound ions, phosphate of high energy, factors of metabolism, and electron microscopic changes in the ion exchange of membranes. These give an opportunity to complete the classical membrane theory in the case of heart muscle. The authors have registered the monophasic action potential by monopolaric (contact) electrodes. The indifferent electrode was fixed on the injured area or in its immediate vicinity, the differential electrode was fixed outside the heart muscle. In the

authors' opinion the injured area becomes positive in the course of excitation caused by heart activity. The demarcation potential and the monophasic action potential are the resultants of the electric fields.

THE EFFECT OF ACTH TREATMENT ON THE CORTICOSTERONE BINDING CAPACITY OF TRANSCORTIN

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As measured by the gel filtration method of De Moor*, 24 hours after the last of 14 consecutive daily injections of ACTH (Cortrophine Z, 3 I.U/day) the corticosterone binding capacity of transcortin was found to be reduced in the plasma of intact, adrenalectomized and thyroidectomized rats of either sex, and in ovariectomized females. The reduction was less in thyroidectomized than in intact control animals. Plasma corticosterone and total protein concentrations were in all experimental groups the same as in the respective control groups. The conclusion is that prolonged ACTH treatment reduces the corticosterone binding capacity of transcortin not at all, or not only, via the adrenals, the thyroid gland, or the ovary.

* P. de Moor: The binding of radiocortisol on transcortin as measured by gel filtration. Pflüger Arch. *285*. 349. 1965.

ULTRASTRUCTURAL STUDY OF THE HORMONE TRANSPORT IN THE ADRENAL MEDULLA OF NORMAL AND RESERPINE TREATED RATS

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According to the "molecular" theory of hormone transport both adrenaline and noradrenaline get in molecular form into the circulatory system by way of active diffusion. The rapid, lasting secretion of the large amount of catecholamines cannot be interpreted by this slow mechanism. A few observations have been made recently on the "granular extrusion" of the catecholamines from the medullary cells into the sinusoids of the adrenal. In the course of electron microscopic studies the author examined in the adrenal medulla of chicken and rat at first in 1963 and later also in many instances sinusoids in which occasionally one or more catecholamine granules were observable. 6, 24, 48 and 78 hours following repeated reserpine treatment (3 $\times$ 2.5 mg/kg) increased cell surface activity and the mass appearance of catecholamine granules were observed in the majority of the adrenal sinusoids. On the basis

of these observations it is suggested that the granular form of hormone transport is the primary extrusion mechanism of the adrenal medulla which supplies the circulatory system with rapidly mobilizable "catecholamine reserve". Further studies are needed to elucidate the mechanism of the "granular extrusion".

SUBMICROSCOPIC ANALYSIS OF THE EFFECT OF OBSTRUCTION ON THE PANCREATIC ACINAR CELLS

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In our earlier investigations the ultrastructural aspect of functional conditions of pancreatic acinar cells of Rana esculenta was examined. We found that the number of secretory granules is connected with the submicroscopical structure of acinar cells. In our present experiments the effect of the obstruction has been investigated and it has been pointed out, that their effect depends on the condition of pancreas before the operation. In the experiments we have studied the dependence of the characteristics of acinar cells upon the time of the artificial obstruction, and it has been found, that in the hypoactive stage of pancreas (starved animal) the number of secretory granules decreases after an initial increase, whereas in the hyperactive stage (fed animal) it remains at a comparatively constant level. Among the earlier functional conditions in a submicroscopic relation considerable changes can be observed in the hyperactive stage of the pancreas, which appears first of all in certain areas of the ergastoplasm and nucleus. In the course of obstruction — as it appears to us — the different intracellular effects play an important role in addition to the mechanical factors of dilatation of the acinar lumen.

THE DISTRIBUTION OF ALPHA-AMYLASE ISOENZYMES OF FROG PANCREAS IN DIFFERENT CELL FRACTIONS

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Water-soluble proteins of frog pancreas were resolved by DEAE cellular column chromatography into four components with amylolytic activity Two of these four fractions are bound on a column equilibrized with 10^{-2} M TRIS/-HCl buffer of pH 8.0 very weakly and they could be eluted by washing the column with the same buffer. By linear chloridegradient elution two further components were separated at ionic strengths of 0.03 and 0.07 pH-optima and K_M-values of the four isozymes are equal.

The absolute and relative amounts of the four isozymes are different in various cell fractions. Approximately the same quantities of the weakly

bound two fractions are present in the microsomal fraction, in the zymogen granules and in the soluble fraction. The bulk of the microsomal amylase was eluted at an ionic strength of 0.03 (3rd fraction), but the three other fractions were also detected. The amylase of the zymogen granules was identical with the isozyme eluted at 0.07 M chloride concentration. In this fraction the amount of the 3rd fraction was negligible. All four isozymes could be found in the soluble cell fraction with the highest amylase activity related to the total protein content.

ALLOSTERIC ENZYMES AND THE REGULATION OF CELL METABOLISM

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The idea that the combination of one molecule (ligand: substrate or any other specific compound) with a macromolecule can influence the binding of another ligand (the same or different) to the same macromolecule has been proposed long time ago. It has been supposed that the underlying mechanism of the effect is a conformational change in the macromolecule induced by the binding of the first ligand. Some years ago Monod has introduced the term: "allosteric protein" to describe proteins in which such effects occur. The introduction of such a term has been valuable in calling attention to a particularly important class of enzymes which potentially at least, provide an explanation for the regulation of metabolic processes in living cells. During the last few years it has been detected that all the main metabolic routes of the cells are regulated by allosteric enzymes.

A characteristic property of the allosteric enzymes is that they have not only substrate binding site(s) but one or more allosteric site(s) for binding the allosteric effector, activator or inhibitor. Consequently the application of such terms of the classical enzyme kinetics as competitive or non-competitive inhibition becomes only formal, and it is impossible to deduce a molecular mechanism from kinetic data alone. One of the most important hypotheses advanced to explain the mode of action of allosteric effectors is that they regulate the activity of enzymes by "allosteric transitions" or conformational changes in the enzyme structure.

During our studies on the properties of the allosteric enzyme of the arginine biosynthetic pathway and on the properties of the allosteric enzymes of the aromatic amino acid biosynthetic pathway we have observed that the temperature very strongly influences certain properties of these enzymes. The activity of the allosteric enzyme of the arginine pathway is inhibited competitively (apparent) by arginine (*K* system). In this case the inhibitor constant (K_i) of the enzyme decreases with decreasing temperature, on the contrary, the apparent Michaelis constant (K_m) of the enzyme for the substrates does not significantly change with changing temperature. The activity of one of the allosteric enzymes of the aromatic amino acid pathway is inhibited non-competitively (apparent) by phenylalanine (V system). In this case the

inhibitor constant (K_i) of the enzyme does not change with the temperature, in contrast to this, the Michaelis constant (K_m) of the enzyme for the substrates sharply decreases with decreasing temperature.

The binding of inhibitor or of the substrates depends on the conformation of the enzyme molecules. Since we could not detect any kind of allosteric site interactions in the presence either of substrate or of inhibitor we cannot claim that the substrate or inhibitor induces any shift in the conformation of the enzymes. On the contrary, it is reasonable to suppose that the shift in conformation is caused by a change in the equilibrium of the fluctuation of the polypeptide chain of the enzyme molecule induced by the temperature. As a result of the fluctuation of the polypeptide chain, in one possible conformation the enzyme may bind the substrate, and in another conformation the inhibitor is bound to the enzyme molecule with certain probability determined by the temperature, the concentrations of the substrates and of inhibitor, and the essential conditions in the broadest sense.

The result of the binding of substrate or of inhibitor is the stabilization of the appropriate conformation of the enzyme molecule and the induction of movement of all groups involved in the binding of substrate or inhibitor to fit the complete binding or catalytic requirement.

In the cases studied, decreasing temperature favors the formation of the inhibitor (arginine) binding conformation of the allosteric enzyme belonging to the *K* system and the formation of the substrate binding conformation of the enzyme belonging to the *V* system.

It seems probable that the molecular basis of the regulation of cell metabolism by the temperature is the temperature induced change of the conformation of the allosteric enzymes observed.

ROLE OF INHIBITION AND ACTIVATION OF ALLOSTERIC PHOSPHORYLASE IN THE REGULATION OF GLYCEROGEN EXCHANGE

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It is known that the limiting enzyme of glycogen mobilization is phosphorylase whose active and inactive forms were demonstrated in muscle, heart, liver and other tissues. Cori (1956) has demonstrated that adrenalin or electrical stimulation transforms phosphorylase *a* into active *b* in the muscle *in vivo*. Later it became known that this transformation is caused by the activation of phosphorylase *b* kinase and that it helps the cyclic production of AMP by adrenalin. On the other hand, the electric stimulation of the muscle does not go together with the cyclic production of AMP. This made understandable the glycogen mobilization effect of adrenalin: its cycle AMP production together with the activation of *b* kinase produces the phosphorylase *a* form. The electric stimulation on the other hand, activates the *b* kinase with the help of Ca^{++} and an activating factor, and this activates phosphorylase. The same process was observed in liver and heart owing to the effect of adrenalin and glucagon.

It seems nevertheless, that only in liver and in smooth muscle is the transformation of the inactive form of phosphorylase into the active form absolutely necessary for the mobilization of glycogen. An other regulation mechanism was discovered as well, in the skeletal muscle and heart muscle. This is based on the allosteric character of the inactive form of phosphorylase.

Lyon and Porter (1963) have demonstrated that the muscle of the so-called "I" mouse stock does not possess phosphorylase *b* kinase enzyme and consequently the phosphorylase *a* form cannot exist either. In spite of this glycogen decomposes in the muscle of these mice also during work. This phenomenon could be explained by such an activity increase of phosphorylase *b* which happened without the transformation into *a*.

According to the investigations of Morgan and Parmeggiani (1964) anoxia also causes quick glycogenolysis in the heart muscle without the transformation of significant amount of phosphorylase *b* into *a*. In this case too, the mobilization of glycogen is caused by the activity increase of the *b* form. There is sufficient AMP present in the heart muscle to ensure the activity of the *b* form. Nevertheless, the *b* activity induced by AMP in aerobic heart is kept low by G-6-P and ATP (allosteric inhibition of the *b* form). In anoxia the concentration increase of AMP and P_i, moreover the quick transmission of G-6-P results in the release of phosphorylase *b* from the allosteric inhibition, its activity increases and thus is able to mobilize glycogen.

The phosphorylase *b* form is made active by its increased affinity towards the substrates (P_i and glycogen) owing to the effect of AMP. On the other hand, the substrates increase the affinity towards AMP. The competitive inhibition predominating the activation effect of AMP is the inhibitory effect of ATM and G-6-P, that is to say, ATP and G-6-P significantly increase the value of AMP K_m to phosphorylase *b*.

G-6-P takes an exceptional place among the intermediates in the regulation of the glycerogen exchange. The concentration decrease of G-6-P promotes the glycogenolysis by moderating the inhibition of phosphorylase *b* and by decreasing the amount of G-1-P necessary for the resynthesis. On the other hand, the concentration increase of G-6-P inhibits the mobilization of glycogen partly by exerting inhibition on phosphorylase, and partly by exerting activation on glycogen-synthetase. At the same time it increases the level of G-1-P and in this way an increased production of UDPG, the precursor of glycogen, becomes possible.

Helmreich and Cori (1965) found by re-examining the glycogenolysis occuring during the activity of the skeletal muscle that the activity of phosphorylase and phosphofructokinase has a basic significance in the regulation of glycogenolysis. During the *stimulation* of muscle the activities of these two enzymes increase parallel and proportionally with each other, and thus the accumulation of G-6-P does not occur even during increased glycolysis. On the other hand, owing to the stimulating effect of *adrenalin*, only the activity of phosphorylase increases significantly, the activity of phosphofructokinase does not, and consequently the level of hexosemonophosphates significantly increases during the glycogenolysis caused by adrenalin. The concentration change of ATP, ADP, AMP and P_i in the muscle is too small for the explanation of the *in vivo* observed increase of enzym activity during the stimulation. The concentrations of G-6-P, F-6-P and FDP do not make probable this change either. Thus, the most important change in close relation with the concentration

and relaxation during muscle activity seems to be the fact that phosphorylase *b* kinase goes through an activation and inactivation cycle which results in the transformation of phosphorylase *b* form and *a* form. Nevertheless, the lactic acid production of the "I" stock cannot be explained by this change.

In accordance with the above discussed, the regulation of the glycogen mobilization takes place according to two mechanisms. One of the mechanisms is characterized by the formation and decomposition of the active form of phosphorylase. This can be rendered parallel with the increased and moderate production of the enzyme proteins. *The main character of the other mechanism consists in the regulation of the activity of the finished enzyme by intermediators and nucleotides, on the basis of the allosteric characteristics of the enzyme.* The two mechanisms play a role most probably in different measure in the glycogen mobilization caused by muscle contraction, anoxia and adrenalin. The different kinds of tissues (skeletal muscle, smooth muscle, heart muscle, liver, etc.) can also differ from each other essentially according to which of the two regulating mechanisms predominate in them, and in what measure.

In the Institute of Medical Chemistry of Debrecen we succeeded to demonstrate that the allosteric effect of the intermediators includes not only the *b* form of the phosphorylase, but the *a* form as well. The activity of phosphorylase *a* can be also inhibited by G-6-P, 6-P gluconic acid, PP_i, and glucose similar to the phosphorylase *b* (in raw muscle extract and in the preparation of crystalline phosphorylase *a* too). AMP delays the inhibition.

The allosteric inhibition of the *a* form of phosphorylase is significant from several of view. It explains the fact that the 5 to 10 per cent phosphorylase *a* present in the resting muscle does not mobilize the glycogen. The inhibition caused by G-6-P makes it possible that the activity of phosphorylase *a* is also under a fine regulation, depending on the concentration of G-6-P. As glycogenolysis goes on the level of G-6-P increases significantly, and it can exert an energetic arresting effect on the *a* form before its transformation into *b* form.

The intermediators and nucleotides regulate not only directly the activity of phosphorylase *a* and *b*, but also the interconversion of the two forms. We have experienced, namely, that G-6-P inhibits the phosphorylase *a* formation in the *b* kinase reaction, on the other hand, it enhances the inactivation of the *a* form in the phosphorylase-phophatase reaction. This can be looked upon as a double feed-back effect. The *b* kinase inhibitory effect of G-6-P proved to be arrestable with AMP and glycogen, but not with cyclic AMP. G-6-P stimulates the inactivation of phosphorylase *a*, its transformation into *b*. This effect is especially conspicuous in the presence of AMP which inhibits the phosphorylase phosphatase in itself. G-6-P delays this inhibition totally. According to this G-6-P enhances the formation of the inactive *b* form and inhibits its return into the active *a* form.

Summary. Some intermediators (G-6-P, PP_i, UDPG), and certain nucleotides (AMP, ATP) of the glycogen exchange play a fundamental role in the regulation of the glycogen exchange. Their concentration change regulates partly the activity of phosphorylase *a* and *b*, and partly alters the interconversion of the two forms. The actual activity of phosphorylase is increased or decreased in an allosteric way. The interconversion of the *a* and *b* forms is influenced by a mechanism which is not quite clear yet. It can be supposed that the phosphorylase serving as substrate is altered.

It has been demonstrated that a close relation and interaction exist between the allosteric control mechanism (the actual activity of the existing enzym) and the mechanism regulating the transformation of the two forms of the enzyme.

REGULATION OF BRANCHED METABOLIC PATHWAYS

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Certain biosynthetic pathways are operating through common enzymes. Due to the action of these enzymes not only the rate of reactions should be regulated but also the ratio of arising endproducts.

The maintainance of an adequate proportion of enzymes requires a *coordinative enzyme synthesis.* An overcharge of the regulatory system by extreme concentrations of an endproduct or one of the substrates may result in *coordinative derepression.*

The first common enzyme might have an important role in these complex regulatory systems, where not only the affinity of different substrates possesses a regulatory role but also the *rate of feedback inhibition might change to a great extent or might cease in the presence of different substrate concentrations.*

In demonstrating the above principles we summarize our results obtained by studying branched chain amino acid biosynthesis.

SOME ASPECTS ON THE CONTROL OF THE FUNCTIONAL BEHAVIOUR OF STRUCTURAL PROTEINS

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The study of the relations between the structure and function of proteins and the discovery of their control mechanisms require again and again a newer aspect of protein construction. Thus, besides the ways of the regulation of enzyme activity detected in the last years, in which the different metabolites are acting as modifiers on the conformation of the enzyme-proteins, more and more enzymes seem to be of complex composition, their activity could be regulated by monomer-polymer transformation, by association of subunits to form hybrids etc. The *in vitro* determined structural and functional characteristics of proteins of complex construction naturally depend considerably on the applied methods of extraction and isolation, as well as on the effects of circumstances. All these statements are especially important in case the protein is closely attached to the cell-structure and the changing of conformation does not result in a change of enzyme activity or does not result only in such a change, but is responsible for a transport function, contraction etc.

The present summary contains the results of investigations concerning the actin-components of the contractible protein-complex of the muscle, i.e. of the actomyosin.

The purified so-called homogenous actin extracted from aceton-dried muscle powder has no universally accepted protein-constants yet, and it has primarily been determined functionally. In the course of our experiments it has been proved that by different methods, namely by butanol and ionexchange treatment the actin gets decomposed into several components. The isolated components are characterized by great conformational lability, which may be recognized e.g. by removal or just by addition of ions. The behaviour of actin towards lipid-solvent materials refers to the fact, that in holding together the components phospholipids may be involved. Hitherto it has not been cleared yet in what way the *in vitro* features of the components are reflected in the functions of contractible proteins composing the muscle structure. Just as a matter of interest it may be mentioned, that among the actin-components there can be found a stimulating and an inhibiting factor to actin polymerization as well. This latter may be significant with respect to regulation, because the actin fraction extracted from the muscles of E-vitamin deficient rabbits in the usual way contains it as a separate component, besides the protein-complex capable of polymerization.

STUDIES ON FACTORS AFFECTING LIPID MOBILIZING ACTION OF ADRENOCORTICOTROPIC HORMONE

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Lipid mobilization is based to our present knowledge on lipolysis brought about by a hormone-sensitive lipase. This process is indicated by the drop of the triglyceride content of adipose tissue, it can also be followed, however, through measurement of free glycerol and free fatty acids (FFA), resp., when incubating adipose tissue. Actual concentrations of these substance are usually considered as characteristics of the hormone-sensitive lipase activity. Recent data, however, show that alteration of FFA content is also affected by other processes of cell metabolism (e.g. resynthesis, oxydation).

The purpose of the present work was to investigate to what extent factors affecting fatty acid resynthesis exert their influence on the effects on fat metabolism of ACTH as the most active lipomobilizing hormone when measured by changes in FFA and glycerol. With rat epididymal adipose tissue effects of glucose and adenine-nucleotides were studied at various ACTH levels. It could be established that glucose inhibits fatty acid liberation is spite of promoting glycerol formation. This inhibition gradually rises with decreasing ACTH concentrations. ATP, ADP and adenosine-5-phosphate, in contrast, decrease to a small extent glycerol formation and very appreciably fatty acid liberation.

Further on relationships between lipolysis and resynthesis were investigated with added "uncoupling" factors. It was found that 10^{-3}–10^{-5} M dinitro-

phenol inhibits ACTH effects as regards liberation of both glycerol and fatty acids. This lipolysis may be, however, restored on addition of the nucleotides mentioned. Unlike these, l-thyroxine or triiodothyronine in 10^{-6} and 10^{-7} M concentrations, resp. while leaving unchanged glycerol liberation strongly increase the FFA producing action of ACTH. d-Thyroxine as well as the above-mentioned thyroid hormones without ACTH are inactive.

Effects influencing lipolysis elicited by ACTH in a negative sense as regards fatty acids may be principally attributed to an increase in resynthesis. Concomitant effects are in the case of glucose an acceleration of lipolytic cycle in fat cells and in the case of nucleotides a direct action on hormonesensitive lipase. It can be concluded from our results that the potentiating action on lipomobilization of thyroid hormone is related to the inhibition of resynthesis of fatty acids formed by lipolysis.

STATISTICAL ANALYSIS OF CONDITIONAL EVOKED POTENTIALS

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The data of Ádám and Kukorelli (Acta Physiol. Hung. *26*. 1965.; 6th Intern. Congr. EEG Vienna, 1965) proved that conditional evoked potentials may serve as a memory model. The aim of the present experiments was the quantitative, statistical analysis of this conditioning process. The electrical stimulation of the splanchnic and sciatic nerves was associated in anesthetized cats. A total of 400 pairings were delivered to each animal in series of 40–40 associations. The establishment of the conditional response was observable during the interserial application of the conditional stimulus presented by itself: these 20–20 conditioned evoked potentials were averaged by a NTA 256-type computer. The waves of the averaged curves were quantitatively characterized by estimation of the area between the isoelectric line and the curve. Data were obtained by statistical processing:

(i) Concerning the separation of the conditional evoked potential from the spontaneous background fluctuations;

(ii) Regarding the dependence of the conditional response from the number of associations and the delay interval between the conditional and unconditional stimuli.

The conditional connection was represented in form of learning curves and a formula was created describing approximately the empirical curves.

COMPUTER EVALUATED EXAMINATIONS TO DETERMINE THE NERVOUS POINT OF ATTACK OF DDT

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The aim of the experiments was to determine the point of attack of DDT within the nervous system. Therefore its effect elicited on sleep induced by hypnotica of different points of attack was observed. The animals were given different doses of DDT and the doseeffect-curve was also established.

In cases of 8 different doses measurements were carried out in 6 instances each. Disappearance time of the cervical and labyrinthine — as well as of the corneal reflex, duration of the disappearance and the total duration of sleep (thus 7 independent data) were established in groups of 10 animals. The rats were, in every case, narcotized with two different anesthetics.

From the data obtained variance analyses were carried out regarding doses and also the days. Then Scheffé's repeated comparative method was applied in order to establish which were the group that significantly differed from one another.

The "Gier" digital computer was used for the calculation.

The results revealed that DDT practically did not antagonize sleep induced by Chloralhydrate affecting mainly the cerebral cortex. Pentobarbital, with the brain-stem as main point of attack, considerably decreased the duration of sleep and its profoundness.

THE ROLE OF THE CORTICAL AREAS IN THE REGULATION OF THE METABOLISM AND BODY TEMPERATURE

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Authors investigated the higher neural functions occurring after effects which disturb the autonomous equilibral state of the organism in intact rats and after destruction of different portions of the cerebral cortex. 150 rats were used for the experiments. As unconditional stimuli the inhalation of a gas mixture containing 6 per cent O_2 was associated with repeated sound signs (1000 Hz). In other experiments quick histamine habituation was built up.

The experiments have shown that the amending autonomous functions regulating the homeostasis of the organism were not manifested against hypoxia if bifrontal cortex destruction was applied. Apparently the development of histamine habituation is not bound to the frontal cortex, only its abolishment is. The lesion of other areas of the cortex does not influence the development of the conditional responses protecting the autonomous equilibrium of the organism. The phenomenon can be fitted into the cybernetical view of the system of the regulation maintaining the homeostasis.

