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EARLY MANIFESTATIONS OF CONDITIONING

Proceedings of a Symposium Organized by the Hungarian Physiological Society

Budapest, July 2 — 3, 1963

NEW TRENDS IN CONDITIONED REFLEX RESEARCH

By

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When the agenda of the symposia to be held this year were determined by the 1961 Congress of the *Hungarian Physiological Society*, it has been decided after careful considerations and deliberations that the problems to be discussed should not be restricted to the most actual fields of research concerned with the central nervous system, but should include the most representative investigations of *Hungarian* neurophysiologist.

In fact, the studies of the development of the temporary connections in concrete structures by electrophysiological, neuroendocrine, psychopharmacological and behavioural methods have in recent years been in the foreground of international interest, and have brought also in Hungary a number of new observations requiring discussion.

Personally, I am delighted to be able to introduce our present symposium, because more than 10 years ago, at the 28th Meeting of the *Hungarian Physiological Society* in 1952, I outlined as relator "The Significance of Central Nervous Structures in Pavlovian Physiology", the trend of research at my Institute. I must admit, at that time my paper was not received with too much enthusiasm, but now we can witness a blossoming-out of this concept and trend of research not only in this country, but also throughout the world.

During the decades following World War II research concerned with central nervous function had been stepped up and expanded throughout the world. As a result, the progress made in this field during that short period brought us nearer to an understanding of cerebral function than did all the work done during the previous century. Even in such countries, including Hungary, where in the past there was up-to-date neuromorphological and pathological, but practically no neurophysiological research, the possibilities offered by the liberation have led to such an advance in neurophysiology that the results obtained are certainly worthy of international attention.

The discovery early in this century of the principle of the conditioned reflex played a significant role in the world-wide advance of neurophysiology. The blossoming-out of the conditioned reflex principle in its whole perspective, its recognition and use throughout the world, so-to-say its second renaissance,

fell to the past decade. The Pavlovian doctrine became a science of general biological, but also of ideological significance, because it is in its every part, in its every conception a profoundly dialectic, advancing, moving, unclosed doctrine. During the few decades of its advance, the science of higher nervous activity has conquered many new fields, deepened and broadened both in and outside. Although the main trends of advance had been outlined by PAVLOV himself, the investigations opened up more than once, quite unexpectedly, new vistas and possibilities. Progress in this field necessitated first of all the use of certain special methods of experimentation, as a result of which specially trained borderline investigations were required. This is how the research concerned with higher nervous activity has assumed an interdisciplinary character. For the physiologist working in a restricted field of problems it is not an easy task to know what is happening in the field of higher nervous activity research, as a whole, and, as a matter of fact, it is not his duty at all any longer.

The investigations into the more complex aspects of higher nervous activity now require training and skill significantly different from those needed in the studies of the more elementary problems of central nervous function, and in both fields the demands are so great that it is virtually impossible for one man to cope with them at the same time. This does not mean that we can neglect the results obtained in distant fields. So for example if in research concerned with the second signal system the progresses made in the field of elementary phenomena are ignored, the worker with his speculations will be led into a vacuum, lose contact with basic physiology, and, in the best case, be compelled to base on outdated views in the interpretation of his results. But the physiologist concerned with elementary phenomena is in the same danger, if he fails to analyse the elementary phenomena in the synthesis of complex manifestations; his analytical facts may then grow into a meaningless mass of data, and his problems may become artificial or formalistic.

The superiority of the synthetic view follows from the dialectic character of the Pavlovian doctrine, and if modern research wants to avoid the danger of dogmatism, the high demands must be met fully. This should be the most important task of the symposia. The enormous rate of advance in research makes it absolutely necessary that such a co-ordinative evaluation should be made from time to time; on the other hand, the information pouring in from the different special branches promotes the synthesis at a higher level.

Although none of the problems now in the centre of interest in modern neurophysiological research may be pointed out as being the one furnishing the essential basis of brain function, yet we may be justified in saying that, in view of the complicated phenomena intermingled in evolution, in the past decades the most important aspects studied have been those of consciousness, attention and the mechanisms of learning. The Pavlovian concept has offered a new and objective method for studying the laws and dynamics of higher nervous activity.

It was often by Pavlovian methods that experimental psychology called attention to the importance of certain problems, for example motivation, in the analysis of behaviour; and the modern electrophysiological methods have allowed an insight into such neuroanatomical structures, where partly laws determined early in the course of evolution, and partly laws of a statistical character prevail.

In the past decades efforts have been made to understand the essence of brain function independently of subjective factors; this was a great step forward after the past century's metaphysical approach. It has become possible to study by concrete scientific methods every aspect of higher nervous activity and to fix the complex manifestations of brain function to certain cerebral activities. In the advance thus achieved the fight between the advocates of the localization doctrine and those of the dynamical stereotypes was a powerful driving force, as a result of which it has been realized with increasing clarity that although some function may be located to some area of the brain at the nervous level of a given phase of evolution, this cannot be generalized at the phylogenetical, and only to some extent at the ontogenetical level. The most general interpretation of central nervous functions has been achieved by the cybernetical trends, as in ASHBY's homeostate, or in the application of the fundamental principles of the information theory to the interpretation of brain function.

In the analysis of the more concrete activities of the central nervous system by means of the methods of experimental behaviour or by electrophysiological techniques, the investigator faces only one definite aspect of the phenomena, which determines the nature of the phenomenon and at the same time the relativity hidden in the laws governing the detail function. Although they have attempted to approach brain function as a whole, such studies have led to the complex terminology. Thus, for example to the differences in the interpretation of the EEG and behavioural arousal or the emotional processes, and have led to the confusion that for instance attention is understood to mean different things by the Pavlovian, the experimental psychologist and the electrophysiologist. A co-ordination and interpretation on a common basis of the results obtained by different methods and by approaching higher nervous activity from different angles are the tasks of the present and of the near future, first of all to allow conclusions as to the essential factors in such fundamental functions as attention, memory, learning, or emotions from the laws of the various projections of brain function.

The aim of our symposium is to study the early manifestations of the mechanisms of learning. At a high cortical level the early manifestations of the "conditioned reflex" can be studied only indirectly, because all the manifestations of ontogenetical life are built on some conditioned reflexes developed earlier, or acquired independently of ontogenesis. This uninterrupted evolution of

cerebral function is composed essentially of unbroken links of the chain of conditioned reflex connections, manifesting themselves either in some form of memory, or in new temporary connections of the signals coming from the environment.

One of the preconditions of learning in the higher animal is the attention reaction, alertness, which is essentially inseparable from consciousness, though the two represent different categories of cerebral activity and cannot be identified with each other. When we raise the neurophysiological problem of alertness we want to answer also the question to what extent the early stages of learning can be traced back to existent intrinsic and extrinsic factors. ADRIAN said that the attention reaction consists in directing consciousness from one thing to another. Without trying to analyse critically the definitions put forward by ADRIAN or others, it is necessary to point out two characteristic features of this form of brain function: attention determines first the relationship of the living to its environment, then, at a certain higher level of consciousness, it always shows some autonomy. This is to be understood to mean that, in possession of existent, previously acquired informations, attention can be directed through the memory mechanisms. These definitions do not, of course, permit one to gain insight into the organization of central nervous activities. As it will be dealt with later, according to the observations made at our Institute, attention and its electrophysiological and behavioural manifestations can be abolished by the destruction of the mesencephalic and diencephalic activation systems, and at the same time in its somatomotor manifestations the animal shows the behaviour of an alert animal.

There is now abundant evidence to show that the attention reaction and the orientation reaction associated with it can be considered to be activities of the whole brain, so of the neocortex and subcortex, although the basal diencephalic structures also play certain determinant activator roles in these activities. The part played by the ascending non-specific diffuse activation system in alert behaviour, attention and the learning reactions described by MAGOUN and MORUZZI, JASPER, as well as by their followers, has now been filled with meaning, and at the same time its original definition has expanded. NAUTA's tegmento-diencephalic cycle assumes the unity of the brain stem and rhinencephalon in emotional and motivated behaviour, and in another direction reciprocal role of the thalamo-cortical connections has come to the foreground in the organization of the attention reaction. The alpha-excitability conception of ADRIAN, CHAO and BREMER, as well as of LINDSLEY, who suggested a correlation to exist between attention and the hypersynchronizating activity of the cerebral cortex, has been revived in the literature of recent years, I might say also that attention has to some extent been focussed again upon such a controlling role of the neocortex. The ingenious experiment of FOX that in the case of sensorily deprived monkeys in the dark the light switch is pressed down

spontaneously when hypersynchronization appears in the cortical and deep leads indicates some close relationship between the spontaneous periodicity of the attention reaction and the development of hypersynchronization. In our cat experiments we have arrived at the conclusion that the periodic change in the attention reaction of the sensorily deprived animal is always accompanied by the appearance of hypersynchronous wave bursts in the cortical and in certain subcortical leads. These periodic changes are composed of relatively short periods, lasting a few minutes each, and the repeated increases in attention are often accompanied by changes in behaviour, locomotion, washing, stretching, *etc.*

The question arises how does attention fit into the mechanism of learning? It is different in character in such conditioned reflex situations where the conditioned reflex is "approaching" and "aversive", respectively. In both situations there is a definite attention reaction, but one might take part in the induction, the other in the inhibition of a certain motor behaviour. The same happens in the course of differentiation. The attention reaction in response to some differential stimulus means always an inhibition, in the Pavlovian concept an internal inhibition. The components of opposite signs of the attention reaction do not belong strictly in the basic category of attention: here an affective motivation factor already plays a role, which determines the direction of the behavioural reaction and serves as its further driving force. According to the Pavlovian view this attention reaction is associated with a differentiation of the environmental stimulus. Differentiation is understood to mean many things; in general, it is interpreted as differentiation between two or more signals. It has to be pointed out that every new environment, or some change in the old one will evoke one or another form of differentiation. Let me mention a simple example to illustrate this: a cat is habituated by presenting a sound for 10 seconds every minute. After a certain number of reinforcements we reach a point where the animal does not respond any more to this at the EEG level. If subsequently we present the signal in intervals 2 or 3-minutes the animal will respond with EEG arousal not only to the signal given in prolonged interval but also to a few subsequent stimuli presented at the original interval.

Essentially, habituation arises through the homogeneity of the relationship between some spatial and chronological environment and brain function. A biologically effective breaking-up of the homogeneity of the spatial and chronological environment will result in a new attention reaction and differentiation. In some cases the stimuli coming from the environment are biologically valuable, in others irrelevant for the animal, which has to decode them from the environment. This decoding of information is closely linked to the attention reaction and to the discrimination of the situation stimuli and we may say that it is the basis of every mechanism of learning. The task of the neurophysiologist is to explore in the concrete unity of structure and function those structures,

which play a fundamental role in the organization of these processes and to elucidate the laws governing that attention reaction, a process of decoding. It is beyond doubt that these processes play the leading role in the development of temporary connections and their role can be understood exclusively in the unity of the extrinsic and intrinsic environment.

THE SIGNIFICANCE OF SUBCORTICAL MOTIVATIONAL MECHANISMS IN THE ORGANIZATION OF CONDITIONAL CONNECTIONS

AN ATTEMPT AT THE PHYSIOLOGICAL INTERPRETATION OF THE BASIC MECHANISM OF MOTIVATION

By

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The basic neural mechanisms of motivation have been investigated in cats by applying electrical stimulations to diencephalic and mesencephalic structures in the background of a dual (alimentary and avoidance) conditional reflex environment.

It has been established that there are two elementary and antagonistic mechanisms (pull and push effects) behind the apparently homogeneous motivational effects elicited by the stimulations. The effect compelling the animal to approach a stimulus or object acting on it at the moment of stimulation, has been termed a *pull effect*. An effect compelling the animal to avoid a stimulus or object, has been termed a *push effect*. It has been established that these two basic effects are inseparably interconnected. The pull effect at a critical level of motivational excitement is regularly transformed into a push effect. The possible neural organization responsible for the complex interrelationships of the two motivational subsystems has been discussed by analysing several experimental facts.

It is pointed out that both the pull and the push effects are able to organize conditional connections corresponding to their nature, with external stimuli acting at the moment of their existence. On the basis of conditional connections produced to the turning on and off of electrical stimulation, an attempt has been made at the physiological interpretation of HULL's drive reduction hypothesis. Attention is called to the importance of the arrest reaction in the formation of instrumental conditional reactions. Finally, conclusions have been drawn concerning the general importance of the elementary drive mechanisms in the organization of the learning process.

The general importance of motivation in the conditioning process is widely recognized. Our knowledge concerning the neural organization of the different special drive processes is, however, still scanty. According to the classical physiological conception drives (unconditional mechanisms in the Pavlovian sense) are composed of chains of simple reflexes. This conception is really physiological in the sense that it is based on the simplest elements of the nervous system. It neglects, however, the important discovery of experimental psychology, according to which during the elaboration of a conditional reflex the conditional effect has a retroactive influence upon connection formation, consequently the system responsible for the establishment of the conditional connection must have a peculiar inner organization. The main event in this feed-back-like mechanism by which the conditional connection is accomplished according to HULL's concept would be a reduction of drive. More concretely, and in a strong form (MILLER 1957) the hypothesis states that drive reduction is the necessary and sufficient condition of reinforcement. This elegant logical construction must be regarded as correct in the general biological sense. Princi-

pally it states that during adaptation such behavioural acts are elaborated by the nervous system by which the living being is able to avoid any external or internal disturbing agents and to ensure a relatively stable resting state. Essentially the same happens during the regulation of any physiological constants.

There are, however, some objections based on experimental facts to the general applicability of the drive reduction theory. They state that it is only valid in the case of strong drives (HEBB 1955). The drive reduction hypothesis is a psychological construction the validity of which could neither be substantiated nor rejected on the basis of investigations carried out directly on neural substrates. Its acceptation from a physiological point of view is made difficult by the fact that it relates the formation of the conditional connection to a general decrease of excitation, which is seemingly contradicted by all the known physiological evidences. Be it said in its excuse, the hypothesis does not state anything about the concrete neural events by which drive reduction might operate.

In the firm conviction that without a better knowledge of drive mechanisms neither the nature of the conditional connections nor the electrophysiological events accompanying conditioning can be understood, it has been decided to re-investigate the question from an aspect different from the conventional one.

The experiments to be reported have been made on cats provided with implanted stimulating electrodes. The investigations were confined chiefly to the diencephalon and mesencephalon, as the best representative regions of the main drives.

Several ways are available for studying the neural mechanisms of motivation. The most wide-spread method is what we may call *instrumental stimulation technique*. One of its versions is when the stimulation circuit is closed by the animal itself pressing a pedal placed in the experimental situation. In the second variety, technically similar to the first one, the stimulation started by the experimenter is interrupted by the animal pressing a pedal. The first variety is suited to show and localize effects with rewarding, the second one with punishing characteristics (OLDS 1963). The two methods may be applied in combination (ROBERTS 1958). One of the main advantages of the instrumental stimulation technique is that the recording of pedal manipulation offers excellent possibilities of quantitative analysis. Its drawback is that the interpretation of data requires the implication of psychological or often subjective notions (reward, punishment, pleasure, *etc.)*

The essence of the method used by us is that stimulations are applied in the background of pre-established conditional reflexes. Stimulations can be applied in several varieties (during different phases of the execution of conditional reflexes, during different stages of elaboration of conditioning, during the application of inhibitory conditional stimuli, *etc.)* These circumstances ensure that the effects of stimulations can be controlled by well-known be-

havioural manifestations. The recognition and separation of indifferent or inhibitory stimulation effects, moreover the analysis of post-stimulation effects (rebound, periodic excitatory and inhibitory processes) is also rendered possible.

The study of these manifestations with the instrumental technique is limited or even impossible. While — as we shall see later — the evaluation of these very manifestations may be indispensable for the understanding of some basic properties of motivational mechanisms.

With the help of our method, in a former series of experiments (GRASTYÁN, LISSÁK, KÉKESI 1956) the following, apparently basic statements were made. Stimulation of certain points of the hypothalamus as well as the mesencephalic reticular formation can elicit in itself — *i.e.* in the absence of the conditional stimulus — conditional reflexes elaborated in the experimental situation. This effect has been termed (and will be termed below) an activation of the conditional reflex. Similar observations have been made by several other authors (LAGUTINA and ROZHANSKI 1949; DELGADO, ROBERTS and MILLER, 1954; COHEN, BROWN and BROWN 1957; NIELSON, DOTY and RUTLEDGE 1958; WYRWICKA, DOBRZECKA and TARNECKI 1959).

A second statement was that the effects activating the alimentary (approach) and avoidance reflexes are organized in a reciprocal antagonistic manner. This latter statement was considered important and at the same time plausible because it would have proved that basic principles of reflex-organization of the spinal cord can manifest themselves in a level representing more complex functions. The validity of the statement was, however, made uncertain by a methodological error, the importance of which became obvious in the course of our later studies only. In the first series of experiments, the effects of stimulations were mostly checked with the two conditional reflexes not simultaneously but in succession. This involved the risk of the establishment of a stable connection between stimulations and the first used conditional reflex, with the possible consequence of a reciprocal influence in the case of the conditional reflex investigated for the second time.

The simultaneous preservation and establishment of both reflexes was made impossible at that time by the fact that the avoidance reaction elaborated with painful electric shocks always depressed the approach reaction. This difficulty was overcome in the present series of experiments by replacing the electric shock with cold water (inundation of the bottom of the experimental cage) *(Fig. 1)* (CZOPF, KARMOS, BAUER, GRASTYÁN 1963).

One of the main problems of the stimulation technique used by us consists in the difficult recording and quantitative evaluation of complex and often not stereotyped conditional locomotor reactions. A satisfactory solution was then found with the introduction of a simple optical recording method. The experiments were carried out in red background illumination and movements of the animal were recorded by photographing the light of a small electric

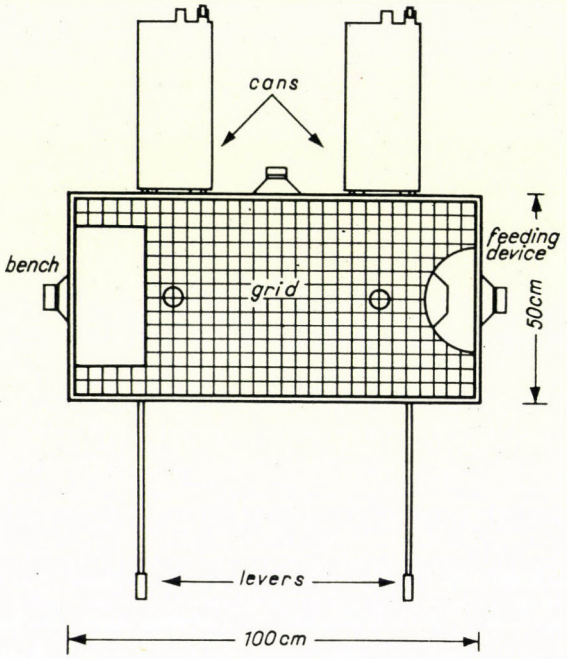

Fig. 1. Schematic representation (view from above) of the apparatus used for the simultaneous elaboration of alimentary and avoidance reflexes. Further details see in text

bulb attached to the head of the animal with a camera fixed to the top of the cage. This method proved useful in recognizing basic movement patterns *(Figs 2, 3, 4, 6, 7)*.

Characteristics of the activating effect

The most important and at the same time most surprising observation made in the present experiments with simultaneously elaborated approach and avoidance reflexes was that on stimulation with appropriate parameters one and the same point could activate both conditional reflexes *(Figs 2, 3, 4)*. This bidirectional activating effect could be produced (with a careful selection of stimulation parameters) by stimulating most points of the regions investigated (hypothalamus, thalamus, mesencephalic reticular formation). Exceptions were structures *(e.g.* n. reuniens, mamillary region) the stimulation of which inhibited both reflexes at any parameters of stimulation *(Fig. 7)*.

On the basis of these observations the important question arose, what factors are determining which of the two reflexes will eventually be activated. Principally, the following three factors should be considered: (i) localization of stimulation; (ii) parameters of stimulation; (iii) stimuli of the environment acting on the animal at the moment of stimulation.

A thorough analysis of the activating effect revealed that, depending on various circumstances, any of the three factors may be determining.

It was established that in cases when stimulation with identical parameters resulted in an alternate activation of both reflexes, the direction of the effects was determined by the actual stimuli coming from the environment. More concretely, that reflex has been elicited with the stimulation the conditional signals (sight of feeding device or flight place) of which acted on the

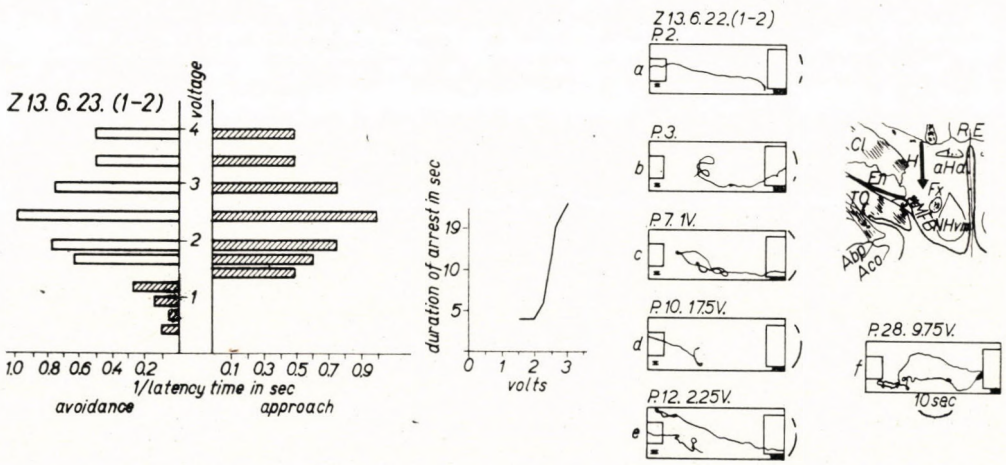

Fig. 2. Effects elicited in the double conditioning apparatus by stimulating the lateral hypothalamic area. Localization of the electrode can be seen in the extreme right side of the figure. The diagram on the left side demonstrates the effects elicited with different voltages (ordinate). Columns show the speed (reciprocal of latency time) of activation in seconds (abscissa). Right side approach, left side avoidance, reactions. Columns are shaded when the activation process was elicited in a position indifferent in relation to the goal places

In the middle of the *figure*, duration of the arrest reactions (ordinate) following the activation process is shown, at different voltages (abscissa). In the right side of the *figure* recordings of conditional reactions as well as activations produced by electrical stimulations are shown. Recordings were obtained by photographing the movements of a small lamp attached to the head of the animal. *a*) Alimentary conditional reaction; *b*) avoidance reaction; *c*) activation of avoidance reaction; *d*) activation of alimentary reaction; *e*) periodic alternation of activation and arrest reactions elicited with a single high intensity stimulation; *f*) stimulation with a voltage around the threshold elicited only diffuse orienting, searching movements

animal at the moment of stimulation. Moreover, it was also established that stimulation parameters able to activate equipotentially both reflexes could be found by stimulating any of the points showing motivational effects. These, so-to-say "optimal" parameters, were, however, characteristically different when stimulating different regions of the investigated structures.

In the course of a systematic investigation of stimulation parameters it was observed that in the case of the hypothalamus and thalamus (aspecific thalamic system) particularly with altering the voltage of stimulation, while in the case of the mesencephalic reticular formation stimulation at different fre-

quencies characteristic differences could be shown in the activation of the two reflexes.

In *Figs 2, 3, 4* data obtained with stimulations in three different regions of the hypothalamus are shown. Stimulation with threshold voltages resulted in every case in an activation of the avoidance reflex. By stimulating with suprathreshold values, different peculiarities were found in the three different regions.

By stimulating in the lateral hypothalamic area (the most effective self-stimulation region in OLDS' (1963) experiments) it was found that every stimu-

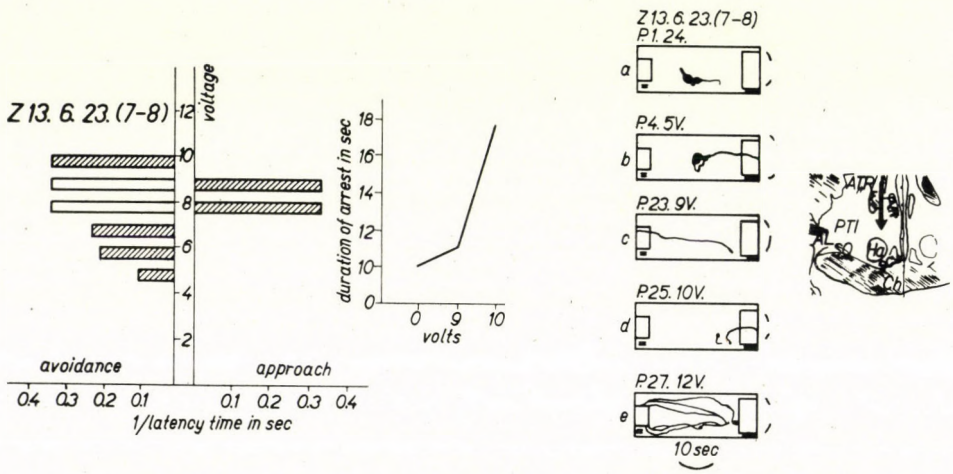

Fig. 3. Effects elicited by stimulating the anterior medial region of the hypothalamus. Construction of the *figure* is identical with that of *Fig. 2*

a) Stimulation with threshold voltage elicited only orientation; *b)* activation of the avoidance reflex; *c)* activation of the alimentary reaction; *d)* higher voltages activated exclusively the avoidance reflex; *e)* uninterrupted strong stimulation resulted in the disappearance of the activating process

lation parameter over threshold can equipotentially activate both the approach and the avoidance reflexes *(Fig. 2)*.

Stimulations in the anterior and medial regions of the hypothalamus with medium intensity, suprathreshold voltages produced equipotential activation. Higher intensities than these activated the avoidance reflex in a dominant manner *(Fig. 3)*.

On stimulation of the medial hypothalamic regions at the level of the hypothalamic ventromedial nuclei under the usual conditions, all voltages activated only the avoidance reflex. If, however, at the onset of stimulation the conditional sound stimulus of the approach reaction was applied for a short time (which in itself was inadequate to elicit the reflex) then by stimulating with threshold value voltages the approach reaction could be activated *(Fig. 4)*.

In the case of the mesencephalic reticular formation, activation of the approach (alimentary) reaction could in some cases only be produced by stimulating at lower frequencies (4—10 c/s) or with short trains of higher frequencies $\left(\frac{100\ c/s}{4-10/s}\right)$. Continuous higher frequency stimulations (100—300 c/s) activated in most cases only the avoidance reaction.

Summing up, with an appropriate selection of the parameters, stimulation of any region showing motivational properties can activate both avoidance and approach reactions. From this it logically follows that similar or identical basic

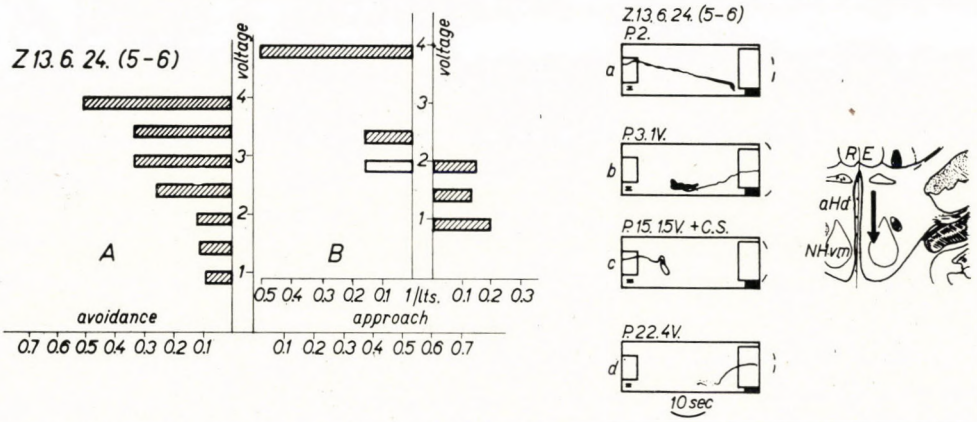

Fig. 4. Effects elicited by stimulating the medial hypothalamic region at the level of the ventromedial hypothalamic nuclei

Left side of *Fig. A :* Stimulations carried out under the usual conditions activated only avoidance reactions at any voltage. *B :* If stimulations were preceded by a short application of the alimentary signal, activation of the alimentary reaction could be produced with voltages around the threshold. *a)* Alimentary conditional reaction; *b)* activation of the avoidance reaction; *c)* activation of the alimentary reaction; *d)* higher voltages activated only the avoidance reaction

mechanisms lay behind the organization of both reactions, despite their seemingly antagonistic character. Moreover, the fact that stimulation with appropriate parameters of certain regions is more favourable for the activation of the avoidance reflex, shows that there must be at least two basic mechanisms behind the activating process and the importance of one of these mechanisms is greater in the avoidance than in the approach reaction. The existence of the supposed basic mechanisms finally became obvious in the course of an observation. Namely it was found that the activating effects could only be maintained in a repeatable fashion if stimulation was interrupted at the moment when the animal had reached the goal of the conditional reflex (feeding device, flight place).

If stimulation was continued, then the animal retreated during the stimulation from the goal and during subsequent stimulations consistently avoided

this object *(Fig. 2 c)*. In several cases the corresponding conditional reflex was simultaneously destroyed. The extinctive property of persisting stimulations could be shown concerning the activation of both reflexes. A difference in the case of the avoidance reflex was found in the higher number of stimulations required for extinction.

The essence of this phenomenon seems to consist in the fact that an effect having originally an approaching character is transformed during continuous stimulation into its opposite, namely into an effect having an avoidance character. Considering that two antagonistic motor tendencies could be attached to different regions of the hypothalamus also with the instrumental stimulation technique (OLDS and OLDS 1963) there remained no doubt of there being a close correlation between these approach and avoidance tendencies and those observed by us. In order to avoid any confusion, the approach and avoidance tendencies have been termed pull and push effects, respectively. This distinction seemed necessary considering that the terms approach and avoidance were conventionally used to denote special conditional reactions, while the postulated two basic effects are inherent components of both of these conditional reflexes. By the term pull the intention was to denote a behavioural form during which the animal comes close to, by push when it moves away from, a stimulus or object acting on it.

The knowledge of these two elementary mechanisms of motivation makes it easy to interprete the above-described facts. It can be established in the case of both reflexes that the final phase of their execution should be built on pull effects. Since before approaching a goal the animal moves away from certain objects *(i.e.* in the case of the avoidance reflex from the place of punishment; in that of approach from places where it does not get food), consequently push (avoidance, punishing) tendencies must also be effective in both behavioural patterns. Therefore the conclusion seems reasonable that activation of both reflexes is built upon alternating sequences of simple pull and push effects. The conditions of elaboration of both reflexes show that the importance of push effects must be greater in the avoidance than in the approach reaction. (During avoidance conditioning, the whole situation except the flight place obtains a definite pushing character.) The importance of pull effects is, however, not necessarily less in the avoidance than in the approach reaction.

Thus, the avoidance reflex dominates in our dual conditioning situation, despite of the apparent equilibrium of the two reflexes. From this it follows that conditions for the activation of the avoidance reflex should by all means prevail. This hidden disequilibrium would make it comprehensible why on stimulation at threshold values it is always the avoidance reaction which is activated. On the other hand, if stimulations of a given region predominantly activate the avoidance reflex we can assume that push tendencies must dominate in the effect of stimulations. This makes it probable that in regions from

which the avoidance reaction can be activated with a greater scale of parameters, neural elements representing push effects are located predominantly.

According to this interpretation of the activation effects, the lateral regions of the hypothalamus represent predominantly pull, its medial regions predominantly push, elements. The validity of this interpretation seems to be supported by observations made with the instrumental stimulation technique (OLDS and OLDS 1963).

It must be admitted that the two basic opposite effects were easier to recognize with the instrumental technique. At the same time it must be pointed out that the relationship of the two effects, and especially their significance in natural adaptation processes (conditional reflexes) is not clearly revealed by the instrumental stimulation technique alone.

The experiment in which under uninterrupted stimulations the transition of pull into push effects was observed has clearly shown that the two effects represent inseparably linked mechanisms. By continuous stimulation of any motivational point the pull effect turned into a push effect after a definite period of time. It could also be observed that the speed of this transition was commensurate with the intensity of stimulation, in other words stimulations at higher intensity produced pulling phases of shorter duration. (The transition of the phases characteristically depended on the location of stimulations. This factor will be considered in detail in the following.)

In the above-described experiments it was only the transition of pull into push effect which could be observed in a directly controllable fashion. The question arose whether a push effect, too, may reverse into a pull effect during continuous stimulation. The activation of the conditional reaction indirectly proved the possibility of a transformation in this direction. The final phase of the execution of the conditional reaction (in both approach and avoidance conditioning), as it was shown previously, is built on pull effects. This final pull effect must (owing to the actual arrangement of the experimental situation) necessarily be preceded by one or several push phases. The question by what factors and how the alternation of the two phases is regulated, will be considered later. Before that we should like to point to an important observation of ROBERTS (1958) which support our data concerning the interlinked character of the pull and push mechanisms.

According to ROBERTS, stimulation in the posterior hypothalamus producing the so-called flight reaction (HESS 1954) have a dual behavioural effect. The onset of stimulations proved to be rewarding, the rest punishing. The real existence of both effects could be demonstrated in cats trained to press a bar to turn on the stimulation and to perform a locomotor escape response to turn off the same stimulation. In another variety, animals could be trained to oscillate back and forth between two of the three arms of a symmetrical Y maze to

turn on the stimulation in one arm and turn it off in the other. These observations have been confirmed (BOWER and MILLER 1958) in the rat.

Our own findings obtained in the same region allow to identify ROBERTS' rewarding and punishing effects with our pull and push effects. In addition, ROBERTS' experiment definitely supports our assumption concerning the interconnectedness of the two mechanisms. Such a general conclusion, however, cannot be derived from ROBERTS' results, because his dual effect was found to be a specific effect of a circumscribed region. An alternative interpretation also arises, namely that the dual effect is the consequence of an anatomical overlap of the structures representing the two effects and in this sense a stimulation artifact. This possibility, however, seems to be invalidated by the fact that the sequence of the two effects is consistently the same. If the artifact hypothesis would be valid, then with certain electrode localizations a reversed sequence of the two effects, namely that in which the onset of stimulation is punishing (pushing) and the rest rewarding (pulling), should also be obtained. Such sequences, however, could not be observed in any of the cases. The most convincing denial of the assumption of an overlap is furnished by our observation that the rewarding—punishing sequence is not an exceptional property of the posterior hypothalamic region, but a common feature of any motivational structure. As it has been shown, pull-push sequences could be demonstrated (with characteristically different stimulation parameters) in every region.

From the above facts some general conclusions may be drawn concerning the neuronal organization of the motivational system. The relative anatomical separability of the pull and push effects, moreover the fact that they represent incompatible motor tendencies suggest that the two functions belong to independent neuronal elements. On the other hand, from the fact that the two effects follow each other in a definite sequence — *i.e.* pull precedes push — we must necessarily conclude that a one-way excitatory connection exists between the two systems representing the two effects. According to the sequence, the elements of the push system would be thrown into action through the pull system. If this is true then only the specific drive afferents innervating the motivational system are connected to the pull system. (See the circuit diagram, *Fig. 11).*

As mentioned above, the speed of transition of pull into push effect is commensurate with the intensity of stimulation. This would show that the setting into action of the push system is bound to a critical excitatory level of the pull system. The further analysis of the organization of motivational mechanisms will, however, be easier after we have discussed some additional experimental facts.

On the basis of the above considerations it is evident that the speed of transition of the two effects does not depend on the intensity of stimulation only but also on the location of the stimulation. If a region containing dominantly push elements is stimulated, then a pull effect of very short duration

can be expected, considering that the threshold of excitation in the push system (owing to the direct effect of the stimulation) is decreased at the beginning of stimulation. (In other words the push system is excited not only in the natural way, through the pull system, but also directly.) Consequently, stimulation at threshold intensities in such areas will elicit a reaction which could have been elicited with a strong stimulation in a dominant pull representation. The acceptance of the interconnected nature of the pull-push mechanism would also explain why it is so difficult to find pure approach or avoidance representations even with the instrumental stimulation technique.

By accepting the functional connection of the two basic mechanisms, a more satisfactory explanation offers itself of the fact that approach reactions can be activated from certain regions of the reticular formation by stimulating at lower frequencies. Stimulation of this area dominantly activates the avoidance reflex, consequently it must represent more push than pull elements; according to this the duration of the pull phase will be extremely short. During continuous stimulation the avoidance reaction has therefore a greater chance to come into operation. Stimulating at low frequencies, or with intermittent short trains of higher frequencies, we have, however, a chance to elicit only pull phases (stimulation is interrupted before a push effect could appear) and, in consequence, the signals of the environment will determine which reaction will be activated, *i.e.* the approach reaction can also be activated. This dissimilar frequency affinity of the two mechanisms may offer a solid starting point for the study of the neuroanatomical organization of the drive systems. The fact that low frequency stimulation is more favourable for eliciting pull than push effects suggests that the pull system is oligosynaptic in relation to the push system.

As a further evidence of the integrated nature of pull and push mechanism let us cite some important observations of HUNSPERGER (1956). By studying di- and mesencephalic affective reactions this author found that with stimulations in a peripheral zone of an unbroken field comprising portions of the preoptic area and hypothalamus and the central gray substance of the midbrain, low threshold stimulation elicited flight reactions, whereas low threshold stimulation in the central part evoked defence reactions. Also, — and this is particularly important for the sake of our own argumentation — strong stimulations of portions of the peripheral zone elicited an affective defence reaction instead of flight. Moreover the same reaction (defence) was obtained by weak stimulation of the peripheral zone, when the animal was hindered in its flight. On the basis of our previous experience there is no doubt that the reaction-sequence described by HUNSPERGER is also indentical with our pull-push sequence. It should, however, be emphasized that the term "flight-reaction" is inaccurate and misleading. The fast locomotion appearing in the course of this reaction may at first sight seem to be a real flight. If, however, the reaction is studied

either with the conditional or with the self-stimulation technique, it becomes clear that pull (approach, rewarding) tendencies dominate in the phenomenon.

The importance of environmental signals in the mechanism of drives

It has been mentioned that according to HUNSPERGER (1956) a flight (pull) reaction elicited with threshold stimulation can be transformed into a defence (push) reaction if the animal is hindered in its flight. This observation clearly

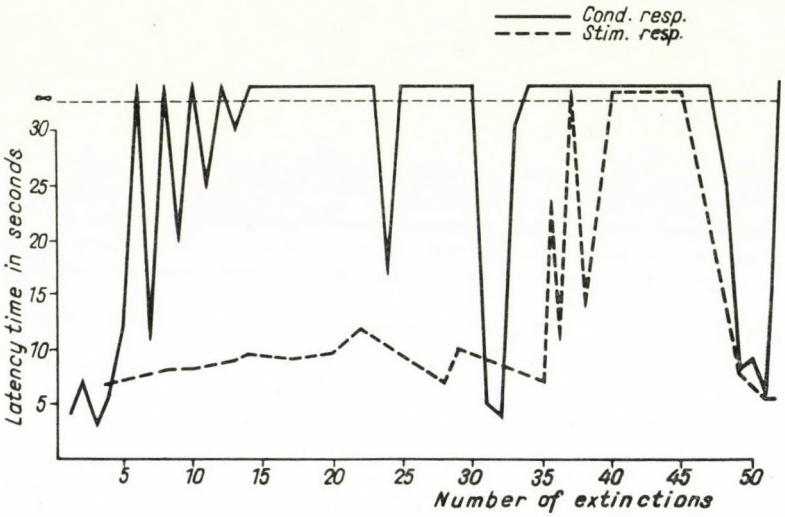

Fig. 5. Effect of the extinction of the conditional reflex on the activating effect induced by hypothalamic stimulation. Heavy line: conditional reaction; broken line: activation. Ordinate: latency time of reactions; abscissa: number of trials. In a definite period during extinction the activating effects begin to oscillate parallel with the conditional reactions

shows that the transition of pull into push effect can be influenced also by external, environmental factors.

A definite evidence of the determining influence of the environment has been obtained also in our own experiments by studying the effects of extinction. It was established that extinction of the conditional reflexes goes together with the disappearance of the activating effect of stimulation *(Fig. 5)*. Locomotion, of course, has been elicited by the stimulations also after the completion of extinction (diffuse orienting, searching movements) but the specific conditional reactions disappeared. It could be established that during locomotion, elicited by the stimulation, the goal of the extinguished reflex has been definitely avoided by the animal. It is consequently reasonable to assume that during extinction connections of a pushing character developed to that place (or signal).

Essentially the same happened in this latter case as formerly when the conditional reaction was extinguished with uninterrupted stimulations (at the

goal). During extinction of the conditional reaction the activating process, during extinction of the activation the conditioned reaction was destroyed. This close interdependence of the two processes convincingly demonstrates that natural drive processes and those elicited by electrical stimulations have a common neuronal substrate.

Both kinds of extinction clearly show that external cues originally having a pulling character turn into push signals if during their actior motivational excitement does not decrease in due time. Accepting this, it is easy to conceive how a given stimulus can obtain a pull conditional character. On the basis of the above considerations the formation of a pull conditional connection is expected if in the presence of a given stimulus the actual motivational excitement decreases or disappears in due time, before a push effect would appear. This assumption would make comprehensible the seemingly contradictory observation that activation of an alimentary conditional reflex (approach reaction) can be maintained without reinforcing the reaction with food. A plausible explanation is that interruption of the stimulation in itself has a reinforcing property. (The same holds also in the case of the avoidance reflex.)

Summarizing the above-mentioned conclusions, it may be stated that the pull effect has a powerful capacity to form conditional connections, corresponding to its nature with the external stimuli in the presence of which it acts. The same statement should of course be valid for the push effect. From the conditions of formation of the pull conditional connection it necessarily follows that a push connection is formed if in the presence of a given external stimulus the motivational excitement persists and the pull effect is transformed into a push effect. With this assumption, however, the problem is not closed. As it has been pointed out, before the appearance of the push phase the conditions for the formation of a pull connection have been given. If after transition of pull into push the final result is a conditional push connection, one has to conclude that the preceding pull connection must somehow be destroyed. Discussion of the possible mechanism of this process must be postponed until having completed our knowledge with some further important observations.

On the basis of the aforesaid facts it is easy to conceive that in a well-known (conditional) environment the direction (pull or push) of a motivational excitement is determined by external stimuli. This is expressed in the activation process of conditional reflexes elicited with direct electric stimulation of the neuronal substrate of drives. (From a neurophysiological point of view, behind the activation effect there is probably a facilitatory process. Stimuli coming from the environment facilitate the function of the motor system driven also by impulses of the motivational system. According to an alternative interpretation of activation, electric stimulation as a strong disturbing factor might simply disinhibit the conditional reflexes. Disinhibition cannot be disregarded but its basic importance in the activation process is excluded by two

facts. First, if the essence of activation would consist in disinhibition, then activation could be elicited only in a restricted number of repetitions, as it must have been extinguished during repetition. In reality it can be elicited unrestrictedly. Second, as already mentioned, activation disappears after extinction of the conditional reaction. If activation would consist in disinhibition, then it should not disappear after extinction.)

Let us now see what may happen in a strange situation, *i.e.* in an environment where stimuli have no conditional connections and consequently no cue functions. If in such a situation an excitement is aroused in the motivational system either by electric stimulation, or in a natural way (hunger, thirst, pain, sex, *etc.)* then first a pull effect should appear. As a consequence of this the animal approaches the stimulus which acts on it at that moment. If during approaching the object the driving force does not decrease or cease, then the pull will be transformed into a push effect and a conditional push connection will be formed to this stimulus. By means of the push effect, however, the animal finally escapes this stimulus and arrives under the effect of a new environmental stimulus. At that moment push will again be replaced by a pull effect — by what mechanism it will be discussed later — and if drive does not cease again, a new push effect will appear and a conditional push connection will be formed again. This process continues up to the time when the animal has reached a situation where the drive decreases or disappears. To stimuli of this last situation a conditional pull connection should evidently be formed.

Thus we can establish that in an unfamiliar environment at the first appearance of motivational excitement the alternation of pull and push is determined by the motivational system itself. During this process, however, the environment becomes known and later — by the next appearance of motivational excitement — external stimuli of the situation will determine the alternation of pull and push in an order corresponding to the stimuli acting on the subject. It must be emphasized, however, that a situation the elements of which are all unfamiliar is practically unconceivable. Therefore we must assume that a determining effect of environmental stimuli is always effective up to a certain measure. However, it is certainly in the discussed way that unfamiliar elements of the environment become "known".

Summarizing the above conclusions we may say that the determining character of environmental stimuli develops in the course of a process essentially identical with the so-called trial and error type of conditioning. It follows that the primary motivational effects are aspecific before the formation of conditional connections. As mentioned before, pull and push effects are implicated in both approach and avoidance reflexes. Pull and push effects on the other hand can — with appropriate parameters of stimulation — be elicited from all parts of the motivational system. According to this, the assumption seems plausible that activation of the alimentary conditional reflex is not neces-

sarily a result of stimulation of the so-called alimentary centre (with the classical terminology, the unconditional feeding centre). This conclusion is of importance because the mechanism of activation has already been interpreted by some other authors as a specific mechanism. WYRWICKA *et al.* (1959) on the basis of experiments carried out in the goat concluded that it is the hypothalamic feeding centre the stimulation of which is responsible for the activation of the instrumental alimentary reaction. The fact that the alimentary reflex can be activated by stimulation of regions distant from that of the feeding centre clearly shows that this interpretation is not necessarily correct. A further counter-argument is the above-mentioned observation that activation can be maintained without reinforcing the reaction with food. These arguments do not deny the existence of a feeding centre, but only emphasize that special motivational centres cannot be identified on the basis of stimulation effects. Considering that under certain circumstances the same movements are required for acquisition of food as well as for avoidance of a dangerous situation, it follows that the primary motivational mechanism cannot be specific. In other words, primary drive effects represent preparative or appetitive rather than consummative motor patterns. Consummative actions, as indicated by a wealth of data, are bound partly to higher partly to lower systems of the central nervous system, but they are not independent from the primary motivational systems of the di- and mesencephalon.

In conclusion we may say that the motivational mechanisms involved into feeding can only be localized by way of their (neural and humoral) afferents. This point is worth considering also in localization studies of other special drives. Inferences gained on the basis of stimulations carried out in a conditional reflex situation may be misleading.

The relationship between statements derived from stimulation experiments and the drive reduction hypothesis

In the course of the discussion of the experimental facts several statements have been made the analogy of which with the postulates of the drive reduction hypothesis is quite obvious. The fact this coincidence showed itself quite unintentionally urged us to check the validity of the hypothesis more thoroughly with our own method. This in fact meant the answering the question, by what particular mechanism the reduction of drive contributed to the formation of a pulling conditional connection.

Above it has been stated that the basic condition of preserving the activation effect (which regarding the final process is a pull effect in both reflexes) consisted in the interruption of stimulation when the animal has attained the goal. Since on the basis of our former reasoning it seemed doubtless that motivational effects elicited by electrical stimulation represent mecha-

nisms essentially identical with those of natural motivational effects, we feel justified in saying that the basic condition of the formation of a conditional pull connection is the sudden decrease of motivational excitement. This statement is essentially identical with that of the drive reduction theory. Another formulation of it would be that interruption of stimulation or reduction of a natural drive has a reinforcing property. This formulation is particularly advantageous for the comprehension of the mechanism of the avoidance reflex.

In the case of the avoidance reflex it is namely still a matter of dispute whether reinforcement is represented by punishment or the escaping from it. The drive reduction theory naturally assumes the latter view, because avoiding of an unpleasant event goes together with a reduction of drive. By means of this interpretation of reinforcement, the drive reduction theory ensures a common ground concerning the basic mechanisms of both the approach and the avoidance reflexes. Our own experiments support also in this respect the drive reduction theory. We could definitely show that interruption of stimulation plays the role of reinforcement in the case of both reflexes.

Let us now see what may be the closer nature of the mechanism by which drive reduction contributes to the formation of a pulling conditional connection. To approach this question, the following simple experiments have been performed.

The experimental animal (cat) was placed into a sound-proof box of a bottom size of 100 cm by 100 cm. Electric stimulation was consistently turned on when the subject occupied a preselected point in the cage, and turned off when by means of diffuse orienting, searching movements elicited by the stimulation the animal had reached another preselected and conspicuously marked region. The purpose of these experiments was to check whether or not stimulation of a motivational structure in itself might produce conditional manifestations. The conditions of our experiments were similar to those of ROBERTS (1958) except that in the latter case stimulation was turned on by a pedal manipulated by the animal itself.

In the case of the validity of the drive reduction hypothesis it could be expected that after several repetitions a conditional connection would be formed to the point of interruption of stimulation. It was thought that, as a consequence of the training, stimulations applied in any points of the experimental situation would result in approaching this "goal", and, also that the animal would stay in this area for prolonged periods.

This prediction was partially proved by the experiments. In the course of the first stimulating complex searching movements appeared. Later they became more and more simple, while finally reactions were elicited passing off in the shortest way in between the start and goal places. It could also be observed that simultaneously with the appearance of this fast final reaction the subject began to cling more and more to the goal place *(Fig. 6)*.

In the knowledge of the activating effects we were not surprised at the further observation that stimulations of different points than those used in the experiment produced at first the same effect.

We could definitely establish that interruption of a stimulation of motivational character has really the property to form a positive conditional connection. This would have been a convincing evidence of the drive reduction hypothesis had at the same time a contradictory observation not arisen. The formation of a conditional connection to the start place was namely also observed. This conditional connection was not as stable as that attached to the goal place, its conditional character was, however, unquestionably proved by the following facts. The start place was often looked for by the animal spontaneously, and it was staying there for longer periods. On the other hand, if stimulation was applied when the animal occupied a different place then the reaction was performed not in the shortest way between the actual place of stimulation and the goal, but always through the original start place.

The fact that not only turning off but also turning on of stimulation proved able to organize conditional connections seemed definitely to contradict the drive reduction theory, or at least to restrict its general applicability. This contradiction might be resolved by assuming that not only the last movement, but a whole series of movements preceding drive reduction are involved into the formation of a conditional action. The reaction established to the start point of stimulation could be regarded as the first member of a complex chain of conditional events, forming a stereotyped reaction. It is well known that such or even more complex stereotyped reactions can easily be elaborated under natural conditions. From a teleological point of view the conditional connection formed to the beginning of stimulation would ensure a safe starting point for adaptation in this situation. This supposition, however, does not explain the closer mechanism of the conditional connection formed to the start point, neither can it be taken for granted that it is the mechanism of drive reduction which is really responsible for the formation of the whole stereotype. The consideration that at the beginning of stimulation neuronal excitation is relatively weak and later it increases progressively would rather favour the view proposed by HEBB (1955) according to which "at low levels an increase of drive intensity may be rewarding . . ." (reinforcing). This view, which in its essence emphasizes that an increase of excitation is the basic condition of the formation of a new connection in good agreement with all the physiological and morphological evidences known so far (ECCLES 1961; SZENTÁGOTHAI and RAJKOVITS 1955).

The question, however, arises why we need drive reduction in the case of strong motivational excitement, where this basic condition of connection formation (simultaneous existence of excitatory processes) is better ensured than in the case of a weak motivational excitement. The answer to this question is easy on the basis of the relationship of the pull and push effects. From the consistent

order of sequence of the two events we have concluded that the push system is thrown into action through the function of the pull system, if the intensity of excitement has reached a critical level in the latter. Moreover, it could also be established that by the appearance of the push effect the stabilization of a preceding conditional pull effect was upset. According to this, the importance of drive reduction would consist in preventing the appearance of a pushing phase during the approach of a given object. In this indirect way drive reduction would ensure the conditions of the formation of a conditional pull reaction to objects or stimuli actually present.

Thus, in this conception drive reduction is an important although passive mechanism in the formation of a conditional connection. In the case of a weak drive it has evidently no importance, as in this case pull is not transformed into push effect. Consequently, the conclusion seems to be correct that the factor always responsible for the formation of conditional connections is an increased excitement. From this point of view start and stop of stimulation have a common significance. The general biological importance of the formed connections would be a protection against all kinds of disturbing agents, in other words the safeguarding of a relatively stable resting state.

On the basis of this seemingly final conclusion — which is in agreement with the general principles of biological adaptation — the problem of drive reduction in the formation of conditional connections appears to be settled. As we shall see later, however, this is not true. Our conclusion namely according to which the role in connection formation of drive reduction is only a passive one, is not perfectly correct. To prove this, we need to discuss a problem which at first seems to be independent of the question of drive reduction.