A SYSTEM-THEORETICAL APPROACH TO MODELS OF METABOLISM

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Earlier mathematical models of metabolism like the description of metabolism as open system may be more generalized taking General Systems Theory (GST) as a base. This procedure, not yet fully worked out in all respects today, would entail that all branches of GST, like those based upon set theory and topology (in the form of relational biology), linear and nonlinear differential equations (especially suited for the description of oscillatory phenomena) probability theory (as a background of stochastic models), Boolean logic (enabling comprehension of trigger phenomena) etc. could usefully be applied. Which form of GST would seem suited best depends on the level of abstraction, as indicating semantic steps, and the need (or freedom) of being more abstract or more concrete. Concrete models are those whose transformation into computer-simulation or even real automaton-construct was successful. Nearly on each level of the mathematical methodologies cybernetic features of the models, like open-loop control, feed-back control, goal seeking behaviour and learning (in the most general sense) can easily be revealed and are in few instances outlined.

THE CYBERNETIC MODEL OF RENAL CIRCULATION

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On the basis of literary data and their own experimental results the authors have attempted to construct a simple block diagram of the regulation of renal blood flow.

For this purpose the following parameters were taken into account: 1. If the total vascular resistance of the organism is increased the blood pressure rises and the renal blood flow decreases. 2. If the total vascular resistance of the organism is increased, but an enhancement of the blood pressure is prevented by a stabiliser, the renal blood flow decreases, but to a lesser extent than in the case of 1., 3. If the value of the blood pressure is raised without enhancement of the vascular resistance renal blood flow must increase to a lesser extent. 4. After elimination of the effects of the reflexes coming from the central nervous system, in the case of changes of the blood pressure the blood flow must not change. 5. It ought to be possible to disconnect the autoregulation of the blood flow characterising the isolated surviving kidney. To make a model of the above principles the authors have constructed a model operating analogously. In addition to the design illustrating the connections authors show the apparatus. The changes of the minute volume of the cardiac output, the renal blood flow and the blood pressure can be recorded on the

instruments of the simply constructed model if the above-mentioned parameters (total resistance, blood pressure, autoregulation, renal resistance) are changed.

EFFECT OF STIMULATION OF THE LABYRINTH ON BLOOD FLOW THROUGH THE CEREBELLAR CORTEX

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The scarcity of data in the literature has tempted us to study the changes of blood flow through the cerebellar cortex caused by stimulation of the semicircular canals of the labyrinth. Blood flow was studied by means of an electric system based on the principle of negative feed back, enabling the changes in thermoconductivity to be measured. In rabbits in chloralose-urethane anaesthesia simultaneous recordings were made of the changes of respiration and systemic blood pressure, and, in the cerebellar cortex, of the electrical activity and blood flow. Stimulation of one labyrinth with cold water resulted in an increase in blood flow through the contralateral cerebellar cortex and in a decrease on the same side; in response to a warm stimulus the changes in blood flow were of opposite character. Galvanic stimulation produced a biphasic change, and rotation an increase in blood flow through both cerebellar hemispheres. — The lecture analyses the differences in effect between the various types of stimulation as well as the correlation between the parameters studied (systemic blood pressure, respiration, electrical activity and blood flow).

HEMODYNAMIC DATA TRANSFORMATIONS FROM THE ANIMAL TO THE MAN BY ANALOG COMPUTER

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Data of our earlier a–v shunt experiments on dogs were fed into our analog computer model (ACM). The blood flow values of the upper and lower part of the body were augmented as if they were human hemodynamic parameters.

The opening of femoral a–v shunts corresponded to a 0.57 fold decrease in vascular resistance in the dog, and it was equal to a 0.54 fold decrease in resistance in the model. Simultaneously the cardiac output increased up to 146 per cent of its original value in the dog, and had to be increased up to 143 per cent in the ACM in order to maintain the same mean arterial blood pressure as it was in the dog.

The shape of the aortic blood pressure curve made by the ACM was in good accordance with that of the dog: the anacrot shoulder of the curve became

peak and the incisure took place lower both in the dog and in the ACM-experiment.

Conclusion: the hemodynamics of the dog and the man proved to be similar to each other by analog computer model.

CHLOROPLAST DEVELOPMENT IN NORMAL AND MUTANT MAIZE LEAVES

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Chloroplasts were isolated from normal leaves grown at different light intensities (5, 25, 100 and 1000 lux) by density gradient centrifugation, and the amounts of pigments and protein per chloroplast were determined.

With higher light intensities chlorophyll content of chloroplasts increased by a factor of 7, carotenoid content by a factor of 5, and protein content by a factor of 3. The number of chloroplasts calculated per unit weight of leaves decreased in the course of differentiation.

Chlorophyll/carotenoid ratios of etiolated and mutant leaf tissues were lower than the ratios found in the chloroplasts isolated from them. On the other hand chlorophyll/carotenoid ratios of normal green leaves and their fully developed, stable chloroplasts were equal.

The differences in pigment ratios indicate that in normal leaves at the beginning of chloroplast development and in the mutants throughout, heterogeneous populations of chloroplast are formed, while fully developed chloroplasts of normal leaves are similar in pigment content.

Differences in pigment composition of the chloroplasts are also reflected in the heterogeneity of electron microscopic structure.

THE REGULATION OF DNA REPLICATION IN LYSOGENIC RHIZOBIUM

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Regulation of DNA replication was studied in rhizobium meliloti infected with temperature phage or carrying prophage. In such cells two systems of replication are in interaction, one controlling the DNA-synthesis of the host bacterium, the other that of the phage. In lysogenic cells the genetic information of the phage is repressed and incorporated into the bacterial genome. Bacteria in this state were induced by mitomycin C in different concentrations. The effect of the antibiotic on the lysis of the cells, on the production of phages and on the inhibition of bacterial replication was measured. In the

presence of mitomycin the replication of the bacterial DNA was inhibited and the prophage became de-repressed. The reaction of mitomycin with DNA is known but no differences were found between bacterial and phage-DNA in structure or composition to explain the dual effect. The concentration dependency and the timing of the induction indicated an interference with the regulation of replication. In the bacteria a diffusible initiator is known to be needed to start a new cycle of replication, whereas in the case of temperature phages the repressor protein has to be inactivated or its action inhibited to start replication. Thus the mitomycin preventing the initiator and the repressor to be bound to DNA can interfere both with positive and negative regulation. This conclusion was tested on synchronously growing bacteria and on phage superinfection.

CYBERNETICAL MODEL OF THE REGULATION OF THE HOMEOSTASIS OF THE ORGANISM

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Neuro-humoral regulation of an autonomous function is performed by a system of superimposed regulating circuits. Their functions can be conceived like this: the regulating mechanism of the underlying regulating circuit composes at the same time the regulating area of the superimposed regulating circuit. The existing-value developing in the regulating mechanism of the more peripherally located circuit represents a stimulus for the superimposed regulating circuit inasmuch as here in the regulating mechanism of the superimposed circuit the must-value differs from the one developed in the still higher located regulating circuit. The vertically superimposed circuits are also connected horizontally and compose closed regulating circuits. In this system the reconnection due to the stimulus is a positive or negative effect and consequently the result of the regulation depends on the target point of the disturbance. The development of a research cybernetical model along these lines is in progress.

HISTOCHEMICAL INVESTIGATIONS ON THE DNA AND RNA CONTENT OF THE ANTERIOR PITUITARY. A CONTRIBUTION TO BIOCHEMICAL ASPECTS OF ENDOCRINE CYBERNETICS

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In cells of the anterior pituitary with a high biosynthesis of proteohormones as castration — and thyroidectomy-cells the DNA of the nuclei is not to be detected with the Feulgen reaction. In the cytoplasm of these cells RNA is markedly increased when comparing it to cells with a normal function.

Cells of the anterior pituitary functionally inhibited by peripheral hormones following estrogen and thyroid hormone overdoses contain but little RNA. DNA stains intensively. Following adrenalectomy and glucocorticoid application the changes are different.

It is suggested that the "Feulgen-negative material" of the nuclei of hyperactive anterior pituitary cells must be regarded as "activated" or "masked" DNA that induces the specific biosynthesis of proteohormones in the pathway "activated DNA", "messenger RNA". In inhibited anterior pituitary cells no "masked DNA" is demonstrable.

DNA and RNA have to be included as reactable factors into the morphological and biochemical concept of endocrine cybernetics.

INFLUENCE OF THYMECTOMY AS WELL AS THYMECTOMY AND SPLEENECTOMY ON THE INCREASE OF CORTICOSTERONE AND TRANSCORTIN LEVEL CAUSED BY THYROXINE TREATMENT IN THE RAT

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In our present study the influence of thymectomy (Thym.-x) as well as Thym.-x and spleenectomy (Speen.-x) on the increase of corticosterone (Cs.) and transcortin (Transc.) level caused by thyroxine (T_4) treatment has been investigated.

The operations were performed on young-adult Sprague-Dawley strain male rats and the T_4 treatment started 3 weeks later. The rats were injected 20 μg/100 g. BW/day, s.c. for seven days and decapitated 24^h after the last injection. The Cs. levels were measured by means of Opton spectrofluorometer and the Transc. were determined after separation on a Sephadex-G 50 column, using trace amounts of C^{14}-Corticosterone.

Although T_4 treatment was found to be able to rise the Cs. level up to 39.05 ± 8.64 μg/100 ml in the sham-operated group, comparable to the control mean value of 4.85 ± 1.01, the same T_4 treatment failed to cause such a high increment in the Cs. level of Thym.-x (20.00 ± 5.93) as well as Thym.-x and Spleen.-x (11.63 ± 1.93) groups.

As compared with the control value (37.25 ± 1.35 μg/100 ml plasma) the Transc. level was found to be significantly elevated by the T_4 treatment up to 85.06 ± 3.89 in the sham-operated, and 81.59 ± 5.03 as well as 74.19 ± 2.31 in the Thym.-x, and Thym.-x and Spleen.-x groups, respectively.

Our results show that T_4 treatment produces a substantially less increase in the Cs. level of the Thym.-x as well as Thym.-x and Spleen.-x rats than in that of the controls, although the increment of the transcortin level was 100 per cent in all T_4 treated groups. Furthermore, it is apparent that the T_4 treatment fails to rise the Cs. and Transc. levels parallel, probably due to their different mechanisms.

SOME ASPECTS OF IODINE KINETICS IN THE ORGANISM STUDIED BY MEANS OF COMPUTERS

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The study of iodine kinetics using radioisotopes facilitates the elaboration of a model of iodine kinetics. Until now several models have been developed both simple ones and very complicated ones. As a model, suitable for practice as well as for experiments can be considered such one, that makes use of the definition of the thyroid activity and the metabolism of thyroid hormones in the quantitative sense, reflects substantial processes in the organism and is not pretentious for the figuring out of the kinetic parameters.

By means of an analogue computer we have studied the behaviour of the most commonly known models, that means after a single as well as a continuous administration of the tracer. It appears that while in a short-timed interval we find only slight differences between the experimental curves and the analogue record, answering the studied models, many models do not suit when confronted with the continual tracer administration.

The data concerning the thyroid function, necessary for clinical and experimental purposes, may be reached even from short-timed examinations and by means of simple models, in which the mathematical statistical correlation of the theoretical and experimental curves is significant.

ABOUT THE ADRENERGIC CONTROL OF MYOCARDIAL METABOLISM

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Blockade of the adrenergic beta receptors significantly reduced myocardial oxygen consumption in the anesthetized spontaneously breathing dog. This effect was brought about by the elimination of adrenergic factors influencing cardiac work (blood pressure and heart rate effect) on the one hand, and probably by a direct inhibition of the myocardial metabolism on the other. This is shown by the observation according to which the phenomenon appears also in the isolated fibrillating heart perfused with a constant volume of blood from a donor animal where the action of adrenergic beta receptor blockade on other factors influencing myocardial O_2 uptake (as on the myocardial contractility, automaticity, blood pressure and cardiac output) is not be taken into consideration. The mode of action of adrenergic beta receptor blockade on myocardial metabolism is discussed.

CHANGES IN THE GENERATION CYCLE INDUCED BY IONIZING RADIATIONS

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Untreated Németh–Kellner ascites lymphoma cells and the same type of cells irradiated with different X-ray doses were used in cell kinetic studies. The labelled mitosis method, micro-autoradiography, carried out after the administration of H^3 thymidine, were applied to determine the generation cycle of tumour cells.

The length of the generation cycle was found to be 20 to 21 hours for untreated ascites cells, out of which G_1 runs to 8, S to 9 and G_2 to 3 hours. A 9-hour G_2 block was observed after whole body irradiation with 500 R. During mitosis inhibition the mitosis index dropped to 1–2% — as compared to that of $15 \pm 4\%$ of the untreated group. After irradiation, the cell polulation was in part synchronized — as proved by the fluctuation of the mitotic — and thymidine indices.

The above changes in cell kinetics could be observed also after irradiation with 10 R.

Partial synchronisation might be explained by the G_2 block and by the different radiosensitivities of the single phases of the generation cycle.

ON THE MECHANISM OF THE INHIBITION OF THE PYRUVATE ACETYL-CoA REACTION BY TRICARBOXYLIC ACID CYCLE INTERMEDIATES

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Earlier we have found that in isolated rat-liver mitochondria the pyruvate → acetyl-CoA reaction was inhibited in the presence of tricarboxylic acid cycle intermediates. To clarify the mechanism of this inhibition the effect of tricarboxylic acid cycle intermediates on the pyruvate → acetyl-CoA reaction has been studied in a system containing oxalate and transaconitate. (In this system pyruvate is metabolized solely via acetyl-CoA and the tricarboxylic acid cycle is blocked simultaneously.)

It was found that 1. malate, fumarate, succinate and 2-oxoglutarate were all inhibitory, 2. the inhibition exerted by the intermediates increased at higher concentrations, 3. there was a direct correlation between the degree of inhibition caused by the intermediates and the number of oxidative steps needed for their transformation to oxaloacetate, 4. succinate was not inhibitory when its oxydation was blocked by malonate, 5. the inhibition caused by the intermediates could be overcome by 2,4-dinitrophenol.

The *in vitro* rate limiting of the pyruvate → acetyl-CoA reaction are discussed. The oxydation of tricarboxylic acid cycle intermediates and the oxydative decarboxylation of pyruvate will compete for the electron-transport

chain if the capacity of the electron-transport chain is limiting. The inhibition of the pyruvate → acetyl-CoA reaction is the result of this competition. The above feedback inhibition may play a role also *in vivo* in the mechanism by which pyruvate utilization and thereby the tricarboxylic acid cycle are controlled.

THE EFFECT OF LEUCINE ON GLUCOSE METABOLISM

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Authors performed acute and long-term experiments in rats to get information on alimentary constituents which influence the blood sugar curve. They found that administration of leucine together with glucose, owing to the insuline like effect of the former, had a marked decreasing influence on the blood sugar.

The effect of leucine on the blood sugar curve can be counteracted by caffeine.

It is suggested that the influence of the investigated substances on the blood sugar is due to their effect on liver and tissue metabolism.

THE EFFECT OF LEUCINE ON THE METABOLISM OF THE LIVER

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It is well known since the studies of Fajans that leucine produces hypoglycemia in subjects treated with sulphonylurea compounds. Similar manifestations were not observed with other amino acids. Thus it seemed to be worthwhile to study whether or not the effect of leucine on the metabolism of the liver is also different compared to other amino acids.

The experiments were performed on isolated perfused rat's liver using the method of Issekutz modified by us. Alanine and glycine decreased the glucose release of the liver perfused with Tyrode-solution, but did not have considerable effect on the glycogenolysis of the liver. Leucine on the other hand, increased significantly the glucose release of the liver, and abolished glycogenolysis. When besides leucine a sulphonylurea compound was also added to the perfusion fluid, the glucose release of the liver did not increase and instead of glycogenolysis significant glycogenesis was detected. Alanine and glycine given simultaneously with sulphonylurea did not exhibit detectable changes.

These studies indicate that in the development of leucine hypoglycemia beside the increase of insulin secretion the effect of leucine on liver metabolism may also have a definite role.

CHARACTERISTIC SHIFT IN THE REGULATION OF TISSUE METABOLISM DURING REGENERATION OF THE LIVER

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Loss in number of hepatic parenchymal cells due to any reason, is followed as a rule by restoration processes of compensatory character. These processes culminate in a massive increase of mitotic activity, aimed ultimately to restore the original cell number.

The sophisticated process of cell division, so intricately organised, requires availability of both energy and matter for its specific purposes. In order undisturbed course of cell division to be secured, quantitative and perhaps qualitative shift in resting metabolism is supposedly needed.

On this ground it was supposed, that differentiated cellular activities are subjected to characteristic modifications, during the massive proliferation seen following removal of cca. 65 per cent of hepatic parenchyma.

To test this hypothesis, urea-synthetic and glycogen storage-capacity of livers, during induced regeneration were determined systematically.

The data obtained showed that early phase of regeneration, which is accompanied unconditionally by highly elevated mitotic activity, presented the expected shift in tissue metabolism. As characteristic findings, significant drop in glycogen storage capacity and alternating change in urea synthetic capacity were found.

The possible mechanism and significance of metabolic changes found during liver regeneration are shortly discussed.

NEURAL CONTROL OF METABOLIC AND MORPHOLOGICAL ALTERATIONS DUE TO ACUTE, EXPERIMENTAL TOXIC INJURY OF THE LIVER

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The question of how the nervous system might participate in controlling different hepatic activities has remained unsettled up to the recent time. It is equally obscure wether neural factors are involved in different manifestations of the acute toxic injury of the liver.

For studying the possible significance of neural involvement, the course of acute experimental toxic injury of the liver has been studied in rats previously chordotomized at different levels of the spinal chord.

For producing hepatic toxic injury rats were given different doses of CCl_4 via a gastric tube. The actual degree of toxic alterations has been evaluated by histological and biochemical means. As biochemical tests determination of total lipid content and changes in lysosomal enzyme activities — both free and fixed — were regularly used.

It was found as if in preventing histological alterations the time interval elapsed between chordotomy and CCl_4 administration was the decisive factor. On the other hand, the possibility of preventing fatty infiltration seemed to be much more related to the niveau of spinal transsection. Dynamics of processes, characteristic of toxic injury of the liver is excellently reflected in the existing degree of lysosomal degradation. Chordotomy proved to be a preventive measure also in this respect.

Prevention of both morphological and metabolic alterations due to the toxic injury of the liver could be achieved by an efficient combination of the three factors, including the niveau of spinal transsection, dose of CCl_4 and the time of administration.

THE FUNCTIONAL ROLE OF THE LIPOPROTEINS OF ACTIN

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In our preceding paper we have given an account of the decomposition of the purified actin extracted from acetonedried muscle powder into lipoprotein, protein and butanolsoluble fraction. This communication deals with the identification, estimation and the functional significance of the lipid-components of actin.

According to our experiments the G-actin capable of polimerizing does not contain only 3 moles P per mole actin, as it could be expected of the ATP content, but 4 moles P per mole protein. The difference can be explained by the presence of phospholipids. The isolation and identification of the phospholipids were successful by using paper, thin-layer, column chromatography and different specific reactions. It has been proved that the greatest part of the phospholipids of actin is lecithin except a few per cent of cholaminphosphatide. A more thorough analysis has shown, however, that in actin there is a far greater quantity of tri- and diglycerides containing comparatively a large amount of unsaturated fatty acids. In the actin extracted from acetone-dried powder there are 10 moles/mole, while in the natural actin, isolated from washed myofibrils there are as many as 19–20 moles/mole of glycerins. The storing period of the acetone-dried muscle powder has an influence upon the glycerin content of actin, i.e. in the course of prolonged storing (at -15° C) the glycerin content decreases and parallel with it the polymerizibility decreases. All this is in full accordance with the supposition, that during storing the structure of actin is getting desintegrated, and correspondingly a change takes place in the ability of G–F transformation and in the myosin-ATP-ase activating effect.

HORMONAL REGULATION OF AMINOACYL-tRNA SYNTHETASE AND "TRANSFER ENZYME" ACTIVITIES IN THE RAT'S SEMINAL VESICLES

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The activities of aminoacyl-tRNA synthetases and "transfer enzyme(s)" have been determined in the 105 000 g supernates made from the homogenate of seminal vesicles of normal castrated and castrated plus testosterone treated adult male rats, respectively. Activities of aminoacyl-tRNA synthetases have been estimated on the basis of the amino acid dependent ATP-pyrophosphate exchange reaction. To follow the "transfer reaction" a rat liver polysome system has been developed and the stimulatory effect of the vesicular supernates on the rate of incorporation of tRNA bound, ^{14}C-labeled amino acids into peptide linkage has been measured. Experimental results were referred to the DNA content of the tissue portions from which the enzymes have been prepared.

Following castration the activities both of the aminoacyl-tRNA synthetases and of the "transfer enzyme(s)" decrease to 20–25 per cent of the normal values within a period of two weeks, but most part of this decrease occurs during the first week. After 12 hours following the intraperitoneal injection of 5 mg. Retandrol to castrated rats, the activities both of the aminoacyl-tRNA synthetases and of the "transfer enzyme(s)" start to increase in direct proportion with time and 48 hours after the androgen administration they reach 3-5 times higher values, than the corresponding values of the control. Activities of various aminoacyl-tRNA synthetases show difference in the extent of increase, but these differences are independent of the characteristic amino acid composition of the secretory proteins. The significance of testosteron induced early increase in the enzyme activites studied, is emphasized by the result, that the seminal vesicles of castrated rats incorporate radioactive amino acids into proteins in vitro 14–20 hours after testosterone injection three times more intensively, than the seminal vesicles of untreated, control animals do. The probability that the aminoacyl-tRNA synthetases and the "transfer enzyme(s)" are synthesized *de novo* after testosterone administration, is discussed.

RNA METABOLISM IN FROG PANCREAS

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The P^{32} isotope uptake by different RNA fractions of frog pancreas obtained by thermal phenolic fractionation was studied. Two hours after the isotope injection both tRNA and rRNA were extracted at 4° C but only tRNA was found to be labelled. The fraction which was extracted at 45° C contained ribosome precursors having ribosomal RNA type base composition. The

fraction extracted at 65° C contained mainly mRNA (base composition of this fraction similar to homologouse DNA). According to the observation in the fractions obtained at the different temperatures (at 4°, 45°, 65° C) the specific activity of RNA fractions showed an increasing tendency.

When RNA was isolated from both starved and refed frogs kept at 0° C the incorporation of P^{32} was found minimal in fractions extracted at 45° C and 65° C as compared to tRNA obtained at 4° C.

On the basis of our earlier experiments and the present results it can be concluded that the synthesis of pancreatic amylase in frog kept at low temperature is the result of the inhibited cytoplasmic degradation of RNAs derived from the nuclei.

REVERSIBLE DISSOCIATION AND REASSOCIATION OF A RIBONUCLEO-PROTEIDE PARTICLE DERIVED FROM THE CELL NUCLEI CONTAINING mRNA

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RNP particles were isolated from purified cell nuclei of rat liver with 0.1 M NaCl solution which contained 10^{-3} M $MgCl_2$ and 10^{-2} M Tris-HCl, pH 8. These particles having a sedimentation coefficient of 30 S contained a greater part of the mRNA content of the cell. In these particles the rate of RNA and protein was 1 : 4. In linear CsCl density gradient the density of the particles was 1.40. The size of the particles was 180 × 180 × 80 Å as measured on electronmicrographs.