The possible inner organization of the pull-push system

As stated above, in the case if a pull effect is followed by a push effect, then despite the fact that the conditions of the formation of a conditional pull connection are given (by the simultaneous existence of motivational excitement and the impulses of environmental stimuli), no pull but only a push connection will be formed. From this experimental fact we must assume that the push system reacts on the pull system in an inhibitory manner. The way by which this retroactive inhibition may hinder the manifestation of the pulling conditional connection can only be determined by the points of attack of inhibition. For this purpose first the points where possibilities for the formation of positive connections are given should be taken into account.

Considering that, in dependence on the actual environmental factors, both pull and push effects activate a great variety of motor patterns and not single specific movements, we must assume that the motivational mechanisms operate

on complex motor systems, which are structurally independent from the — we may say — primary drive systems. If, according to our former statement the condition of the formation of a durable new connection is the lasting coexistence of excitatory processes, then principally there are at least two points where the formation of conditional connections may take place.

Conditional connections can be formed on the one hand between the impulses produced by the environment and the system producing the primary motivational effects, and on the other between these and the impulses of the motor system. The consequence of the realization of the first connection would consist in inducing an increase of motivational excitement by previously indifferent environmental stimuli. The consequence of the realization of a conditional connection between the primary drive system, environmental stimuli and the motor system would result in that previously indifferent stimuli would produce definite and appropriate motor actions. The establishment of an instrumental conditional reaction would principally correspond to this second coupling mechanism.

According to these possibilities the reactive inhibition of the push system manifests itself partly in the pull section of the motivational system itself, partly in a point where motivational and environmental stimuli reach the motor elements. Let us consider in both cases the possible consequences of these inhibitory functions.

If inhibition is effective on the pull system, it may principally result in a cessation of the push effect itself, because it may decrease excitement in the pull system below the critical level necessary to excite the push system. Consequently this feed-back-like inhibition may be responsible for the transition into pull of a push effect. This process can well be observed during actions elicited by stimulating motivational structures, but previously we could not find a satisfactory explanation of its mechanism. The assumption of an inhibitory process acting directly on the pull system thus seems necessary also on the basis of this requirement. But at the same time it should also be admitted that this form of inhibition cannot be effective in the prevention of the formation of the unnecessary pulling conditional connection, because it lags behind the excitatory process to be connected. (Pull effect always precedes push.)

Let us now see what will be the consequence if inhibition acts in a point where motivational as well as environmental impulses reach the motor elements. As it has been deduced formerly, this possibility seems to meet the requirements of the realization of an instrumental conditional refiex. But there is still some incongruence concerning the simultaneous appearance of excitatory and inhibitory processes. At the moment namely when the pushing effect appears, the impulses of the pull system have already reached the motor elements, thus the conditions of conditional coupling are already present. The fact that in spite of this incongruence the push effect impedes the formation of

the pull connection, necessarily proves that the formation of the conditional connection depends not only on a simple coincidence but also on a lasting simultaneous persistence of the excitatory processes.

The retroactive inhibition of the push system probably acts on this persisting excitation. With this assumption, however, new difficulties arise, especially concerning the mechanism of drive reduction. It is evident that it is at the moment of drive reduction that the conditions for the persistence of the excitatory impulses are ensured in the slightest degree. As a consequence it is difficult to conceive how a conditional connection can be formed. The contradiction is obvious since our own experiments yielded convincing evidence that the most advantageous conditions of the formation of a conditional connection are given in the very moment of drive reduction (interruption of stimulation).

This contradiction can only be resolved by assuming that the motivational system is able to set into action a further system capable of delaying reverberating impulses until the primary motivational excitement has already ceased. It is known that the adequate morphological basis of such a reverberating function is given in almost any structure of the central nervous system, but especially in cortical formations. It was partly this consideration which has led us to include the hippocampus in the circuit diagram *(Fig. 11)*.

By accepting the reverberatory function the conditions of conditional coupling have been ensured only on the side of the sensory processes. Still, the formation of a special conditional motor act evidently requires a simultaneous persistence of the motor act to be connected. This condition is ensured, as we shall see later, by the arrest reaction which regularly accompanies drive reduction. Before analysing this factor let us return to some other facts proving the existence of the reactive inhibitory function of the push system.

In self-stimulation experiments, — which will be discussed in detail later,— it was observed that interruption of stimulation during a strong push effect may be followed by a marked rebound-like pull effect, composed of reciprocal movements of the push effect. Had the pull system not been inhibited by the push system, this marked rebound-like pull effect could not have appeared.

The most convincing evidence of the existence of a reactive inhibitory function of the push system has been presented by OLDS (1962), namely that stimulation of points in the mesencephalic tegmentum producing effects of aversive character inhibited the effects of hypothalamic rewarding points. On the other hand, stimulation of the latter facilitated the effect of the mesencephalic aversive points. Both facts are in perfect agreement with our own findings, only to this the statement concerning the relationship of the two (pull and push) systems should so be completed that there be an unidirected facilitatory and inhibitory connection between the pull and push systems. The push system is set into action through the pull system, and the latter is inhibited by the former.

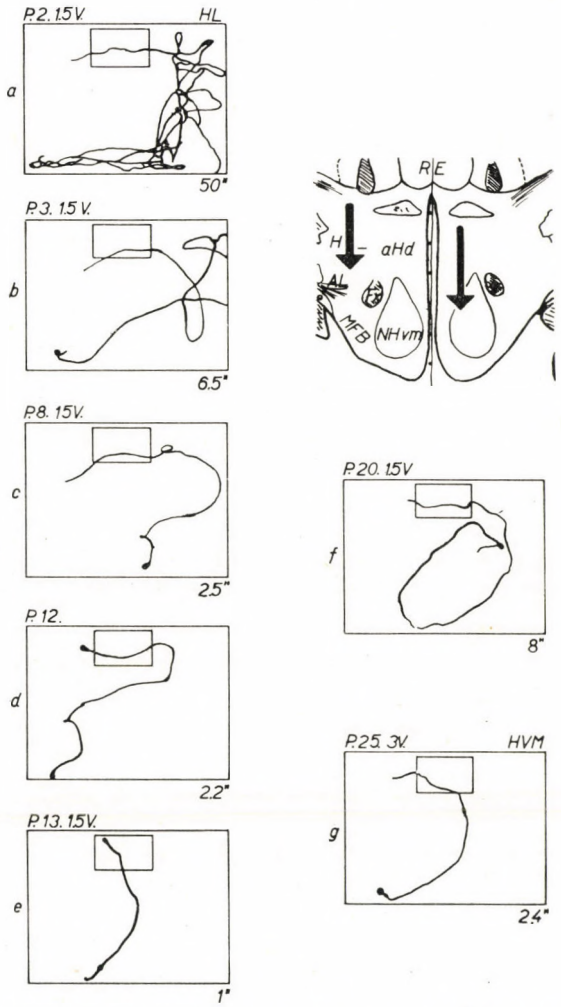

Fig. 6. Conditional reactions produced to the turning on and off of hypothalamic stimulation. Stimulations were consistently turned on when the animal resided in the left lower corner of the apparatus, and turned off when the animal in the course of searching movements produced by the stimulation had reached the region marked by the rectangle. *a)* Diffuse searching movements elicited by the first stimulation; *b—d)* the approach of the marked region becomes progressively simpler; *e)* after about 10 repetitions of stimulation a fast, simple reaction is accomplished in the shortest way between start and goal; *f)* if stimulation is turned on with the cat staying in an unusual place, the goal is approached through the start place; *g)* if a point not used in the preceding procedure is stimulated, the same stable conditional reaction appears as in the former cases

Some additional considerations, however, are necessary in view of a further contradictory finding concerning the inhibitory effect of the push system. In the above-mentioned instrumental stimulation experiments *(Fig. 6)* a conditional connection of a pulling character developed at onset of stimulation, in spite of the fact that the initial pull effect has been transformed into

push effect during stimulation. This was in contradiction with the statement according to which the appearance of a push effect impedes the formation of a pulling connection. The contradiction seems to be resolved by assuming that the neuronal excitement induced by stimulation is at first relatively weak and it increases progressively during stimulation. From this it follows that in the first period of stimulation the pulling effect may reach the critical minimal duration necessary for the formation of the conditional connection. In the course of a relatively weak motivational excitement the push effect probably also remains below its possible maximum intensity. If we assume that the appearance of the inhibitory function of the push system — like the transition of pull into push — is bound to a critical level of excitement of the push system, then it can be imagined that a weak push effect does not destroy the preceding pull connection. Thus, principally, there are two possibilities by which external stimuli acting at the beginning of stimulation might assume a dual (pull-push) conditional property. Such ambivalent connections would offer an acceptable interpretation of the fact that the conditional connection formed to the interruption of stimulation is more stable than that formed to the start of stimulation. In the case of drive reduction, the connection has naturally exclusive pull character.

According to our opinion, this interpretation is valid also in the case of the paradoxical finding by ROBERTS (1958), that stimulation in the posterior hypothalamus motivated prompt learning of escape responses but did not induce learning of avoidance responses. According to ROBERTS' interpretation, the rewarding onset of stimulation interfered with the elaboration of the avoidance reaction. This assumption—provided the rewarding effect can be identified with our pull effect — does not principally differ from our own interpretation.

Besides, our interpretation seems to be more advantageous because it omits the use of notions reflecting subjective factors, like reward or punishment, and it also seems to offer a more exhaustive interpretation of that experiment of ROBERTS in which the rewarding and punishing phases of stimulation were identified by using two separate pedals. According to ROBERTS the approach and pressing of the first pedal would be motivated by rewarding (pull), the second one by punishing (push), effects. According to our interpretation a pull effect should operate in the approach and manipulation of both pedals. A push effect must be effective in the moment when the animal is leaving the first pedal. This push effect, by means of the above discussed negative feed-back mechanism, turns again into a pull effect when the animal arrives into the action-sphere of the second pedal.

In seeming contradiction with this consideration, in a case of a typical flight reaction (first phase rewarding) we succeeded in elaborating an avoidance response to the stimulation. The contradiction is only apparent because a care-

ful analysis of the animal's conduct supported our former reasoning. The responses elicited with the conditional sound stimulus remained labile even after a high number of associations. On the other hand, the peculiar and unusual manner of the reaction's execution (hesitating, creeping motions) suggested that the conditional stimulus had been connected to conflicting motivational tendencies.

In a sharp contrast to this after a limited number of stimulations a stable and fast conditional reflex was established to natural stimuli of the flight place. Finally, the interesting picture could be observed that the conditional sound stimulus elicited long latency, unstable responses, and at the same time fast, stable spontaneous responses appeared in the intersignal periods.

When interpreting these manifestations, the following should be emphasized. The conditional stimulus enters into contact with both pull and push effects if it precedes or covers the application of the electric stimulation, because the sound is relatively independent from the place of reinforcement. That is the reason why this connection always remains unstable. In contrast, natural stimuli attached directly with the reinforcing place enter into contact exclusively with unambiguous (pull) motivational effects, therefore the connection developing to them will be stable.

The importance of the arrest reaction in the mechanism of drive reduction

Let us now return to the heart of the problem of drive reduction. Formerly the formation of a pulling conditional connection was thought to be ensured by the drive reduction by preventing the appearance of a push effect. It was also shown that the push effect by way of an inhibitory mechanism (inhibiting the delayed reverberating impulses induced by the pull system) prevented the establishment of a pulling conditional connection.

The role of drive reduction remains, however, also with these additional facts a passive one. When analysing the inhibitory component of the push mechanism we argued that in order to connect motivational and environmental stimuli with a given movement pattern, all the impulses to be connected must simultaneously persist for a definite period of time. As far as the movement to be connected is considered, we may also say that it is the last movement, elicited by stimulation, and just preceding drive reduction, which must persist throughout the postulated critical period of time. The question is whether such a manifestation can really be observed and if so, by what mechanism is it organized.

A phenomenon corresponding to this criterion has actually been observed in our stimulation experiments. Its role has, however, only been suspected when its existence must have been postulated in the course of the above-mentioned considerations. By analysing the activating effects of stimulations it was consist-

ently observed that at the termination of stimulation the animal remains frozen in its momentary posture, for a surprisingly long period. This even occurred in cases when, in view of the relatively great energy of locomotion, a continuation of movements could have been expected. The reasoning that an active inhibitory process might be responsible for this peculiar braking effect seemed to be sound.

In the basis of its overt manifestations the phenomenon could be identified with the so-called "arrest" reaction. Its relatedness to stimulation was

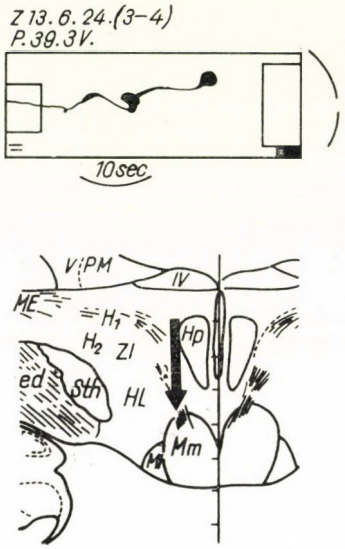

Fig. 7. Stimulation carried out in the supramamillary region inhibited the execution of the approach reaction. (It also inhibited the avoidance reaction.) The inhibitory effect is represented by the large black point in the middle of the recording line. After termination of stimulation the conditional reaction has been accomplished

proved partly by its rebound character, partly by the dependence of its duration on the intensity of stimulation *(Figs 2, 3)*.

The arrest reaction, as a direct product of stimulation of certain thalamic regions, was first described by HUNTER and JASPER (1949). Such a direct (not rebound) arrest reaction was elicited in our own experiments by stimulating the n. reuniens in the thalamus, and the mamillary region in the hypothalamus *(Fig. 7)*. With regard to the neuronal organization of motivation and arrest, it seems important to mention that in several cases a definite reciprocal relationship was found between structures producing the arrest reaction as a direct and as an after-effect. Stimulations producing marked motivational effects were regularly followed by rebound arrest reactions, while stimulations producing marked arrest reactions were often followed by rebound motivational excitement. This reciprocity quite clearly demonstrates the existence of a

mutual inhibitory connection between the structures representing these two different effects *(Fig. 11)*.

Returning now to the problem of the conditional reflex, we can say that at the moment of drive reduction a mechanism able to maintain the last motor pattern produced by the actual drive is started as a release phenomenon. By this mechanism a possibility is offered for the movement having the closest relation to the reduction of drive to be connected to actual environmental stimuli.

The function of this conditional connection will be that certain environmental stimuli will induce not only general motivational excitement but also the specific motor act which definitely leads to a reduction of drive. Thus, by means of the mechanism of drive reduction the selection of peculiar motor patterns, not existing formerly as independent actions, can be envisaged. Moreover, a multiple coupling of different selected patterns may result in perfectly new configurations of movements.

Our earlier statement that drive reduction has an importance only in the case of strong drives still remains valid. The significance of the mechanism is, however, increased by the recognition that by means of drive reduction such special combinations of movements can be attached to external stimuli which did not belong to the original movement repertory of the animals. In the case of weak motivation, which principally does not constrain the animal to execute phasic movements, such new elements do not play a role in accommodation.

The general importance of the conditional reflex in adaptation has often been questioned on the basis that, in contrast to habits formed under natural conditions, on the motor side no new elements are involved into a new conditional connection. The fact that in both classical and instrumental conditioning the conditional stimulus is associated with an existing habit seems to substantiate that objection. The criterion of a new adaptation process would evidently require the formation of new motor patterns, as it really happens in the course of acquisition of new skills. The novelty of a motor pattern must naturally consist of a new sequence or combination of simple motor acts. This is the very point where our conception may offer a reasonable explanation. With the mechanism of the arrest reaction the possibilities for the selection and combination of any movements are given. Remembering that the arrest reaction is a product of drive reduction, the final conclusion that drive reduction is a fundamental mechanism of a complete adaptation (learning) process seems still warranted.

It seems necessary to discuss some additional characteristics of the arrest reaction which are important in its neuronal organization. At first the arrest reaction seems to consist of a homogeneous inhibitory mechanism. Examination of its overt manifestations, however, reveals that this is not the case. The essential characteristic of the arrest reaction is that a definite posture is maintained for a protracted period. (In the case of arrest elicited by direct stimulation the

animal is frozen in the position which it had assumed at the beginning of stimulation; in the case of a rebound reaction, in a posture which it had assumed at the termination of stimulation.) These postures may be highly uncomfortable and their persistence can only be explained by supposing a continuous firing of the original neural impulses producing it. This persisting firing is maintained by the formerly discussed reverberating process. Thus we are now compelled to postulate the existence of a reverberatory function between the motivational and the motor system. At the same time it is also necessary to suppose the presence of an inhibitory mechanism impeding the intervention of other movements during arrest. Thus, we may assume the occurrence of selective inhibitory and excitatory mechanisms behind that particular phenomenon. The neural structures possibly involved in its organization will be considered later *(Fig. 11)*.

Inhibitory effects, periodical manifestations

It was already mentioned that marked arrest reactions can be elicited by stimulating the mamillary region in the hypothalamus and the n. reuniens in the thalamus. These stimulations powerfully inhibited the execution of both alimentary and avoidance reflexes. This two-way inhibition excluded the possibility that push effects have been confounded with inhibition. (In the case of a true inhibitory effect the quality of the effect does not change on applying different parameters.) It was established that stimulations applied at any phase of both conditional reflexes stopped their execution. After the termination of stimulation the acts were completed in an undisturbed manner *(Fig. 7)*.

The advantages of the conditional method became particularly conspicuous when examining the complex after-effects of stimulations. It was possible, *e.g.* to show that, similarly to the direct effects, rebound arrest reactions on the one hand inhibit and, post-stimulatory excitatory reactions on the other, activate conditional reactions.

An additional important phenomenon, reflecting accurately the complex dynamic interrelations of excitatory and inhibitory processes, was observed at stimulation with higher intensities. After the termination of a suprathreshold stimulation activating one of the two reflexes, a self-sustained periodical process composed of progressively shortening arrest and activating processes was launched. The duration of the whole periodic process was roughly commensurate with the intensity of stimulation *(Fig. 8)*.

(Similar periodic alternation of vegetative as well as motor facilitatory and inhibitory phenomena elicited from the hypothalamus in lightly anaesthetized cats have been reported on previously; GRASTYÁN *et al.* 1953).

These findings obviously support the formed assumption of a reciprocal inhibitory relationship between the mechanisms of motivation and of the arrest

reaction. The fact that this conclusion has actually been arrived at on the basis of stimulation after-effects has a particular importance. Namely, considering that the unavoidable artificial effects of a direct stimulation cannot play any

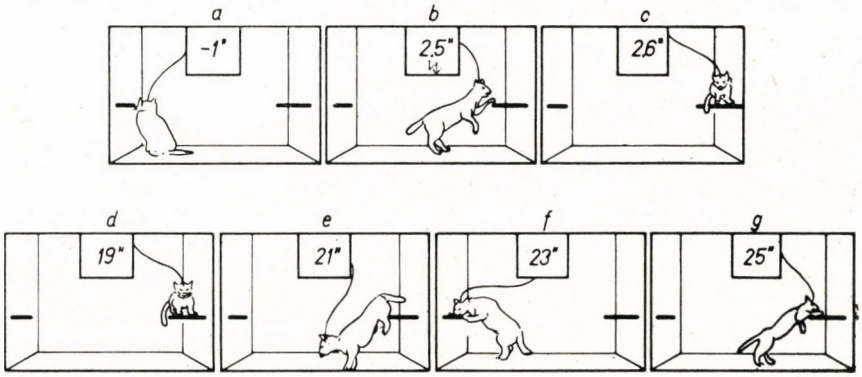

Fig. 8. Periodic alternation of activations and arrest reactions elicited by strong hypothalamic stimulation in the double conditioning situation. *a)* The animal at rest; *b)* activation of avoidance reaction; *c—d)* arrest reaction (15″); *e)* spontaneous approach reaction; *f)* arrest reaction of short duration; *g)* spontaneous avoidance reaction

role in the after-effects, they must be regarded as even more reliable indicators of the physiological connections and organization than the direct effects of stimulation.

A dual, activating and inhibitory effect of the same thalamic locus

In an earlier paper (KOPA, SZABÓ and GRASTYÁN 1962) it was shown that stimulation with identical parameters of the region of the centrum medianum and points in the pretectal region may induce diametrically opposite effects in two psychologically different situations of the avoidance conditional environment. If stimulation was applied at the place of the application of painful electric shocks, an activation of the avoidance reflex resulted. If stimulation of the same point with the same parameters was carried out when the cat was sitting on the place of flight, then in a marked contrast with the former effect a relaxing or even sleep-inducing effect could be observed. This relaxing effect was not preceded by any signs of excitation *(Fig. 9)*.

There are two facts available proving that the effect produced on the flight place is really quieting. First, at the termination of stimulation an arousal reaction as well as orienting reactions of a rebound character were observed. Second, in the course of simultaneous EEG recordings the appearance of slow waves and sleep spindles in the neocortex could be registered. During the activating effect, the appearance of diffuse neocortical desynchronization was recorded *(Fig. 9)*.

The first important conclusion to be drawn from these manifestations is that the effect of stimulation is determined by the actual environment. We have no reason to doubt that in the activating part of the dual effect the same mechanism is involved as that elicited by stimulating other motivational structures (*e.g.* the hypothalamus). The relaxing effect, however, is a perfectly new phenom-

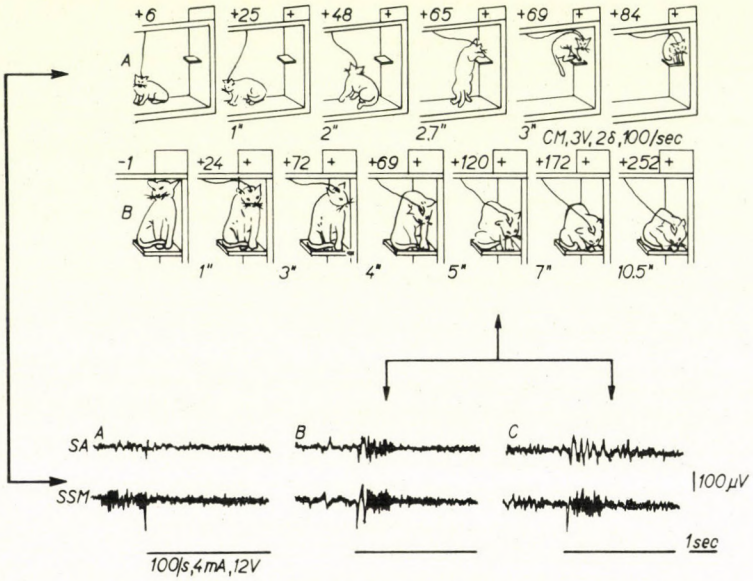

Fig. 9. Stimulations of the region of the central median nucleus or some points of the pretectal region with identical stimulus parameters elicited diametrically opposite effects depending on the situation they had been applied

A : Stimulation applied at a moment when the cat was staying on the metal grid used for painful shocking produced rapid activation of the avoidance reflex. Stimulation is accompanied by marked neocortical desynchronization. *B :* Stimulation applied when the cat was staying on the flight place elicited relaxation and sleeping posture. At the same time slow waves and sleep spindles appeared in the neocortex. Redrawn pictures from a cinematographic recording. Figures on the top of the pictures show the number of copied picture, below the time in seconds after the onset of stimulation

enon which has never been met with on stimulating other structures. It therefore seems to represent a specific thalamic mechanism.

Considering the conditions of elicitation of the relaxing effect, it may be established that it is determined by an environment which does not contain any definite stimuli of motivational character. During the approach of the flight place a pull effect must be at operation. Subsequently, however, motivational excitement disappears on the bench, and consequently the new configuration of stimuli existing there cannot come into contact with any definite driving force. The validity of this interpretation seems to be supported by an interesting accidental observation. Namely, if the experimental box was over-

heated, then, instead of the relaxing effect a typical heat-regulating effect, panting, was elicited by the stimulation. This effect could not be elicited in an indifferent temperature by any kinds of parameter. From this we may conclude that the panting does not represent a direct effect of the stimulated structure. It is well-known that panting can be elicited at a low threshold from definite regions of the hypothalamus. Thus, this effect is probably elicited from the thalamus by facilitating the function of that hypothalamic region. We think that a similar interpretation holds also in the case of the conditional activating effect of stimulation. With a generalization one may say that the thalamic system producing these dual effects always works in the direction of the actual motivational state. With a definite motivational excitation or in the presence of motivational signals the system increases the actual motivational excitement. In want of motivational cues it induces generalized inhibition.

From a neurophysiological point of view the dual effect can only be explained by assuming that in the area in question excitatory and inhibitory neural elements are located in an overlapping manner. This assumption is supported by electrophysiological findings (TISSOT and MONNIER 1959). The setting into operation of the two effects might so occur that in an environment having motivational cues the threshold of facilitatory elements, in an indifferent situation and in the lack of motivational excitement the threshold of inhibitory elements, is lowered. Consequently, stimulation always increases the process the function of which is actually dominant.

In the regulation of the functional tone of the facilitatory elements impulses of the primary motivational systems must play an important role. The known anatomical connections of the activating system are in agreement with this assumption. At the time being, we have no definite information concerning the origin of impulses regulating the functional tone of the inhibitory elements. The facilitatory and inhibitory neurons are probably organized in a reciprocal antagonistic manner, and the function of the inhibitory elements appears at the moment of the decrease of excitatory processes as a released function.

Earlier some facts have been presented supporting the existence of the reciprocal interrelationship between drive mechanism and arrest. The question whether the inhibition belonging to the arrest reaction and that of the dual effect (relaxation, sleep) are bound to common or separate neural elements cannot be answered on the basis of the available data.

Summing up, the dual effect represents a higher level of integration of motivational processes than those of the hypothalamic effects. It represents a level which by influencing the continuum of sleep-wakefulness, consequently the whole behavioural domain, seems to be identical with MAGOUN's activating system, reflecting its peculiar, inner functional organization.