In the presence of 0.7 M KCl, 2.5 M NaCl or 4 M urea the particles were dissociated to mRNA and protein. The sedimentation coefficient of the two components sedimented in sucrose gradient ranged between 4 to 10 S. If the KCl, NaCl or urea were removed by dialysis reassociation of the particles was observed. These reassociated particles upon examination by sucrose or CsCl density gradient centrifugation as well as electronmicroscopy proved to be identical with the original ones.

Moreover, under adequate experimental conditions similar particles were formed from proteins of 30 S RNP particles and homologous mRNA obtained by thermal phenolic fractionation.

THE ROLE OF TONIC AND TETANIC MUSCLE IN THE GLYCOGEN METABOLISM OF THE SCELETAL MUSCULATURE

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In our previous investigation striking differences had been stated in the carbohydrate metabolism of tonic and tetanic muscle forming the sceletal musculature. According to our results the specific biological function (antigravitic and locomotoric) of these muscles can be held responsible for these characteristic differences. In the present work the role of two kinds of muscle in the carbohydrate metabolism of sceletal musculature was studied and first of all the changes during the function of sceletal musculature was investigated. For this purpose adult rats were made run at a velocity of 8–10 m/min. for 4 hours and the effect of muscle work was estimated on the glycogen metabolism of the soleus (tonic) and the gastrocnemius (tetanic) muscle. In the control animals the amount of glycogen and lactate of tetanic muscle was greater than that of the tonic one. Under the effect of gentle muscle work more glycogen is utilized in the tonic muscle having smaller glycogen content, than in the tetanic one. At this time the amount of lactate decreases, as compared to control animals. This decrease is more striking in tonic muscle, than in the tetanic one. This supports our previous assumption, that the utilization of lactate in tonic muscle is greater than in the tetanic one. The changes observed in glycogen content and glycogen metabolism of two kinds of muscle during function of the sceletal musculature yield further evidence for the different role of tonic and tetanic muscle in the carbohydrate metabolism of the sceletal musculature.

PINK SPOT IN SCHIZOPHRENIA AND PARKINSONS DISEASE

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About 10% of schizophrenic patients and about 80% of Parkinsonian patients excrete in their urine, a substance similar to the pink spot first described by Friedhoff and Van Winkle(1). This pink spot material has been isolated from the urine of our patients and identified by chromatographic, electrophoretic, fluorimetric, ionexchange, spectroscopic and mass-spectroscopic means as 4-hydroxyphenylethylamine (p-tyramine). Although other pink spot substances have been seen in addition to p-tyramine they were present in very small amounts (less than 5% of p-tyramine). Apart from showing that they were not β-3,4-dimethoxyphenylethylamine, monoacetylcadaverine or monoproprionylcadaverine they have not yet been further identified. The effect of gut sterilisation on the excretion of p-tyramine, p-hydroxyphenylacetic acid and dopamine in a single Parkinson patient maintained on a constant diet over a period of 24 days has been studied. The results of these studies will be illustrated and discussed.

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IS THERE ANY HEXOSE RELEASE FROM FIBRINOGEN BY THROMBIN?

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Carbohydrate content of fibrinogen (F) and fibrin (Fn) as well as the significance of the carbohydrate component of F in the coagulation has been confirmed by several authors. A controversy exists, however, regarding the differences between the carbohydrate content of F and Fn, respectively. There is neither direct proof, nor direct refutation concerning the hexose release from F by the action of thrombin (T). The present paper deals with an approach to the problem discussed by trying the isolation of possibly released hexoses. Starting with pure F samples containing at least 10 mg of possibly releasable hexoses the conversion of F into Fn was carried out at 37° C, with different pH values and T concentrations in the presence of LL-factor. Volatile buffers were used in all experiments. Clot liquors were obtained partly by lysing the Fn-polymer and thereafter by deproteinization partly by mechanical desintegration and filtration. Hexoses were determined by the orcinol-, anthron- and the modified Molisch method, respectively. Results of the experiments show that the hexose content of the fractions isolated from the clot liquors previously desalted and highly concentrated in vacuo is not significantly higher than that of the control samples containing T previously inactivated by heat. The limited proteolysis of F carried out by the specific T-protease does not release peptides containing hexoses. Thus a direct proof was obtained against the hexose release during the conversion of F into Fn.

CHANGE OF METABOLISM IN HAEMOLYTIC SHOCK AT A BLOOD PRESSURE KEPT ON CONSTANT LEVEL

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According to the literature, in shock besides the general failure of circulation, the blood circulation of certain organs does not get injured to the same extent because of redistribution. The blood supply of the heart is relatively good. This can be attributed to the dilatation of the coronary arteries and to the decrease of the resistence of the veins. Nevertheless the activity of the heart muscle gets injured sooner or later because of the insufficient blood supply and the changed metabolism.

The oxygen consumption of the heart muscle increases in the initial phase of the shock, later it decreases. The oxygen saturation of the venous blood is low and the percentage of the oxygen consumption is high.

The problem is the following: to what extent do all these changes take place if the decrease of blood pressure is prevented after the introduction of blood establishing the shock.

The experiments were carried out on 10 dogs. The fall of blood pressure caused by the effect of the suspension of human red blood cells was eliminated with an apparatus constructed by us.

Owing to the effect of heterolog blood the circulation of the coronary artery increased significantly and the oxygen and sugar consumption by the heart muscle increased also. The decrease of the a–v. O_2-difference refers to an increased speed of flow and to a short circuit circulation. The blood supply of the brain increased also, but it decreased in the hind legs.

On the bases of the experiments we suppose that the redistribution in shock is established even if the fall of the blood pressure is prevented. The maintenance of the blood pressure prevents the decrease of the blood supply of the heart muscle and brain, but does not hinder the decrease of the peripheral circulation. In the case of maintaining the blood pressure the period of the changes of shocks is short.

CORRELATION BETWEEN THE ATHEROMATIC LESIONS AND THE LIPID FRACTIONS OF THE AORTIC WALL IN THE EXPERIMENTAL ATHEROMATOSIS INDUCED BY LOUSTALOT DIET IN RATS

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I have been prompted by the insufficient knowledge due to the correlation between the atheromatic lesions and lipid fractions in the aortic wall to carry out my experiments.

The experiments were made on 150 female and male animals. Atheromatosis was produced by a Loustalot diet during 80 days. The extension of the aortic lesions was measured with the aid of a light microscope after depriving from the adventitia and cutting the aorta along the long axis. The explanation of the atheromatic lesions has been expressed in the percentage of the surface of the aortic wall. An aliquot of the methanolchloroform extract of the aortic wall homogenate was used for determining cholestanol, phospholipids, triglicerides and long chain free fatty acids.

It has been found: 1. The extension of the lesions of the aortic wall was of the same extent in both sexes. After 80 days of diet it ran to 4 per cent. 2. The cholesterol of the aortic wall rised in the same measure and proportionally with the time of the diet in both sexes. The aortic wall of the control animals. contained 7.9 mg% of cholesterol, that of the dietic rats after 80 days 18 mg%. 3. Also the phospholipid content was increased. It was 11 mg% in the controls. After 80 days diet the aorta of the females contained 26 mg% of phospholipids and that of the males 17 mg%. 4. The triglicerids decreased during the diet. They fell from 37 mg% to 18 mg% in both sexes. 5. The quantity of free fatty acids diminished too. It dropped from 8 mg% to 4 mg% in the aorta of the females and from 9.5 mg% to 5 mg% in that of the males.

EFFECT OF IMIPRAMINE ON THE GAS EXCHANGE AND METABOLIC EFFECT OF SOME SYMPHATOMIMETICAL AMINES IN RAT

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Very few data can be found in the literature concerning the effect of imipramine on the gas exchange. The study of the problem seems to be very interesting knowing the influence of several psychotrop drugs on the regulation of gas exchange, and taking into consideration certain co-effects of imipramine with the neuro-vegetative pharmacons, especially with the adrenergic amines.

In the experiments the effect of different doses of imipramine on the oxygen consumption of white rats was studied on the one hand, and the influence of the drug on the effect of amphetamin, adrenalin, and noradrenalin on gas exchange on the other. The oxygen consumption of the animals was measured in an instrument of Belák–Illényi type and the measurements were done according to the conditions of determination of basic metabolism. After determining the basic value, the effect of the drugs was observed for 2 to 3 hours. The experiments were performed on rats awake, and on rats narcotized with 0.7 g/kg ethyluretane.

It was established that imipramine doses of 0.5 to 25 mg/kg does not influence gas exchange uniformly, oxygen consumption is slightly increased by it in certain cases and in other cases it is decreased.

Imipramine doses of 7.5 to 15 mg/kg decreased significantly the metabolism increasing effect of 2.5 mg/kg amphetamin. In contrast to this, imipramine, 2 to 3 hours after its introduction slightly increased the raised metabolism caused by 0.3 mg/kg noradrenalin. The metabolic increase caused by the same dose of adrenalin was influenced by imipramine only slightly, at the beginning it was rather moderate, and later slightly increased.

According to the above results it can be established that duality may be observed in the effect of imipramine exerted on gas exchange. On the one hand imipramine shows an anti-hyperbolic effect (e.g. against amphetamin) like tranquillens, on the other hand it reveals a compensating effect of adrenergic mediators in the relation of gas exchange.

ROLE OF STOMACH AND INTESTINAL TRACT IN CONTROLLING THE WHOLE POTASSIUM CONTENT OF THE ORGANISM

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The authors have investigated the conditions under which potassium is absorbed and excreted in the stomach and intestinal tract.

On human beings given a breakfast containing caffeine they found that the more sour gastric juice contained less potassium, but the relation could not be evaluated statistically. 200 ml of 70 and 140 mEq/l hydrochloric acid,

respectively, were introduced by stomach pump into dogs and at the same time 0.05 mg/kg histamine was injected under their skin. 30 minutes later a reversed ratio was found in the content of the stomach between the gastric acidity and the potassium content. This was statistically significant.

The amount of potassium excreted into the intestine was investigated on a separated loop of intestine of narcotized rats. The investigation was carried out on a group of untreated animals, on a group of animals pretreated with KCl, and on a group in which the kidney veins were tied down. The experiment showed that the increase in the whole potassium content of the organism did not influence considerably the excretion of potassium into the intestine.

It was investigated with the same method whether the absorbed amount of potassium is the function of the whole potassium content of the organism. According to the observations if the potassium amount is greater in the organism of the animals the absorption becomes moderate, in fact, that amount of potassium which was tolerated well by the unharmed animals destroyed those ones which had their kidney veins tied down.

INVESTIGATION OF Zn^{65} INCORPORATION AND THE EXCRETION-SPEED IN RATS BY WHOLE-BODY COUNTING

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Zn^{65} can be induced in persons working with neutron-generators and different accelerators (e.g. cyclotron). The possibility for the incorporation of Zn^{65} is greater, because it is produced as a fission product in the reactors or by nuclear tests. On account of the rapid development of the nuclear-industry and that of the renewal of the experimental explosions large amounts of Zn^{65} can be introduced into the biological circulation. The above mentioned facts include the hazard of the cumulation of this isotope in the human organism.

The author has investigated the effect of different pharmacological pretreatments upon the Zn^{65} incorporation, furthermore on the varieties of the excretion-speed.

The measurements were carried out by the aid of a large single crystal animal whole-body counter in a lead chamber designed by the author.

THE SIGNIFICANCE OF PERI- AND EMPERIPOLESIS IN THE FUNCTION OF THE THYMUS

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Recent investigations consider the thymus as the source of cells possessing immune competence and — though not without opposition — they support that function of the thymus gland in numerous experiments. Author studied those factors which confirm the above hypothesis on cellular and subcellular

level. The close connection of the thymocytes and the cells of the epithel reticulum can be observed in tissue cultures and in observations made in electron microscopic pictures. The phenomenon of peripolesis and emperipolesis manifests itself in the moving of the thymocytes, in the significance of cellcontact, in the intracellular digestion-process and in the enzymatic function of these cells. These specific phenomena characterize to a certain extent the function of the thymus.

CHANGE OF ABILITY OF SERUM ALBUMIN TO FIX CONGO RED IN PREGNANT WOMEN AND NEW-BORN BABIES

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Authors investigated the ability of serum albumin to fix Congo red in 146 persons (25 healthy adults, 30 pregnant women shortly before confinement, and 91 new-born babies). The method is based upon the ability of serum albumin by which it bids Congo red and prevents in this way the precipitation of Congo red by NaCl. The average values are the following: in control cases 0.33 ± 0.05 unit of extinction (UE), in pregnant women: 0.20 ± 0.05 UE, in new-born babies, in the blood of the navel string: 0.26 ± 0.04 UE. The difference between the values of the three groups is statistically significant. The ability of serum albumin to fix Congo red decreased during the first days of life, and on the fifth day the measurable minimum was 0.17 UE. This was followed by a slow rise which reached the 0.20 UE value on the tenth day.

The decrease of the mentioned ability of albumin facilitates the crossing of barriers for some substances and this can be the reason for the supersensitivity of some pregnant women to certain drugs, endotoxin and exotoxin. The difference of the fixing ability of maternal and embryonic serum albumins has a biological importance in the transplacentic transport processes, which take part in the embryo's supply. The changes in the fixing ability of serum albumin might be in correlation with the hyperbilirubinaemia of the newborn baby during the first days of life.

INVESTIGATIONS CONCERNING THE TIME COURSE OF THE NEUROHUMORAL MECHANISM OF ERYTHROPOESIS REGULATION

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Authors have investigated on adult male rats in which phases of the anaemic hypoxia can the erythropoetic reaction be influenced by increasing the oxygen content of the inhaled air, or by dosing such air in different time periods.

If rats were made to inhale 96 to 99 per cent O_2 in the first 24 hours after bleeding (loss of blood, 1 per cent of the body weight), and immediately after this 55 to 58 per cent O_2 was inhaled, the usual reticulocyte reaction did not occur. If the inhalation of oxygen started only 12 hours after bleeding, the reticulocyte reaction appeared later. When the inhalation of air with increased oxygen content started 48 hours after the loss of blood, the erythropoesis was influenced insignificantly. The inhalation of gas mixtures enriched in oxygen (58 per cent of O_2) weakens the reticulocyte reaction following blood loss. On the other hand, if the loss of blood is 2 per cent of the body weight, even the inhalation of pure oxygen cannot keep back the erythropoetic reaction.

The experiments have shown that tissue hypoxia lasting only for a few hours is sufficient to set this neuro-humoral erythropoetic mechanism in function. The reticulocyte crisis of different degrees induced by the inhalation of increased oxygen, and the deferments in time of this according to the period of oxygen inhalation, can be related with the time course of the exciting agents of erythropoetin mobilization. It is known, that the level of plasma erythropoetin starts to rise 6 to 8 hours after venesection or some other acute hypoxia, and reaches its maximum after 24 hours. According to this time course, after reaching the increasing erythropoetin plasma level, the reticulocyte crisis can be influenced only to a small degree or not at all by oxygen inhalation.

THE EFFECT OF WHOLE BODY IRRADIATION ON THE ^{86}Rb TRANSPORT OF ERYTHROCYTES

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The ^{86}Rb uptake of circulating erythrocytes of rats irradiated with different doses was investigated immediately after irradiation both under *in vivo* and *in vitro* circumstances. By means of simultaneously employing ^{42}K and ^{86}Rb we compared the effect of X-ray irradiation on the K and Rb transport. Our results show that the change in the values in the case of *in vitro* incorporation is greater than that *in vivo*. While the ^{42}K uptake of erythrocytes in irradiated animals is smaller than that in control animals, the ^{86}Rb uptake significantly increases due to the effect of irradiation. This increase appears in the case of 25 rad already, with 50—100 rad it is pronounced, but with higher doses the efficiency curve reaches saturation. If blood is irradiated *in vitro* a smaller deviation but of a similar trend will take place only in the case of much greater doses.

The different behaviour of Rb from K can presumably be explained by the fact that Rb has greater affinity to the carriers of the active transport than K, thus causing an increased Rb transport incidental to decreased K influx.

EFFECT OF DRUGS AFFECTING PERMEABILITY ON THE BLOOD CELL ACETHYLCHOLINESTERASE-ACTIVITY OF RATS, IN VITRO

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The role of the acethylcholinesterase enzyme (AChE) in membrane permeability has not been clarified as yet. It is supposed, that an unidentified esterase type enzyme, with similar characteristics as AChE has, does exist in experimental inflammations. Greig and Holland (1949), Taylor (1952) and others suggested AChE to be a regulator of ion penetration. Others, as Zajicek (1957), Auditore et al. (1959) could not confirm this theory. Authors examined therefore the effect of different drugs influencing permeability, on the activity of AChE.

The activity of AChE was determined in rat's blood cell haemolisates, washed blood cells and crude membrane preparations with mecholyl as substrate, by the modified method of Hestrin. Aldosterone, $CaCl_2$, both 10^{-4} M, added to the incubating medium proved to be ineffective, histamine had only a slight effect, but this depended highly on the circumstances. Prednisolone Na succinate, suprastin (chloropyramin), pipolphen (promethazin), hibernal (chlorpromazin), epinephrine, norepinephrine (I_{50} with all of them was about $5 \cdot 10^{-4}$) inhibited, Sandosten (thenalidin) did not affect the activity of AChE. Histamine, administered simultaneously with antihistamines did not influence their inhibitory effect. However some of the drugs mentioned above proved to be AChE inhibitors. No close correlation was found between AChE activity and the ability of these drugs to influence permeability and inflammation.

DNA IN THE HUMAN ERYTHROCYTE MEMBRANES

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In 1963 it was proved that DNA is bound to mitochondria and in 1964 DNA was detected in human erythrocytes. Based on these results our aim was to study membrane-bound DNA. Red blood cells were separated from leucocytes by sedimentation in Dextran solution. After haemolyzation the membrane was isolated by high power centrifugation and dissolved in a 5 per cent solution of Na-dodecyl sulphate. After deproteinization DNA was characterized by UV absorption and base composition. By this method the membrane-bound DNA of normal erythrocytes and of patients with haematological disorders were compared.

ON THE NON-ELECTROLYTE PERMEABILITY OF ERYTHROCYTES

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In previous experiments marked differences were observed in the glycerol and thiourea permeability of erythrocytes of various species. Characteristic differences were also found between the non-electrolyte permeability of foetal and adult, normal and abnormal human erythrocytes.

In the present investigations the effect of proteolytic and lipolytic enzymes, detergents, SH-agents, complex-forming substances and metabolic inhibitors on non-electrolyte permeability of the erythrocytes was examined. By means of various *in vitro* membrane modifications, the different types of abnormal penetration curves, observed in various haematological diseases, could be produced.

STUDIES ON THE ELECTROPHORETIC MOBILITY OF ERYTHROCYTES

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Cell membranes are lipid–protein–polysaccharide complexes. The main obstacle of studying them in more detail resides in the fact that even the mildest physical and chemical examination methods damage these sensitive biointerfaces. Cell electrophoresis provides information concerning the physicochemical alterations of the external surface of the cell membrane without considerable damage to it.

The migration velocity of the erythrocyte in the electric field is determined by its surface charge, i.e. by the chemical nature and arrangement of its surface structure. The electrophoretic mobility of the cell is defined as the velocity per unit field strength. The measurements were carried out on an Opton cytopherometer. The electrophoretic mobility of adult and fetal human erythrocytes as well as of erythrocytes of animal species were examined. Data are presented on mobility changes observed with *in vitro* modified normal human erythrocytes and with red cells of patients suffering from various haematological diseases.

ON THE DIFFERENCES IN MEMBRANE STRUCTURE OF FOETAL AND ADULT ERYTHROCYTES

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Foetal erythrocytes contain Hb F, while adult erythrocytes contain Hb A as the major Hb-component. The enhanced O_2-affinity of foetal erythrocytes is, however, not due to foetal Hb, but is a function of the interaction of foetal

membrane and foetal Hb. Foetal and adult erythrocytes differ in their ultrastructure, in their osmotic-, heat-, mechanic- and acid-resistance. We have observed marked differences between adult and foetal RBC in non-electrolyte permeability and in several enzyme-activities. The lipid composition (cholesterol, esterified fatty acid, lipid-P, phospholipid classes and the fatty acid profile of phospholipid, as revealed by means of gas chromatography) of the foetal and adult erythrocyte membrane was studied in detail. The differences observed in lipid structure are discussed with regard to other structural and functional differences of the two cell-types.

THE EFFECT OF SYNTHETIC VASOPRESSIN ON INULIN SPACE IN THE DOG

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The extrarenal inulin space of anaesthetized dogs subjected to ureteral blockade has been found to be 24 per cent of body weight 90 min. after the injection of inulin. The intravenous infusion of synthetic vasopressin caused the extrarenal inulin space to increase to 50 per cent of body weight. Without ureteral blockade, the inulin space of anaesthetized dogs attained 34 per cent prior to, and 39 per cent after, the infusion of vasopressin.

In these experiments the amount of inulin found in the kidneys was not substracted, which accounts for the larger inulin space.

The changes in the inulin content of various organs in response to vasopressin have been determined.

INFLUENCE OF VERATRINE ON THE ION TRANSPORT OF FROG SKELETAL MUSCLE

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Recent work by Varga et al. has suggested that the depolarizing action of veratrine may be due both to the increase of sodium permeability and that of Na_i in muscle cell. The aim of the present experiments was to analyze the effect of veratrine on ^{24}Na-transport in isolated sartorius muscle of frog. The method applied was similar to that described by Keynes and Swan (J. Physiol. *147*. 591. 1959).

Influx was increased 3.5–4.5 fold in Ringer-solution containing 0.1 mM veratrine. In normal and Li-Ringer ^{24}Na-efflux was also increased by veratrine, however, the enhancement took place only for a brief period and even in the presence of the drug the efflux diminished to the resting level. Veratrine failed to increase the efflux when sodium was replaced by choline in the

Ringer-solution. It appears likely that veratrine does not interfere directly with sodium extrusion and the increase of ^{24}Na-efflux observed only in normal and Li-Ringer was due to the depolarization induced by veratrine. The transitory enhancement of the efflux and a long-lasting increase of the influx resulted a markedly higher Na_i in muscle fibre treated with veratrine. The netto gain of Na and Li was accompanied by an equivalent amount of K loss.