Self-stimulation experiments

Our self-stimulation experiments evolved accidentally from one of the possible variants of stimulations carried out in the background of conditional reflexes. A possible version was to apply stimulation in the moment when the animal has reached the goal of the conditional reflex (feeding device or flight place). Technically this was so arranged that pressing of the feeding device or the bench (below: pedal) turned on stimulation, its release turned it off. It was

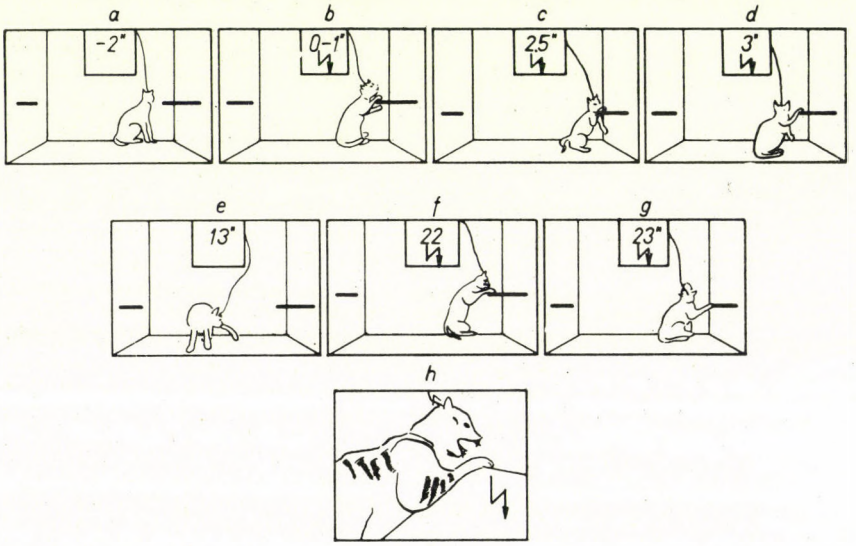

Fig. 10. Self-stimulation process elicited from the medial region of the hypothalamus (localization see in *Fig. 4*). Marked manifestations of rage (growling, hissing, piloerection *etc.*) (h) during stimulation

ascertained that under such conditions a self-stimulation process, meeting the criteria of the classical self-stimulation process (OLDS and MILNER 1954) is established. The only difference was that the animal approached and pressed the pedal not during a spontaneous orientation process but under the effect of a conditional signal. On the other hand pressing of the pedal produced not a predetermined impulse train of definite duration but a continuous stimulation. It was interrupted by the release of the pedal.

It was surprising that under such conditions a self-stimulation process appeared on the stimulation of points producing marked overt manifestations of rage *(Fig. 10)*. Similar observations were reported by BROWN and COHEN (1959). We have been urged particularly by this observation to investigate systematically the factors involved in the mechanism of self-stimulation. The relatively low frequency of self-stimulation (250—1000 presses/hr) offered conditions favourable for the separation of the different factors. (The low

frequency of self-stimulation is explained by the fact that pedal pressing is more clumsy under our conditions than in an apparatus devised directly for such purposes.)

According to our observations, the process of self-stimulation consists of the following components. The pedal is pressed by the animal for a definite period of time, depending on the parameters of stimulation, then it releases the pedal and retreats. Subsequently in part of the cases the animal returns rapidly to the pedal and presses it again. In other cases a definite arrest reaction occurred after retreat.

Considering that pressing the pedal under our experimental conditions resulted in continuous stimulation, it is unquestionable that retreat from the pedal was the consequence of stimulation.

Under the classical conditions of self-stimulation there are two possible versions. The pedal is released either during the stimulation or after its termination, in the latter case as a consequence of an undefinable factor. In our own experiments the approach and pressing of the pedal — because of its conditional character — is certainly the product of a pull effect. From the fact that the pedal is pressed for a definite period of time we may conclude that the pull effect is increased during the first phase of stimulation. The retreat of the animal must necessarily be a consequence of the fact that the character of the effect has changed, *i.e.* the original pull effect became a push effect. Simultaneously, however, stimulation is terminated. Two possibilities may be envisaged in this phase of our observations. If stimulation is interrupted during the push effect then a pull effect appears composed of reciprocal movements of the push effect and this compels the animal to press the pedal. If in the course of retreat a new pull effect develops, then the interruption of stimulation is followed by an arrest reaction. The arrest reaction prevents the abandonment of the environment of the pedal. After the termination of arrest, in the course of the hyperactive pulling phase the animal is compelled again to approach and press the pedal. Thus, according to our interpretation and under the circumstances of our experiment, self-stimulation is a periodic process of forced character, from which the animal cannot be released. This would explain why self-stimulation can be continued with the complete physical exhaustion of the animal.

Our interpretation is based on the consistently emphasized fact that the basic motivational mechanisms (pull and push effects) are functions interconnected in a definite sequence. According to this, our interpretation seemingly fails if the pedal is released after the termination of stimulation. It is evident that no push effect could develop in this case as a direct effect of stimulation. Thus we cannot answer what factor has made the animal to release the pedal. It is possible that the minimum duration of stimulation necessary to sustain a self-stimulation process just reaches the threshold of the pushing effect. It is conceivable, too, that at the termination of stimulation neuronal excitement

continues and finally leads to a push effect. Interpretations built on subjective emotional factors accompanying or following stimulations are not acceptable. Though we have no doubt that stimulations are accompanied by complex subjective manifestations, these in our opinion are consequences and not causes, therefore they cannot replace neural mechanisms even temporarily.

The interpretation of motivation with the help of a "conceptual" nervous system

The complexity of the relations discussed in the course of this study on the one hand, and their poorness in relation to reality on the other, convincingly shows that to construct a circuit diagram of neural mechanisms of even the simplest behavioural act is a vain hope. We were fully aware of this when preparing the present diagrams and our purpose was only to facilitate the survey of our data and the verifications of our conclusions. It was also hoped to get new working-hypotheses, to be controlled experimentally.

Relationships in the circuit diagram have been simplified to a possible minimum. Connections have been constructed on the basis of the functional data; in the anatomical sense these are hypothetical, in HEBB's (1955) terminology they represent a "conceptual" nervous system. Connections have not been identified with concrete anatomical pathways even in the case if it would have been justified by functional and morphological data. Structures representing essentially identical basic mechanisms have been integrated into a common system, *e.g.* the hypothalamus represents all the structures contributing to the basic motivational mechanism.

Considering that the connections postulated on the basis of our observations could not be unified without contradiction in a single scheme, we decided to construct two alternative schemes.

Starting from the recognition that the same basic mechanisms (pull and push) are involved in the different specific drives produced by different specific afferent neural or humoral impulses (hunger, thirst, pain, sex, *etc.*) only one unspecified drive was incorporated in the scheme. The extremely complicated and uncertain relationships of the different drives have been omitted lest they confuse the most important relations.

As it can be seen, the afferents (DS) responsible for the inducement of a driving force are only connected to the pull system, on the basis of the finding that the primary effect of stimulation is consistently a pull effect. The push system is thrown into action by the pull system (2) if excitement in the latter reaches a critical level. This activation threshold is marked by the vertical hatching. Both systems are connected to the corresponding motor system. It seems highly probable that both the pull and the push effects are built on some specific and elementary motor patterns. It must be assumed that these motor

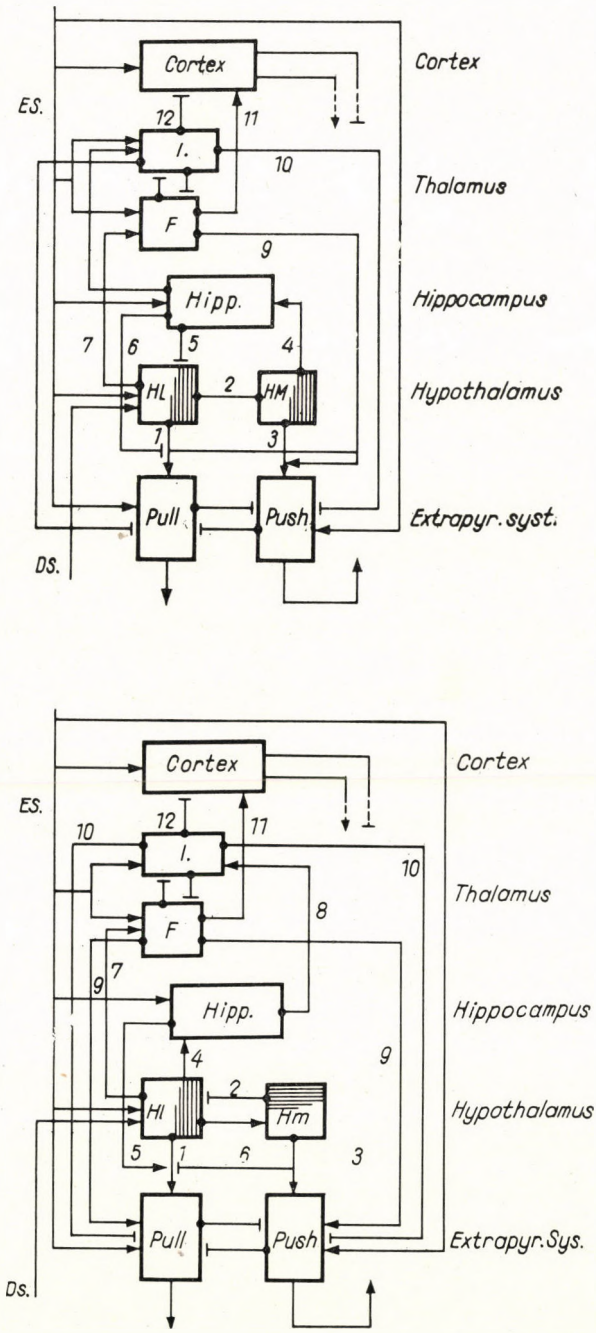

Fig. 11. (I and II) Schematic representation of the basic mechanisms underlying motivation, constructed on the basis of the described facts. Detailed explanation see in text

patterns, owing to the modifying environmental effects are highly complex. In the last analysis they should, however, be related to the simplest manifestations of approach and avoidance laid down in the spinal cord, with extension and flexion. Attention is called at the same time to the similarities showing themselves between the organization of the medullary respiratory centre (relationship of in- and expiratory neurons) and those of our pull-push system. But a detailed discussion of the motor organization of the pull and push effects had also to be omitted because it would have involved a survey of the whole hierarchy of motor integration.

Let us now follow the mechanisms of motivation with the help of the scheme. Essentially, it suffices to analyse three conditions: (i) the appearance of a pull effect in the case of a weak motivational excitement; (ii) the transition of pull into push effect; (iii) the mechanisms of drive reduction or, in other words, the effect of the cessation of drive in the case of an intensive pull effect.

(i) In the case of an excitement of definite strength under the influence of the pull system the animal approaches the stimulus actually acting on it. Simultaneously with the start of the motor act, a system able to maintain firing after the cessation of the original drive impulses is also set into action. According to the first scheme *(Fig. 11A)*, this structure fired simultaneously by motivational (7) as well as by exteroceptive impulses is the facilitatory system of the thalamus (F). As a consequence of these two kinds of afferentation, facilitatory impulses (9) are sent to the efferent projections of both the pull and push systems. The condition of the formation of a conditional connection according to our scheme is the simultaneous convergence of two excitatory processes. Such a connection may be neutralized by an active inhibitory connection. A conditional coupling under the actual circumstances is given by the pull system, considering that the push system is momentarily inactive.

According to the second scheme *(Fig. 11B)*, the structure responsible for the reverberating impulses is the hippocampus, which is brought into action by impulses of the pull system (4) as well as by environmental impulses. The role of the hippocampus in respect of the reverberatory function has been taken into account on the basis of its inner structural organization (McLardy 1959). According to the scheme, hippocampal impulses should facilitate the pull effect. On the basis of this assumption a facilitation of the pull effect might be expected from direct electrical stimulation, and its decrease from destroying the hippocampus. This expectation was, however, contradicted by our own earlier findings (Grastyán *et al.* 1959). The contradiction, as it will be shown later, can be resolved by assuming a connection between the hippocampus and the inhibitory system of the thalamus (8).

(ii) If excitation in the pull system reaches a critical level, pull is transformed (2) into a push effect. According to the first scheme, in the moment when excitation in the push system reaches a critical level, impulses go to the

hippocampus (4); this structure, with the help of impulses coming from the environment, inhibits (6) the impulses (1) going from the pull system to the corresponding motor system. In this way the manifestation of the pull connection is hindered. At the same time the impulses of the push system (3) converge with those of the facilitatory system (9) and thus the conditions of the formation of a connection are given at the motor system innervated by the push system. On the very impact of the push effect, the animal finally turns off from the external stimulus which had acted on it and gets under the influence of a new environmental stimulus. Simultaneously, however, the pull mechanism must again be effective, because at a critical level of the push excitement the inhibitory feed-back mechanism exerted through the hippocampus (5) is set into action and therefore the push effect will be transformed into pull effect.

In the second scheme the inhibition (6) of the efferent impulses of the pull system and the inhibition acting on the pull system itself (2) are exerted by the push system. The final effect is, however, the same in both cases.

(iii) If in the course of an intensive pull effect the animal arrives at a situation where the impulses responsible for motivation cease to act (drive reduction) the following processes may take place.

The thalamic facilitatory system is already set into action and these impulses facilitate the impulses of the pull system. At the moment of drive reduction, however, the facilitatory system also ceases functioning and thus the inhibitory system is released. This inhibition is destined to protect the actual movement pattern, by hindering the appearance of any other movement. In this way are ensured the conditions of conditional connection formation between this movement and the environmental impulses. A conspicuous failure of our first scheme is, however, that the reverberating impulses are attached to the function of the thalamic facilitatory system which, as we have seen, ceases to function at the moment of drive reduction. Therefore, neither the excitatory reverberating impulses, nor the inhibitory impulses required to preserve the specific movement pattern, are ensured.

The second scheme gives a more satisfactory solution.

The hippocampus set into action by the impulses of the pull system (4) sends impulses (5) to the corresponding motor system. At the same time the thalamic facilitatory system ceases to function and, as a consequence, the inhibitory system is released, the excitation of which is increased by the reverberating impulses of the hippocampus. This system inhibits (10) all the movements which are not under the effect of reverberating impulses and thus the conditions are ensured to the formation of a conditional connection between the actual movement and the peripheral impulses.

This second scheme has been criticized on the grounds of the disagreement between the postulated facilitatory effect of the hippocampus and observations made on the basis of stimulations (GRASTYÁN *et al.* 1959) as well as

lesions (KARMOS and GRASTYÁN 1962). It is, however, possible that not the expectations derived from the scheme, but rather the interpretation of our earlier findings was incorrect. Stimulation of the hippocampus elicits an arrest-like reaction and not generalized inhibition. Following destruction of the hippocampus the animal becomes hyperactive. The conditions of eliciting by electrical stimulation an arrest reaction are more exactly shown in the second than in the first scheme. Impulses (5) going from the hippocampus to the motor system maintain the actual movements while the impulses of the simultaneously activated thalamic inhibitory system (10) prevent the intervention of any other movement.

The hyperactivity following hippocampal lesion may be regarded as a consequence of dropping out of the inhibitory function of the thalamic structure.

On the basis of these considerations the second scheme probably stands nearer to the reality than the first one. A common fault of both schemes is that the inhibitory function involved in the arrest reaction as well as that involved in the dual effect (general relaxation) is attributed to the same system. The inhibitory process of the arrest reaction is an independent function and belongs to a structure *(e.g.* the corpus striatum) not investigated in these experiments; it will be checked in the course of further studies.

In the last two decades there were numerous debates on the importance of cortical and subcortical processes in conditioning. The debates, in the lack of a necessary amount of concrete information, were mostly sterile. In the last years the discovery of MAGOUN's activating system has turned the scale slightly in favour of subcortical processes. The functional principle of the activating system, however, does not justify any one-sided conception. The extreme importance of the discovery of the activating system can be seen in the introduction of a dynamic principle into the functional neuroanatomy of the brain against the static classical concept.

The most essential characteristic of the principle is that the integrative action of the central nervous system is bound to a continuous functional interaction of two anatomically and functionally well-defined systems. The features of the specific and diffuse projection systems in this interaction are not subordinated but co-ordinated.

In the light of the presented conception let us finally draw some general conclusions concerning the relative importance of the specific and diffuse projecting systems in the establishment of conditional reflexes.

The functional state of the facilitatory (F) and inhibitory (I) systems located at the thalamic level is determined by impulses (7) originating from the mesencephalic reticular formation and the hypothalamus. At the same time, the functional state of the cortex depends on the thalamic systems (11, 12). From the point of view of the conditional connection this would mean that it is the primary motivational system which determines whether external stimuli

reaching these higher levels reach the excitatory or inhibitory, or the push or pull connections. These determining effects of the subcortical system are naturally only effective in the course of the establishment of conditional connections, in other words in unfamiliar situations. After the establishment of appropriate conditional connections, *i.e.* in a familiar situation, it is the function of higher formations which determines the function of the motivational systems by way of their direct descending projections. This determining effect of the environment seems to manifest itself also at the electric activation of conditional reflexes.

Summing up, we may state that during the development of conditional reflexes it is the functions of the subcortical activating systems and after the establishment of conditional reflexes, that of the specific systems, which chiefly dominate. Since, however, a perfectly unknown situation practically does not exist, it follows that either of the functions can be absolute.

We are fully aware that the interpretations put forward in this study are often oversimplifying and mechanistic. The purpose of the study was, however, intentionally to reduce the neural factors involved in conditioning and learning to a minimum level, for the sake of a clearer view of their organization. This intention necessarily involves oversimplification. At the same time it is promising that the most fundamental properties of the conditional process can successfully be interpreted on the basis of neurophysiological findings. It is also admitted that many of the often highly sophisticated requirements of different learning theories are not met by this conception. It is well known that several of the current behaviour theories deny the importance of motivation in learning (perception or non-reinforcement theories). It seems worth while to consider that the majority of the unanswerable objections can be found in conceptions which represent pure psychological constructions and also reject the competence of physiological aspects in theorizing. Instead of considering these discrepancies we should bear in mind that the validity of many of these theoretical postulates are being seriously questioned by new achievements of neurophysiology.

The findings of the present study as well as of a number of other authors have clearly shown that direct manipulations in the nervous substrate of motivation result in manifestations reflecting the postulates of the so-called stimulus-response or reinforcement theories (PAVLOV, THORNDIKE *etc.)*. In that classification the closest correlation was offered by the so-called drive reduction theory proposed by HULL (1943). Opponents of the general importance of motivation in conditioning might object that this correlation could only be found because the manipulations were restricted to motivational structures. The question arises whether manipulation in other systems would not support the postulations of the rival theories. After the discovery of the principle of activation, however (MORUZZI and MAGOUN 1949), that possibility can hardly

be substantiated. It seems to be well-established (LINDSLEY 1951; GASTAUT and ROGER 1960) that the function of the ascending activating system, which anatomically coincides with the structures showing motivational properties, is essential in the organization of every higher process. Even in the light of recent findings (DOTY *et al.* 1959; ADAMETZ 1959) according to which the critical structures are not restricted to the mesencephalic reticular formation, it is certain that a minimum degree of excitement in this system is an indispensable condition of the integrative action of the whole brain.

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CONDITIONED EVOKED POTENTIAL, A MODEL EXPERIMENT OF LEARNING

By

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On presenting a combination of various somatic and visceral stimuli, conditioned evoked potentials have been obtained in unanaesthetized, curarized cats. From the character of the changes of the evoked responses conclusions have been drawn as to certain elementary laws of the process of learning. The conditioned evoked response, in the lack of reinforcement by a second stimulus, showed a regular extinction curve; this inhibition could be suspended by the introduction of a new stimulus, *i.e.* disinhibition was brought about. The conditioned evoked response appeared in the cortical representation areas of both afferent nerves involved in training.

Our symposium is concerned with the earliest signs of the development of the temporary connection. Research workers usually approached this most decisive phase of the learning process in two ways. On the one hand, they studied the early motor, vegetative or electric phenomena accompanying the development of a classical alimentary or defensive conditioned reflex, and, on the other, they subjected to electrophysiological analysis the activities taking place in the single central neurones, to elucidate the simplest cellular processes.

Both methods have produced excellent results. Still, in our opininion, decisively new insight might be obtained also from the analysis of complex cellular organizations, just as in the case of classical conditioning, if we create a learned phenomenon by the simultaneous presentation of two afferent stimuli but eliminate the efferentation of the conditioned reflex arc. In this way the greatly inert multineuronal effector apparatus cannot overmask certain processes, as it often happens in the course of classical conditioning.

The essence of the electrophysiological model used by us is a kind of sensory-sensory conditioning. Stimulation is carried out by applying a combination of various somatic and visceral stimuli, and the changes in the duration and amplitude of the cortical evoked potentials are used as the indicator. Thereby we have obtained a temporary connection model, allowing excellent quantitative control.

Our aim was to follow up the dynamics of the development of learned processes and to subject these early phenomena to precise electrophysiological analysis.

Methods

Forty unanaesthetized, curarized cats were involved in the acute experiments. At operation under light ether-hexobarbital anaesthesia we exposed the right ectosylvian gyrus, described as the representations of both the splanchnic and sciatic nerves, as well as the right temporal cortex, the auditory centre. We applied spherical silver electrodes to both areas. Then we exposed the left splanchnic and sciatic nerves, divided them, and placed stimulating electrodes on their central stumps. The parameters of stimulation were, 0.5 msec, 1.5 V, square impulses. For auditory stimulation we used clicks, conducted by a rubber tube directly into the outer auditory meatus of the animals. Cortical activity was recorded by EEG, and the evoked potentials were recorded from unipolar leads by a cathode oscillograph.

Results

In the first experiments we presented 0.5 msec splanchnic shocks together with single sciatic stimulations. After 30 to 80 reinforcements of this type, carried out at 5-second intervals, we observed a peculiar facilitation: the amplitude of the cortical potential evoked by splanchnic stimulation increased

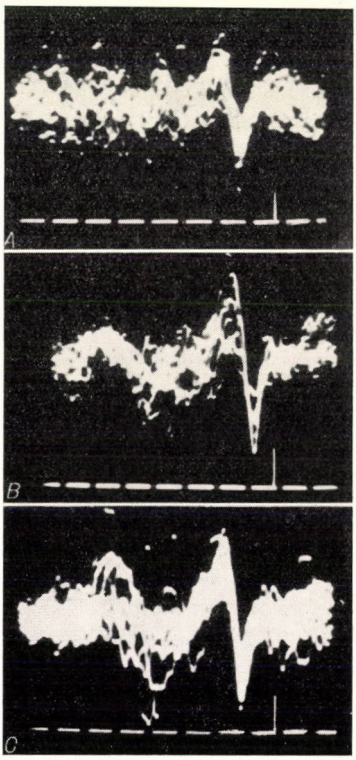

Fig. 1. Conditioned evoked potential. *A:* potential evoked by splanchnic stimulation. *B:* combined splanchnic and sciatic stimulation. *C:* response to splanchnic stimulation alone after 80 trainings. Note the increase of amplitude. Recorded from ectosylvian gyrus. Each section shows 10 superimposed curves. Read curves from right to left. Time signal, 20 msec. Vertical line: time of presenting the stimulus

to almost twice the control value; this increased response could be recorded for minutes *(Fig. 1)*. Thus, this peculiar phenomenon is a typical learned reaction, possessing the criteria of a conditioned response.

If the response evoked through splanchnic stimulation is presented alone for minutes, *i.e.* it is not "reinforced" by sciatic stimulation, then, to use the Pavlovian terminology, the splanchnic stimulus loses its signal significance and a typical extinction results. *Fig. 2* shows such experiments on two cats. In the

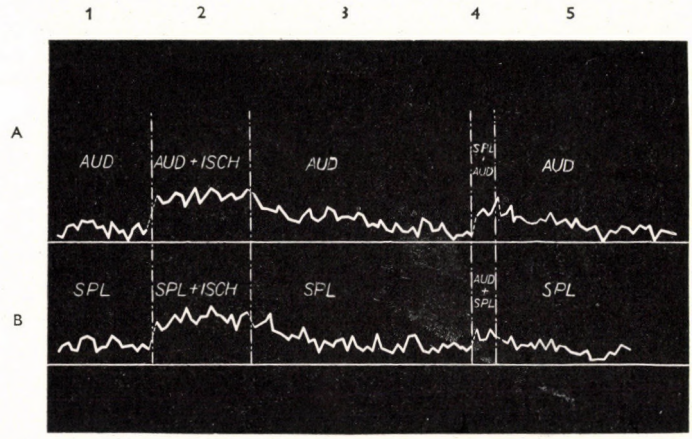

Fig. 2. Extinction and disinhibition. Each point of every curve represents the amplitude of one evoked potential. *A:* potential evoked by auditory stimulation. *1:* before reinforcement, *2:* click + sciatic stimulation, *3:* after training, potential evoked by click, without reinforcement. Note extinction curve. *4:* increase of amplitude in response to the presentation of a new (splanchnic) stimulus in the phase of extinction; disinhibition has resulted. *5:* this is again followed by extinction. *B:* the same in the case of a potential evoked by splanchnic stimulation, reinforced by sciatic stimulation *(2)*, with disinhibition in response to auditory stimulation *(4)*

first, auditory stimulation was applied together with sciatic stimulation. On reinforcement the amplitude of the evoked potentials increased. This increase of amplitude, in a learned way, persisted also when the auditory stimulus was presented alone. On the other hand, the amplitude gradually decreased to almost the pre-reinforcement level, when reinforcement had been discontinued: a typical extinctive inhibition resulted.

If we present a new stimulus during the phase of extinction, for example splanchnic stimulation in this case, inhibition of the inhibition results: the amplitude increases again. Thus, our model experiment has proved electrophysiologically the occurrence of extinctive inhibition and disinhibition.

We have been devoting particular attention to the topography of the foci of excitation created by the afferent impulses. We have found that in case we presented a combination of stimuli whose cerebral cortical projections were different, for example auditory stimulation with sciatic stimulation *(Fig. 3)*, conditioned evoked potentials appeared both in the sciatic represen-

tation area and in the auditory cortex. This was a remarkable evidence, as it has always been claimed that with the development of the temporary connection one dominant focus arises, presumably at the site of the reinforcing, uncon-

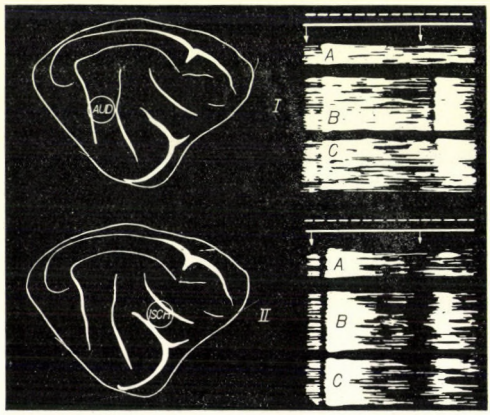

Fig. 3. Topography of the conditioned evoked potential

I: Conditioned evoked potential induced by auditory + sciatic stimulation, recorded from the auditory cortex. *II:* the same, recorded from the cortical representation of the sciatic nerve. For training, the clicks are presented 200 msec before the reinforcing sciatic stimulation. The records have been made by KOZHEVNIKOV's technique, *i.e.* the cathode oscillograph was operated with modulated ray brightness. Each line represents one evoked potential. Dark spots indicate positive, light spots negative wave phases. *A:* response to auditory stimulation before reinforcement. *B:* responses to auditory + sciatic stimulation (note the positive and negative phases of the pontentials evoked by the clicks, as well as by the sciatic stimulation 200 msec later). *C:* conditioned evoked potential to sound, after reinforcements (note the marked evoked responses not only at the site of the auditory stimulation, but also at that of the previous sciatic reinforcement). Time signal, 20 msec

ditioned stimulus. Our data indicate that the principle of the one dominant focus is untenable. We have found foci of excitation in the projection zones of both afferent stimuli presented simultaneously.

Discussion

As we have seen, by conditioning evoked potentials we have constructed a relatively simple temporary connection model, by means of which we have already succeeded in studying some fundamental laws of the process of learning.

The basis of learning seems to be a peculiar state of facilitation arising and persisting for a long time in the central neurones, as a result of training (reinforcement). We feel more and more inclined to think that this peculiar facilitation is not different from the phenomenon described late in the past century by SCHIFF [3], called hysteriosis by VEDENSKY [4] early in this century and now known as post-tetanic potentiation after LLOYD [2] and Ecc-

LES [1]. The pattern is undoubtedly peculiar: after training the evoking stimulus maintains foci of excitation for hours not only in its own place, but also in the place of the reinforcing stimulus, which is not presented any longer. This conditioned focus of excitation follows the laws of extinction and disinhibition.

In our electrophysiological model experiments two foci of excitation have invariably arisen in topographically different areas when a reaction was being learnt.

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CLASSICAL CONDITIONING AND RETICULAR UNITS

By

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The rapid development of electrophysiological microtechniques has made it possible to extend the analysis of conditioning to the cellular level (JASPER, RICCI and DOANE 1960). In the present paper it has been attempted to follow the elaboration of a conditioned response in reticular neurones using computer processing of electrophysiological data.

Experiments were performed in 44 adult albino rats aged 3 months. The animals were immobilized by d-tubocurarine, fixed in a stereotaxic apparatus and maintained on artificial respiration 60/min. Using a hydraulic microdrive, steel or glass microelectrodes were introduced through a large trephine opening over the cerebellum to the ponto-bulbar reticular formation. A loudspeaker mounted 20 cm from the animal's head was used for acoustic stimulation (conditioned stimulus), while electric shocks were applied through a pair of silver wire electrodes to the sciatic nerve (unconditioned stimulus). Cortical primary responses to the sciatic stimulation were recorded from the contralateral somatomotor cortex. Unit activity was recorded in first, and the primary responses in the second, channel of a dual beam oscilloscope. After a well isolated unit exceeding at least three times the maximum peak to peak noise had been found in the reticular formation, its response to the auditory and sciatic stimuli was tested, using a simplified version of an average transients computer built in our laboratory (TŮMA and BUREŠ 1963).

The apparatus *(Fig. 1)* consists of ten decadic counters with a capacity of one million bits each, with a maximal counting rate of 100 kc/sec. Spikes exceeding the preset minimal amplitude pass from the oscilloscope through a shaping circuit and through a system of ten parallel gates to the individual counters. Normally all gates are closed. Their opening is controlled by an electronic switch. Switching pulses applied at regular intervals ranging from 10 microsec to 10 sec are obtained through a decadic reductor from a 1 Mcycle oscillator. The electronic switch does not only open the gates to the counters but may also be used for synchronizing the applied stimuli. The function of the apparatus is controlled from a control panel. The electronic switch always starts from position one and keeps gate one open until the next clock pulse opens gate

two, *etc.* When switching from position ten to one, the switch is automatically disconnected and the apparatus is prepared for another cycle. A predetermined number of cycles can be automatically given by using the automatic control.

A definite section of the unit activity is divided into ten consecutive intervals of equal duration and the spikes in these intervals are counted in the corresponding counters 1—10. Spontaneous activity is considered to be a

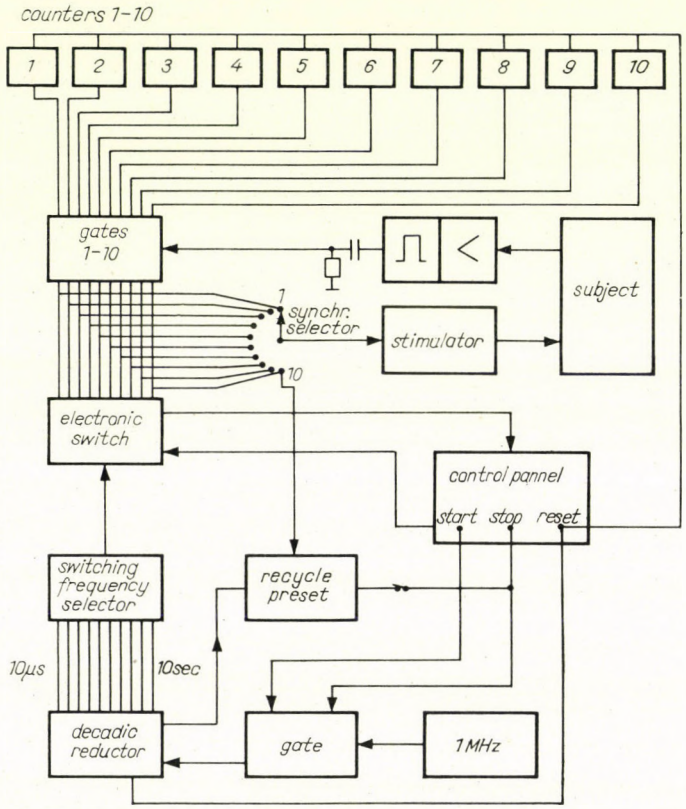

Fig. 1. Block scheme of the electronic analyser of unit activity. For details see text

random process characterized by rather stable statistical parameters (GERSTEIN and KIANG 1960). When the stimulus synchronized with a certain analysing interval does not affect the activity of the neurone and when the number of average cycles is high enough, spikes occur in all counters with the same probability. Statistical methods must be used to decide whether deviations from the control level are related to the stimuli.

A switching interval of 2 seconds was used throughout, giving an overall cycle duration of 20 sec. A continuous acoustic stimulus (200 or 2000 c/s) was maintained during the third interval and a single electric shock was applied to

the sciatic nerve at the beginning of the fourth interval. Averaging was usually made in blocks of ten cycles repeated in 45 sec intervals. Only units showing a clear-cut reaction to the unconditioned stimulus (sciatic nerve stimulation) but not reacting to the acoustic stimulus (conditioned stimulus) were used in the conditioning experiments. During the conditioning procedure, the acoustic stimulus was combined with sciatic nerve stimulation up to 100 times.

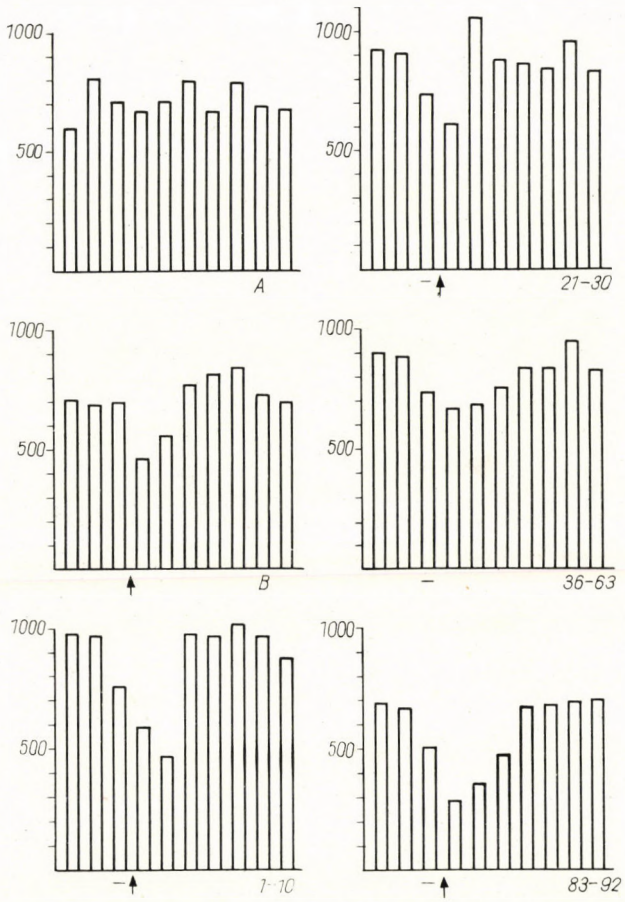

Fig. 2. Post-stimulation histograms of reactions of a reticular unit to the conditioned stimulus (horizontal bar — *A*), unconditioned stimulus (arrow — *B*) or to combinations of both stimuli. For details, see text

A typical experiment is illustrated by *Fig. 2*. Histogram *A* shows no statistically significant reaction to the acoustic stimulus while the sciatic nerve stimulation has a clear-cut inhibitory effect *(B)*. In the first ten reinforced trials the acoustic stimulus developed an inhibitory effect, which can also be seen in trials 21—30 and 83—92. Thus, as the result of conditioning, the neurone began to respond to an initially indifferent stimulus. The conditioned reaction

could still better be demonstrated by omitting the unconditioned stimulus. To prevent extinction, every third unconditioned stimulus was dropped out. The histogram of 10 presentations of the isolated conditioned stimulus scattered among trials 36—63 shows changes very similar to those induced by combination of the conditioned and unconditioned stimuli.

The conditioned reactions demonstrated in the above figures imitated the responses to the unconditioned stimulus. In some cases, however, conditioning resulted in an opposite change. In this case the conditioned stimulus prob-

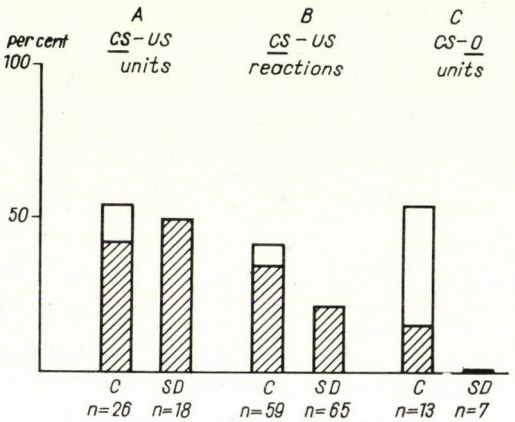

Fig. 3. Incidence of conditioned reactions in the third interval ($CS - US$), in the fourth interval ($CS - 0$)

Imitatory reactions — shaded part of the columns.
Inverse reactions — empty part of the columns.
C — control group; *SD* — spreading depression group.
For details, see text

ably started activity opposing əqɪ unconditioned stimulus effect on the given neurone, an activity which could best be revealed by omitting the sciatic nerve stimulation.

In another group of rats conditioning was performed during bilateral functional decortication induced by repeated waves of cortical spreading depression. Impairment of electrical activity of the cerebral cortex was checked by the disappearance of primary responses to sciatic nerve stimulation. In spite of suppression of cortical function, conditioned reactions of reticular units could be established similarly as in normal conditions. Certain differences between the normal and functionally decorticated rats are illustrated by *Fig. 3*, summarizing all experimental data.

Statistically significant results of conditioning were observed in about 50 per cent of the examined units both in normal and functionally decorticated animals *(Fig. 3 A)*. The conditioned reaction usually imitated the change induced by the unconditioned stimulus. In functionally decorticated animals

the conditioned reactions were observed during a shorter period of time than in the normal rats as indicated by the less frequent incidence of positive reactions in the first fifty reinforced trials *(Fig. 3 B)*. The difference between normal and functionally decorticated animals was best expressed when the result of conditioning was tested by omitting the unconditioned stimulus *(Fig. 3 C)*. No positive reaction was found under these conditions in the spreading depression group although more than 50 per cent units were found to be conditioned in the normal rats.

The experiments described in the present study can be regarded as a cellular counterpart of the conditioned arousal. The responding units are links of complex neural chains normally activated by the unconditioned stimulus and after conditioning also by the conditioned stimulus. As the plastic change may occur at some input element of the circuit, probably far away from the point of recording, it must be stressed that it is scarcely possible to use this technique to detect the neurones primarily involved in formation of new connections. For this purpose more localized unconditioned stimuli (possibly affecting the recorded unit only) may give more relevant results.

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ИССЛЕДОВАНИЯ КОНСТРУКЦИИ ВИСЦЕРАЛЬНОЙ КОРЫ

Э. Ш. АЙРАПЕТЬЯНЦ

ИНСТИТУТ ФИЗИОЛОГИИ ИМ. И. П. ПАВЛОВА АН СССР И ЛЕНИНГРАДСКИЙ УНИВЕРСИТЕТ, ЛЕНИНГРАД, СССР

В течение многих лет лаборатория автора, применяя различные методики стремится описать те структуры головного мозга — корковые и подкорковые, благодаря которым осуществляется информация о висцеральной (resp., интероцептивной) сигнализации, анализ и регуляция процессов в висцеральной сфере организма. В настоящее время накоплен значительный экспериментальный материал, позволяющий составить эскизы корковой локализации висцеральных анализаторов и роль в завершении афферентной проекции таламического коллектора. Мозговой аппарат любого анализатора складывается из множества компонентов, расположенных на разных интегральных уровнях головного мозга, включающие межкорковые, взаимно-корковые-подкорковые нервные связи. Однако связь настолько взаимообусловленная, что повреждение одного из них влечет за собой органическое разрушение других. Представляет интерес в этих обязательных соподчинениях, явление замещения — викарирования функций и структур.

Кора мозга собак и висцеральные условные рефлексы

Полная декортикация исключает возможность образования оборонительного и пищевого условного рефлекса несмотря на более чем 200 сочетаний. Гистологический анализ подкорковых образований полностью декортицированных собак показал глубокие и обширные повреждения.

Односторонняя декортикация не препятствует сохранению и образованию условных сигналов, выработанных с непарных внутренних органов.

О висцеральной проекции в двигательной корковой области

Билатеральная экстирпация сигмовидной извилины вызывает исчезание висцеро-механических, висцеро-химических условных сигналов и их анализа сроком от 2 до 7 месяцев. Следовательно, эти участки относятся к аппарату висцерального анализатора. Однако факт последующего восстановления висцерального анализа указывает на наличие аналогичных или

гомологичных проекционных аппаратов и вне двигательной корковой области, благодаря чему и возможна компенсация (викарирование). Предварительные эксперименты показали, что экстирпация некоторых полей переднего полюса коры не влияет на состояние висцеральной сигнализации.

Запись биотоков коры при висцеральных раздражениях обнаружила специфические изменения электрокортикограммы преимущественно в двигательной области.

О роли таламических ядер

Экстирпация сигмовидной извилины вызывает ретроградное перерождение ядер таламуса, большей частью клеток вентрального, частично медио-дорзального, центрально-латерального. Значит, несмотря на указанные повреждения висцеральная сигнализация спустя определенное время оказывается возможным. Подобная же экстирпация сигмовидных извилин у щенков угнетает висцеральную сигнализацию на срок не более 3-х недель, хотя в таламусе происходит перерождение ядер аналогичное описанному у взрослых собак. Следовательно, афферентная корковая проекция возе можна и без указанных ядер. Есть основания полагать о наличии в аппарат-таламуса «висцерального коллектора» с механизмом замещения выпавших структур.

О роли лимбической коры

У собак при значительной по площади билатеральной экстирпации после передней лимбики резко и длительно угнетаются висцеральные условные рефлексы. После же экстирпации ограниченных участков, равно как и билатеральная экстирпация задних отделов лимбики условные рефлексы угнетаются в слабой степени и на короткий срок. При этом во всех вариантах на длительный срок нарушается адекватная условно защитная реакция на насыщение организма растворами кислоты и щелочи.

Запись электрокортикограммы на кроликах с отведением с двигательной коры, передней и задней лимбики при висцерохимическом раздражении обнаружила сходную картину отражения. Введение аминазина снимает указанные эффекты. Вместе с этим билатеральная экстирпация в одном варианте двигательной коры, в другом — лимбической коры исключает возможность висцерального отражения ЭКГ в оставшихся участках коры. Двустороннее разрушение задней области лимбической коры не снимают типичные висцеральные отражения КГР в передней области лимбики и в двигательной коре.

В условиях интактного мозга выявляются различия в характере изменения ЭКГ лимбической коры в зависимости от концентрации ряда вводимых в кровь химических веществ.

Афференты лимбической коры

Методикой дегенераций волокон в опытах на кошках показали, что афферентные связи между передними и задними лимбическими областями взаимны, но неравнозначны: передний посылает в задний большое число волокон, чем задний к переднему. Опыты на кошках и собаках показали, что 4 и 6 поля посылают в лимбическую область значительно меньше волокон, чем лимбическая в двигательную. При разрушении 32 и 24 полей обнаруживается обильная и территориально обширная дегенерация волокон в 6 и 4 полях и распространяется на поля 1, 2, 5, 7, 19. Гораздо в меньшей степени эта картина повторяется при разрушении задней области лимбики. Таким образом исследования показали значительные лимбико-моторные афференты.

Заключение

Можно считать правомерным морфофизиологическое выделение висцеральной коры, осуществляющей функции высшей интеграции висцеральной афферентной сигнализации. Стратиграфически это и есть месторасположение ансамбля (комплекса) центральных концов висцеральных анализаторов — корковых проекций. Их объединение осуществляется как путем перекрытия разбросанных вдали от ядер анализаторов их элементов, так и в форме динамической констелляции самих ядер. Локализационно висцеральная кора включает двигательную и лимбическую области. Дальнейшие исследования выявят роль других районов коры в системе висцеральных анализаторов.

Нет основания для обозначения «лимбической системой» структур, выходящих за пределы лимбической коры, так и ее идентификации с «висцеральным мозгом». Последнее представляет совокупность корковых и подкорковых областей, осуществляющих в целом на церебральном уровне висцеральный анализ и висцеральные регуляции.

THE ROLE OF BASAL FOREBRAIN STRUCTURES IN THE AVOIDING CONDITIONED REFLEX ACTIVITY IN RATS

By

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During the past decade many attempts have been made at the clarification of elementary processes in the organization of temporary connections, such as the early manifestations of conditioning, orienting reaction, habituation. Among these the orienting reaction, as the first step of the learning process, is an early manifestation of temporary connections. It has a close relationship with the function of consciousness, with the discrimination of environmental stimuli and motivation.

The present report deals with some features of the functional and neuroanatomical organization of the conditioned reflex.

The experiments were performed on adult albino rats. The animals were trained to jump onto a bench in a training box measuring 27 cm by 35 cm to avoid the electric shock given through a grid on the floor when the sound of a bell was presented as the positive signal. Under similar experimental conditions a plexiglass maze was also applied. In this experimental situation the animals had the possibility to approach the bench through several different routes. Bilateral electrocoagulation of subcortical structures was made by means of a stereotaxic apparatus. The methods used in these investigations have been published previously [1].

In both the "free" and the "maze" situations the first step was the learning of the somatomotor pattern. In the "free" experimental situation the rats always followed the same routes and jumped onto the bench at the sites experienced during the first trials. The learning of the somatomotor pattern in the "maze" system was similar, the animals took the same route of escape during the consecutive trials. Development of the somatomotor pattern preceded the stabilization of the temporary connection *(Fig. 1)*. These findings agree well with those of ENDRŐCZI and LISSÁK (see the paper of E. ENDRŐCZI in this issue).

The question of somatomotor memorization in the development of temporary connections was answered in the following way: the conditioned reflex responses mentioned before were extinguished in non-reinforced trials and after a week the rats were reconditioned in the same experimental situation. The somatomotor pattern concerning the route performance of the re-establish-

ed conditioned reflex reactions was then studied. It was observed that the escaping routes and the places of jumping onto the bench resembled closely in the first as well as in the second conditioned reflex activity. During the extinction only the temporary connection could be inhibited, but part of the conditioned reflex activity imprinted into the brain mechanism in the form of somatomotor memory was preserved *(Fig. 2)*.

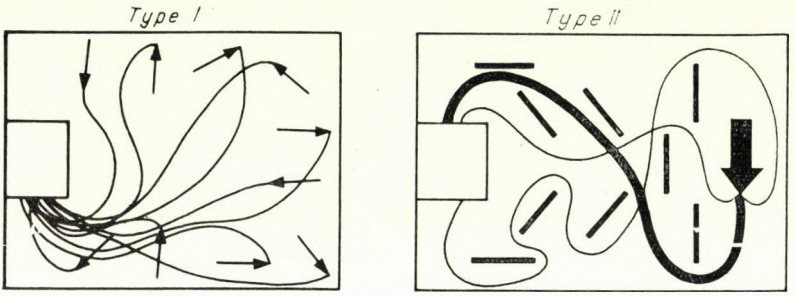

Fig. 1. Spatial orientation and escaping routes in "free" (type I) and in "maze" (type II) experimental situation in the course of avoiding conditioned reflex activity

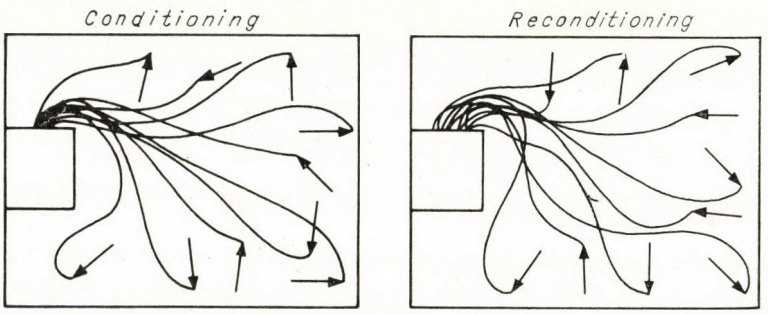

Fig. 2. Spatial orientation and escaping routes in a "free" experimental situation during conditioning and reconditioning

The long-term memory of a conditioned reflex as a somatomotor pattern should not be regarded as due to the primary functions of sensory analysers. According to the observations of AIRAPETIANZ [2], the sensory analysers are responsible for the integration of spatial orientation in the development of conditioning, but their removal cannot influence the somatomotor memory following the stabilization of temporary connections. On the other hand, on the basis of the above mentioned experimental data, a rapid setting up of the conditioned reflex during reconditioning is the result of the function of somatomotor memory.

In the next experimental series we studied the role of the diencephalon and striopallidum in the organization of temporary connections in the rat. Recent findings have shown that the septal area, the striopallidal system and

the medial forebrain bundle are forming a complete functional unit with the non-specific ascending diffuse activating system of the brain stem [3]. The meso-diencephalic activating system plays an important role not only in the maintenance of consciousness, but also in the discriminating processes related to conditioned reflex behaviour. On destroying these structures the conditioned reflex was abolished; at the same time a marked impairment of the self-preservative functions occurred.

It has been pointed out by FULTON and INGRAHAM [4], KING [5], BRADY and NAUTA [6], KAADA [7], and other authors that lesioning the forebrain structures resulted in a serious change of behaviour.

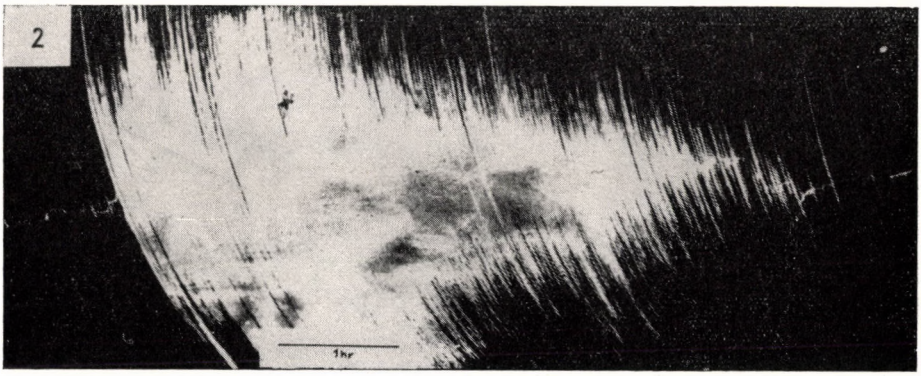

Fig. 3. Typical actogram following bilateral lesion of the medial forebrain bundle

An extreme increase in spontaneous somatomotor activity featuring orienting and searching characteristics was observed in our experiments after having lesioned the basal septal area, *Broca*'s diagonal bundle and the medial forebrain bundle. The hypermotility of several hours duration was followed by deep somnolence. However, the animals did not show any goal-directed or adequate reactions to the environmental stimuli but displayed a great number of typical orienting and searching movements in the absence of environmental signal. Lesions destroying the rostral septal region including the descending fornices did not result in similar behavioural changes *(Figs 3, 4)*.

To explain the described behavioural changes we have assumed that the basal forebrain area, as a part of the meso-diencephalic activating system, has an important role in decoding and transferring the meaning of environmental signals into adequate reactions. Under normal circumstances the impulses of specific afferentation arriving *via* the sensory analysers, reach and activate this decoding system. In association with cortical structures, the system decodes the signals important for the animal, and integrates co-ordinated, goal-directed reactions in behaviour.