The results suggest that the depolarization of muscle fibre induced by veratrine can be attributed to an increase in P_{Na} and Na_i as well as a decrease of K_i

EFFECT OF TRYPSIN ON THE CA-UPTAKE AND ENZYMOCHEMICAL PROPERTIES OF SARCOPLASMIC RETICULAR FRACTION

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In earlier experiments authors demonstrated that during storage that is "ageing", cholinesterase and ATPase activity of sarcoplasmic reticular fraction (SRF) obtained from fish muscle increased while its Ca-uptake decreased proportionally to the increase of enzyme activity. It was assumed that the molecular rearrangement of SRF structure was responsible for this phenomenon. In the present experiments trypsin was applied in different protein/trypsin ratios. Digestion was stopped by trypsin inhibitor Lima bean. For measuring ATPase activity incubation mixtures containing 2 mM ATP, $MgCl_2$, 50 mM KCl, 20 mM TRIS-maleate buffer (pH. 7) were applied and for measuring cholinesterase activity those containing 1.5 mM ACh, 2 mM $MgCl_2$, 50 mM KCl, 20 mM TRIS-maleate buffer (pH 7). Ca-uptake was measured by application of a perfusion procedure and isotope techniques using cellulose columns (Acta Biochim. Hung. *1.* 159. 1966). It was established by the authors that both cholinesterase and ATPase activity increased up to a multiple of the original activity with proceeding time of digestion. Activities reached maximal values by about the twentieth minute. Ca-uptake of SRF ceased after digestion lasting a few minutes (3–10′). It has further been pointed out that trypsin reduces the Ca activation of SRF-ATPase and even an inhibiting effect of Ca can be observed after about the 10 minutes of digestion.

STUDIES ON THE EXTRACELLULAR SPACE IN FROG SKELETAL MUSCLE

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Comparative studies were carried out on the volume of extracellular space (ECS) using different tracer compounds: $Na_2{}^{35}SO_4$, ^{131}I-human albumin and di-^{131}I-benzoic-azo-human albumin prepared by the authors.

It was found that the gain of radioactivity in an isolated sartorius muscle of frog exposed to a Ringer-solution containing tracer compounds at +4° C was in direct proportion with the soaking period. The equilibrium was not reached even within 16 hours of exposition. It is reasonable to assume that the "conventional substances" used for the estimation of ECS or their products of decomposition could enter the muscle cell. However, there is a possibility that a special adsorption takes place which is powerful enough to hold the molecules of the studied compounds on the surface.

The time course of the movement of radioactive substances from muscle into a nonradioactive Ringer-solution was also studied. Experiments have shown that there are more than two exponential processes having different rate constants. Estimation of the "real" volume of ECS was carried out with the aid of compounds of more stable chemical structure and a kinetic analysis of tracer loss from isolated muscle.

The method presented was found more suitable for the determination of the volume of ECS than those described previously.

THE POTASSIUM AND SODIUM TRANSPORT OF ERYTHROCYTES

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The unequal K^+–Na^+ distribution between erythrocytes and plasma is the result of a dinamic equilibrium, for the maintenance of which complicated physicochemical and biochemical processes are responsible. The "uphill" transport processes occurring against the electrochemical potential gradient, i.e. the Na^+-outflux and K^+-influx, require active work and a suitable source of energy. Energy is provided by the macroerg phosphate bonds of ATP while the transformation of energy into ion movements is the result of the function of the "transport ATPase" system localized in the cell membrane. This "transport ATPase" enzyme or enzyme system can be specifically inhibited by ouabain and activated by $K^+ + Na^+$, its enzymechemical characteristics, however, are so far not known in detail.

The "downhill" transport occurring towards the electrochemical potential gradient is virtually a passive process requiring no energy. The rate of cation movements, however, even in the case of downhill transport is under a metabolic control. The present report deals in detail with this question, first of all on the basis of own experiments.

The cessation of glycolysis, induced by inhibitors or by lack of substrates, results in the breakdown of ATP. As a consequence of this, the active cation transport ceases, K^+ begins to flow out of the cells and simultaneously Na^+ enters the cells. The rate of this ion transport is relatively low, 1.5–2.0 meq/l erythrocytes/hour. In some cases, however, e.g. under the influence of Pb^{++} ions, NaF or iodoacetate + purine nucleosides a high rate K^+-outflow (5–20 meq/l erythrocytes/hour) takes place. The main characteristics of this artificially induced K^+-outflow is that it is not accompanied by equimolar Na^+-influx, but uptake of H^+ ions instead of Na^+ ions occurs in the biological system.

Our earlier experiments proved that the prerequisite of K^+/H^+ exchange is the presence of free Ca^{++} ions in the plasma. The elimination of Ca^{++} ions either by washing or by chelating agents results in the cessation of this transport process. The rate of ion transport can be decreased by the inhibition of the enzyme phosphoglycerate-kinase, as well: if the action of this enzyme is abolished by the activation of the enzyme reactions of the 2,3-diphosphoglycerate shunt, the rapid K^+- outflow cannot occur. Recent observations on the role of N-containing compounds, e.g. NH_4Cl, hydrazine, histidine and histamine, influencing the rate of ion exchange deserve attention. Out of these compounds the most effective is histamine which can significantly enhance the rate of cation transport even in a relatively low concentration. This permeability increasing effect of histamine can be prevented by antihistamines as well as by inhibitors acting at different stages of carbohydrate metabolism. On the basis of these experimental data a working hypothesis was elaborated to explain the mechanism of cation transport.

It is known that histamine — though in a low concentration — is a permanent constituent of the normal plasma. It is likewise known that in shock the histamine level of the plasma may show a 100–200-fold increase. Therefore, it may be presumed that histamine or some metabolic product of it may act as a physiological regulator of the rate of downhill transport processes in the blood. On the basis of these findings it seems conceivable that the mechanism of permeability alterations we brought about experimentally in blood, is identical with the mechanism regulating physiologically the gastric H^+ ion secretion.

RENAL TRANSPORT PROCESSES

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The transport of various substances in the kidney is governed by essentially the same laws as those regulating transport processes in other organs, the differences being rather quantitative than qualitative and reflecting the adaptation of the kidney to its biological role. Owing to lack of time, selection of the material is necessary which inevitably implies arbitrariness at the same time. To illustrate the renal reabsorptive processes, the tubular handling of Na^+, i.e. the transport of electrolytes and water will be dealt with, whereas of the tubular secretory processes the transport of para-aminohippurate (PAH) has been chosen for discussion.

Under non-diuretic conditions approximately 99.0 to 99.5 per cent of the filtered Na^+ is reabsorbed in the tubules. This means that about 5 to 10 per cent of the total exchangeable Na^+ of the organism is lost in the urine. Thus, apparently insignificant changes in the amount of Na^+ reabsorbed may lead to considerable loss, or retention, of Na^+.

In the proximal tubules, the concentration of Na^+ in the tubular and peritubular (interstitial) fluid is essentially the same, still, there is a potential difference amounting to about 20 mV between the two compartments, the tubular fluid being electronegative. Thus, the electrochemical potential of

Na^+ is greater in the peritubular compartment. In the proximal tubule about 70 to 80 per cent of filtered Na^+ is reabsorbed against an electrochemical potential gradient, pointing to active transport of the Na^+ ions in this portion of the renal tubule. The active phase of this transport process is that forwarding Na^+ from the tubular cell into the peritubular fluid, the movement of Na^+ ions from the tubular fluid into the cell being entirely passive, along the electrochemical potential gradient.

Increases in the amount of filtered Na^+ (load) through alterations of glomerular filtration rate (GFR) lead to similar increases in reabsorbed and excreted Na^+, fractional reabsorption and excretion remaining unchanged. If, however, Na^+-load is increased by raising plasma Na^+-level, the amount of Na^+ reabsorbed increases less than the amount excreted, hence fractional reabsorption diminishes, while fractional excretion increases. From this it is concluded that the rate of Na^+-transport is augmented by increased load of Na^+, but its efficiency is limited by a critical difference in concentrations (electrochemical potentials). This concept has received a strong support from tubular perfusion experiments.

In the proximal tubule water is transported passively across the permeable tubular epithelium, since the active transport of Na^+ generates an osmotic gradient along which water is reabsorbed.

The thick segment of the loop of Henle is inpermeable to water, therefore the marked active Na^+ transport occurring at this segment renders the tubular fluid hypo-osmotic, and the interstitial fluid hyperosmotic. By means of the countercurrent systems of the loop of Henle and the vasa recta the Na^+ actively reabsorbed in the thick segment is distributed in a way that its concentration increases from the cortex-medullary border towards the renal papilla. This ensures that, in the presence of ADH, water be reabsorbed along the distal tubule and the collecting ducts, as far as the osmotic gradient existing between the tubular fluid and the interstitium is brought into equilibrium. Thus, osmolality of the urine is increased to reach the hyperosmolality of the renal medullary interstitium. In the absence of ADH, however, the distal tubular epithelium and the collecting ducts are inpermeable to water and the hypo-osmotic fluid elaborated in the thick segment is excreted in the urine.

The transport of Na^+, thus, not only allows the reabsorption of equivalent amounts of water but owing to the structural characteristics of the kidney it secures the elaboration of concentrated urine, i.e. the preservation of water. Therefore, the rate of water reabsorption in the renal tubules depends, even in the presence of ADH, on the transport of Na^+.

The transport of Na^+, on the other hand, is a prerequisite also for the excretion of K^+ and H^+ ions, since the secretion of K^+ and H^+ in the kidney is effected through the exchange processes Na^+–K^+, and Na^+–H^+, respectively. Thus, the mechanisms governing the transport rate of Na^+ exert a control also on the urinary excretion of water, and K^+ and H^+ ions.

A better understanding of these mechanisms has become possible as the disturbances in electrolyte and water household observed after adrenalectomy or in conditions associated with deficient adrenocortical function were more thoroughly studied. The most active corticoid hormone in this respect proved to be aldosterone. This compound enhances the transport of Na^+ not only in the renal tubules, but also in the epithelium of the bladder, colon, and the

toad skin. Most probably it exerts its action by affecting the rate of protein synthesis: the formation of a hitherto unknown enzyme (carrier molecule) is augmented. It is also assumed that in the renal tubules aldosterone enhances the passive phase of the movement of Na^+, i.e. the diffusion from the tubular fluid into the epithelial cells (facilitated diffusion), while the rate of active transport is increased by the augmented Na^+-load itself.

The homeostatic adaptation of the kidney is clearly demonstrated by the fact that the excretory capacity is greatest for several "foreign" substances not present in the organism under physiological conditions. Some of these substances have been extensively studied, a classical example of them is para-aminohippurate (PAH). At relatively low plasma concentrations of PAH, the amount excreted in the urine may be four or four and a half times larger than that of filtered PAH, hence under appropriate conditions the tubules may secrete practically all the PAH that have escaped filtration. The tubular secretion of PAH shows all the characteristics of active transport. It is exceedingly susceptible to damaging impacts, therefore it constitutes the most reliable and sensitive indicator of the integrity and functional capacity of the tubular epithelium. The secretion of PAH can be studied also *in vitro*, and the results obtained with isolated tubules or kidney tissue slices can be compared with data obtained *in vivo*; a quantitative checking of the values will allow the informations gained *in vitro* to be extrapolated for conditions *in vivo*. Also, from changes in the secretion of PAH it is possible to conclude to intrarenal redistribution of blood if impairment of the transport mechanisms in the tubules can be excluded with sufficient certainty.

TRANSPORT FUNCTION OF THE INTESTINE

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Research on transport from the intestine has nowadays three main objectives: 1. the synthesis of our knowledge on the fine structure and function of the intestinal epithelial cell; 2. investigations into the mechanism of transport — carried out mainly by *in vitro* experiments; 3. studies on nutritional, hormonal and circulatory factors influencing this transport by *in vivo* methods. The above aspects serve as basic problems to be dealt with in the present review.

1. In the introduction the author aims to give a survey on the fine structure of the intestinal epithelial cell, discussing the morphology of microvilli, organella and endocytosis.

2. Problems of transport mechanism are introduced by a critical review on the methods adopted which is followed by a survey on intestinal transport of carbohydrates. It seems most probable that the absorption of hexoses occurs in two steps. The first of these phases is a facilitated diffusion by which glucose enters the cell, the second is a process of great energy requirement inducing the intracellular transport from the lumen to the capillaries. Several hypotheses have been put forward to find an explanation for the mechanism of transport function but neither the intracellular metabolism of the transported

substrate nor the presence of phosphorylation process nor the mutarotase activity of the brush border could give a satisfactory explanation. The author and his collaborators indicated as early as 1956 that the ATP content and the ATP-ase activity of the intestinal mucosa have a role in the transport mechanism. Recently the general importance of membrane-ATP-ase has been recognized and with it the problem has become conspicuous again. The relationship among sodium ions, membrane ATP-ase and glucose transport has been proved beyond doubt but a more exact mechanism is still unclarified. The absorption of amino acids has many characteristics in common with the absorption of carbohydrates. Both a facilitated diffusion and a great energy requiring intracellular transport can be differentiated during the process, although competitive inhibitory experiments have proved that at least two pathways exist. The first is that for methionine and leucine, the other for proline and glycine. Another feature of the common characteristics is the indispensable presence of Na^+ both for amino acid and for glucose absorption. Lipoids are transported in the form of free fatty acids, mono-, di- and triglycerids, the latter by endocytosis. The active transport mechanism of fatty acids and monoglycerides is not yet known. Investigations on the transport of calcium, iron and cyanocobalamine have contributed further knowledge on the mechanism of absorption. A detailed survey of the results is, however, beyond the scope of this review and only a general outline can be given.

3. Although *in vitro* experiments are of great importance for research on transport process, *in vivo* experimental results are more suitable to reproduce real physiological conditions. Besides, *in vivo* methods can be best adopted to clinical research too. Author discusses the effects of different diets, the role of insuline, thyroxine and that of the hypophysis and intestinal circulation in the mechanism of absorption. The author concludes reporting on the experimental analysis of two pathological conditions, i.e. intestinal damages induced by irradiation and by dosage of bacterial toxins.

MICROBAL TRANSPORT PROCESSES

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By "microbal" it is meant here those microbes and inferior plants which are not included in the classical microbiological discussions because of their sizes. The classical permeability problem (summarized by Collander, 1959) will not be dealt with among the transport processes although it has contributed to a high extent to the understanding of the basic characters of biological membranes at the turn of the century. To support some conclusions of this presentation, a few experiments carried out on Streptomyces aureofaciens, Saccharomyces carlsbergensis and Scenedesmus obstusiusculus will be shown, made in the Plantphysiological Institute.

The research work on transport phenomena of microbes started with the investigation of the exchange of inorganic ions and of the osmotic characteristics of cells in the early fifties. Applying the methods of biochemical genetics, the investigations of Monod et al. (1955 to 1960) have brought a new trend

in this field. The isolation of transport mutants inherited monofactorially and the inductibility of some transport systems allowed for the reasonable assumption that specific protein-like carriers (permease) may play a role in transport processes. Recently the regulation of transport processes has been demonstrated and a number of attempts has been made to isolate and biochemically characterize the permease proteins.

Escherichia coli is the best known microbe but the study of at least a dozen of other species is in process. Nothing is known from this point of view about actinomyces, about several specialized microbe groups, and about blue algae which are closely related to the microbes.

The investigation of transport problems started with yeast in the twenties with Pulver's and Verzár's observations and till now has produced about 300 articles. In most cases commercial cultured yeast (Saccharomyces cerevisiae lines) was used. Although the biochemistry of S. cerevisiae is almost as known as that of E. coli, a serious disadvantage of the former is our ignorance about its cytogenetic and genetic aspects. This can explain the preferential use of Neurospora crassa in transport investigations in the latest years. Its large cells render possible bioelectrical measurements, it is, however, a drawback that the experimental substance is not homogen owing to the differentiation in the mycelia. In the case of yeast there are a number of established facts about the transport of alkali cations, phosphate monosaccharids and disaccharids and in the case of Neurospora the results of the research of K^+ and amino acetate transport deserve attention.

The investigation of transport processes in algae started with the analysis of the composition of vacuola fluid and with bioelectric measurements. From these investigations the active accumulation of a few ions was inferred. Because of marine coenocita algae were available only in a few research stations, the fresh-water (and brack-water) Chare algae were used for the investigations.

The second group of studies on algae transport phenomena was carried out on thalloid marine algae (green red, and brown algae) mostly performed in the fifties. The application of respiratory inhibitors is especially characteristic to this research work.

It is very surprising, but the study of transport processes of the fresh-water monocellular green algae (Chlorella, Scenedesmus) flourished only in the latest years. Essentially the same methods were used here as with microbes and yeasts. The transport of certain organic substances was experienced only in this group of algae. Even now there are a number of algae stocks (Euglenophyta flagellata, Bacillariophycease, etc.) which are unknown regarding their transport.

The comparison of transport processes of different organisms reveals the general occurence of both the active and carrier mediated transport types. In addition to these passive transport processes ("promoted diffusion") can be also demonstrated in single cases. The two types differ monofactorially in few cases analyses. Taking this as a starting point, it can be supposed that more or less specific proteins take part in certain transport processes. The function of these specific proteins in transport processes is not known. It is probable that transport processes utilize the energy of ATP, but at the present we have no data which would indicate the role of specific transport ATP-ase's in the microbal transport processes.

TRANSPORT OF WATER

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Problems: osmoregulation in fresh water, water transport in plants, secretion of saliva, origin of oedema, production of urine, etc. more precisely: What is the mechanism of the mobilization of fluids under hypotonic conditions or against pressure? Some authors attempt to unite the different theories by means of thermodynamics of nonequilibrium, irreversible processes e.g. Katchalsky and Curran 1965. In this theory the "active" or "carrier"-mechanism caused chemical reactions (e.g. Goldacre 1952, Scholander 1960) can also play a role together with "passive" transport processes produced by pressure, temperature, electrical and chemical potential differences. The problem can be solved by thermoosmosis caused by temperature difference in itself. As a physical phenomenon this was proved frequently in model experiments (e.g. Ernst 1936, Alexander and Wirtz 1950, Haase and Steinert 1959). The biological importance of this effect was discovered explicitly by Ernst in 1936. The results of this kind of experiments are as follows: 1. Temperature gradients of 1° C/mm have given rise to significant differences in the water content of different vegetable tissues, so that, after 20 hours, the amount of water contained in the cooler part of the tissues was about 5 per cent higher than that in the warmer portion. At the same time hypotonical fluid ($^1/_3$ concentration) flowed together which amounted to a few ml. at the cooler site. 2. The concentration of the bleeding sap of sunflower plants was found to be five times lower than of the sap of the root cells. 3. According to the literature the osmotic pressure of the yolk surpasses that of the white of hen's egg by 2 atmospheres for weeks. The temperature of the yolk was about 0.06° C degrees higher than that of the white. Yolks heated selectively by diathermy and kept in hypotonical Ringer solution swelled singnificantly more slowly than the non-irradiated controls. 4. Erythrocytes illuminated with intensive light probably become warm selectively. This can cause a decrease of volume due to water loss. The value of haematocrite decreased by 6 per cent significantly after an illumination for half an hour compared with the values of the controls kept in the dark. A haemolysis occurred in a small measure, but there was no correlation between the degree of haemolysis and the measure of volume decrease. The diameter of erythrocytes illuminated for 4 minutes in a microscope decreased by 4 per cent significantly according to 600 measurements. The consequence may be drawn on the basis of measurements and theoretical considerations, that a temperature difference of 0.01° C is able to produce vapour pressure difference equal to about 1 atm osmotical pressure in adequate structure.

ELECTROPHYSIOLOGICAL ANALYSIS OF THE DEPOLARIZING EFFECT OF VERATRINE

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The mechanism of the depolarizing effect of veratrine has chiefly been studied on neural structures. In order to understand the veratrine effect which induces contraction of the skeletal muscle, however, it was necessary to gain a knowledge of the depolarization brought about by it on the muscle. The experiments were carried out on sartorius muscle of frog using Ling Gerard type electrodes and Keithley electrometer.

0.01 mM of veratrine does not induce depolarization though it markedly potentiates K contracture. The cause of this potentiating effect, therefore, cannot be a summation of veratrine and K-depolarization. 0.02 mM veratrine brings about depolarization which reaches its maximum relatively slower than in the case of 0.05 mM and 0.1 mM veratrine concentrations, respectively. The latter and also 1 mM veratrine in a few experiments, likewise depolarized the muscle membrane to 35–40 mV.

Substituting choline for the total Na content of the Ringer solution, the depolarization failed to come about. Hence, the presence of sodium ions or the Na-influx is presumably necessary to elicit depolarization. The latter is a consequence of the increase of P_{Na} in addition to the known iongradient. Substituting Li for Na the depolarization reaches its maximum in approximately double the time, in close agreement with the observations of Keynes according to which the Li-influx is about a half of that of Na.

Finally, if the already depolarized muscle is washed with choline-Ringer, the muscle repolarizes despite the further presence of veratrine, presumably because it extrudes the Na in the choline-Ringer.

STRUCTURAL CORRELATES OF SYNAPTIC MEMBRANE PERMEABILITY

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Though not a real synapse in the strictest sense of the word, yet, due to its simply built and relatively large proportions, the myoneural junction offers a useful model of neurochemical impulse transmission. Electron microscopic localization of synaptolemmal esterolytic enzymes has been studied in order to get a more detailed information on the steps of impulse transmission in the myoneural junction.

According to light microscopic histochemical and biochemical data, it is mainly the post-synaptic membrane of the motor end plate that exerts a high acetylcholinesterase (AChE) activity. Electron histochemical studies performed in this laboratory, however, prove that both pre- and post-synaptic

membranes contain equal amounts per surface area of *AChE*. On the other hand, the activity of *non-specific arylesterase* is located within the synaptic gap (in the "middle dense layer" of the synaptolemma) whereas *butyryl esterase* is located mainly within the pre-synaptic terminal, in between the intervesicular space of the motor axon. Finally, the enzyme hydrolyzing *thiol acetic acid* (comprising various esterases as well as the proteolytic enzyme Cathepsine C) is bound to coarse structural units attached to the internal surfaces of both pre- and post-synaptic membranes.

Based upon these findings, the idea is put forward that it is not only acetylcholine, but at least more than one specific or non-specific membrane-active substances that take part in the regulation of synaptic membrane permeability. It appears that, due to a local liberation and a local destruction, acetylcholine is able to change permeabilities of both pre- and post-synaptic membranes, possibly even without traversing the synaptic gap. Synaptic membranes, rendered permeable by acetylcholine, may let through other substances, too, the hydrolysis of them being marked by the activity of enzymes splitting indoxyl acetate and butyrate, respectively. A shift, or relay of these substances in the successive layers of the synaptolemma may thus result in those ionic processes that realize synaptic transmission.

An analogous electron histochemical structure ("enzyme sandwich") can be envisaged in central (muscarinic) cholinergic synapses, too. Such an assumption is in harmony with recent findings of neurophysiology on the ineffectiveness of acetylcholine upon cerebellar neurones. Also the strange fact that both excitatory and inhibitory synapses exert an AChE activity can readily be interpreted on the basis of this working hypothesis.

ALLOSTERIC CONTROL OF MUSCLE PHOSPHORYLASE AND ITS ROLE IN GLYCOGEN METABOLISM

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We reported at the meeting of the Hungarian Physiological Society (1966) that G-P-6, glucose and other intermediary products influence the phosphorylase-*a*-activity in the muscle. In our present experiments the nature of the inhibiting effect of G-6-P and glucose, and its conditions were examined. We have established that the G-6-P inhibition is competitive against G-1-P, the substrate of phosphorylase. In addition, the inhibition brought about by G-6-P can also be suspended by AMP, the allosteric activator of phosphorylase. This indicates that G-6-P displays an allosteric effect on phosphorylase as well. The inhibiting effect of glucose is similar and can be suspended by AMP.

A considerable difference was experienced between the effect of the two blocking materials and the phosphorylase-*a* with changing pH. At a low pH (6.0) the enzyme can be inhibited with G-6-P to a high extent, with glucose to a lesser extent, than at a pH 6.8. At pH 7.6 the inhibiting effect of G-6-P decreases, and that of glucose increases. According to this a minor change in

the concentration of G-6-P and AMP as well as pH, can increase or decrease the glycogen metabolizing effect of phosphorylase.

We tried to influence the G-6-P sensitivity of the crystallized phosphorylase-*a* with heat treatment and using oxygen or iodine. Heat treatment decreased the G-6-P sensitivity of the enzyme but not consistently. Cysteine kept even increased sensitivity. Cysteine enhanced the relative low G-6-P sensitivity of some phosphorylase-*a* preparations considerably.

THE EFFECT OF DIFFERENT PHOSPHORUS LEVELS IN THE DIET ON THE NUCLEOTIDE METABOLISM OF ERYTHROCYTES

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For three weeks young male rats were kept on two different diets, one with normal salt, high Ca, normal P levels, the other rich in Ca and poor in P. The P and Ca values in the serum, as well as the adenine nucleotide contents in the erythrocytes of the animals have been investigated.

It has been established that the Ca rich and P poor diet decreased excessively the P value in the serum, but when the Ca content of the diet was high and the P content normal the P level in the serum was also significantly lower than in the controls. Between the serum P level and the adenine nucleotide content of erythrocytes a correlation could be observed. With diets with high Ca and normal P contents the ATP level of erythrocytes has been significantly lower, than in the controls and significantly higher than in animals on Ca rich and P poor diets.

In animals on Ca rich and P poor diets ADP levels were significantly higher than in the control animals, showing even on a Ca rich but normal P diet an increasing tendency. In the three animal groups AMP levels showed no marked differences.

It seems that by way of the P level of the serum dietary Ca and P affect the adenine nucleotide content of erythrocytes.

CHANGING OF THE ORGANIC IODINE BINDING AND MOBILIZATION COEFFICIENT OF THE THYROID GLAND BY RHEOPYRIN TREATMENT

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In our experiments we investigated the by effect of Rheopyrin on the organic iodine binding coefficient (α) and the mobilization coefficient (σ) of the thyroid gland. These coefficients can be calculated on the basis of the equation $dT/dt = \alpha S - \sigma T$ using the activities (T) measured in the thyroid

gland. α is calculated from the ascendent part of the iodine uptake curve, σ is calculated from the mobilization part after 24 hours. The deviation of the steepness of these two parts from the normal one is to the effect of medicine on the function. The doses used in the experiments were between the therapeutic and half-toxic doses.

The impending effect of a single Rheopyrin dose administered simultaneously with radioactive iodine on the value of α is very pronounced for 6 hours. The effect will gradually cease an the iodine uptake of the thyroid gland reaches the control level in 48 hours generally. But if the Rheopyrin treatment is repeated every 24 hours after administering iodine, a considerable decrease can be observed in the values of α and σ.

In one group the animals had been given a preliminary Rheopyrin treatment for seven days and then the iodine uptake of the thyroid gland was examined after 24 hours following the last Rheopyrin dose. No significant change could be detected in the value of the coefficients.

TRANSPORT OF WATER AND ELECTROLYTES IN MUSCLES OF ANURIC ANIMALS

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Changes in the content of water and electrolytes were studied in striated muscles of rats 2–3 days after nephrectomy. After having the muscles soaked in cold Ringer's solution a swelling occurred in both the "red" M. soleus and "white" M. extensor dig. 1., the extent of which was greater in those of the uremic animals than in those of the control ones.

In the Na-uptake and K-outflow of the soaked muscles there is a similar difference between ill and normal rats. During swelling chloride ions are accumulated by muscles in a smaller quantity than sodium ions. The ratio Cl^- uptake /Na^+-uptake seems to be constant (also according to calculations based on others' data)/both in normal and anuric animals.

The following may be a reasonable explanation of the above fats. When muscles swell on account of an inhibition of metabolism *in vitro*, part of the sodium gets into the cells together with chloride and there appears an uptake of an additional quantity of Na instead of an equivalent K-outflow. A higher swelling capacity and the exchange of electrolytes of uremic muscles in this system is probably the result of a change in the permeability or "binding" of ions rather than of a simple disorder in metabolism.

THE ULTRASTRUCTURE OF THE EMBRYONIC RAT ADRENAL CORTEX TRANSPLANTED IN THE ANTERIOR EYE CHAMBER

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An answer was sought to the question whether the ultrastructure of the cells of the rat adrenal cortex, principally of the cristate mitochondria and the granular-surfaced endoplasmic reticulum, as it appears on the fifteenth day of embryonic life is definitively determined, respectively whether the structure characterizing the adult cells is quicker to develop when cultured *in vivo* than in situ. To this end, 15-day-old embryonic rat adrenals were transplanted in the anterior eye chamber of intact and adrenalectomized adult rats of either sex.

As early as 24 hours after transplantation, even in the intact animal, the number of mitochondria and the endoplasmic reticulum were found to have increased, particularly the latter. Most mitochondria turned vesicular and tubulovesicular. Cristate mitochondria or a few cristae in the afore-mentioned types were rare. The endoplasmic reticulum became smooth-surfaced and vesicular.

These alterations were more pronounced in the 4-and 6-day-old transplants, especially in those derived from adrenalectomized hosts.

ROLE OF ADRENAL CORTICAL HORMONE IN CONTROLLING THE LEVEL OF HISTAMINE AND 5-HYDROXYTRYPTAMIN (5-HT) OF RAT TISSUE

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According to our investigations the dose of cortison given once (20 mg/body kg i.m.), or prolonged cortison treatments (7 times 10 mg/body weight kg, twice daily, i.m.) significantly decreased the histamine content of the stomach and the skin in rats, moderately increased the histamine level of the blood, and did not bring about considerable change in the histamine content of the liver and the lungs. The same treatment caused a considerable decrease in the 5-HT level of the blood, the liver, the lung and the skin, but the 5-HT level of the stomach did not change considerably.

4 and 14 days after adrenalectomy of both sides the histamine level of the blood, the liver, and the lungs greatly increased, while the histamine content of the stomach and the skin significantly decreased. After adrenalectomy the 5-HT content of the blood, the lungs and stomach significantly increased, at the same time the 5-HT content of the skin decreased while the 5-HT level of the liver became normal after an initial decrease, during the 14 days of the experiment.

Experiments are in progress to explain the mechanism of action influencing these amine levels of the adrenal cortical hormone by the activity changes of histaminase, histidin decarboxylase and monoaminooxydase.

DATA ON THE MECHANISM OF GRANULE-FORMATION IN MAST CELLS

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Granule formation of mast cells was investigated in model experiments. Thymus tissue cultures obtained from normal and cortisone-pretreated rats were observed parallel by both histochemical and electron microscopic methods after the addition of some components of the mast cell granules, namely, heparin, histamine, and serotonin. It was established, that heparin itself was unable to form mast cell granules alone — but serotonin by binding heparin of the culture media could do so. Histamine behaves in the same way — it binds heparin and produces granules — but it can bind the excess heparin only in the presence of glucocorticoids. The glucocorticoids do not alter the structure of the granule, although the authors pointed out its presence in the mast cell granules of mice by electron microscopic autoradiography. Authors discuss the possible mechanism of granule-formation and attempt to solve the contradictions between the chemical and biological data.

DATA ON THE PROPERTIES OF THE SARCOPLASMIC RETICULAR FRACTION OF FROG MUSCLE

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The sarcoplasmic reticular fraction (SRF) of muscles from frog hind limbs was prepared as previously described (Acta Biochim. Biophys. Hung. *1.* 233. 1966). It was found that the degree of Ca-uptake is 0.7–1.3 μm Ca/mg protein in the presence of 5 mM oxalate and 0.04–0.09 μM Ca/mg protein in its absence. The SRF ATPase can be activated by Ca in media both with and without oxalate. The influence of Ca, Mg and ATP applied in different concentrations on the Ca-uptake and ATPase activity of SRF, respectively, was also studied. During storage of the preparations, i.e. so-called "ageing", the degree of Ca-uptake in a media both with and without oxalate decreases while at the same time ATPase activity markedly increases. Cease of Ca-uptake coincided with the appearance of the inhibiting effect of Ca upon SRF ATPase.

Studying the effect of tryptic digestion on SRF it was found that ATPase activity is increased as digestion proceeds but the Ca activation of ATPase as well as the Ca-uptake of SRF is decreased.

THE ANTISEROTONIN EFFECT OF BENZCYLAN (HALIDOR)

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Authors examined the effect of serotonin on the systemic blood pressure by direct measurement in the carotid artery in 135 male rats (180–200 g body weight) in urethan i.p. anaesthesy. With pretreatment of 60 mg/kg hexamethonium i.p. the blood pressure curve showed always a positive reaction.

Injecting several antiserotonin compounds into the femoral veins, authors stated, that the blood pressure raising effect of 30 μg/kg serotonin will be extinguished by 75–100 μg/kg of Delyside, 50 μg/kg of BOL, 1–2 μg/kg of Deseryle, or 500 μg/kg of Halidor.

The rise of blood pressure caused by $BaCl_2$, based on peripheral vasoconstriction is eliminated neither by the antiserotonin agents mentioned above, nor by the given amount of Halidor. The whole antiserotonin effect of the Halidor lasts for 30–40 minutes.

Though the antiserotonin effect of Halidor in comparison with the other drugs can be achieved only by relatively high doses, this effect of the compounds is valuable, because its toxicity and by-effects are negligible.

A STUDY OF SPATIAL ORGANIZATION OF THE VISUAL INFORMATIONS IN THE CAT'S VISUAL CORTEX BY VIRTUE OF THE ANALYSIS OF RECEPTIVE FIELD CHARACTERISTICS AND THAT OF LATENCIES OF ELECTRICALLY EVOKED NEURONAL RESPONSES

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In locally anaesthetized and immobilized cats the electrical stimulation of optic radiation and LGB, respectively, produced a number of response patterns with differently distributed latencies in single neurones of area Br. 17. The afferent fibres having concentric field organizations showed a latency range from 0.49 to 2.22 msec. Units with simple receptive fields responded with one or rarely more discharges at latencies ranging from 1.31 to 5.94 msec. The difference in latency of responses of simple field neurones and concentric field axones (0.90 msec) was highly significant. Smaller proportion of neurones showing complex receptive field organizations fired with 1 or 2 discharges at latencies over 4 msec. Most of these neurones did not give short latency response at all, but demonstrated periods of diminished or absent spontaneous activity for up to 425 msec.

According to their late activation patterns, neuronal responses were grouped into five classes: *a)* release, *b)* late activation with short latency discharge,

c) late activation without short latency discharge, *d)* total inhibition, and *e)* unchanged spontaneous activity. Interrelations between the receptive field organization and the type of inhibitory phenomena following electrical stimulation were found.

The experimental results are consistent with the notion (Hubel and Wiesel, 1962) that neurones with simple receptive fields have relatively direct input from the lateral geniculate and constitute the first phase in the cortical processing of visual input. Visual information is probably transferred to other cortical neurones from neurones with simple field organization. Thus units having complex receptive fields may participate in the more complicated cortical integrative processes.

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Hubel, D. H. and T. N. Wiesel 1962, J. Physiol. *160*, 106–154.

BIOCHEMICAL EXAMINATION OF PROTEINS IN THE PERFUSATE OF ISOLATED KIDNEY

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Recently it has been shown that the transplanted kidney releases a strongly histocompatible antigen, which seems interesting biochemically in connexion with kidney homotransplantation.

The-above mentioned antigen can not be found immunologically or biochemically in the peripheral blood. Only recently Najarian et al. (1966) could demonstrate it immediately in immunological experiment from the effluent blood of the transplanted kidney.

It was supposed that the isolated perfunded kidney in an acute experiment would excrete some sort of protein or proteins.

Indeed, in experiments performed on rabbits the biochemical examination of the kidney perfusate showed that the kidney continually was excreting a substance of protein character in small amounts. After this substance beeing concentrated with Sephadex G 25 a further biochemical examination has become possible.

On starch- and agarose gel this protein showed the same characteristic electrophoretic mobility.

After tryptic digestion the thin-layer fingerprint of this protein showed almost the same picture even through several hours.

The uncoupling of the oxydative phosphorilation with DNP or potassium iodide has not altered the fingerprint, though the amount of protein excreted by the kidney has decreased.

FEEDBACK EFFECTS OF INTERMEDIATES AND NUCLEOTIDES ON THE ACTIVATION AND INACTIVATION OF MUSCLE PHOSPHORYLASE

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Previously (Bot *et al.*, 1966; Acta Physiol. Hung.: *30*, 376) we observed that G-6-P inhibits the activity of phosphorylase *a* and consequently a change in the concentration of G-6-P has a regulatory effect on glycolysis. In the present investigations we report that G-6-P as an intermediate of glycogen metabolism has a feedback effect on the enzymatic inactivation of phosphorylase *a* and on the activation of phosphorylase *b*.

We estimated the inactivation of phosphorylase *a* by incubation of crystalline phosphorylase *a* with purified phosphorylase phosphatase. AMP inhibits the effect of phosphorylase phosphatase in physiological concentration, while G-6-P and glycogen can accelerate it. G-6-P not only accelerates the effect of phosphatase, but also abolishes the inhibitory effect of AMP. So phosphorylase phosphatase at increasing concentrations of G-6-P can inactivate phosphorylase also in the presence of AMP. Glycogen and glucose cannot abolish the inhibitory effect of AMP on phosphatase.

G-6-P can regulate both activation and inactivation of phosphorylase. According to the authors' examinations G-6-P inhibits the phosphorylase *b* kinase in low concentrations and therefore it can abolish the development of active phosphorylase. AMP abolishes the effect of G-6-P.

So both AMP and G-6-P have a contrary effect on the function of enzymes playing a role in the activation and inactivation of phosphorylase and they also have a feedback effect on the regulation of glycogen mobilization.

FLUOROMETRIC DETERMINATION OF THE COMPONENTS OF B_6-VITAMIN AS THEIR DANSYL-DERIVATIVES

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A number of new analytical processes are known for the determination of the components of B_6 vitamin. In spite of this it seemed fruitful to apply the "Dansyl" technique to measure the components of B_6 vitamin. Following the method of Gray and Hartley and Boulton and Bush DNS-pyridoxamine and DNS pyridoxal compounds have been formed by the reaction of 1-dimethylaminonaphtaline-5 sulphochloride. The obtained new Dansyl derivatives are not light-sensitive, but stable compounds having intensive yellow fluorescence (activation maximum: 365 mμ, emmission maximum: 540 mμ in ethanol).

* I am grateful to the Wellcome Foundation for the provision of a Research Travel Grant.

DNS pyridoxamine and DNS pyridoxal can the most suitably be isolated in a system of light petroleum (100–120° C))-tolueneacetic acid-water (133 : 66 : 170 : 30) on a Whatman N° 4 paper in about 14 hours. Chromatograms were evaluated by a "Fluorescence Chromatographic Scanner". In a range of 0–5 micrograms the calibration curve is linear. We can even measure sub-micro amounts, when the paper chromatogram strips are heated to 80–105° C before scanning. The difference in the fluorescence intensity of cold and hot strips is much more than 200%. The marked difference in Rf, the fluorescence intensity of the heated strips, the high stability of the compounds make it possible to determine the components of B_6 vitamin.

EXAMINATION OF THE ANTAGONISTIC BARIUM-CALCIUM EFFECT ON THE ISOLATED HEART OF MOLLUSCS

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The author has carried out examinations on local terrestrial mollusc species, as well as on those from the Mediterranean and Red Sea. After examining the effect of barium ions on the functioning of the heart, he antagonized the developed change of effect with calcium ions.

The performed examinations have demonstrated that in the case of land snail species (Helix pomatia L.) the increase of tonus and decrease of amplitude, caused by barium ions (1 mg/ml), are perfectly antagonized by $CaCl_2$. The increase of tonus, produced by $BaCl_2$, can be compensated only by setting a strictly definite calcium ion concentration (2 mg/ml and above), on the other hand, a change of amplitude and frequency can be equalized even with a dose smaller than that. In addition, the efficiency of $CaCl_2$ is influenced by seasonal factors, as well. That is intelligible as the Ca-level in the haemolymph of the animal changes even under natural physiologic conditions. (In winter, in the period of rest, it increases.)

In the case of sea molluscs, a heart stopped with $CaCl_2$ again begins functioning under the influence of barium ions. An increase of tonus caused with $BaCl_2$ can, however, be compensated with calcium ions but to a very low degree. It is demonstrated by all these that the efficiency of the calcium ions in question can considerably be modified by the isotonic ion concentration that is different in several organisms. The results obtained are confirmed also in the case of edible snails by the changes observed in the Ringer solution which is free from K^+ and Ca^{++}.

THE CHANGE IN CONFORMATION ACCOMPANYING THE FUNCTION OF ALLOSTERIC ACETYLGLUTAMATE-PHOSPHOTRANSFERASE

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We have found previously that in Chlamydomonas reinhardti the catalytic activity of the enzyme acetylglutamate-phosphotransferase, the first enzyme in the arginine pathway, may be inhibited by arginine. The inhibition against the substrate acetylglutamate seems to be a competitive one. Since the inhibitory effect of arginine may be reversibly suppressed by urea without any effect on the catalytic activity of the enzyme, it has been proved that the substrate-binding site and the inhibitor-binding site(s) of the enzyme are different.

However, it has remained a question, what kind of change in the conformation of the enzyme accompanies the binding of the inhibitor, making the binding of the substrate more difficult or impossible. It has been found that the inhibitor decreases the velocity of inactivation caused by different denaturating agents, especially non-polar solvents (for example dioxane). On the contrary, in most cases the enzyme cannot be protected against inactivation by the substrate. It seems probable that the enzyme, binding the inhibitor, has a more compact structure than the free or catalytically active enzyme, and under these conditions the catalytic centre becomes inaccessible to the substrate.

DATA ON THE LOCALIZATION OF THE INHIBITORY SYSTEM IN THE CEREBRAL CORTEX

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As it was earlier demonstrated, gamma-amino-butyric acid (GABA) inactivates the upper layers of the cat's cerebral cortex and enhances the amplitude of the acoustically evoked potentials in both surface and deep leads. The circular isolation of the gyrus ectosylvius anterior or the horizontal isolation of the upper three cortical layers from the rest of the cortex at a depth of 600—800 μ enhances the amplitude of the evoked potentials especially at higher stimulus frequencies. Cortical evoked responses produced by the stimulation of the medial geniculate body are augmented by GABA as well. On the basis of these facts the authors assume that there exists in the cerebral cortex an inhibitory system, which is localized in the upper three layers of the cortex. This system becomes activated by the afferent impulses synchronously with the nervous elements producing evoked cortical responses. This activation of inhibition can be demonstrated also by the recording of unit cell discharges in the auditory cortex.

ALTERATION OF POTASSIUM DISTRIBUTION IN THE STATE OF POTASSIUM DEFICIENCY

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It can be supposed that the reasons causing potassium deficiency, according to their particular effect may decrease the potassium (K) content of different organs and tissues in different measures and time. To solve this problem it was investigated how the K-insufficient food or the DOCA caused K-insufficiency influence the K content of the red blood cells and the muscle tissue of rats.

The K content of the muscle significantly decreases ($P < 0.01$) after 7 days and remains nearly on the same level to the end of experiment (21 days) owing to the effect of both the K-insufficient food and the DOCA dosage. In contrast with this the Na content increases ($P < 0.01$). The K content of the blood plasma decreases parallel with the K-content of the muscle ($P < < 0.01$). The Na content does not change in an evaluable way in the case of K-less food, but significantly increases to DOCA treatment ($P < 0.01$). The K-content of the red blood cells slightly decreases in the case of K-insufficiency of food ($P > 0.05$) and significantly increases after DOCA dose ($P < 0.01$). The Na content of the red blood cells does not alter in a characteristic way.

The following consequences may be drawn from the results: 1. In K-insufficiency caused by K-insufficient food or DOCA dose the K-content of the plasma indicates the K-insufficiency and its degree. 2. The K-determination of red blood cells is not suitable for the demonstration of the insufficiency. 3. The measure of K-loss in different organs is not equal. 4. Owing to the effect of the factor causing K-loss in some organs, the excrement of the delivered K is not complete, some other organs are taking it up, and thus the stored K brings about a new state of K-distribution.

EFFECT OF DIETARY INDUCED HYPERLIPEMIA ON THE PHAGOCYTIC ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM

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Little is known on the regulation of serum cholesterol level in man and only hypotheses and statistical correlations may indicate the causes of an elevated cholesterol level. Even the mechanism of dietary induced hypercholesterolemia in animals has not been quite clarified yet. An attempt was made to investigate the role of the RES as a possible factor.

The effect of a thrombogenic diet inducing marked hyperlipemia and that of the S 65 (Sós) cardiopathogenic diet enducing only a slight elevation in cholesterol level were investigated in mice and rats, resp. RES function was tested by the method of Cr^{51} labelled chicken red cell clearance and of Au^{198} colloid clearance.

The particle clearance of the animals on both of the dietary regimens markedly decreased as soon as after 3 days. No further changes could be observed during the following 3 weeks.

Liver colloid uptake of the mice fed a thrombogenic diet did not follow the changes in the particle clearance values. Such discrepancy, however, could not be observed in the rats fed on a S 65 diet.

Previous experiments of the authors also indicated that changes in the RES function could not have an important role in the pathomechanism of dietary hyperlipemia. The present experiments confirmed this finding. Further experiments are needed to find the common factor in thrombogenic diet inducing marked hyperlipemia and in cardiopathogen diet without such effect causing an impaired RES function.

CORRELATION BETWEEN THE ACTIVITY OF THE SODIUM CARRIER SYSTEM AND THE RESTING POTENTIAL IN THE CAT HEART

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It is generally accepted that the activity of the sodium carrier system of the excitable cell membrane is a function of the resting potential, the maximal depolarization rate of the action potential being considered as the most reliable electrophysiological indicator of the carrier activity. In a study of the transmembrane potentials of the cat auricle we have shown that increasing the extracellular potassium concentration from 2.7 mM/l to 6.7 mM/l results in a very considerable decrease of the maximal depolarization rate (from 90 V/sec mean value of the control to 36 V/sec), while the mean resting potential undergoes only a slight change (from 66 mV to 60 mV). If the cells of both the control and high potassium series are grouped according to the value of the resting potential, it becomes evident that in the presence of 6.7 mM/l external potassium the rate of depolarization of the cells is at any resting potential level considerably lower than that of the corresponding controls.

We suppose that the so called sodium carrier system is not specific for sodium, but depending on the external potassium concentration, it transports also a certain amount of potassium into the cell.