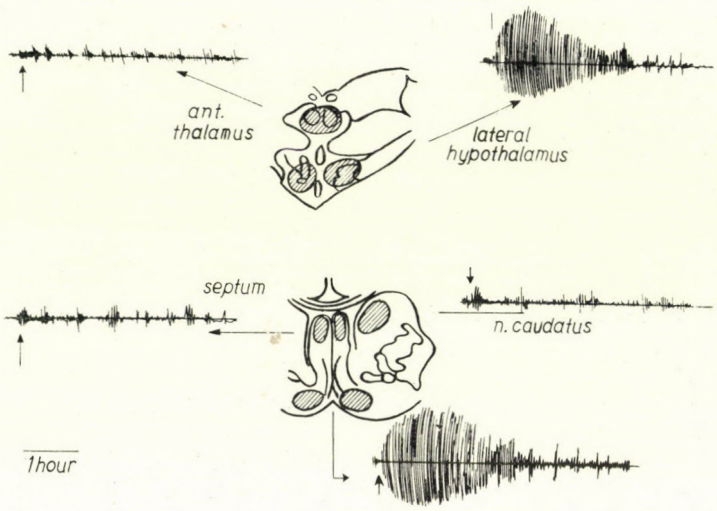

Fig. 4. Actograms following electrolytic lesions of different brain areas

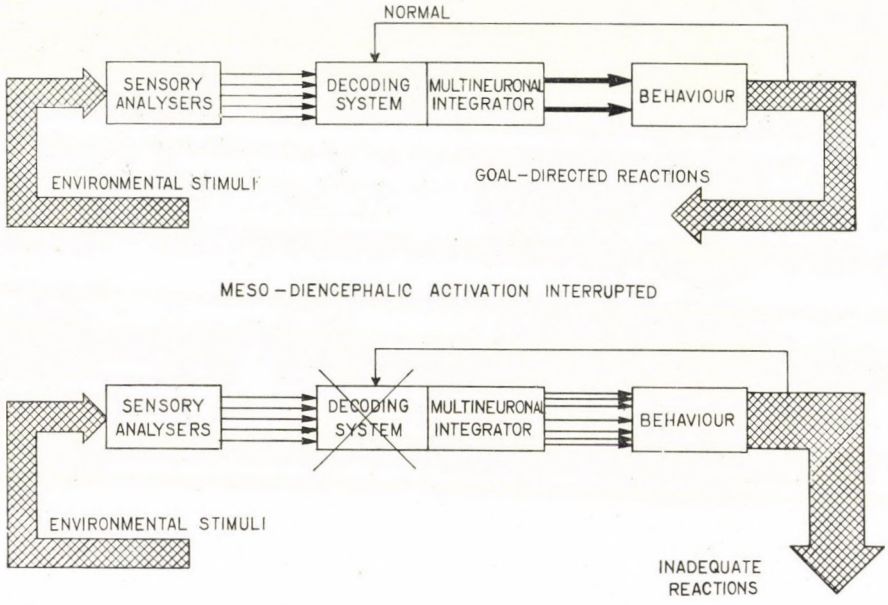

Fig. 5. Schematic presentation of animal behaviour concerning the decoding in normal animal and following the interruption of meso-diencephalic activating system (for details, see text)

Thus an interruption of the meso-diencephalic activating system seems to result in an impairment of decoding and the animal loses its ability to "understand" the biological meaning of environmental stimuli. The decoding inability results in sequences of increased orienting and searching reactions, because of the uncontrolled, undiscriminated actions of various, biologically neutral

stimuli *(Fig. 5)*. Further evidence of this assumption could be found in experiments where the area below the anterior commissure and/or the medial forebrain bundle was destroyed and the conditioned reflex performance was completely abolished. Similar behavioural changes did not ensue following lesions to the rostral septum. This means that the descending fornices or rhinencephalic connections running at the level of the rostral septal area have no importance in the phenomenon. The same impairment of conditioned reflex activity as described before, could be observed following the destruction of the striopallidal system, if the lesions had reached the inferior thalamic peduncle and the lenticular ansa. In contrast with basal septal destructions, no spontaneous hypermotility followed these operations; the animals were somnolent and there was a disturbance in the self-preservative functions (feeding, drinking, avoidance, *etc.)*.

Concerning the localization of the so-called "feeding centre" in the area where our lesions had been placed, MORGANE [8] has recently reported that lesions to the globi pallidi and/or the medial forebrain bundle acted by destroying pathways rather than centres. Such lesions led to catastrophic impairments; the animals lost their sense of self-preservation and died in spite of the fact that they were fed artificially. In our experiments frontal leucotomy, bilateral lesions of the caudate nuclei, nucleus ventralis posterolateralis thalami, amygdala, and the amygdalo-hypothalamic fibres did not result in similar behavioural changes.

Summarizing the results, the somatomotor memory which develops in the early stage of the learning process plays an important role in the organization of temporary connections. The neuroanatomical basis of this phenomenon is hardly understood. Lesions to the forebrain structures, *e.g.* basal septal area, medial forebrain bundle and the thalamo-strio-pallidal connections result in a disturbance of perception of environmental stimuli and hereby abolish the temporary connections. This means that these structures take part in a decoding of environmental stimuli by means of discrimination and have a decisive role in the development and maintenance of facilitatory and inhibitory processes between the cortical and subcortical structures.

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THE ROLE OF THE MESO-DIENCEPHALIC ACTIVATING SYSTEM IN EEG AND BEHAVIOURAL AROUSAL, MOTIVATION AND CONDITIONED REFLEX PROCESSES

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Abundant evidence accumulated over the last fifteen years has shown that the non-specific activating system of the brain stem plays an important role in the integration of behavioural and EEG arousal reactions (Moruzzi and Magoun 1949; Lindsley, Bowden and Magoun 1949). Since these fundamental discoveries numerous data have pointed to the part of the mesencephalic reticular formation and the midline thalamic nuclei in the maintenance of the conscious state, the development of the orienting reaction, emotional behaviour, and the formation of temporary connections (Morrell 1957, 1958, 1960; Roitbak 1960; Livanov 1960; Anokhin 1960; Kreindler 1960; *etc.*). Numerous observations in the past decade have confirmed the complex involvement of the limbic structures in the control of behavioural processes, and the former idea of mosaic-like representations of the subcortical structures in these events has been replaced by a more dynamic view, which considers higher nervous activity in its evolution and as the reflection of environmental changes in the biosphere.

In our former experiments it was found that stimulation of certain points in the mesencephalic reticular formation, the central grey matter as well as in the diencephalon elicited the conditioned reflex reactions in the absence of a positive conditional signal. Electrical stimulation of the brain stem always facilitated those conditioned reflex reactions which appeared in dominant way in a given environmental situation (Endrőczi, Lissák, Yang and Medgyesi 1958, 1959). The elicitation of a conditioned reflex response by the electrical stimulation of some of the subcortical structures may be considered to be due to an artificial driving force, which is the principal element of motivation. In connection with such intrinsic tendencies of brain function we pointed out a few years ago that the spontaneous goal-directed motor responses observed during the interval between two consecutive conditional signals may be regarded as the somatic manifestation of drive and an objective index of the intensity of motivation. The above mentioned experiments yielded some evidence that the reduction of driving force in the course of the development of conditioned reflex processes could be considered a consequence of the discriminative inhibi-

tory processes and that the rostral forebrain structures had an important role in these events (ENDRŐCZI and LISSÁK 1961). The present paper deals with the role of the meso-diencephalic activating system in the integration of EEG and behavioural arousal, attentive behaviour and habituation.

Methods

The observations were carried out on 45 cats and 40 albino rats. Implantation of silver ball cortical, and stainless steel depth-electrodes in cats was performed by means of a stereotaxic apparatus under pentobarbital anaesthesia. Enamel-insulated bipolar electrodes were fixed to the calvary by means of acrylate and the leads were soldered to the socket of a miniature radio-tube. All details of the implantation techniques used in this experiment have been described earlier (ENDRŐCZI and LISSÁK 1962; ENDRŐCZI, KORÁNYI, LISSÁK and HARTMAN 1963). For the time of observation the cats were placed in a sound-proof and electrically insulated room and the sound stimuli were administered through a loudspeaker. Recording of electrical activity was done by an 8-channel electroencephalograph and stimulation of the subcortical structures was performed by means of an impulse generator having independent controls of frequency, intensity and impulse duration. Bilateral electrocoagulations of the subcortical structures were made stereotaxically by means of 3 mA anodal polarization lasting for 10 seconds.

In these conditioned reflex experiments the cats were trained to jump onto a bench to obtain food when the light of a 15 W bulb above the feeder was switched on. The compartment, measuring approximately 4 m^2, contained three similar feeders, spaced at a distance of 45 cm, and each feeder was supplied with a light signal of its own. The conditional signal was presented at intervals of 2 minutes, each time for 10 seconds. The animals jumping onto the bench spontaneously during the intersignal periods were permitted to stay there for 15 seconds and then were pushed off with a special device. As an unconditional stimulus, a piece of fresh meat was used for reinforcement and ten trials were given in every session.

Behavioural processes related to the avoiding conditioned reflex were studied in 40 rats. The animals, staying in a box measuring approximately 40 cm by 25 cm, could avoid the painful electric shock given through the grill on the floor by jumping onto a bench placed at a height of 15 cm. A bell sound given for 10 seconds at 2 minute intervals served as the conditional signal. The electric shock of short duration was administered during the last three seconds of the conditional signal. In each session ten trials were given. In both the alimentary and the avoiding conditioned reflex experiments extinction of the conditioned reflex was achieved by means of non-reinforced trials without any change in the experimental situation.

At the end of the experiments the brains were perfused with a stock solution of formol injected through the carotid artery and the placement of electrodes or lesions was checked in frozen sections.

Results

Periodic changes in resting EEG activity and behaviour

The resting EEG activity of a non-motivated cat placed in a sound-proof room showed periodic changes. Each period lasting for 5—8 minutes started with a desynchronization of cortical activity and the appearance of theta rhythm recorded from the dorsal hippocampus. The low voltage fast activity of the neocortex was gradually replaced by synchronized high voltage slow waves and instead of the regular theta rhythm of the hippocampus irregular slow waves of 2—3 cps could be observed. In the later phase of the period the highly synchronized EEG activity, showing spindles even in the records of different subcortical structures (septum, midline thalamic nuclei, caudate nucleus), suddenly turned into a desynchronized state as seen at the

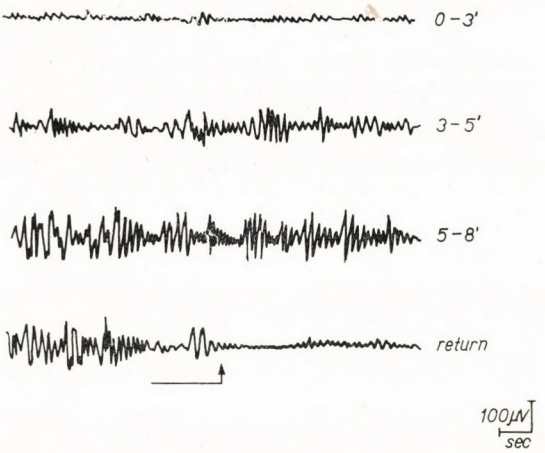

Fig. 1. Periodical change in neocortical electrical activity of a cat in non-motivated state. A period lasts approximately 8—10 minutes, the arrow shows the beginning of a new desynchronized period

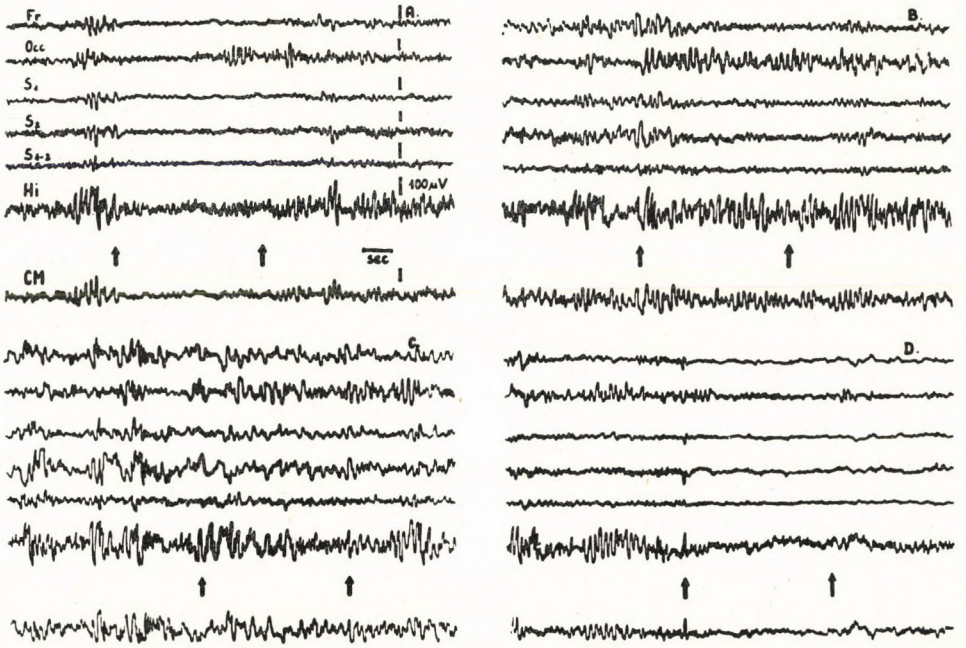

Fig. 2. The EEG desynchronizing activity of a sound stimulus during the development of habituation. Records from neocortex, septal region and hippocampus were taken in consecutive intervals *(A, B, C, D)* of a period shown in *Fig. 1.* The sound stimulus was lasting 8 seconds and given in 80 second intervals. The records demonstrate the electrical activity during administration of the 40th, 41st, 42nd and 43rd trials

beginning. The administration of a sound stimulus which was strong enough to attract the animal's attention blocked this periodicity and resulted in an EEG arousal of various duration.

The periodicity observed in resting EEG activity has raised the question

whether or not the attentive reactivity of the animal can display parallel changes. To answer this question the habituation of a novel stimulus was used, a bell sound or a tone of 700 cps which was given at intervals of 80 seconds each time. Approximately the first 20—30 trials resulted in an EEG and behavioural arousal lasting for 15—30 seconds. In each stage of the above-mentioned EEG periodicity a total of 12 cats was used. In the later phase of habituation, after the administration of 40—50 trials the test stimulus induced EEG arousal only in the first two thirds of each period but failed to influence the highly synchronized last third of it. In the later progressive stage of habituation the test stimulus showed slight desynchronization in the first third of each period only, and, finally, at the completion of habituation, usually after the administration of 200—250 trials, the sound induced the facilitation of high amplitude slow waves from the neocortex and the septal region in a proper phase of the period. These findings revealed that the animal's attentive reactivity cannot be regarded as the sole product of environmental changes, and that attentive reactivity has a characteristic intrinsic aspect which reflects the periodic changes of excitatory and inhibitory states within the central nervous system.

The administration of a differential signal, a tone of 400 cps or another bell sound, in the sequence of a repeatedly given but habituated test stimulus resulted not only in temporary desynchronization of EEG activity but produced a deshabituation to the test signal lasting throughout several trials. Similar deshabituation of a previously habituated sound signal could be observed in response to stimulation of the mesencephalic reticular formation for 15 seconds, which elicited a moderate temporary desynchronization of the cortical EEG activity. Concerning the resting EEG activity in the intersignal periods of the deshabituation stage, it could be assumed that the subcortical structures have a property of maintaining the latent excitatory states caused by the environmental changes or electrical stimulation of the brain stem reticular core. Such a latent excitatory state as mentioned before may be considered to be due to such postsynaptic excitatory processes within the cortical structures which the macroelectrode technique has failed to reveal in these investigations. Nevertheless, the mesodiencephalic activating system, as a multineuronal relay of afferentation between the environment and the cortex, may also be regarded as the site of these events.

The role of basal forebrain structures in EEG and behavioural arousal and learning processes

Bilateral lesions in the basal septum and/or in the anterolateral hypothalamus, destroying the lateral preoptic area and the medial forebrain bundle resulted in a lack of EEG response to different environmental stimuli. The cortical EEG records of such animals displayed high voltage slow waves often

accompanied by a state of hypersomnolence. Acoustic stimuli or painful electric stimulation of the hind legs did not change cortical EEG activity and failed to elicit attentive behaviour. Destruction of the basal forebrain structures produced not only a marked impairment of EEG arousal and attentive

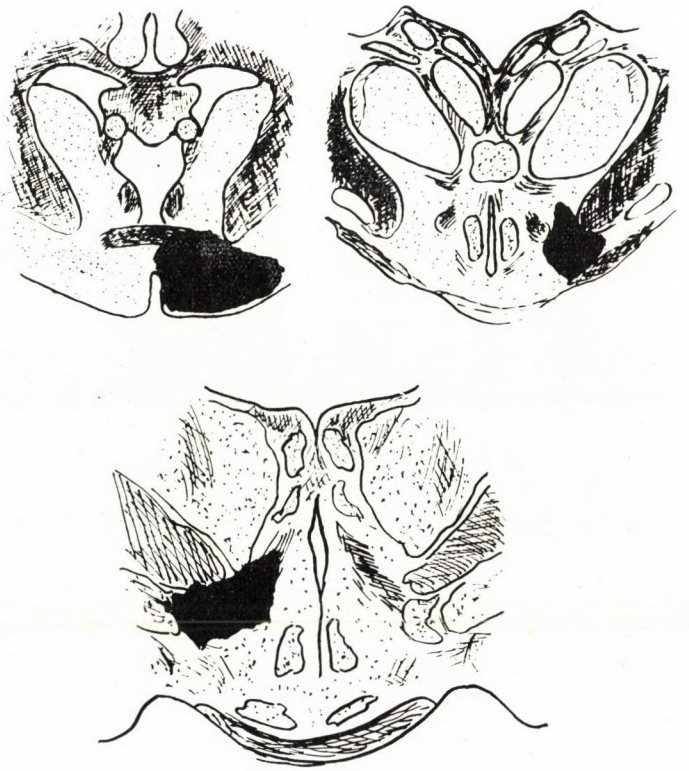

Fig. 3. Schematic demonstration of lesions located in the basal septal and subcommissural area (upper left), lateral preoptic region (upper right) and in the lateral subthalamic area (center). All lesions were made bilaterally

behaviour but also resulted in a complete lack of self-preservative functions. Because of the impaired food intake, such animals had a survival time of approximately a week. Some of them walked round the cage without avoiding harmful situations, but did not show any failure of co-ordinated locomotor activity. A similar impairment of the EEG and behavioural arousal could be observed when the lesions destroyed the ventromedial part of the globus pallidus including the inferior peduncle of the thalamus. There was no change related to the EEG and behavioural arousal reactions in cats bearing extensive bilateral destructions of the rostral septum, the amygdaloid complex of nuclei or nucleus of the ventrolateral part of the thalamus.

Concerning the lack of behavioural and EEG arousal seen in the animals following destruction of the basal forebrain structures, the general view is that they cannot decode the biological meaning of environmental changes. In addition to the classic non-specific diffuse activating system of the brain stem our

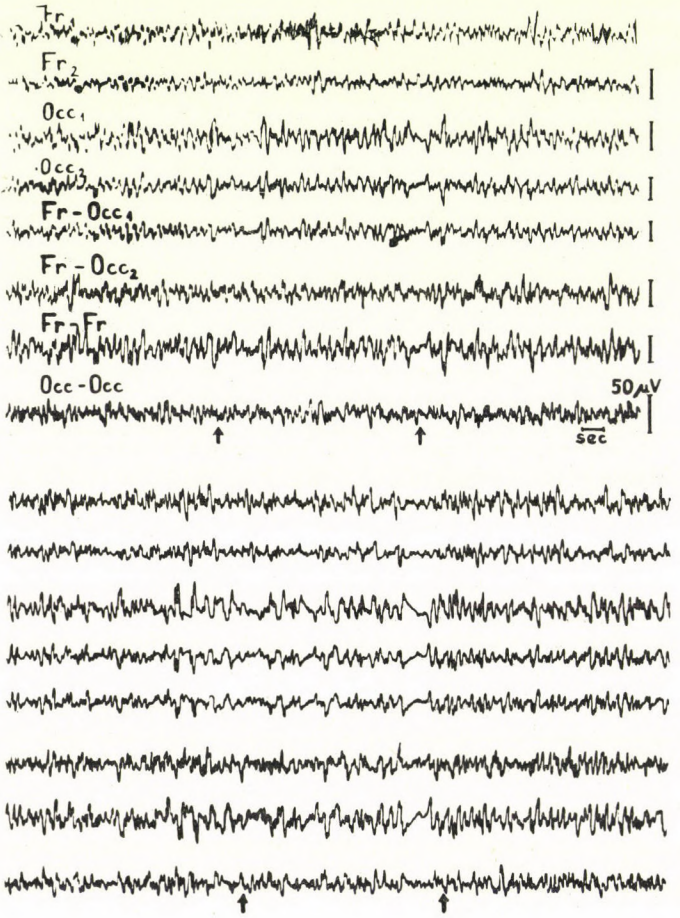

Fig. 4. Shows the absence of EEG arousal reaction during the administration of painful stimuli applied to the hind legs after the electrolytic destruction of antero-lateral preoptic region and medial forebrain bundle (upper records) and that of lateral subthalamic projections. Stimuli were given between the two arrows. Records were taken 4 days after surgical intervention

experimental results suggest that certain basal forebrain areas and the subthalamic strio-pallidal connections have also an important role in these events. *Fig. 5* shows schematically the neuroanatomical structures involved in the non-specific activation of the neocortex and the archicortex, caused by environmental changes. According to our assumption, afferent impulses arriving from the specific pathways induce an excitatory state of the meso-diencephalic

activating circuit, the frame of which is formed by the mesencephalic reticular formation, the thalamic midline nuclei — the strio-pallidal connections *via* the inferior peduncle of the thalamus — the basal septal region including *Broca*'s diagonal band — the medial forebrain bundle — and finally, to close the activating circuit, again the brainstem reticular core. The excitatory state of the meso-diencephalic activating circuit, assumed by us on the basis of the

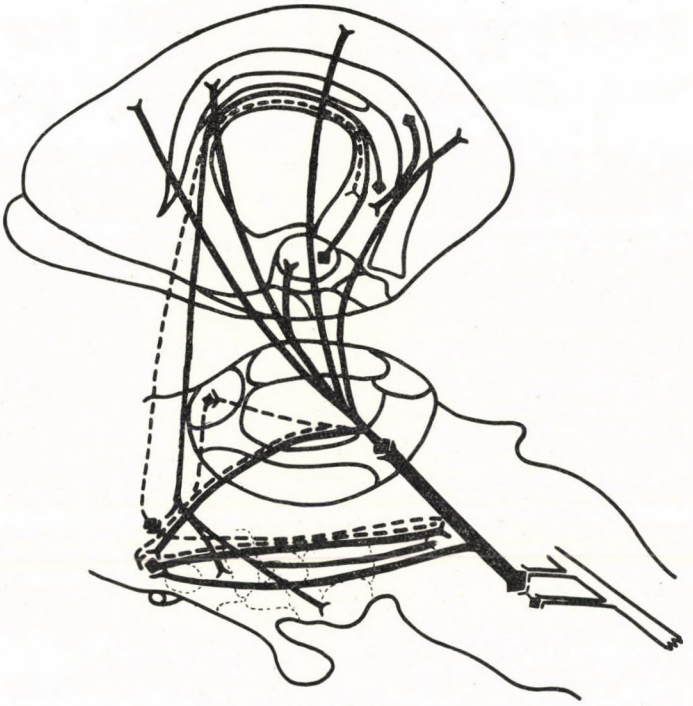

Fig. 5. Schematic representation of main connections involved in formation of meso-diencephalic activating system and its relations with forebrain structures

mentioned experimental findings, has its most rostral closure at the level of the basal septal region, but a large number of posterior closures within this circuit predisposes it to discrete participation in the integration of a wide scale of somatic and visceral processes as well as in the activation of cortical arousal. The ample connections between such an assumed activating circuit and the limbic structures allow for extensive facilitatory and inhibitory influences in both directions. On the other hand, there is no doubt that the neocortex can inhibit or facilitate this activating system through its ample corticofugal and corticopetal projections.

The assumption that cortical EEG arousal requires an excitatory stage of the whole meso-diencephalic activating circuit involving the rostral forebrain

structures, has been confirmed in further experiments, with stimulation of the mesencephalic reticular formation in cats bearing extensive lesions in the basal septal area and the medial forebrain bundle. Stimulation (5—15 V, 0.5 msec, 60 Hz) of the reticular formation failed to induce EEG or behavioural arousal reactions in the lesioned cats. The animals, however, responded to electric stimulation with somatic manifestations. This observation shows that interruption of the meso-diencephalic circuit, even at points other than the brainstem reticular core, prevents the development of an excitatory state which is fundamental to the integrated EEG and behavioural arousal of the neocortex and the archicortex.

The role of the meso-diencephalic activating system in drive reduction and in the organization of internal inhibition of conditioned reflex activity

There is a feature common to all kinds of conditioned reflex activity, manifesting itself with locomotor performance, namely that the animals show a number of spontaneous goal-directed motor response (a spontaneous "conditioned response" in the absence of a positive signal and reinforcement) in the intersignal intervals. In alimentary conditioned reflex experiments on cats we studied the characteristics of spontaneous goal-directed motor activity and arrived at the conclusion that such a motor response corresponds to the somatic manifestation of a driving force in a given conditioned reflex situation. The number of spontaneous motor responses during the acquisition of a conditioned reflex is fairly high and is gradually reduced in the course of stabilization. Without going into the details of spontaneous goal-directed motor activity described in detail elsewhere (ENDRŐCZI and LISSÁK 1961; ENDRŐCZI 1962), our findings may be summarized as follows.

1. During the establishment of a conditioned reflex the number of spontaneous motor responses decreased. Such a drive reduction may be regarded as being due to discriminative internal inhibition. In the absence of a differentiating signal in the stereotype, the number of spontaneous motor responses never dropped to zero level but the introduction of a differential signal, requiring a higher intensity of discriminative inhibitory processes, resulted in a complete abolition of spontaneous goal-directed responses.

2. During the extinction of conditioned reflex activity in cases where spontaneous motor activity has a constant base level in a highly stabilized stage of conditioning, its number is reduced long before the conditioned reflex has been suppressed. The preceding reduction of spontaneous motor activity during the extinction suggests that internal inhibitory processes are brought into play primarily by the elimination of drive and are followed by the inhibition of conditioned reflex activity.

3. Spontaneous goal-directed motor activity takes part in the memorization of the somatomotor pattern, which plays a fundamental role in the early phase of learning processes. (For details of this aspect of spontaneous goal-directed motor activity the reader is referred to the paper by L. KORÁNYI in this issue.)