ON THE ORIGIN AND ROLE OF OPTICAL ISOMERY IN LIFE

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In the experiments in which organic molecules are produced from a simulated reducing atmosphere both L and D isomers have been synthetized and no factor has been found which favors a particular isomer, or which would make

possible the selection of one of them. The discovery of the violation of parity principle suggests a possibility. As it is well known the electrons emitted in β–decay are polarized and it seems to be warranted, that this fact is associated with the occurrence of left handed isomers in living beings.

In order to prove this possibility the destruction of D and L isomers has been studied under the influence of polarized β–particles. It turned out, that the D isomer decomposes in shorter time. On the basis of the suggested reaction mechanism the origin of life and the intermolecular electron transport will be discussed.

VIBRATIONAL HYPERTENSION OF THE RAT

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It has earlier been established, that one hour lasting horizontal vibration with a frequency of 3 Hz and an amplitude of 28 mm induces hypertension in rats. Hypertension may be inhibited by previously administered regitine, and by ingesting potassium and magnesium, respectively.

In recent experiment the effect of changes in frequency and amplitude on the development of vibrational hypertension was examined.

Vibration in all the experiments lasted one hour and was of horizontal direction.

In case of a frequency of 2.5 Hz raising of the amplitude (5, 15 and 25 mm) proportionally increased the tensiogenic effect. The same experiences were made with a frequency of 5 Hz (amplitudes: 5, 15 and 25 mm) and a frequency of 8 Hz (amplitudes: 5, 15 and 25 mm).

Relying upon comparative examinations of vibrational effects of identical amplitude and different frequencies it was found, that increasing of the frequency also intensified the tensiogenic effect.

It may be established from the foregoing that both the amplitude and the frequency-values are important factors in the development of vibrational hypertension.

EFFECT OF NEONATAL TREATMENT WITH RESERPINE ON FSH, LH AND LTH PRODUCTION OF THE PITUITARY IN RAT

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Literary data indicate that reserpine treatment after birth induces permanent endocrine alterations: delays sexual naturation, disturbs oestrous cycle, modifies hypophyseal LH production.

Authors injected one group of newborn rats with a single dose on the 4th day of their lives, while the other group received five injections s.c. of 50 μg

reserpine solution in two days intervals. The weight of the treated animals was measured daily, and the time of the vaginal opening and — later the course of the oestrous cycle — were registered. Some of the control and/or the treated male and female rats were brought into parabiosis, others were hemicastrated to register and compare the compensatory gonadal hypertrophy. Pituitary LTH content of the last group of rats was determined by the pigeon crop-sack micro method, STH and LTH by acrylamide-gel electrophoresis. The endocrine organs and accessory sex organs were weighed and examined in histological sections.

Reserpine injection lowered body temperature and decreased intake of food in newborn rats causing serious somatic developemental disorders responsible for the delay in maturation. In the 4th month of their lives, however, the reserpine treated animals showed no disturbances of endocrine function.

DETERMINATION OF THE PERCENTAGE OF FREE THYROXINE IN SERUM BY GEL-FILTRATION

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Recently the determination of free thyroxine content of serum, applying labeled thyroxine has been widely used. Several methods have been described, one of them is based on gel-filtration. The so called free thyroxine percentage, obtained by this method, is influenced by several factors. The authors therefore studied, how can the results be influenced by the size of the gel particles, the length of the column, the pH, the dilution and the concentration of thyroxine. The experiments were carried out using Sephadex G-25 gel. The reliability of the method was controlled by means of $Na^{125}J$ and ^{131}J-albumine, too. On the basis of their results the authors consider the method suitable for determination of the free thyroxine percentage of serum.

INCORPORATION OF $^{14}CO_2$ INTO PROTEINS OF NORMAL AND MUTANT LEAVES DEPENDING ON THE DEVELOPMENT OF CHLOROPLASTS

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$^{14}CO_2$ incorporation was studied in normal and mutant maize leaves illuminated for 12 hours with different light intensities (5, 100, 1000, 10 000 lux) prior to $^{14}CO_2$ exposure.

With higher light intensities $^{14}CO_2$ incorporation into normal leaves was enhanced logarithmically while for the lycopenic mutant a maximum at 1000 lux could be observed. CO_2 assimilation of the ζ-carotenic mutant was not affected by illumination.

Labelling of amino acids, organic acids and sugars separated as bulk fractions by ionic exchangers from alcoholic extracts of different strains was similar when the leaves were kept in the dark. At higher light intensities, however, labelling of sugars showed a marked increase, which was less pronounced or missing in the mutants.

Light intensity had a considerable effect on the specific activity of proteins eluted from the residue with 1 N NaOH. In normal leaves with a light intensity of 10 000 lux saturation values of labelling were obtained. In lycopene containing mutants proteins of maximum activity were produced at 1000 lux, in the ζ-carotene containing mutant the highest labelling of proteins was found at 100 lux.

A comparison of the ratios calculated from specific activities of amino acids and proteins indicates that protein synthesis of normal and mutant leaves differs in both qualitative and quantative aspects.

MEASUREMENT OF IONIC MOVEMENTS IN THE ISOLATED VENTRICLE OF THE FROG HEART BY MEANS OF ISOTOPE WASHOUT-CURVES

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The movement of K^+ ions in the isolated frog ventricle has been studied by Rb-86 washout-curves. The rate of exchange of K^+ ions has been established by the single-injection technique and the results compared with data obtained after sufficient equilibrization. Mathematical analysis of the washout-curves has revealed that the exchange of intracellular K^+ ions takes place in several phases. On the basis of the intracellular component of the curve it has been calculated that the amount of exchanged K^+ per min. attains 0.70 μeq per g of tissue, which is in satisfactory agreement with earlier results obtained by a different method.

APPROACHING THE PROBLEM OF THE GROWTH OF PLAQUE-SIZE BY MEANS OF A MATHEMATICAL MODEL

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The activity of the phage-bacterium biological complex is characterized by several quantities. One of these is the kinetics of the growth of plaque-sizes. Under highly standardized experimental conditions (reproductive difference 1.5%) the determined time-functions of plaque-size yield a quantity which can be characterized as growth velocity. In its first approach we considered the question as a diffusion-problem from the view-point of a mathematical

model in order to make the biological factors having a part in the growth of plaque quantitative. From the difference curve of the curves obtained on the one hand experimentally and on the other hand through the mathematical treatment of the model, conclusions have been drawn regarding the biological behaviour of the phage-host cell complex. The difference curve was namely brought into connection with the change in time of the number of phages taking part in the transport process.

On this basis — in conformity with experience — the relation of slopes determined from the difference curve at the initial as well as a later (3.5 hours) stage of the growth of plaques is in due accordance with our other results obtained by purely microbiological methods (burst size) regarding the average phage-producing capacity of bacteria.

INHIBITION OF ACID HAEMOLYSIS IN NORMAL ERYTHROCYTES PRETREATED WITH PROTEOLYTIC ENZYMES AND IN PNH CELLS

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By treatment of normal erythrocytes with proteolytic or lipolytic enzymes, changes similar to those observed in erythrocytes in paroxysmal nocturnal haemoglobinuria (PNH) were produced. These enzyme-treated red cells exhibit also marked acid- and complement-sensitivity, extremely low acetylcholinesterase activity and decreased electrophoretic mobility. The factors inhibiting or promoting acid-haemolysis were studied parallel in membrane modified normal cells, in immunologically sensitized erythrocytes, and in erythrocytes of five PNH-patients. The function of some inhibitors of haemolysis may give an insight into the understanding of the mechanism of abnormal haemolysis.

THE EFFECT OF HINDERED DIFFUSION OF THE SOLUTE MOLECULES ALONG THE MEMBRANES ON THE TRANSPORT OF WATER AND OF SOLUTE SUBSTANCES

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Between two parchment membranes of identical surface-area water-solution is enclosed in the experimental system. On the outside of the membranes there is clean water on each side. The diffusion of the solute substance is hindered to a greater extent along one of the membranes, because one of them is covered by plexiglass sheet on a larger surface-area than the other. Water transport takes place across the system in the direction of that membrane which is covered on a larger surface-area. The water transport is great

if gum arabic solution or $K_4Fe(CN)_6$ solution is used. It is small if Na_2CO_3 and even smaller if NaCl solution is used. In many cases the slowly diffusible gum arabic and the quickly diffusible anorganic substance solution is enclosed together between the membranes. In such a case the ration of the anorganic substance compared to the gum arabic is higher outside the more covered membrane, than at the less covered one at the end of the experiment.

The series of experiments seems to be useful for transport-processes in the interstitial space e.g., since the initial part of the capillaries (metacapillaries) is more covered than the further sections of the capillaries.

EFFECT OF THE SPECTRAL COMPOSITION OF LIGHT ON NITRATE ACCUMULATION

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The effect of the spectral composition of light on metabolism can be attributed, according to the examinations in recent years, to the different spectral sensitivity of the two photochemical systems of photosynthesis. The different activation of the two system, apart from decreasing the amount of the reduced pyridine nucleotides and of ATP, results in the alteration of their proportion to each other.

We have examined under controlled conditions how the nitrate accumulation in some organs of the bean plant changes in the case of an activation of the two systems in different degrees.

According to our examinations, there accumulated in the root and stem less nitrate in blue light than in red one. This finding is in line with the results of Lundegardh and others who have mentioned a higher effect of blue light upon reducing NADP than that of red light.

In the leaf there could not be observed any difference. This we explain with a nitrate transport from the stem.

The different nitrate accumulation, produced in blue and red light in our experiments shows that the activation of the two systems of photosynthesis in different degrees appears in the post-photosynthetic processes, as well.

INVESTIGATION OF THE LOCALIZATION OF IN VIVO SUPPLIED $^{32}PO_4$ IN CROSS-STRIATED MUSCLE BY ELECTRON MICROSCOPIC AUTORADIOGRAPHY

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PO_4 labelled with ^{32}P was supplied with food and its localization was investigated in bee's muscle by electron microscopic autoradiography. Ultra-thin sections were made of the thorax muscle of bee and they were covered

with a film emulsion which contained a monolayer of silverhaloid grains. For the preparation of the film twice diluted Ilford L-4 was used. After the exposure and the photographic process the sections were investigated in electron microscope.

The percentage distribution of the grains for the individual bands of the cross-striated muscle was calculated from the number of the grains over the muscle in experiments repeated several times. The experiments show good agreement concerning the distribution. The distribution of the grains originating from *in vivo* supplied $^{32}PO_4$ was the following: 75 per cent was found over the A-band, 16 per cent over the Z-line, and 9 per cent over the I-band. The inhomogen distribution found in the experiments may have importance in the process of muscle-activity.

MECHANISM OF THE GASTRIC SECRETION INHIBITING EFFECT OF THE MECHANICAL VIBRATION

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In their earlier examinations authors have shown, that a vibrational effect lasting 5 hours, in rats operated according to Shay, decreases gastric secretion, and that the α-sympatholyticum (Regitine) does not suspend this effect.

In their present experiments, using the most effective frequency and amplitude (3 Hz; 4 cm; horizontal direction) authors examined the mechanism of the secretion decrease.

A comparison to the controls revealed, that in rats, which were during 6 weeks twice a day exposed to 1 hour lasting vibration and which 24 hours after the last vibrational effect were subjected to Shay's operation, the gastric secretion remained unchanged. Under identical experimental conditions, but upon application of a 5 hours lasting vibrational effect following Shay's operation secretion decreased. Acute — 2 hour lasting — vibrational effect prior to Shay's operation proved to be ineffective regarding decrease of the secretion. These data led us to the conclusion, that the secretion decreasing effect is a transitory one and prevails during the time of vibration, presumably parallel to the adrenalinemia. This surmise is corroborated by the fact that administration of adrenaline (50 μg/100 g i.p.), or of MAOI (Nialamid, 5 mg/100 g) and 2 hours lasting vibrational effect together significantly decrease the secretion.

STUDIES ON THE MECHANISM OF POLIOVIRUS PENETRATION THROUGH THE CELL MEMBRANE

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Cells of a permanent monkey kidney tissue culture were infected in suspension with type 1 poliovirus. The final yield was ten times higher in 0.2 per cent bovine albumin (BA) supplemented Hanks' medium than in Hanks' medium alone, as shown by one-step growth experiments. The presence of albumin was required only during the first 30 minutes following adsorption. The active principle in Cohn fraction V BA was fatty acid.

The activity of 16 C to 24 C saturated fatty acids was studied. When added prior to the adsorption, each of them was either inactive or inhibitory with respect to virus penetration. Added within 1 hour following the adsorption, they had 50 to 100 per cent activity (as referred to BA) depending on the chain length and concentration. Dose response curves exhibited clear-cut peaks within half log unit, the localization of the peak being dependent on the chain length.

Pinocytosis-stimulating activity of the same fatty acids appeared to be less dependent on chain length and concentration.

Enhancement of virus penetration by fatty acids appeared to be the result of their orienting as well as pinocytosis-enhancing effect.

CHANGES IN BLOOD FLOW THROUGH THE CEREBELLAR CORTEX IN CARDIAZOL AND STRYCHNINE CONVULSIONS

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Favourable conditions for the study of 1. the correlation between the activity of the brain and cerebral blood flow (= CBF) and 2. between systemic blood pressure and CBF are offered by the extreme increase in activity during epileptic convulsion, which is associated with a more or less pronounced change in systemic blood pressure. Our earlier experience showed that the changes in blood flow through the neocortex, as against the subcortical structures (thalamus, hypothalamus, mesencephalon), during Cardiazol and strychnine convulsions were of opposite directions, namely the blood flow through the areas primarily involved in the paroxysmal activity increased, whereas it decreased through the other areas. No data are available on the changes in blood flow through the cerebellar cortex in response to Cardiazol and strychnine. The methods mentioned in the preceding lecture (Poór, Kopa, Molnár: "Effect of Stimulation of the Labyrinth on Blood Flow through the Cerebellar Cortex") were used on anaesthetized rabbits. According to our present experiments Cardiazol and strychnine equally cause an increase in blood flow through the cerebellar cortex. The lecture deals, first of all, with the relationships in time of the variables studied (systemic blood pressure, respiration, electrical activity, blood flow).

DATA ON THE ADRENOCORTICAL FUNCTION IN CHICKEN BY SIMULTANEOUS DETERMINATION OF THE URINARY EXCRETION OF FREE AND CONJUGATED CORTICOSTEROIDS

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In the literature there are no data concerning the corticosteroid concentration in the urine of Gallus domesticus, though both the acute and the chronic method of collecting the urine are known. A method was developed by which the total corticosteroid (both free and conjugated) could be determined. Our experimental animals were 11 Leghorn, and Rhode-Island cocks, respectively.

The average of the *normal values* in 15 experiments was 8.2 μg/h (0.72 SE) of corticosteroids (range 4–10 μg/h).

In 5 cases for two consecutive days 3 IU/100 g body weight ACTH (Exacthin) was given i.m. and in the urine collected 5 hours after the second injection the average of the corticosteroid concentration was 22.6 μg/h (4.8 SE). The values received after ACTH administration were in all cases significantly higher, ($p < 0.01$) than the normal data before the ACTH injection.

Knowing the normal data and those received after ACTH, *thermal stress* has been provoked in 8 cases. By bathing in water of a temperature of 14–16° C (for 30 minutes) hypothermic coma (rectal temperature +28° C) and through immediate warming hyperthermia (intensive panting at +41° C) were provoked. Urine was collected 4-6 hours and 23-24 hours after the stress. An average of 16.5 μg/h (0.83 SE) of corticosteroid concentration was observed that is to say significantly higher than the individual normal values ($p < 0.02$). In the thermal stress group increased values were found 4 hours after the stress in 4 cases and 24 hours after the stress in 4 cases.

Our method seems to be applicable for investigation of the adrenal function of chicken both in normal and in stress states.

THE EFFECT OF BRADYKININ ON THE CIRCULATION INTRARENAL

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Synthetic bradykinin was infused at a rate of 0.4 μg per min. into the left renal artery of dogs anaesthetized with chloralose. It has been established that bradykinin exerts a direct vasodilator effect on the renal vessels and renal blood flow increases by about 25 per cent. During the infusion of bradykinin the clearances of creatinine and PAH remain unaltered, while the extraction ratio of PAH diminishes. The rate of urine flow increases in face of unaltered sodium output and reduced osmotic concentration of the urine.

It is assumed that bradykinin enhances first of all the medullary blood flow in the kidney and it plays a role in the regulation of the circulation intrarenal.

SEXUAL FUNCTION OF RETARDED RATS FOLLOWING EARLY RADIO-THYROIDECTOMIZATION

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Inhibition of thyroid function is generally accompanied by a decreased gonad production. *Hohlweg* and co-workers (1962, 1965) have, however, proved that thyroid and STH are not needed for maturation and gonad function in rats having previously reached a certain stage of development.

Authors administered to 21 to 26 days old male and female rats 500 μCi carrier free Na^{131}-J isotope. The animals thus thyroidectomized remained retarded in growth compared to controls of the same litter. One group of rats received testosterone shortly after birth and were subsequently thyroidectomized ("androgenized-thyroidectomized" group). Each group was submitted to fertility tests. Male rats proved to be fertile and sexual cycle of the females did not show major changes, but labour and lactation were heavily disturbed. Androgen-treated females remained infertile. Animals were dissected at one year of age; liver, heart, spleen, kidneys, endocrine, organs and accessory sex-organs were measured and examined histologically. Testes and male accessory sex-organs of the thyroidectomized rats showed a double relative weight compared to the controls, the same being the case with the androgen-treated ones. Hypophysis LTH content was determined by the pigeon crop-sack method and LTH and STH by starch-gel electrophoresis. No STH and a decreased LTH content were found in the hypophysis of thyroidectomized rats.

THE ROLE OF THE THYROID IN EXPERIMENTALLY INDUCED SKELTON- AND ALIMENTARY HYPERTONIA

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Of the endocrine glands the pituitary, the adrenal gland and the thyroid play an important role in the development of hypertension. In their previous examinations the authors have shown that renal hypertonia induced by Grollman operation ceased in consequence of thyroidectomy. Following the administration of thyroid-hormones (Thyroxin, Trijodthyronin) blood-pressure again increased.

In the experiments it has been established, that thyroidectomy inhibited the development of both Skelton's and alimentary hypertonia. After Skelton's operation in the case of intact thyroids blood pressure values of 152 ± 12 mmHg were found and in thyroidectomized groups such of 111 ± 10 mmHg. In the fifth week of the cardiovasopathogenic diet and with maintained thyroids the blood pressure values were: 159 ± 16, and in the thyroidectomized group: 116 ± 8 mmHg.

THE LOCALIZATION OF NATURAL URANIUM IN THE RED BLOOD CELLS. STUDIES MADE BY ALPHA MICROAUTORADIOGRAPHY

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The chemical interaction between uranium and certain elements of blood has been investigated in persons exposed to uranium by means of alpha microautoradiography. Alpha microautoradiography was found to be a suitable method for studying blood smears. After an appropriate pretreatment tracks could be observed in the smears. The majority of the alpha tracks was found in the plasma, less in the red blood cells and none in the white blood cells. On the basis of distribution of alpha tracks it may be supposed that uranium becomes bound only to hemoglobin of red blood cells. The present findings and the literary data being at disposal seem to suggest that uranium might be bound to globin. From the chemical point of view it could be assumed that the carboxyl group of globin would be the active group involved in this binding.

EFFECT OF THE ONTOGENETICAL DEVELOPMENT OF THE NERVOUS SYSTEM ON THE REGULATION OF THE METABOLISM

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Parallel with the ontogenetical development of the central nervous system the regulation undergoes a characteristic change. Usually at the lowest age depending on the animal species, below the age of about 26–30 days, in the course of the associated repetition of the unconditional stimuli upsetting the vegetative equilibrium of the organism, not the amendable conditional response becomes dominating in the central nervous system, but a vegetative change imitating an unconditional reaction. During the transitory period both types of reactions may be observed simultaneously: the conditional response begins with a change in the same direction as the unconditional one turning subsequently into the opposite direction. On overcoming the "mimicking" phase of the development the regulation induces an amending mechanism which renders it independent of the effects of the environment. The effect of the regulation improves parallel with the development.

SOME FACTORS IN THE CONTROL MECHANISM OPTIMALIZING WORK METABOLISM

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Due to neuro-endocrine regulation, several organic functions show an identical tendency in activation and desactivation, resp., during muscular exertion. So the excitation and attenuation processes of the respective cardiovascular, respiratory and motor functions also run an apparently similar course.

However, we have found certain divergence in the functional dynamics of these systems during a complex test series applying four gradually increasing work-loads, which might be due to discrepancies of the enzymatic and energetizing processes though no changes have occurred in the transport mechanism of oxygen.

In the initial phase of work graded motor activity elicited first the activation of pulse frequency. The rise in respiratory minute volume was fast, too, while O_2 consumption increased relatively slower. After 60 seconds of the first load level the percentual exploit of oxygen rose sharply, preceding in tempo the increase of other values. From the second level of load, ventilation and O_2 consumption proceeded in a similar manner.

The cardio-respiratory functions investigated reached their peak activation level at the same point of time, i.e. in the second minute of the highest load. In the following period of attenuation, the curves of pulse beat, ventilation and oxygen consumption decreased sharply and almost parallel. From the 2nd minute of the recovery period, however, the restoration of the initial pulse frequency levelled off, though ventilation kept further decreasing; meanwhile, O_2 consumption showed the most marked fall. The diminution of percentual oxygen exploit occurred last of all, but then it fell quite steeply; from the 4th minute of recovery its curve ran below the base line. This undershoot, or counter-regulation, failed to cease even in the 7th minute of recovery, so this value did not return to the initial one.

Thus, within the scope of integrated control trend, the dynamics of activation and desactivation of certain organs may behave different depending on their own functional state.

SEPARATION OF ISOTOPES BY THERMODIFFUSION

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The separation of isotope solutions by thermodiffusion was continued in our Institute: the separation of $^{39}K - ^{42}K$ and $^{40}Ca - ^{45}Ca$ isotopes was studied. The value of the separating factor derived from concentration, activity, and specific activity is higher than one in most of the experiments.

The obtained values for the separating factors of the specific activity in the experiments were remarkably high. These experimental facts called our attention to the precise evaluation of the results of the tracer method performed in biological systems. On the basis of the experimental results obtained in our Institute and according to the data of the biological literature we raised the question of thermodiffusion in biological systems. It is possible that the existing concentration difference inside the cell is brought about with this mechanisms by the Golgi-apparatus. Further, it may be supposed that the concentration of the urine in the Henle-loop is maintained by capillar thermodiffusion.

THE PROTEINS OF THE mRNA-CONTAINING RIBONUCLEOPROTEIN COMPONENTS OF THE CELL NUCLEUS

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The proteins of the ribonucleoprotein, containing mRNA, isolated from rat liver nuclei could be analyzed very well in polyacrylamide gel, in a discontinous buffer system, containing 6 M urea, at pH 4.5. For the preparation of proteins RNP-bound RNase was activated by 6 M urea, or pancreatic RNase was added. The splitting products of nucleic acids were removed by dialysis, the remained proteins were washed by 5 per cent TCA. Three main components of these proteins were found by gel electrophoresis in polyacrylamide, moreover, several additional bands were present depending on the quantity of histones and other contaminants. As a result of the washing with TCA the amount of the additional components decreased, but the distribution and relative quantities of the three main components remained unchanged.

The proteins of the 30 S RNP-particles of nuclear source, purified by resedimentation in sucrose gradient separated into three main and two to three additional bands by gel electrophoresis in polyacrylamide. The separation does not depend on the method of the preparation of proteins. The proteins of the 30 S particles showed a characteristic picture and they could be easily distinguished from histones and ribosomal proteins, which have essentially more basic character.

STUDY ON DYNAMIC PROPERTIES OF CHANGES IN ADRENAL BLOOD FLOW AND ARTERIAL PRESSURE DURING STOCHASTIC PERIPHERAL NERVE STIMULATION

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Dynamic parameters of changes in adrenal blood flow and mean arterial pressure have been studied during stochastic stimulation of afferent (brachial and ischiadic) and efferent (vagus) nerves on anesthetized dogs of both sexes.

The stochastic stimulation of the nerves has been carried out by square-wave impulses randomly interrupted by a relay, controlled by binar noise of a random signal generator, using a bipolar platinium electrode. The analogue signals of changes in stimulus, adrenal blood flow and arterial pressure have been registered simultaneously on the magnetic tape of a correlator, and on a polygraph. Before tape-recording the periodic components of the signals were damped.

Amplitude- and power-density spectra, as well as auto- and cross-correlograms of the signals have been regularly determined by an analogue computer. The correlograms were used for calculating dynamic parameters like time constant of second-order, T; damping factor, ζ; delay-time, T_d, and transfer coefficient, A. Details of the method had been previously published by the authors (1st All-Union Symposium on statistical problems in engineering cybernetics, Moscow, 1967).

The characteristics of the aforementioned parameters as a function of the bandwidth of stimulus, individual variations of the animals, mode of anesthesia, change in hormonal condition following acute hypophysectomy and hemorrhagic hypotension, have been described. The method seems suitable for studying different transfer functions of some circulatory control processes.

SUBMICROSCOPIC ANALYSIS OF REGENERATING CELL ORGANELLES AFTER INJURY BY CHRONIC DISTENSION ON THE EXOCRINE PANCREAS

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In an earlier communication we reported that the chronic gastric distension had first a positive and later on a negative effect, which had been shown both in changes in the number of secretory granules and in other submicroscopic characteristics which referred to general activity of pancreatic acinar cells of Rana esculenta. In our present experiments we have found that stopping of artificial gastric distension in its negative stage, brings about a regionary regenerating tendency of the acinar cells. The number of secretory granules in comparison with the negative stage of the distension shows a significant growth. With this parallel, vascuolation of the cytoplasm gradually disappears, the ergastoplasm becomes compact, in addition numerous intracytoplasmic inclusions appear reminding to the embryonary developing stages. In the nucleus there are apparent changes in the nucleolus in the period of regeneration. General conclusion: the degenerative effect of the gastric distension points towards regeneration, if we have stopped the artificial gastric distension.

INTERACTION OF CELL POISONS AND MINERAL SUBSTANCES IN THE REGULATION OF THE BODY TEMPERATURE OF RATS

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The metabolic processes taking place at a cellular level were damaged by several days' pretreatment with thyroxin or alpha 2–4 dinitrophenol or by the single injection of alpha 2–4 dinitrophenol or monoiodine acetic acid. The effect of alpha 2–4 dinitrophenol (DNP) and monoiodine acetic acid (MIA) was investigated by recording the temperature of the rectum of rats in the control groups. After this the influence of 40 mg/100 g KCl and 40 mg/100 g $MgSO_4$ was separately determined on the development of the temperature of the rectum in the pre-treated group of 10 rats.

In rats treated with thyroxin for 10 days and every second day with 100 gamma/100 g doses, neither Mg nor K had temperature decreasing effect.

KCl injected 120 minutes after the administration of 1 mg/100 g DNP exerts its effect despite the fact that the drug itself causes temperature increase. Although Mg decreases the oxygen consumption exactly like K, in rats treated daily 1 mg/100 g DNP for 5 days it has a temperature decreasing effect. Mg has temperature decreasing effect even if KCl is injected into the animals in small doses during the period while DNP exerts its effect.

Both KCl and $MgSO_4$ injected 180 minutes after the administration of MIA decrease the temperature of the animals in such measure which surpasses the effect of MIA and the other mineral substances one by one. At the same time KCl proved to be very toxic, in this group most animals die during the experimental time.

EFFECT OF MINERAL SUBSTANCES ON GAS EXCHANGE

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The uneven spread of ions in the intracellular and extracellular space against the concentration gradient can be maintained only by energy liberation. The upset of equilibrium causes the change of the polarisation state of the cell membrane and the energy requirement of the cell. To elucidate this problem rats' oxygen consumption and the temperature of the rectum were measured after injecting a large dose and several small doses of mineral substances one after the other.

It was established that 40 mg/100 g KCl, 125 mg/100 g NaCl, or 40 mg/100 g $MgSO_4$ decreased the body temperature and the oxygen consumption of the animals. The effect of NaCl was the strongest and most permanent. By injecting double quantity of the above described doses in five portion, in every 30 minutes, the KCl increases the body temperature with almost 1° C, while the NaCl and $MgSO_4$ decreases it more and more lower.

After injecting the KCl in five small doses, the 125 mg/100 g NaCl decreases the temperature, while 40 mg/100 g $MgSO_4$ has no effect under such circumstances.

THE EFFECT OF CHANGES IN THE POTASSIUM LEVEL ON SOME DRUGS INFLUENCING THE BODY TEMPERATURE

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Authors gave 2 × 4 ml 7.5 per cent NaH_2PO_4 daily to rats through a stomach tube for 10 days, and other groups of animals were treated with 40 mg/100 g KCl 30 minutes before the investigation. The effect of different drugs was measured on the temperature of the rectum in the control group.

The temperature influence of caffeine and benzedrine has a more moderate effect on animals treated with NaH_2PO_4, while the effect is unchanged in animals treated with KCl in the control groups. At the same time the temperature decreasing effect of urethan ceases completely after the administration of NaH_2PO_4, but exerts its effect unchangedly after KCl treatment. The effect of phenobarbital lessens after NaH_2PO_4 dosage and increases very much after KCl dosage.

The experiments indicate that the upset of the mineral equilibrium of the organism changes the sensitivity for drugs.

PHOSPHATE FRACTIONS AND NUCLEIC ACIDS IN THE GASTRIC WALL OF RATS TREATED WITH NEOSTIGMINE CHRONICALLY

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The stomach wall was analyzed biochemically by the authors in rats treated with Neostigmine Methylsulphate ("Stigmosan") for two weeks with a daily dose of 2 × 0.2 mg/kg administered i.p. The extraction was carried out according to *Schneider–Schmidt–Thannhauser's* method from the whole, glandular stomach and the rumen. The biochemical analysis was performed at the end of the treatment and one month after the cessation of the Neostigmine treatment. The acid-soluble inorganic and organic phosphates, the phospholipid phosphates were measured according to *Brigg's* method, the nucleic acids were estimated on the basis of their sugar and phosphorus components. It has been established that: 1. After Neostigmine treatment only the weight of the rumen decreases and within this the acid-soluble inorganic phosphates ($P = 0.02$), the acid-soluble organic phosphates ($P = 0.04$), the phospholipid phosphates ($P < 0.001$). RNA and DNA ($P > 0.05$) decreased likewise. 2. The weight of the glandular stomach did not change after Neostigmine; the acid-soluble inorganic phosphates ($P < 0.001$), the phospholipid

phosphates ($P > 0.05$) and the RNA ($P = 0.02$) decreased in the glandular stomach, while the acid-soluble organic phosphates ($P < 0.001$) and the DNA ($P > 0.05$) increased. 3. One month after the cessation of the Neostigmine treatment the weight and all biochemical constituents of the rumen, the acid-soluble inorganic phosphates, the phospholipid phosphates and the RNA in the glandular stomach showed the same results as at the end of the Neostigmine treatment, whereas the acid-soluble organic phosphates and the DNA reached pretreatment level in the glandular stomach.

The authors' conclusions are as follows: 1. The cholinergic dominance of the autonomic nerves involves different biochemical changes in the glandular stomach and in the rumen. 2. After a chronic Neostigmine treatment "short term" and "long term" biochemical changes can be observed in the glandular stomach. 3. Vagotomy and Neostigmine treatment resulted in the same changes in gastric biochemism (Acid-soluble inorganic phosphates, phospholipid phosphates, RNA).

CHLOROPHYLLASE ACTIVITY IN NORMAL AND MUTANT MAIZE LEAVES

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Hydrolytic activity of chlorophyllase prepared from normal and mutant leaves was studied with purified chlorophyll *a* and chlorophyll *b* as substrates.

It has been found that chlorophyllase reparations solubilized by acetone of various concentrations attached chlorophyll *a* and *b* to a different extent. Michaelis constants determined in Lineweaver-Burk plots were different for the two substrates.

Chlorophyllase prepared from the ζ-carotanic mutant had a higher activity, while preparations from lycopenic mutant showed a lower activity than that of the normal leaves.

Determinations performed at different stages of chloroplast development revealed an increase in chlorophyllase activity up to the period of granum formation. A further illumination resulted in a decrease of chlorophyllase activity of the mutants.

COMPARATIVE INVESTIGATIONS ON ERYTHROPOETIN ACTIVITY BY MEASURING RADIOACTIVE IRON (^{59}FE) INCORPORATION

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Authors have investigated the activity of erythropoetin in rats by measuring radioactive iron incorporation. The erythropoetin was taken from the plasma of ten patients with developing polycytaemia vera and ten other patients

with decompensated cor pulmonale. The activity value of the found erythropoetin was expressed in per cent of the incorporated radioactive iron, measured on the control group treated with physiological NaCl. To make the results more comparable the activity of erythropoetin prepared from human blood haemolysate was determined and the activity of the erythropoetin of the polycytaemic plasma and of cor pulmonale plasma was compared to this.

According to the investigations the human haemolysate increased the incorporation of radioactive iron by 56 per cent on the average, the cor pulmonale plasma by 149 per cent, the polycytaemic plasma by 193 per cent, respectively, during 24 hours, compared to the control group. The values are correlated to the mass of red corpuscules contained in 1 ml blood of the experimental animals. The results are significant within 0.1 per cent.

CYBERNETIC ASPECTS IN THE MOTOR MECHANISM OF LIFTING THE WEIGHT

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Lifting of the weight was studied under laboratory conditions by the photokinographic method devised by us, and useful for the analysis of micro-effects occurring in the motor action. In this way an arrythmic modulation of movement velocity could be demonstrated in periods proceeding at a relatively slow or medium speed, while during the fastest motions an oscillation of the acceleration values could be measured. The periodicity of the movement process can be traced back to a pulsatile supply of force which, in our opinion, is the consequence of an integration of agonistic and antagonistic forces. The periodically operating antagonistic effect could be demonstrated during the deceleration phases in the EMG records.

On the basis of motor control characteristics 10 different periods can be discriminated in the motion process instead of the usual four. According to the analysis of these periods, we consider the motion process as being controlled by six types of motor forces which join to form three force couples. In the static phase these are the fixator and antifixator forces that become effective. In the dynamic phase the main direction of the displacement is determined by the kinetor and antikinetor forces though movement direction is influenced also by two other antagonistic muscle groups, i.e. by the couple consisting of the modulator and the antimodulator.

ON ADRENERGIC MECHANISMS INFLUENCING ELECTROPHYSIOLOGICAL PROPERTIES OF THE MYOCARDIUM

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It has been shown that in the heart *in situ* of the anaesthetized dog electrophysiological changes underlying the decrease in fibrillation threshold due to reflex sympathetic stimulation elicited by bilateral carotid occlusion, further to direct electrical stimulation of the left stellate ganglion, as well as to intravenous of adrenaline or noradrenaline are related to the excitation of adrenergic β receptors. The decrease in diastolic threshold, the shortening of the refractory period, as well as the increase in asynchrony of recovery of excitability — all due to the experimental procedures mentioned — were prevented by 1-INPEA, a compound possessing highly selective β receptor blocking properties and practically no quinidine-like action, whereas Phenoxybenzamine — an α receptor blocking agent was without effect. — It has also been shown that the mechanism of the arrhythmogenic effect of increased adrenergic activity and that of Ouabain are different. While asynchrony of recovery of excitability was increased by both sympathetic stimulation and toxic doses of Ouabain, a significant increase in asynchrony of impulse propagation could be observed only after Ouabain. — The effect of Ouabain to increase asynchrony could not be inhibited — in contrast to the same action of symphatetic stimulation — by selective blockade of the adrenergic β receptors.

TRANSPORT CAPACITY OF LYMPH TRUNKS IN DOG

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1. The abdominal and thoracic part of the intact lymph system was perfused with diluted plasma via the intestinal lymph trunk. Pressures were measured with electromanometers in small tributaries near the cysterna chyli and the entrance into the venous system. When the perfusion rate was varied a linear pressure-flow relationship was found.

2. When perfusion was maintained for one hour, the lymph system transported volumes 2 – 3 times more than that of the maximal lymph production measured after plasmapheresis. No disruption of lymph trunk wall was found.

EXAMINATION OF THE CHANGES PRODUCED IN PLANT METABOLISM BY THE GAMMA IRRADIATION (CO^{60}) OF THE SEEDS

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The aim of the present experiments was to ascertain the ontogenetic changes produced by the stimulatory and inhibitory effects, respectively, of gamma irradiation given before sowing on the nutrition and carbohydrate metabolism of different plants.

These experiments have been made on maize, bean, tomato, and sunflower plants of different age, exposed to the same growing conditions in a climate chamber. The scope of the investigation covered the movement of the reserve nutritive material (N, P, carbohydrate), the carbohydrate contents in the different organs as well as the uptake and incorporation of labelled phosphorus. The dose-values applied for the treatment varied between 500 to 10,000 r.

The results obtained so far indicate that for the investigation of the positive, and negative effects, respectively, of the irradiation of the seeds on the growth and development of the plants, the quantitative change in the carbohydrates, within this the modification in the ratio of the total and soluble carbohydrates as well as the examination of the uptake and incorporation of P^{32} are promising in the recognition of further correlations.

EXOCRINE SECRETION OF FROG PANCREAS AND ACETYLCHOLYNE

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In our experiments we have investigated the relationship existing between the exocrine secretion of frog (Rana esculenta) pancreas and acetylcholine by analyzing the changes of the following three functional parameters: We have examined on fasting and fed animals, 1. the changes in the rates of cellular activity making use of neutral red absorption method, 2. quantitative changes in the zymogen secretory granules and, 3. alterations of the quantitative characteristics of secreted pancreatic fluid under the effect of acetylcholine in various doses. It has been established that acetylcholine produces effects only in minute doses in all of the three examined parameters while in higher doses this substance has no effect. The cellular activity level shows a 29 per cent growth in fasting animals, at the same time this increment with fed frogs is equal to 33 – 37 per cent. The number of zymogen secretory granules in both starving and fed animals shows a significant diminution following acetylcholine treatment. After acetylcholine injections the quantity of secreted pancreatic fluid doubles in fasting experimental animals while in fed frogs there is an inversion in this trend, since we observed a 52 per cent diminution related to the elevated level of secretion of pancreatic fluid. On the basis of

the above mentioned facts it seems highly reasonable to consider the regulatory role of acetylcholine as a mediator in the control of exocrine functions of pancreas.

FINE STRUCTURE OF VERTEBRATE PHOTORECEPTORS IN DARK- AND LIGHT-ADAPTED STATE

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The formaldehyde-glutaraldehyde-osmium complex fixation introduced by us in the electron microscopy of the retina made it possible to follow the fine structural changes of photoreceptors in dark- and light-adaptation. In rod outer segments of dark-adapted frogs and rats the two adjacent membranes of the receptor discs were closely apposed containing an intermediate dense layer of varying thickness. A 700 to 900 Lux illumination for several minutes made the apposing membranes of the discs to separate and the intermediate dense substance to disappear.

Difference absorption spectra received by cytophotometry of isolated and fixed rod outer segments from dark- and light-adapted frogs showed in dark-adapted photoreceptors the presence of substances having absorption maxima at 480 and 380 μm. The osmiophilia of the dense substance in the receptor discs and the absorption properties of aldehyde-fixed outer segments seem to indicate the identity of the dense layer of the discs with the chromophor of rhodopsin, the retinal.

THE COMBINED EFFECT OF IONIZING RADIATION AND MAGNETISM ON THE GROWTH OF VICIA FABA ROOTS

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In continuation of earlier studies on the mutual biological effects of magnetism and ionizing radiation, the growth rate of Vicia faba roots under the combined effect of a magnetic field and X-irradiations was studied.Resting and pregerminated seeds, respectively, were put into a homogeneous, permanent magnetic field of about 3000 oersted for 24 to 72 hours. Next, the groups were divided into two parts. The half of each group was irradiated with a dose of 200, 400 or 600 R, respectively. The other halves constituted the unirradiated controls. Finally, the mean growth rate for 10 days was determined for both the irradiated and control groups. The irradiation and magnetic effect, respectively, were also determined — as compared to the normal control. The lecture will report in detail on the statistically evaluated results, as 1. Magnetising of resting seeds is ineffective, their irradiation

inhibits growth. 2. Magnetising of germinating seeds promotes growth, their irradiation inhibits growth, parallel to the dose. 3. Magnetised, resting seeds are less damaged by radiation than germinating ones. 4. After irradiation with 200 to 400 R of magnetised, germinating seed, their growth rate approaches the normal value. Some slight growth might be detected even after 600 R, while the controls do not display any growth. Accordingly, magnetising to a certain extent counterbalances the bionegative effect of ionizing radiations. The mechanism of this protective effect is unrevealed as yet.

THE USE OF NEUTRON ACTIVATION ANALYSIS FOR STUDIES ON METABOLIC AND TRANSPORT PROCESSES

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The role of microelements in metabolic and transport processes was studied by activation analysis. Stable, and port-activated isotopes were built into the molecule of different pharmacons, and it was possible by this method to follow up the metabolic changes of the drugs.

For the activation 14 MeV neutrons were used from a neutron-generator, the counting equipment consisted of a NaI (T 1) crystal with a 128 channel analyzer. By using this method it was also possible to determine the oxygen content of organs previously dehydrated. A new and quick method was used for the physical and chemical elimination of the disturbing materials.

The I-metabolism of the thyroid can be determined by means of this method more sensitively, without giving active I. One can examine the ATP metabolism more exactly with 18 labelled water by using activation analysis, than with 32 isotope. The pathological processes in the muscular tissues, and the changes in the concentration of Na, K, Cl, P in connection with these pathological processes can be also measured by this method. When a very small amount of sample is available in the case of transport across cellular membrans — the concentration of sodium and potassium could be determined more reliable by this method, than by flame photometry. — The significance of selenium, as trace element, was proved in this manner. With a suitable stable isotope, a great number of metabolic investigations were performed even on human material (e.g. during pregnancy, or in fetuses).

THE EFFECT OF DENERVATION ON CA^{++} UPTAKE AND ATPase OF SARCOPLASMIC RETICULUM

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An interesting aspect of the neural regulation of muscle structure and function is the reported change in the membranous structure of the sarcoplasmic reticulum (SR) in denervated muscle (1, 2) and the reported increase

in the Ca^{++} transport of fragmented sarcoplasmic reticulum (FSR) (3, 4). We undertook a further study of this phenomenon, particularly with a view to correlating transport, ATPase activity and ultrastructure. The Ca-uptake of FSR in the presence of oxalate increased after denervation for 12–14 days and returned to its initial value in about 30 days. During the same time ATPase activity steadily increased. Thus in the third and fourth weeks after denervation the $\Delta Ca/\Delta ATP$ decreased, indicating a decreased efficiency of the FSR. Electron microscopic examination of negatively stained preparations of FSR from denervated muscle showed a loss of tail-like structures attached to normal vesicles. In the absence of oxalate no increase in Ca-uptake was observed. The ATPase activity increased 4-fold and the efficiency expressed as $\Delta Ca/\Delta ATP$ was decreased from the beginning. Further studies will be needed to establish the mechanism of these changes.

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ALLOSTERIC REGULATION OF THE BIOSYNTHESIS OF AROMATIC AMINO ACIDS

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In E. coli K 12 the first step in the biosynthesis of aromatic amino acids is the condensation of PEP (phosphoenolpyruvic acid) and E4P (erythrose-4-phosphate) to DAHP (2-keto-3-deoxy-araboheptonate-7-phosphate). There are three DAHP-synthase isoenzymes in the cells. The catalytic activity of these enzymes is the same, only their sensitivity to allosteric inhibition is different.

In the course of our investigations the PHE (phenyl-alanine) sensitive DAHP-synthase was partially purified and the reaction mechanism cleared by kinetic analysis. It has been found that the free enzyme reacts first with REP then inorganic phosphate (the first product) is released prior to the addition of the second substrate E4P.

The essence of allosteric regulation is the change in the conformation of these enzymes. The change in energy due to the formation of the enzyme-substrate or that of the enzyme-inhibitor complexes may be related to this change in conformation. We have found that the formation of enzyme-inhibitor complex is connected only with a very small change in energy. However, the formation of enzyme-PEP complex is accompanied by —49500 cal. change in free-enthalpy.

EFFECT OF DETERGENTS AND PHOSPHOLIPASE-C ON THE PROPERTIES OF SARCOPLASMIC RETICULAR FRACTION

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According to the data of Martonosi (Fed. Proc. *23*. 913. 1964) phospholipids play an important role in the building up of the structure of sarcoplasmic reticulum (SR). In the present experiments authors have shown that the ATPase and cholinesterase activities of SR-fraction obtained from fish muscle is increased upon its digestion with phospholipase-C, at the same time the Ca activation of ATPase as well as the Ca-uptake of SRF decrease. Their data suggest that removal of phospholipids results in a rearrangement of protein structure of SR. The relatively low activities of ATPase and cholinesterase indicate a higher structural organization while the relatively high enzyme activities show a lower one in SR.

The authors have demonstrated that enzyme activities are increased by detergents (desocycholate, Triton X-100) in a different degree dependent on species. For example desocycholate increases the cholinesterase activity of fish muscle SRF by about 8–16 fold, the ATPase activity 3–4 fold, the cholinesterase activity of rabbit SRF 3 fold, and the ATPase activity 1.5 fold. An increase in enzyme activity in the similar direction but of a lower degree can be observed in the case of both SRF's when Triton X-100 is being applied.

The use of detergents to disorganize SRF and then the removal of detergents by gel filtration seem to be a convenient procedure to isolate cholinesterase from SRF.

DATA ON THE RELATION OF THE TRANSPORT OF LIPIDS IN BLOOD SERUM AND THE RESPIRATION OF LIVER CELLS IN THE RAT

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Diabetes was induced in one of two groups of rats by 6 mg/100 g alloxan given intravenously. The other group was neurotized by sound, light and vibrational stimuli and by conflicting unconditional reflexes. These stimuli were combined according to a previously scheduled program. According to the recent investigations both diabetes and neurotization by the above-mentioned technique give rise to hypercholesterolaemia. Serum cholesterol, free fatty acid and phospholipoid levels were measured, and the oxygen uptake of untreated mitochondria and those treated with ultrasonic vibration in the presence of succinate was observed both in diabetic animals with blood sugar levels of about 300 mg per cent and in neurotized rats. Finally, the $NADH_2$: : NADP oxydoreductase enzyme activity of particles obtained by ultrasonic vibration was determined.