The neuroanatomical basis of goal-directed motor activity in cats was made the subject of a study in alimentary conditioned reflex circumstances. Bipolar electrical stimulation with parameters (0.2—05 V, 0.5 msec, 12—30 Hz),

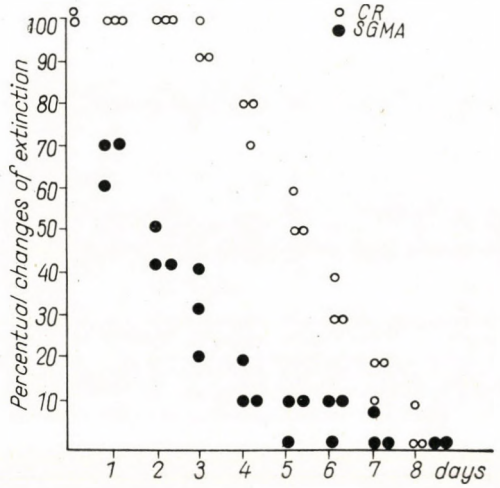

Fig. 6. Extinction of conditioned responses and spontaneous goal-directed motor activity in alimentary conditioned reflex situation in cats

which according to electrode placement generally induced moderate but obvious changes in attentive behaviour or orienting reactions elicited the inhibition or facilitation of spontaneous goal-directed motor activity, without any impairment of the conditioned reflex performance. *Fig. 7* shows that stimulation of the basal septal area below the anterior commissure, the anterolateral part of the hypothalamus including the medial forebrain bundle, resulted in the depression of spontaneous motor responses while stimulation of the posterolateral part of the hypothalamus, the ventrotegmental area, the midline thalamic nuclei and the mesencephalic reticular formation produced an increase in spontaneous goal-directed motor activity. Such facilitation could be observed even during extinction, in a period when the positive signal failed to elicit conditioned responses in most of the non-reinforced trials. In these cases the electric stimulation of positive points for 15—30 seconds not only increased the spontaneous intersignal activity but also restored the effectiveness of the positive signal by inducing conditioned responses throughout several trials.

The depression of spontaneous goal-directed motor activity by stimulation of the rostral forebrain structures raised the question of the physiological meaning of this phenomenon. It is known from the literature that low frequency stimulation of some of the diencephalic structures including the rostral fore-

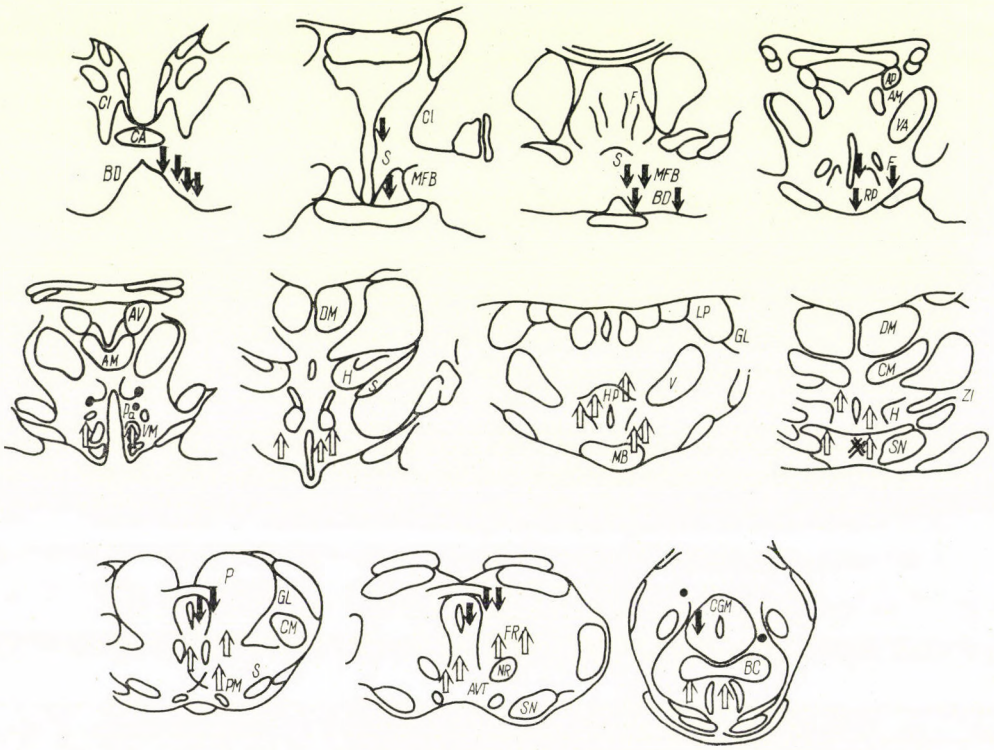

Fig. 7. Facilitatory and inhibitory influence of electrical stimulation of the diencephalic and mesencephalic structures on spontaneous goal-directed motor activity in alimentary conditioned reflex situation in cats. Arrows correspond to the sites of electrode placements and show the inhibiton or facilitation of activity (↓ resp. ↑)

brain area results in a sleep-like condition (HESS, 1949; STERMAN, CLEMENTE 1961; HERNANDEZ-PÉON 1961).

Several authors have pointed to the role of the rostral forebrain structures in the organization of sleep and wakefulness, showing that the stimulation of these structures resulted in synchronized cortical electrical activity (STERMAN, CLEMENTE, WYRWICKA 1963). From the above mentioned experiments we came to the conclusion that virtually different behavioural phenomena elicited by stimulation of the rostral forebrain structures (the basal septal and the preoptic region) might be due to the different manifestations of internal inhibition. It was found that habituation of a novel sound stimulus in sound-proof circumstances was significantly enhanced by stimulation of the basal septal area.

Electrical stimulation of this region elicited high voltage slow waves in cortical EEG activity and irregular slow waves in the dorsal hippocampal records. The animal became drowsy under the influence of electrical stimulation and the repeated administration of the test stimulus (a bell sound or a tone of 700 cps). In contrast to the control experiments where the test stimulus became habituated following the administration of 200—250 trials, its combination with the electrical stimulation of the rostral forebrain area led to an enhanced habituation completed after the presentation of 30—40 trials. The attentive reactivity of the animals did not change under the influence of electrical stimulation of the rostral forebrain area, whereas the administration of a differential sound, though much weaker in intensity than the test stimulus applied for habituation, resulted in normal behavioural and EEG arousal. Some other manifestations of the electrical stimulation of the rostral forebrain structures seemed, however, to contradict the described findings. Following habituation of the test stimulus stimulation of that area for 30—60 seconds namely induced a temporary deshabituation of the test signal. The contradictions between the results obtained under different experimental circumstances by stimulation of the rostral forebrain structures may be explained as follows. In all types of experiments electrical stimulation of the rostral forebrain area enhanced internal inhibition; this manifested itself differently, depending on the experimental situation. Internal inhibition takes part in the decoding and habituation of external signals by means of discriminative internal inhibition. In conditioned reflex experiments the same process participates in drive-reduction and has an important role in the early phase of the learning mechanism. The assumption that the rostral forebrain area might be a particular part of the subcortical structures triggering behavioural and EEG sleep, as several authors have concluded from similar experimental findings, seems to be the consequence of an enhanced habituation in a "sensory deprived" situation. Concerning the deshabituating action of the electrical stimulation of the rostral forebrain area in habituated states, we may assume such phenomena to be due to the rebound effect of the same mechanisms. Such a "rebound-like" manifestation which appears after a relatively short-term stimulation of the rostral forebrain structures and causes temporary deshabituation of a habituated test stimulus, could be regarded as an operating factor in self-stimulation (OLDS 1961, BRADLEY 1959). Self-stimulation may be regarded as the consequence of an unfinished discriminative action: the electrical stimulation for a relatively short duration activates those structures which play a fundamental role in decoding of information content of the environmental stimuli but such intracerebral stimulus does not supply the neural net with biological information. Such failure of information content in the stimulus given intracerebrally results in a behavioural rebound action which aims at obtaining information from the environment where the intracerebral stimulation had been performed. This rebound action

manifests itself by the same motor pattern which evoked the intracerebral stimulus. Such an interpretation of self-stimulation is closely related to our assumption that the meso-diencephalic activating circuit has a fundamental role not only in the integration of sleep and wakeful state but forms the basis of triggering mechanisms playing role in the discrimination of environmental signals. There is no doubt, the brain functions as mentioned before require the integrated co-operation of brain mechanisms at all levels, but we suppose the priority of the excitatory state of meso-diencephalic activating system in these events. If we consider that this activating machinery is implicated in a great amount of feed-back control developed in the course of evolution, we can understand how it works highly selectively as a scanning device in decoding of environmental signals and to encode the cortical closures for creation of adequate biological reactions.

ELEMENTARY TEMPORARY CONNECTION IN THE MESENCEPHALIC CAT

By

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It has been attempted to set up temporary connection over a few days following total transection at the level of the superior colliculus (high decerebration) in cats. The respiratory changes evoked by the direct electrical stimulation of the central vagal stump were reinforced by stimulation of the pelvic nerve. Before training, pelvic stimulation alone caused no change in the amplitude and frequence of breathing. After 100 reinforcements the amplitude and frequency of respiration had changed in response to pelvic stimulation alone, *i.e.* a conditioned respiratory change had developed. After 200 to 300 trainings this reaction became established and persisted for as long as 24 hours. The phenomenon may be considered to be an elementary form of temporary connection inducible at the mesencephalic level.

On the basis of PAVLOV's fundamental work [1] it has been accepted that in higher animals the learned reflexes are formed in the cerebral cortex. Meanwhile, since ZELYONY's paper [2], many workers have been studying the role of subcortical structures, and attempts have been made to set up motor conditioned reflexes in spinal cats and dogs [3, 4, 5, 6, 7, 8]. The conditioned stimulus was a tactile or electrical stimulation of the skin, the unconditioned one was the electrical stimulation of the leg, to which the animals responded by a defensive reaction. The reflex responses induced by such trainings were considered by SHURRAGER *et al.* [8] to be conditioned reflexes. Other authors, however, deny that spinal conditioned reflexes could be set up, claiming that such responses were transient, atypical phenomena, belonging to the so-called "summation" reflexes, that can be elicited by any afferent stimulus, in sharp contrast to the conditioned reflexes, which are chronic, gradually established characteristically cortical phenomena.

There are few data in the literature concerning conditioned reflexes connected subcortically above the spinal level, thus at the medullar, mesencephalic or diencephalic level. There is some information concerning conditioning in decorticated animals. In such animals, according to some authors [9, 10], no real conditioned reflex can be set up whereas others [11, 12] claim that temporary connections can be established which do not essentially differ from the conditioned reflex activities of intact animals.

In the present investigations we have endeavoured to determine whether the conditioned reflexes might be formed without the involvement of the tel-

encephalon and diencephalon at the brain stem (mesencephalic) level. We have therefore studied the possibilities of setting up temporary connection in mesencephalic animals.

Methods

Under ether—hexobarbital anaesthesia, mesencephalic transection was performed in 30 adult cats, by the following technique. After opening the skull, bilateral occipital lobectomy was done, exposing thereby the corpora quadrigemina, then the brain stem was divided at the level of the colliculus superior, using a high-frequency electrocoagulator. The wound was closed, and the animal placed into a thermo-cage, to keep body temperature at a constant

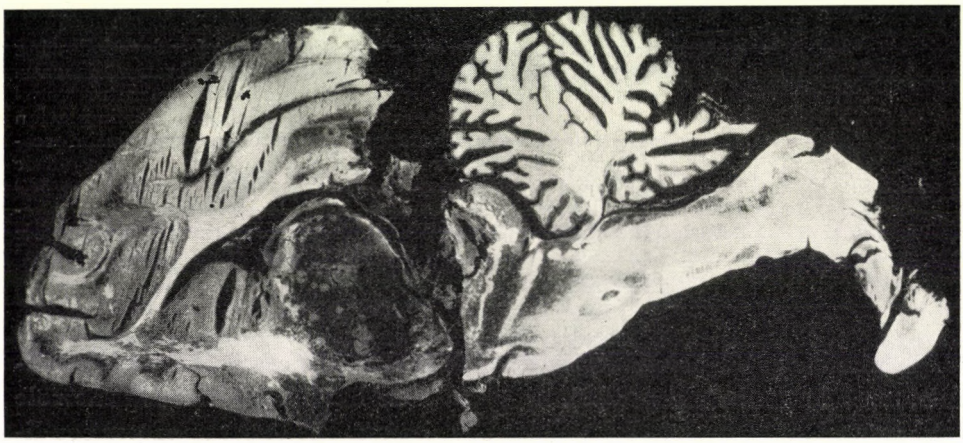

Fig. 1. Sagittal histological section. The brain stem has been cut completely at the level of the colliculus superior

level. By adequate treatment (vitamins, antibiotics, proper feeding, fluid administration) the mesencephalic preparations could be kept alive for a maximum of 9 days. After death the completeness of the transection was checked histologically (*Fig. 1*).

The experiments were grouped as follows.

1. In 11 cats we started respiratory reflex conditioning on the day of the operation, *Sherrington* electrodes were placed on the central stumps of the cervical vagus and the pelvic nerve, and electrical stimulation (1 to 2 V, 50 Hz, 1 to 30 sec) of these afferent nerves was applied. The unconditioned stimulus was the stimulation of the central vagal stump, the unconditioned reflex response (the changes in the amplitude or/and frequency of respiration) was recorded kymographically. The conditioned stimulus was the stimulation of the pelvic nerve, that by itself caused no change in respiration. The interval between trainings lasted 1 minute; in one experiment 100 to 200 reinforcements were applied. When evaluating the results, amplitude and frequency of the respiratory response were equally taken into account. The size of the responses was expressed in percentage of the spontaneous respiratory amplitude and frequency changes $\left(\frac{dA \times 100}{A} = V_a\right.$, where dA = change of amplitude in mm, A = normal amplitude, in mm, V_a = change in amplitude in response to vagal stimulation), and the arithmetic means were represented graphically.

2. By the method described above, we built up temporary connection in 12 animals with intact nervous system, then we transected the brain stem and attempted to elicit the pre-operatively set up conditioned reflex by pelvic stimulation alone, without reinforcement.

3. In 7 mesencephalic animals a motor defensive reflex was conditioned 2 or 3 days after transecting the brain stem. The conditioned stimulus was the stretching of the gastric or rectal wall by means of an inflatable rubber balloon, and the unconditioned stimulus the electri-

cal stimulation of the forelimb (1 to 3 V, 50 Hz, for 1 to 2 sec), to which the animal responded by a defensive leg movement. In each experiment we applied 50 to 100 reinforcements in intervals of 1 minute.

Results

1. Conditioned respiratory changes in mesencephalic animals

A temporary connection was set up in 11 mesencephalic animals. Before training, presentation of the pelvic stimulus alone had no influence on respiration. When this indifferent stimulus was presented together with vagal stimula-

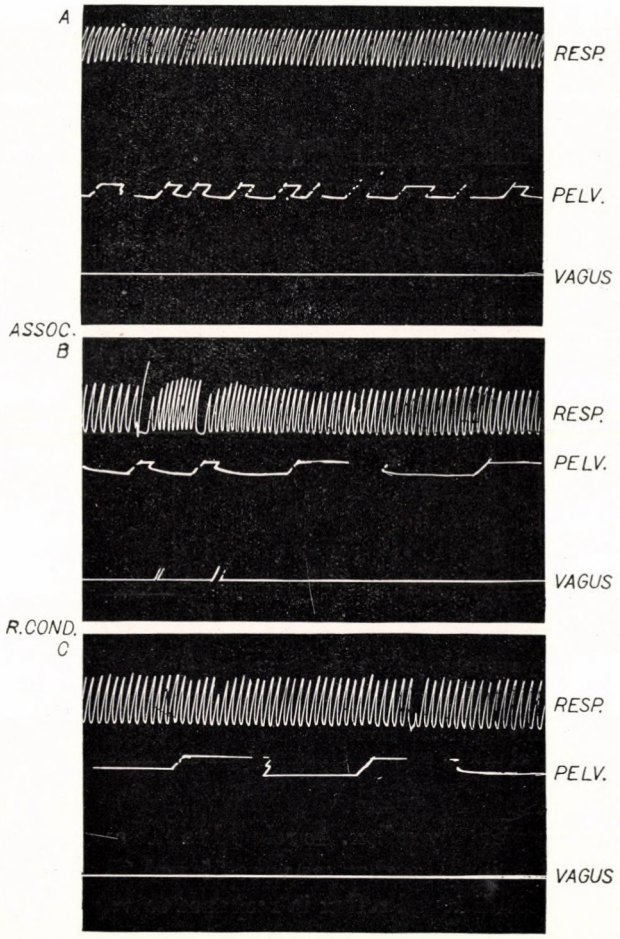

Fig. 2. Setting up of the conditioned reflex. In downward order: spontaneous respiratory movements, duration of pelvic stimulation, duration of vagal stimulation. *A :* the pelvic stimulation before reinforcements had no influence on respiration. *B :* on the left side in 2 cases unconditioned vagal apnoea, to further two isolated pelvic stimuli (41st and 42nd) spontaneous respiration does not yet change. *C :* conditioned apnoea in response to pelvic stimulations (120th and 121st)

tion, after an average of 100 such reinforcements the conditioned respiratory reflex response appeared: change in respiration was evoked by the pelvic stimulation in itself. *Fig. 2/C* shows the decrease of respiratory amplitude in response to pelvic stimulation alone, without vagal stimulation. The conditioned reflex response showed smaller changes in amplitude and frequency than

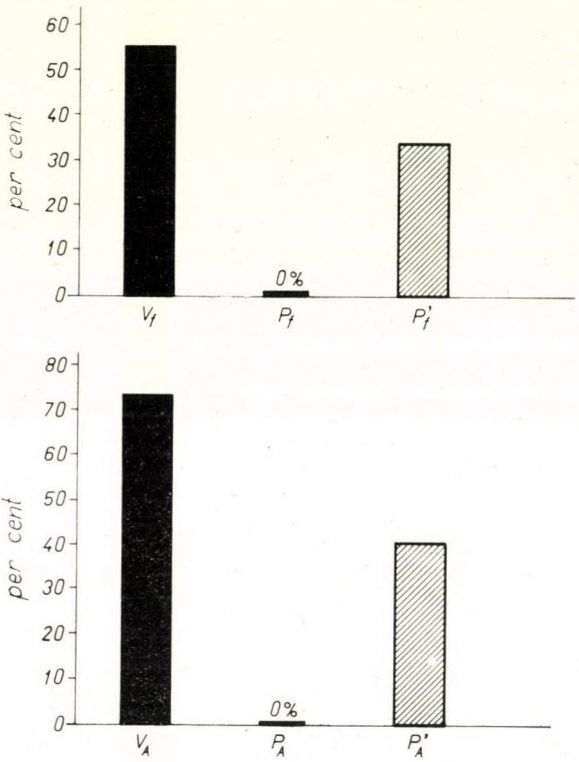

Fig. 3. Mean respiratory response values. Above: changes in frequency, in response to vagal stimulation (V_f) black column; in the middle 0 per cent (P_f) in response to pelvic stimulation before reinforcement, shaded column (P_f) in response to pelvic stimulation after reinforcements. Below: respiratory amplitude in the same sequence. (The arithmetic means were computed from a total of 613 data obtained from 11 experimental animals)

the unconditioned respiratory reflex. As related to the amplitude and frequency of spontaneous respiration, the changes were 55 and 73 per cent, respectively, in the case of the unconditioned reflex in response to vagal stimulation, and 34 and 40 per cent, respectively, in the case of pelvic stimulation after reinforcements *(Fig. 3)*.

In our experiments the conditioned respiratory reflex was built up gradually and became increasingly firmer. After the first trainings isolated pelvic stimulation did not alter spontaneous breathing, and it was not until after

about 100 trainings that the first conditioned change had appeared. This then disappeared, only to become established after 130 to 150 trainings, and reached 100 per cent after 210 to 220 trainings. The response proved to be durable: after an interval of 2 to 3 hours, then 24 hours, the pelvic stimulus by itself evoked changes, without vagal reinforcement.

Extinction could also be induced in these animals. The kymogram shown in *Fig. 4* represents a significant episode of conditioned respiratory change in

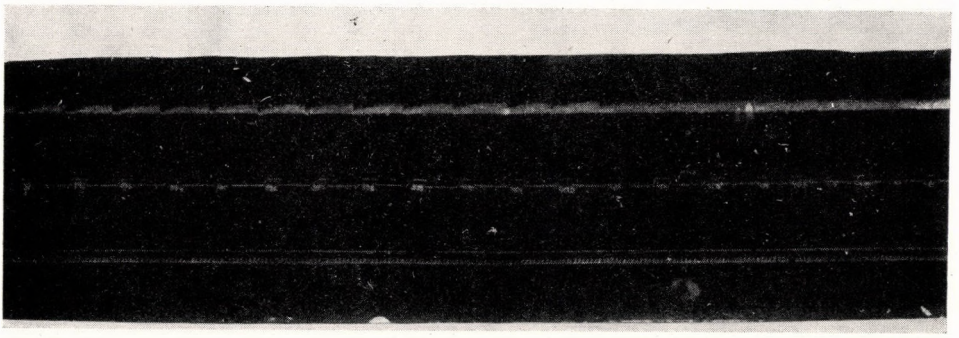

Fig. 4. Induction of extinctive inhibition. Signs as in *Fig. 2*

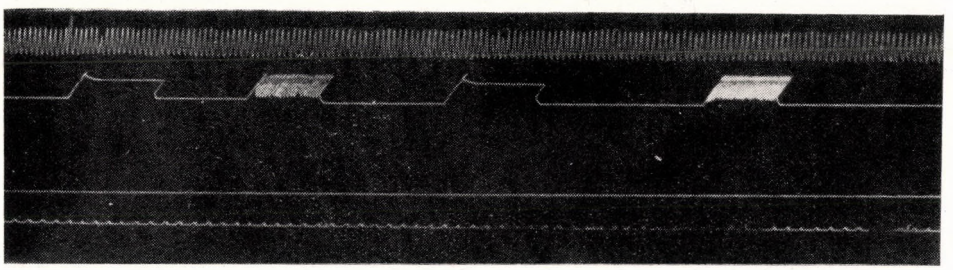

Fig. 5. Skin stimulation tests. Signs as in *Fig. 2.* (In the second row the high-frequency sign indicates the duration of dermal stimulation)

response to pelvic stimulation, that became gradually extinguished without reinforcements and disappeared after the 426th presentation of the stimulus.

Conditioning could not be elicited by applying other afferent stimuli, indifferent from the point of view of respiration. As *Fig. 5* shows, the amplitude decreased in response to isolated pelvic stimulation, while stimulation of the skin caused no change in respiration.

In the control experiments 5 animals were subjected to 100—500 pelvic stimulations, not reinforced by vagal stimulation. Spontaneous respiration did

not change even after pelvic tetanization, because no reinforcement had been applied.

The tests with pelvic tetanization and stimulation of the skin were merely done to check whether pelvic conditioning would result exclusively in the course of reinforcements, or, else, it would represent an atypical reflex response due to a hypersensitivity resulting from tetanization, based on the phenomenon of hysteriosis described by VEDENSKY.

2. Persistence of conditioned respiratory reflex after decerebration

The conditioned respiratory reflex set up in 12 intact cats could be elicited by the first pelvic stimulation and became gradually extinguished without reinforcements following mesencephalic section.

3. Attempts to set up a defensive motor conditioned reflex in mesencephalic animals

In 7 mesencephalic animals, combined stimulation (stretching the gastric wall + electrical stimulation of the forelimb) induced no marked defensive motor conditioned response. Although there was a marked flexor response to the unconditioned electrical stimulus, no similar motor response developed to the stretching of the gastric wall even after 300 trainings.

Discussion

In mesencephalic animals a readily recordable temporary connection can be set up in a short time by simultaneous stimulation of two afferent nerves carrying interoceptive impulses. As opposed to this, no conditioned reflex based on the motor defensive reflex is set up. Thus, the visceral conditioned reflexes may be formed also in the mesencephalic structures, without the participation of the cerebrum, and the conditioned respiratory reflex set up in an animal with intact cerebrum persists after the removal of the higher centres.

The temporary connection established in mesencephalic cats shows the following characteristic features.

(1) It is set up gradually in the course of reinforcements, and becomes more and more stable.

(2) Without reinforcement it is gradually extinguished, *i.e.* it is temporary in nature.

(3) It is relatively durable; it can be elicited even on the next day by the first conditioned stimulus.

(4) It cannot be elicited by the tetanization of a single nerve (pelvic nerve), or by some afferent stimuli which were not involved in the trainings, *i.e.* it is a special temporary connection.

It remains to be seen into which category of the temporary connections should be classified this respiratory learned response on the basis of the classical terminology. Is it a transient, so-called "summation" reflex, or a real conditional reflex? Our experiments did not answer this question, they have merely proved the possibility of conditioning at the mesencephalic level.

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ONTOGENETIC DEVELOPMENT OF TEMPORARY CONNECTION

By

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Paper not submitted

THE ROLE OF THE FRONTAL LOBE IN CONDITIONED REFLEX ACTIVITY

By

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The scheme of the formation of conditioned connections, the connections between the conditioned reflex and unconditioned reflex centres were outlined by PAVLOV 40 years ago. It is now well known that the centres are extensive structures, the development of nervous connections is controlled by facilitatory and inhibitory mechanisms, and that in the formation of conditioned reflexes cerebral cortical and subcortical centres are interacting. The mechanisms through which the temporary connections arise are, however, still not quite clear.

Evidence is also scarce as to the function of the frontal lobe. In phylogenesis it almost overgrows other areas of the cerebral cortex: its enormous rate of growth is one of the most outstanding properties of the human brain. As regards the functions of the premotor parts, apart from some isolated symptoms, only the "highest motor co-ordinator" role and the even more obscure "associative" character of the premotor area are known.

We have subjected to investigation the changes in the motor conditioned reflexes following the extirpation of certain parts of the premotor frontal cortex. We used dogs in the conditioned reflex cage, standing on the Pavlov stand. After presenting the sound stimulus (deep generator sound or buzzer) the right leg of the animal was placed on the feeder, then food was offered. After having repeated this several times, the animal spontaneously carried out the movement in response to the stimulus: a positive motor conditioned reflex had developed. Once this has become firmly established, the animals were not fed following the presentation of other sound stimuli such as a high pitched sound or the ringing of a bell 5 seconds before the presentation of the positive buzzer sound. After an adequate number of trials the motor reaction evoked by the initial generalization was not carried out: negative motor conditioned reflexes, differentiation and conditioned inhibition had developed. The whole course of the experiment was recorded kymographically. In this way both the reflex activity and the lack of reflexes could be recorded precisely.

When the dogs in 2 months had responded to 200 consecutive presentations of the positive stimulus by the conditioned leg reaction in at least 95 per

cent and to 100 negative stimuli they reacted in not more than 10 per cent, we removed a cerebral area by suction, under intravenous potentiated anaesthesia.