Increased cholesterol levels were associated with elevation of free fatty acids and phospholipoids; the increment was different in the two groups. The oxygen uptake of liver mitochondria increased and the $NADH_2$: NADP oxydoreductase enzyme activity also decreased in both groups. The results were analysed statistically.

EFFECT OF A COMBINATION OF PROCAINE, PHENYLETHYLBARBITURIC ACID AND ATROPINE ON PULMONARY OEDEMA

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The effect of the "Cocktail" of procaine, phenylethylbarbituric acid and atropine (0.1 g procainum hydrochlor., 0.015 g acidum phenylethylbarbitur., 0.3 mg atropinum sulfur., 10 ml aqua dest.) was tested on experimental lung oedema produced either by epinephrine (0.8 mg/100 g intraperiton.) or by ammonium chloride (0.7 ml/100 g of 6 per cent solution intraperiton.) in 79 white rats. In these experiments the effect of the drug was compared to that of procaine (0.5 ml/100 g of 1 per cent solution intraperit.). In the experiments with both types of oedema the percentage of surviving animals and the survival time were increased essentially, and also a certain protection against pulmonary oedema was observed. In both test series the "Cocktail" (0.5 ml/100 g of 1 per cent solution intraperit.) was more effective than procaine.

7 out of 10 patients with severe lung oedema and simultaneous diseases of the circulation, respiratory tract, or central nervous system were treated successfully by injections of 10 ml of the "Cocktail". In 14 cases the effect on bronchospasms was tested: each of the patients had to undergo spirographic tests before "Cocktail" injection and after. The spirographic results were compared with the "epinephrine reaction" of the individual patients. In 12 of the total 14, "Cocktail" injection increased the vital capacity.

CONNECTION BETWEEN CATION TRANSPORT AND SHAPE MAINTENANCE OF ERYTHROCYTES

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Straub and Gárdos proved that the active cation transport of erythrocytes is an ATP requiring process. Nakao and co-workers demonstrated that the maintenance of the biconcave shape of erythrocytes is also a function of the cellular ATP level. Whittam raised the question whether the shape maintenance is a direct ATP utilizing function or it requires ATP only secondarily through the ion migrations and the changes in shape occur only as a result of changes in ion composition and osmotic relations.

The connection between active cation transport and shape maintaining function has been investigated in the course of ATP utilizing and generating reactions (glycolysis inhibition, fermentative and oxidative ATP resynthesis). The two processes were influenced by ouabain in an opposite way which suggests the independence of the two mechanisms and their competition for ATP. In addition to the K^+–Na^+ exchange, the K^+–H^+ exchange, induced by NaF, which results in a significant decrease in volume was investigated. The results of these experiments have shown that cation transport in itself is but of little importance in the shape changes. On the other hand one has to consider the ATPase inhibitory and the so called direct membrane effect of NaF. The differences of the direct membrane effect on cation transport and on shape maintenance will be discussed, compared with the characteristics of ouabain-sensitive and insensitive ATPase fractions of haemolysates and isolated erythrocyte membrane preparations, respectively.

EFFECT OF PURE LOW PRESSURE OXYGEN-ENVIRONMENT ON THE METABOLISM OF RATS

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In their first experimental series, authors placed albino rats for 8 hours in low-pressure-chamber containing pure, low pressure (260 mmHg) oxygen. The control animals were kept under normal atmospheric conditions. Beginning with the experimental 8 hours the urine was collected during 24 hours and the Na, K and Ca content, as well as the total-N and amino acid-excretion determined. The LDH activity of the blood serum and of the myocard, as well as the glycogen-content of the hearts were also examined.

It could be established, that:

1. Ca-excretion significantly increases, Na-voidance slightly rises, K-excretion remains unchanged. The value of the Na/K ratio increases;
2. The quantity of total-N increases in the urine;
3. Excretion of some amino acids remains unchanged (aspartic acid, glutamic acid, arginine) voidance of certain amino acids (leucines, phenylalanine proline, alanine) is increased.
4. LDH activity of the serum and the myocard was identical in both groups;
5. In the course of acute charge glycogen content of the hearts did not change.

CHANGES IN LYOSOMAL ENZYME ACTIVITY IN LYMPHOID ORGANS OF GUINEA PIGS OF VARIOUS AGE

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The phagocytotic activity of thymus and spleen has been studied in early developmental stages of guinea pig. As an indicator for measuring the process the changes in specific activity and histochemical localization of acid-para-nitrophenylphosphatase were observed. The experimental results of neonatal thymus show a readiness to physiological involution. The specific activity of acid pNPP-ase increases compared to the previous embryological stage; in the histochemical picture macrophag activation is accentuated, production and growth of Hassall's corpuscles are elevated. In spleen both enzyme activity and the number of phagocytic cells rise in even greater degree. After two-three weeks the phagocytotic activity in the thymus emphatically decreases — similarly to a regeneration. In spleen this change takes place only to a slight extent. While by the fifth week of the development of the guinea pig enzyme activity increases again; in the thymus it reaches only the embryological level, in spleen it is much more pronounced.

INVESTIGATION OF ANAPHYLAXICAL PHENOMENON IN FROGS

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According to our investigations antibody production can be observed against soluble antigens in frogs at a suitable environmental temperature.

To elucidate the anaphylaxic reactions, the reactions to homologous and heterologous antigens by sensibilizated Rana esculenta were investigated. The experiments were carried out on isolated frog hearts and on mesenterial microcirculation. According to the results, no specific reactions were obtained to homologous antigen on passive sensibilized isolated frog hearts by anti-bovine, γ-globulin and antiovalbumin rabbit serum.

In experiments performed on the peritoneum of passive sensibilized Rana esculenta prolonged contraction of the mesenterium arteriols was observed after the application of homologous antigen. This effect proved to be specific on the basis of the control experiments.

According to our results among the investigated biogen amines (adrenalin, acetylcholin, histamine, serotonine) the histamine seems to possess similar effect to the microcirculation reaction of antigens–antibody.

INVESTIGATIONS ON THE METABOLIC RATE IN CAPSAICIN DESENSITIZED RATS

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According to previous experiments the hypothalamic warm receptors can be irreversibly desensitized by capsaicin. Such desensitized animals are not able to regulate their body temperature against heat. (Jancsó, Jancsó-Gábor and Szolcsányi 1966). The experiments were extended to the examination of the regulation of the metabolic rate in desensitized rats at different ambient temperatures.

At temperatures of 30° C (neutral) or 20° C (cool) the O_2 consumption and body temperature of the desensitized animals did not differ significantly from that of the controls. Hence, capsaicin desensitization did not influence the basal metabolic rate and the chemical temperature regulation. However, capsaicin desensitization could prevent in rats at 20° C the fall of rectal temperature and the decrease in O_2 consumption induced by sc. injection of capsaicin.

At a warm ambient temperature of 35° C the body temperature of the desensitized animals exceeded in 45 minutes by 1.5° C that of the controls, whereas the rise in O_2 consumption was practically the same in both groups (23 per cent and 25 per cent, respectively). Consequently, the rise in metabolic rate during hyperthermia is not evoked by the stimulation of the hypothalamic warm receptors and does not either follow the van't Hoff's rule in desensitized rats.

BIOCHEMICAL EXAMINATION OF FREE AMINO ACIDS IN THE PERFUSATE OF ISOLATED KIDNEY

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Authors examined biochemically the free amino acids in the perfusate of rabbit kidney perfused with artificial plasma.

It was found that the isolated perfunded kidney continually released free amino acids. In the perfusate all the amino acids building up the structure of natural proteins were detected, and other amino acids, too.

Even in the first hour the perfusate contains the above-mentioned amino acids in significant amounts. The ratio between the amino acids in the medium is approximately similar to that of the blood plasma. Some of the amino acids (e.g. leucine and valine) may be found in greater amount in the perfusate, than in the blood plasma.

The uncoupling of the oxidative phosphorylation increases to a great extent the release of almost all the amino acids.

The possible biochemical mechanism of the release of the free amino acids by the isolated perfused kidney will be discussed.

INVESTIGATION OF ^{125}I INSULIN-PROTEIN COMPLEX BY GEL FILTRATION IN VARIOUS FORMS OF DIABETES MELLITUS

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It is well-known that antibodies are produced in man upon repeated exposure to insulin. This research was undertaken with the aim of learning more about the appearance and properties of these antibodies. Serum samples were incubated with ^{125}I insulin to establish the antigen–antibody binding. Insulin–protein complex was fractionated by gel filtration using Sephadex G 100 and Sephadex G 200 columns.

In cases of normal sera and of diabetic patients never receiving insulin (freshly discovered or Tolbutamide treated adult cases) the binding of radioactive insulin to proteins was about 10 per cent. The binding in cases of insulin treated diabetics was about 50 per cent. In sera from patients with insulin resistance this binding to antibodies was very high, approximately 80 per cent. Thus these antibodies can play a part in the formation of the resistance.

The insulin–antibody complex, according to its molecular size, belongs to 7S globulins but we found complex between the 19S and 7S protein peaks, too.

Our procedure perhaps makes it possible to subject antibodies of badly controlled diabetics to routine investigation and to characterize them further.

EFFECT OF SOFT BETA-RADIATION ON THE EXCITATION PROCESSES OF THE MUSCLE

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The semiconductor hypothesis developed in our laboratory suggests a possible shift in the excitation phenomena caused by treatment with ionizing radiation. During the last years, different kinds of radiations with wide energy spectra were investigated from this point of view, and many such experimental data were gained which seem to support the above hypothesis.

The effect of the beta-radiation of tritium (max. energy 18 keV) was studied in the present experiment. The isolated frog sartorii were incubated in a normal Ringer solution containing tritiated water with a specific activity of 10 mC/ml. The temperature was 2° C. The changes of the threshold stimulus voltage were measured as a function of irradiation time i.e. the radiation dose. Our results showed that the threshold stimulus voltage decreased in absolute and relative sense as well in the tritium treated muscles compared to the controls.

The electric resistance of the muscle tissue (at 10^4 Hz) showed no significant change either in longitudinal or in cross direction. The potassium content also remained constant during the period of the experiment.

Our investigations seem to support the hypothesis according to which a minute change in the electrical structure can produce a significant change in the excitation process.

EFFECT OF PITRESSIN ON THIOSULPHATE SPACE IN THE RAT

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The distribution of $Na_2S_2O_3$ administered intravenously to rats subjected to ureteral blockade and nephrectomized rats has been investigated. Thiosulphate space has been found to increase progressively during the period of observation, attaining 41 and 40 per cent of body weight 90 min after the injection in the animals with the ureters ligated, and in the nephrectomized animals, respectively.

A priming intravenous injection of 50 mU of Pitressin which was followed by further 50 mU muscularly caused thiosulphate space to increase to 57 per cent in the first group, 90 min after the first injection of Pitressin.

It seems reasonable to assume on the basis of these findings that posterior pituitary extracts increase the permeability of cell membranes not only in the kidney.

FURTHER ELECTRON MICROSCOPICAL STUDIES ON THE "GLOMERULAR BODY" IN THE TROPHOBLAST CELLS OF RATS AND MICE

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In earlier investigations we found a peculiar cytoplasmic structure consisting of numerous twisted threads in the trophoblast cells of the rat and mouse placenta. We termed it "*glomerural body*" (1). The average size of these structures measured 1 μ in rats and 1.5 μ in mice; the number of them was approximately 10 to 15 per cell (the trophoblast cells in question are syntitial in both animals).

The glomerular bodies consist of dense, helical threads, 400 to 500 Å in diameter. In spite of the lack of any membrane around the bodies they are distinct of the surrounding cytoplasm: the threads are embedded in a clear, structureless ground substance extending over the whole body.

The glomerular bodies are topographically in close connection with ribosomes found in great number around them.

Supposing that the glomerular bodies are somehow in relationship with the metabolism of nucleic acids or proteins, experiments were carried out using electron microscopical histochemistry and autoradiography. The glome-

rual bodies were exposed to DNA-se, RNA-se and trypsin digestion, and incorporation of uracil-C^{14}, and tritiated adenine, phenylalanine, tyrosine, leucine, were investigated. Approaching the question from an other angle we observed the effects of Actinomycin D.

The results will be discussed in the lecture.

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A STUDY OF GLYCOPROTEINS IN RATS TREATED WITH METHYLCELLULOSE

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In rats treated with methylcellulose the serum-mucoprotein rose, as compared to the control, to double the amount (from 23 mg per cent to 46.5 mg per cent), and neuramine acid and hexosamine also rose significantly. The protein-bound hexose did not change. The amount of alpha and beta globulins among the protein fractions increased considerably. The hepatic and renal hexosamine concentrations also increased after administration of methyl cellulose. The acidic mucopolysaccharide content of the kidney hardly changed during methyl cellulose treatment. Of the serum proteins the level of albumin in hypophysectomized animals did not sink to the same degree after methyl cellulose treatment as in the hypophysectomized controls. Of the globulins it is only the amount of the alpha globulins that increased. The level of protein-bound hexosamine and neuramine acid also rose considerably after treatment. The hepatic hexosamine and neuramine acid content did not change upon treatment, nor did the renal hexosamine and neuramine acid contents of the kidney increase after methyl cellulose treatment.

METABOLIC STUDIES ON YOSHIDA SARCOMATOUS RATS

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Very few reliable data can be found in the pertinent literature about investigations on changes in the basic metabolism of tumourous animals. According to the assumption of the authors the metabolic level of the host organism influences the development and growth of tumour to a great extent, therefore the clarification of this problem may be of great significance. For

this purpose they made a study of the metabolic changes in rats possessing solid tumours subcutaneously inoculated with Yoshida sarcoma cells. It was established that in the investigated two series (5 animals each), the basic metabolism rose considerably, parallel with the growth of the tumour, the initial value increasing by 100 per cent in the 3rd week of the development of the tumour. This cannot be explained solely by the incidental increase of the metabolism of the growing tumour tissue, because its mass will always remain essentially smaller compared to the weight of the whole animal. In control experiments it was ascertained that neither the subcutaneously inoculated piece of liver, nor the heat-treated (60° C) mass of tumour, nor a simple incision influenced the metabolism during an observation period identical with that of the tumour. Finally the authors analyze briefly the conclusions that may be drawn from the obtained results.

THE PROBLEMS OF REGULATION OF PHOSPHORYLASE BY INTERMEDIATES IN THE LIVER

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According to the literary data phosphorylase, the limiting enzyme of the glycogen breakdown in the liver is in possession of 60 per cent of total activity, which would make possible the breakdown of the total glycogen reserve in 20 minutes or so. Therefore we suppose that *in vivo* inhibiting factors reduce the effect of phosphorylase. It seemed to be interesting to examine whether intermediates regulating phosphorylase in muscle also have an influence on the function of the phosphorylase system in the liver.

G-6-P has a relatively unimportant effect on liver phosphorylase in case of great concentration which is consistent with the fact that G-6-P is less of an end product in the liver than in the muscle. The glucose, end product of glycogen breakdown in the liver inhibits the activity of phosphorylase considerably. This inhibition differs from that of the musclephosphorylase so much that it cannot be suspended by AMP. It corresponds to the fact that liver-phosphorylase cannot be activated by AMP. Thus the changing of glucose concentration may play a role in the regulation of the activity of phosphorylase, that is, in the mobilization of glycogen. Liver differs from the muscle in its phosphorylase inactivating system too as far as G-6-P does not increase the phosphorylase phosphatase function of the liver, and on the other hand, it is unable to suspend the inhibiting effect of AMP. Further we are studying the effect of additional intermediates on the activity of liver-phosphorylase, and on the change between its active and inactive forms.

SOME ASPECTS OF THE QUANTITATIVE DETERMINATION OF FREE FATTY ACID (FFA) IN BLOOD PLASMA

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Regarding its diagnostic value in different metabolic disturbances, more and more attention has been paid recently to the assumed significance of the FFA content of the plasma.

Dole's micromethod, developed for the exact determination of micro-quantities of FFA in the plasma, proved highly unreliably under our working conditions. On the other hand, *Mosinger*'s modification of the method was found excellently suitable for clinical purposes. In this modification of *Dole*'s method, changes in colour of a phenol-red indicator, dissolved in a n-heptane-ethanol system in the presence of Na-barbital buffer, is measured spectrophotometrically. We found the method reproducible, quick and specific enough. Its sensitivity was found to be around 10 μU.

Control values, obtained by this method satisfactorily covered the range which is accepted as normal in the pertaining literature.

REGULATION OF HOMOSERINE-DEHYDROGENASE ACTIVITY

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Homoserine-dehydrogenase is the feedback regulated enzyme of the synthesis of amino acids belonging to the aspartic acid family. The enzyme plays an important role in the regulation of the synthesis of lysine, threonine and isoleucine. Studying the reverse reaction of the enzyme prepared from Chlamydomonas reinhardti (homoserine-aspartic acid semialdehyde) it was found to be able to operate with both NAD and NADP. The enzyme can be inactivated by PCMB which indicates that it may be an SH-enzyme. The feedback inhibitor of the enzyme is threonine, the inhibitory effect of which proved to be apparently competitive for homoserine, non-competitive for NAD. The inhibitory action of threonine is diminished, resp. eliminated by potassium ions, as the latter are decreasing the K_m of NAD and reasing the K_i of threonine. The potassium effect seems to be specific, other monovalent cations do not exhibit similar effect, they actually reduce the effect of potassium. The relative effect of potassium ions is pH dependent, yet in the presence of potassium the pH optimum of the enzyme is unchanged. There is reason to suppose that in the regulation of the activity of homoserine-dehydrogenase some role may be ascribed to potassium ions.

TEAR AND SALIVA SECRETION AND BEHAVIOUR OF DOGS AFTER ELECTRICAL STIMULATION OF CERTAIN HYPOTHALAMIC NUCLEI

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It is well known that in the hypothalamus there are regions, the stimulation of which changes the food and water regulation. This was observed on rats, cats, sheep, dogs and monkeys, by the authors Anand and Brobeck, 1951; Grastyán, Lissák and Kékesi, 1956; Delgado, 1962; Kostenetskaya, 1963; Bogach, 1966; Dobrovolskaya, 1967 and others.

In the present work six dogs with tear and saliva fistula were investigated The secretion reflexes and the behaviour during the eating before and after the stimulation of various nuclei of the hypothalamus (supraopticus, paraventricularis, lateralis) were studied. We used the morphological classification of O. Sager, 1962. The histological control of the brain was made by dr. Obuchova. The introduction of steel bipolar electrodes (0.1 mm in diameter, glass isolation) changed the secretion reflexes ipsilateral, during 3–4 weeks in one animal. The direct electrical stimulation of deep structures of brain (10–20 mAmp) resulted tear and saliva secretion, but only on one side and caused licking and smelling reactions. It was found that after electrical stimulation of the supraoptic and paraventricular nuclei the chemical components of tear and saliva (proteins and mucopolysaccharids) are changed. The behaviour of the dogs became extraordinary. In 3 dogs out of 6 the food intake increased from 200.0–300.0 gramms to 1500–1700 gramms. The protein content of tear markedly rose (from 8.0 ± 0.3 to 20.4 ± 0.9 in one ml of tears; $p < < 0.001$). The data suggest that the anterior and lateral hypothalamic nuclei are involved in the regulation of food intake and take part in the regulation of chemical components of tear and saliva.

EXCITABLE ARTIFICIAL LIPIDIC MEMBRANES

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A drop of linseed oil spreads on an oxidizing solution (MnO_4K 1 p. 1000) and forms a thin flexible membrane (1 μ). This membrane inserted between salt solutions (for instance KCl M/10), exhibits various properties analogous to that of living excitable membranes: cation selectivity (with a marked preference for K^+ over Na^+), nonlinear current-voltage relation, responses to current characterized by fast transient increases in conductance of which the frequency augments with current intensity (MONNIER and al., 1965a, b). Recent work has confirmed and extended these above results. Intercationic specific permeability of the above membrane has been studied through the observation of bi-ionic potential. Permeability increases in the following order: Li^+, Na^+, K^+, Rb^+, Cs^+, the permeability towards Li^+, the most hydrated cation, being the lowest. Organic cationic drugs can permeate easily the membrane (GOUDEAU, 1967). Permeability increases with the number and length of the alkyl chains. Tetrabutyl ammonium chloride on one side of the membrane, and LiCl on the other at the same concentration, produce a high bi-ionic potential, close to 200 mV. With ephedrine chloride the potential is about 138 mV. Thus the permeability of the membrane towards respectively ephedrine and tetrabutylammonium is more than 100 and 1000 times that towards Li^+. Furthemore the conductance of the membrane increases more than twenty fold when tetrabutylammonium is present, even only on one side of the membrane. The large organic cations appear: *a)* to displace all inorganic cations from the membrane, *b)* to possess in the latter a very high mobility. This feature could be explained by the assumption that inorganic cations are restrained, in their motion, by hydrogen bonds linking some water molecules of their hydration shell to groups fixed upon the membrane reticulum. On the contrary, the motion of the large organic cations may involve only the weak VAN DER WAAL's forces that occur between their alkyl chains and the lipidic reticulum.

SANCHEZ and REYNIER (1967) have confirmed that the resting membrane can be described formally as a capacitance and a resistance in parallel. Capacitance is usually of about 4×10^{-9} farad $\cdot$ cm^{-2}. The average resistivity is 8×10^9 ohms $\cdot$ cm, that is about 1000 times smaller than that of the original linseed oil. The resistance decreases markedly with temperature ($Q_{10} = 4$). This indicates that the membranes are complex structures in a labile condition into which large transformations occur. The dielectric constant is small (about 15) as compared to that of water, indicating a rather limited hydration of the membrane.

The responses of the membrane are of two types according to the intensity of the current. Currents under about 30×10^{-8} amp $\cdot$ cm^{-2} elicit a steady increase of conductance. With stronger currents the increases of conductance are transitory and repeated at a frequency which augments with the current intensity. The conductance during the response can be twenty times that of the resting membrane.

Other lipidic membranes can show excitation processes. Glycerol monooleate is known to form aqueous gels. When to this substance about 10 p.

100 of oleic acid is added, the superficial aqueous gel spread upon a wet cellophane sheet shows repeated change of conductance when traversed by a current.

The essential features which characterize the various lipidic membranes, as model of excitable structures are: repeated transient increases of conductance when traversed by a current, different selective permeability towards the various cations. However, excitability occurs with the same cations on both sides of the membrane. The main conditions for the appearance of these features are: *a)* gel-like elastic structure, moderately hydrated, *b)* fixed charges induced either by oxidation or addition. *c)* temperature between 15° and 30° C, *d)* pH between 5 and 7.

The mechanism of response is still open to speculation. The most promising hypothesis actually investigated is the rupture, under the applied electric field, of the hydrogen bonds between the water molecules of the cationic hydration shell and fixed groups on the membrane reticulum.

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