Our aim was to remove premotor areas without damaging the motor cortex. Therefore we had mapped this area in six dogs. While the stimulation of the lower part of the posterior sygmoid gyrus (to the line of the cruciate sulcus) invariably resulted in jerks of the contralateral leg, in agreement with the data in the literature the stimulation of the anterior sygmoid gyrus evoked merely adversive movements of the head and eyes. The damaged state of the motor area was proved also by the fact that we never found any sign of paresis. We made four kinds of extirpation:

I. Orally from the frontal plane laid across the precruciate sulcus to the base, leaving the olfactory bulb intact, the proreus (frontal) and the orbital gyri were removed together.

II. The cortex of the proreus (frontal) gyrus was sucked out in a depth of about 3 mm, leaving the orbital gyrus intact.

III. The lateral one-third of the anterior sygmoid gyrus was extirpated from the sagittal line drawn from the lateral end of the cruciate sulcus laterally to the coronary sulcus and backwards to the frontal line drawn about 1.5 mm before the end of the cruciate sulcus.

IV. The medial part of the anterior sygmoid sulcus was removed orally from the line 1.5 mm anterior to the cruciate sulcus to the precruciate sulcus, medially to the interhemispheral fissure, laterally to the line drawn sagittally from the end of the cruciate sulcus, leaving the lateral one-third of the anterior sygmoid gyrus intact.

Fig. 1 shows the effect of bilateral extirpation. In the 20 experiments carried out prior to operation the conditioned reflex response was given in almost 100 per cent of the cases to the positive conditioned stimuli, and only very few animals reacted to the negative stimuli. There was hardly any intersignal reaction.

Following the operation type I two of the dogs responded to the negative stimuli in the first 5 experiments by the leg reaction in 70 per cent of the cases, and the number of intersignal reactions increased significantly. Both these facts were indicative of an impairment of inhibition. During the next experiments desinhibition gradually ceased and the conditioned reflex activity corresponding to the character of the stimuli was restored. This type of extirpation did not influence the positive conditioned reflexes.

Before us, ALLEN and KONORSKY carried out the same operation with similar results. It was remarkable that after the operation type II, when we removed the cortex of the proreus (frontal) gyrus, *i.e.* about 8 times less nervous tissue than with the operation type I, the changes were absolutely similar to those obtained with the procedure type I. It seems that with the operation

type I, too, the damage to the inhibitions was due to the extirpation of the cortex of the proreus (frontal) gyrus.

The changes resulting from the operation type III (extirpation of the lateral one-third of the anterior sigmoid gyrus) were just the opposite of those

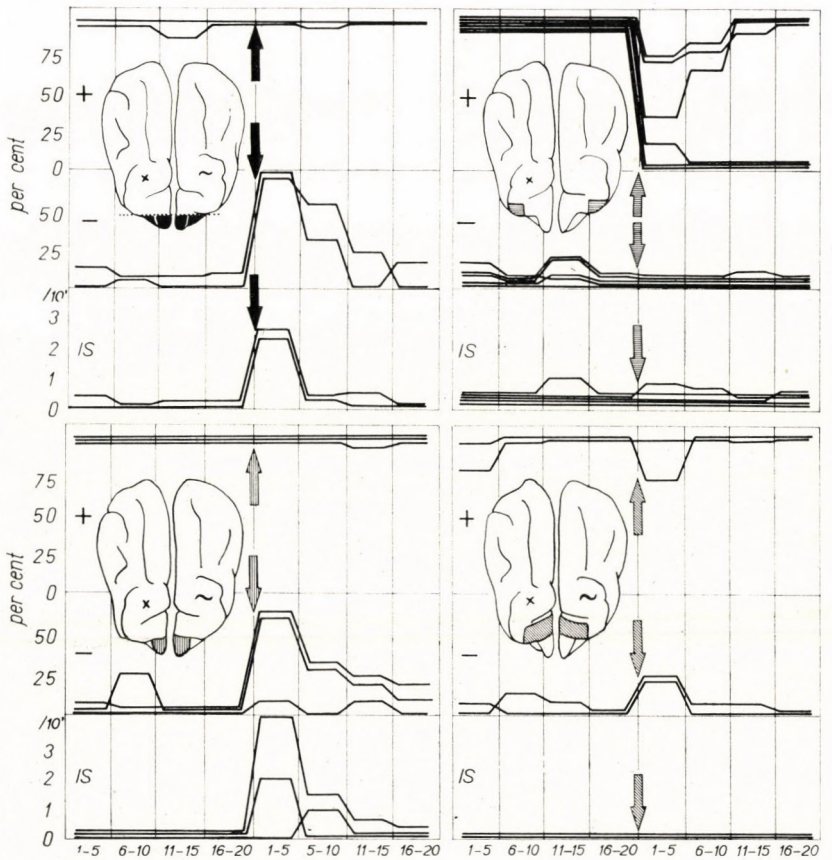

Fig. 1. Effect of bilateral extirpations on conditioned and intersignal reactions. On the ordinate, percentage of positive conditioned leg reactions (+ above), percentage of leg reactions in response to negative conditioned stimuli (— centre) and number of intersignal reactions in 10 minutes (IS, below). On the abscissa, the responses in 20 preoperative and 20 postoperative experiments; one interval five experiments (30 positive and 15 negative stimuli with each animal). Effects of the extirpation of the proreus (frontal) and orbital gyri (on the left, above), of the proreus (frontal) gyrus (on the left, below), of the lateral third of the anterior sigmoid gyrus (on the right, above), and of the medial two-thirds of the anterior sigmoid gyrus (on the right, below). The arrows indicate the time of operation

outlined above. While the extirpation of the proreus (frontal) gyrus and that of the proreus (frontal) and orbital gyri together did not influence the positive conditioned reflexes, after the operation type III the negative conditioned reflexes and the intersignal reactions, *i.e.* the inhibitory activities, remained

intact. On the other hand, the positive conditioned reflexes could not be evoked at all in 3 animals. These did not respond to even hundreds of positive stimulations for months, while in another 3 animals the positive conditioned reflexes were gradually restored after a loss of from 25 to 80 per cent. Thus, extirpation type III resulted in a damage to the positive, *i.e.* excitatory, reactions.

The area removed by the operation type IV (medial two-thirds of the anterior sigmoid gyrus) was about twice as great as that of the proreus (frontal)

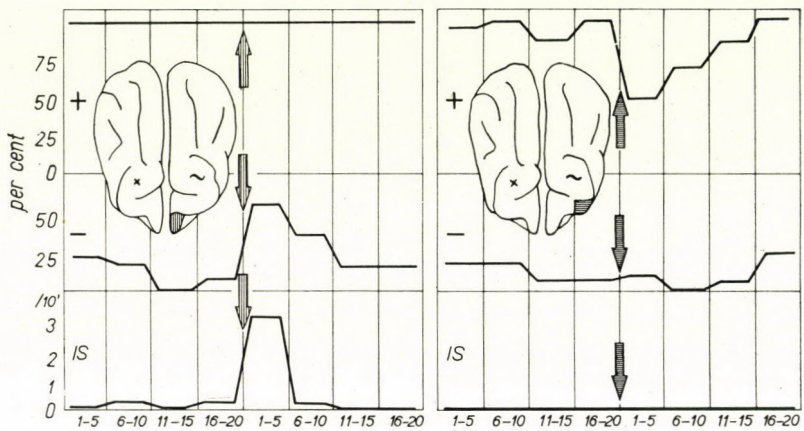

Fig. 2. Effect of unilateral frontal proreus (left) gyrectomy and unilateral extirpation of the lateral third of the anterior sigmoid gyrus (right) on conditioned and intersignal reactions. Signs as in *Fig. 1*

or of the lateral one-third of the anterior sigmoid gyrus. In one dog the positive conditioned reflexes remained intact, in the other they were somewhat impaired. In both dogs the negative conditioned reflexes were temporarily inhibited. The impairment of reflex activity was shorter in duration and was less severe than in the other types of operation. It is possible that these effects were one to secondary lesions involving adjacent areas; such lesions are difficult to avoid when operating on the medial two-thirds of the anterior sigmoid gyrus.

Fig. 2 illustrates the unilateral (left side, the one contralateral to the reacting leg) extirpation of the proreus (frontal) gyrus and the lateral one-third of the anterior sigmoid gyrus. Impairment of positive conditioned reflex activity resulted from the unilateral extirpation of the lateral third of the anterior sigmoid gyrus, and weakening of inhibitions was observed after removal of the proreus (frontal) gyrus.

At the 1959 meeting of the *Hungarian Physiological Society* we have shown that the total loss of reflex activity lasting for months after the extirpation of the lateral third of the anterior sigmoid gyrus ceased when the proreus gyrus

of opposite activity had subsequently been extirpated or when the conditioned reflex had been built up anew and, also, that conditioned reflex activity became gradually normal after the area of opposite activity had been removed. We concluded that the cause was not a direct injury of the reflex pathways, but a fall-out of higher suppressor and facilitatory structures. This we could now confirm by new observations.

A. Parallel with the setting up of conditioned reflexes to sound stimuli we set up in the same dogs conditioned reflexes to light. It is known that in

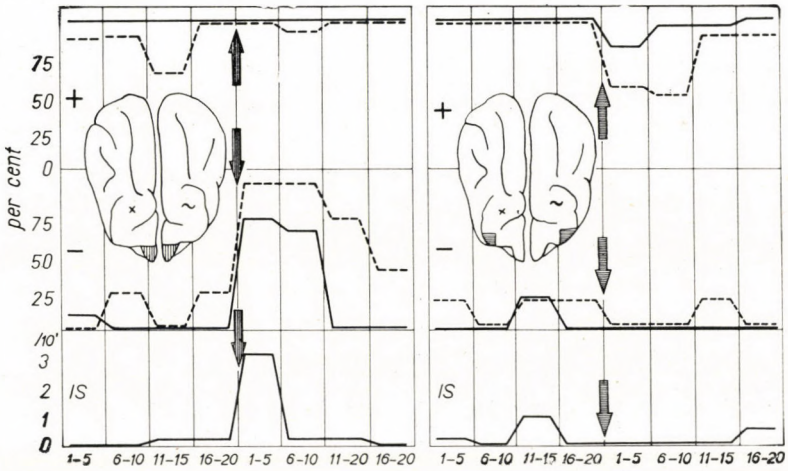

Fig. 3. Changes in conditioned reflex set up to sound (solid line) and light (broken line) and the intersignal reactions following extirpation of the proreus (frontal) (left) and anterior sigmoid gyrus (right)

dogs it is more difficult to set up conditioned reflexes to light than to sound and that the temporary connections which arise in response to light are less stable than those elaborated to sound. The postoperative loss of conditioned reflexes, too, is governed by this law *(Fig. 3)*. After the extirpation of the facilitatory area in the lateral one third of the anterior sigmoid gyrus the inhibition of light stimuli, and after the removal of the suppressor area in the proreus (frontal) gyrus the desinhibition of light stimuli, is more marked than in the case of conditioned reflexes elaborated to sound stimuli.

B. The temporary connection is weaker when the very first stimulus of the experiment is presented. In the case of salivary reflexes for example the number of drops of saliva secreted in response to the first positive stimulus is the smallest. In the case of an inhibited conditioned reflex the extinction, and after the presentation of the negative stimulus the after-inhibition, makes it more difficult to evoke the next positive conditioned reflex. Following the extirpation of the lateral one third of the anterior sigmoid gyrus, when the loss

of reflexes is not total and the positive reflexes have already begun to reappear, we often see in these areas, critical from the point of view of the temporary connection, the inhibition of the conditioned reflex. After the positive conditioned reaction has been created, it is uncommon to find an inhibition of the conditioned reflex.

Following removal of the proreus (frontal) gyrus, disinhibition mostly results in response to the first negative stimulus, representing a difficult task from the point of view of inhibition. Disinhibition can be observed three times less often when the first adequate inhibitory reaction has made the inhibitory connection more stable *(Table I)*.

Table I

Distribution of faulty conditioned reflex responses among the stimuli representing various grades of difficulty in the period of restoration of reflexes

Inhibited positive reaction in the phase of lysis of inhibition	Total	115	100 per cent
	To initial positive stimulus	36	31 ,, ,,
	After inhibited initial positive stimulus	25	22 ,, ,,
	After negative stimulus	43	37 ,, ,,
	After positive response	11	10 ,, ,,
Disinhibited negative reaction	Total	43	100 per cent
	To first negative stimulus	30	70 ,, ,,
	After disinhibited negative reaction	3	7 ,, ,,
	After adequate negative reaction	10	23 ,, ,,

C. It is known that if the differential inhibition is stable, the conditioned inhibition is less easy to set up and is easily disinhibited. After proreus (frontal) gyrectomy the readily damageable conditioned inhibition was disinhibited 2½ times more often than differentiation.

D. Following extirpation of the lateral third of the anterior sigmoid gyrus the positive conditioned reflexes were lost in 3 dogs. In this phase of postoperative disturbances of the temporary connections, extinction due to a lack of reinforcement might have played a role. This was indicated also by the fact that following proreus (frontal) gyrectomy the disinhibition of negative conditioned reflexes is merely temporary. Unlike in the case of the positive reflexes, the non-reinforcement of the disinhibited negative reaction promotes the restoration of inhibition.

All these confirm the contention that the frontal areas in question exert their facilitatory and inhibitory activities through lower structures, and after their removal their role is taken over by other areas. The role of these areas is probably to determine the positive or negative effect of the reaction evoked by the conditioned stimulus. The fact that the lost reflexes mostly reappear and the course of their restoration depends on how they had been built up indicates that the stability of conditioned connections is ensured by other mechanisms.

Finally, we have investigated how the conditioned reflex activity would change after a two months interval in experimentation in normal dogs and in dogs in which reflex activity has apparently completely normalized following extirpation of the proreus and the lateral third of the anterior sigmoid gyrus. The conditioned reflex activity of the intact animals was more precise after the interval; they responded somewhat more often to the positive stimuli and less

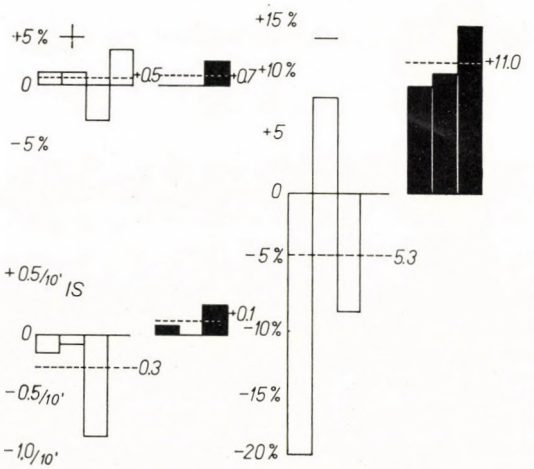

Fig. 4. Effect of 60-days interval on conditioned and intersignal reactions of non-operated (empty column) and operated animals (black column). Above on the left: positive conditioned reactions (+), on the right: negative conditioned reactions (—); below on the left: intersignal reactions (IS). The ordinates show the changes in the conditioned reactions to stimuli, as well as the number of intersignal reactions in 10 minutes in the 20 experiments after the interval (120 positive and 60 negative stimuli per animal). The broken lines and the figures next to them indicate the means for the groups

often to the negative ones, than before the interval, and also the number of intersignal reactions diminished. In the three operated dogs, however, disinhibition in response to the more labile negative conditioned stimuli could be noted significantly more often than before the interval. The number of intersignal reactions also increased in these animals *(Fig. 4)*. Thus, in this way some information may perhaps be obtained concerning latent disturbances of the conditioned reflex activity apparently normalized after frontal extirpation.

On the basis of the above results, the studied areas of the frontal lobe seem to determine whether a conditioned stimulus will evoke a positive or a negative, inhibitory effect. It may be assumed that the enormous phylogenetical development of the frontal lobe serves just the fine and adequate control, refined precision and many-sidedness of excitation and inhibition observed in man, among others.

THE ROLE OF THE FRONTAL LOBE IN THE FOOD-ACQUIRING BEHAVIOUR OF THE DOG

(WITH CINEMATOGRAPHIC ILLUSTRATION)*

By

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Experience obtained in physiological studies, the clinical observations and observations made in connection with brain operations prove unequivocally the role of the frontal lobe in behaviour and psychic activities. Our data obtained by studying conditioned reflexes have shown that certain areas of the frontal lobe had a part in the excitatory and inhibitory conditioned connections. This method has made it possible to analyse in detail nervous mechanisms, but requires experimental conditions in several respects different from the usual ones and restricted to the most essential features of the signals and responses.

In studies of animal behaviour a picture close to natural activity can be gained by analysing the vital food-acquiring reactions performed without previous training. Even under natural conditions any dog may be observed to use its paw or paws in an effort to get food, if so required. Making use of this, we have placed pieces of meat on one side, and led the dog to the other side of a screen. At first, the animal tried to reach the meat with its nose, then to pull it in its mouth with its tongue. As the screen did not permit the animal to pass its head through, it failed to reach the meat with its tongue. Therefore soon the dog tried to use its forelegs, and, reaching over through the screen, it pulled the meat over. After a few trials the attempts involving the nose were inhibited and the animal performed instantly, continuously and in rapid succession the food-acquiring leg reaction. This is illustrated in *Fig. 1.*

This objective method allows to record

(1) the number of attempts made by the dog to reach the food with its nose, the subsequent inhibition and eventual reappearance of this activity;

(2) the latency period of the food-acquiring leg reaction, *i.e.* the time elapsed between the dog arriving to the screen and its reaching through with a fore leg;

*The motion picture was made by Dr. L. Vaday, chief of the photolaboratory, State Institute of Neurosurgery.

(3) the chronological course of the consecutive reachings-over with the forelimb. In our experiments this was characterized by the time elapsed between arriving to the screen and the tenth leg reaction;

(4) whether the animal uses its right or left leg in the reaction;

(5) the duration of leg activity by cinematography.

During the first experimental days the time till the first reaching-over, as well as the total time of performing 10 reachings-over are long and the dogs try several times to reach the food with their nose. After a few days the time

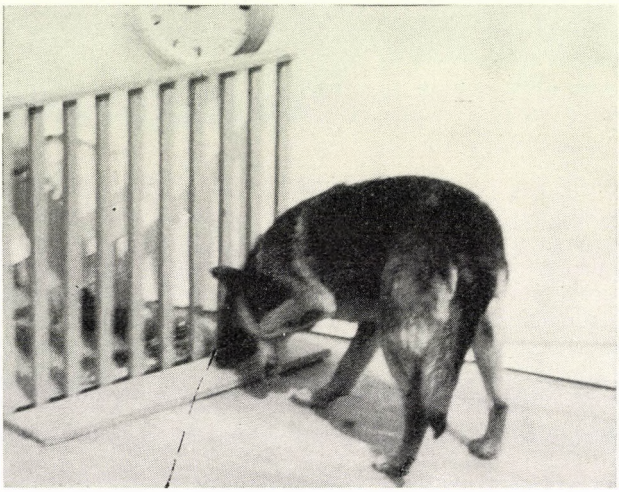

Fig. 1. The experimental situation

till the first reaching-over is reduced to a few seconds, no attempts are made with the nose, and the time required for 10 reachings-over is reduced to 20 to 40 seconds. After the 10th experiment under aseptic conditions, in potentiated intravenous anaesthesia, the premotor areas of the frontal lobe were removed without damaging the motor area. Success of the operation was proved by the fact that a few days later the dogs were active, lively, showing no palsy or motor disturbances. We performed three kinds of extirpation.

I. The cortex of the proreus (frontal) gyrus was sucked out to a depth of about 3 mm.

II. The cortex of the lateral third of the anterior sigmoid gyrus was extirpated, from the line drawn from the lateral end of the cruciate sulcus to the coronary sulcus laterally, and to the line drawn about 1.5 mm frontal to the end of the cruciate sulcus posteriorly.

III. The cortex of the medial part of the anterior sigmoid gyrus was sucked out orally from the line drawn about 1.5 mm anterior to the cruciate sulcus to the precruciate sulcus, medially to the interhemispheral fissure,

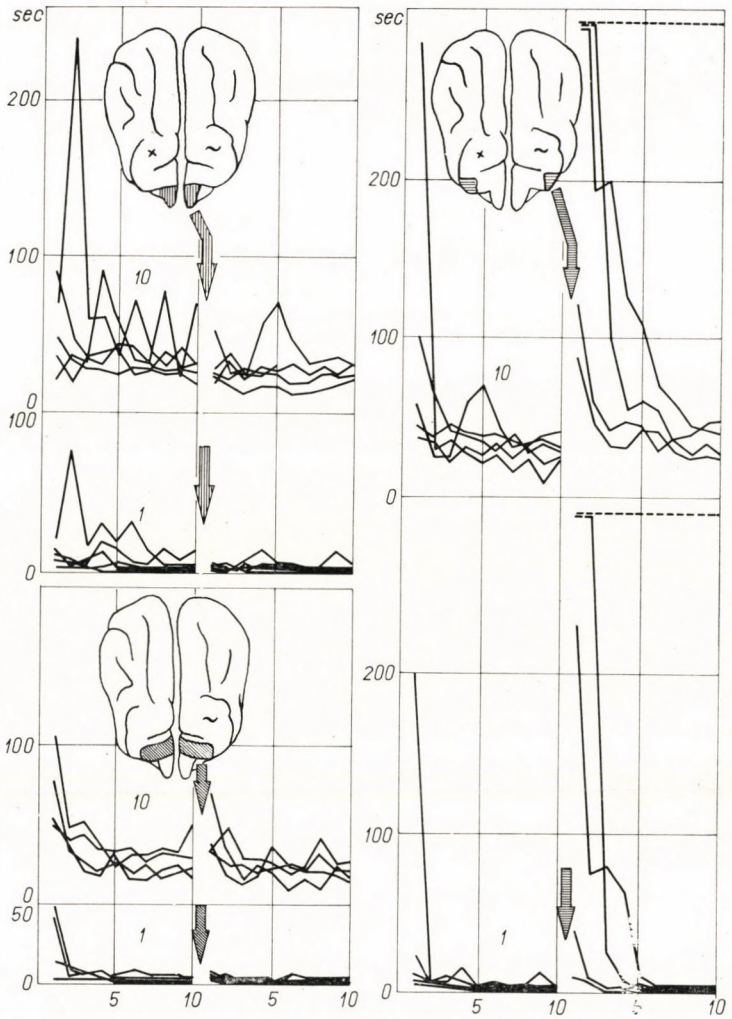

Fig. 2. Effect of bilateral extirpation on the food-acquiring leg reaction. Ordinates: time in seconds of the first (1) and tenth (10) leg reaction. Abscissae: single experiments. Effects of the extirpation of the proreus (frontal) gyrus (on the left, above), of the medial two-thirds of the anterior sigmoid gyrus (on the left, below), of the lateral third of the anterior sigmoid gyrus (on the right). Arrows: time of operation

laterally to the line drawn sagittally from the end of the cruciate sulcus. Thus the lateral third of the anterior sigmoid gyrus was left intact.

Fig. 2 shows the results of the bilateral extirpations. Following extirpation of the proreus (frontal) gyrus, as well as that of the medial two-thirds of the anterior sigmoid gyrus we found no significant change from the preoperative values in either the first reaching-over time or in the time of ten reachings-over. However, a significant change resulted after extirpation of the lateral

third of the anterior sigmoid gyrus. In the first postoperative experiments two animals could not perform the first food acquiring leg reaction in 300 seconds, and made several attempts at reaching the food with their nose. After 300 seconds we terminated the experiment so as to avoid the development of neurosis. In the other three dogs, too, both the first reaching-over time and the time of ten reachings-over were significantly prolonged. When the experiments were continued, one animal failed to reach over in 300 seconds even in the 10th

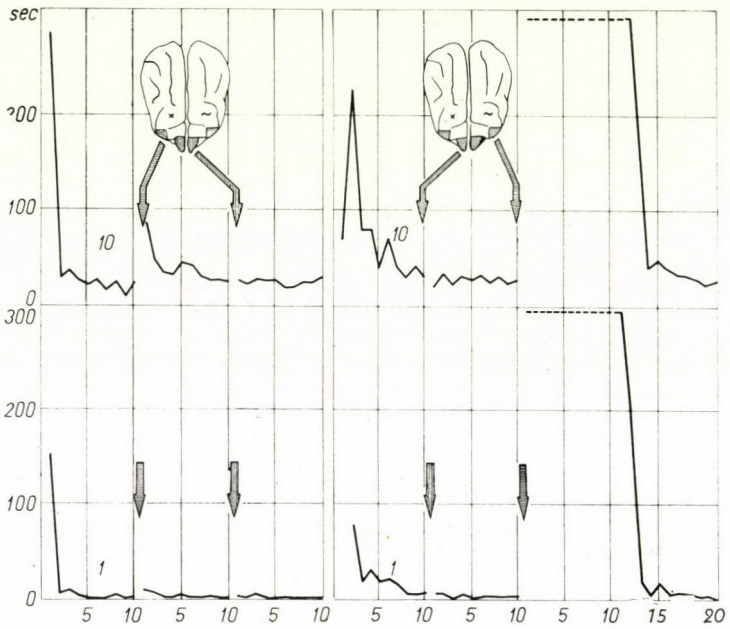

Fig. 3. Effects of different operations in the same dog. On the left; extirpation of the lateral third of the anterior sigmoid gyrus, followed by the extirpation of the proreus (frontal) gyrus. On the right: extirpation of the proreus (frontal) gyrus, followed by the extirpation of the lateral third of the anterior sigmoid gyrus. Signs as in *Fig. 2*

experiment. In the rest both the first reaching-over time and time of ten reachings-over decreased gradually and the attempts at reaching the food with the nose were soon abandoned.

In view of the potential individual reactions to operation, we have examined the effects of the different extirpations in the same dog. The results of the operations, performed in different orders, are illustrated in *Fig. 3* and the motion picture shows the experiments involving these dogs. We clearly see the behaviour of the animals in the initial experiment, the leg reaction is performed without delay, in rapid succession during the days before operation. In the first experiment following extirpation of the lateral third of the anterior sigmoid gyrus, the first dog tried to reach the food with its nose; the time of the first

reaching-over and the time of the ten reachings-over were both significantly prolonged. In subsequent experiments the animal made no attempt at reaching the food with its nose, the times for the first reaching-over and the ten reachings-over were reduced, and no change resulted from the subsequent removal of

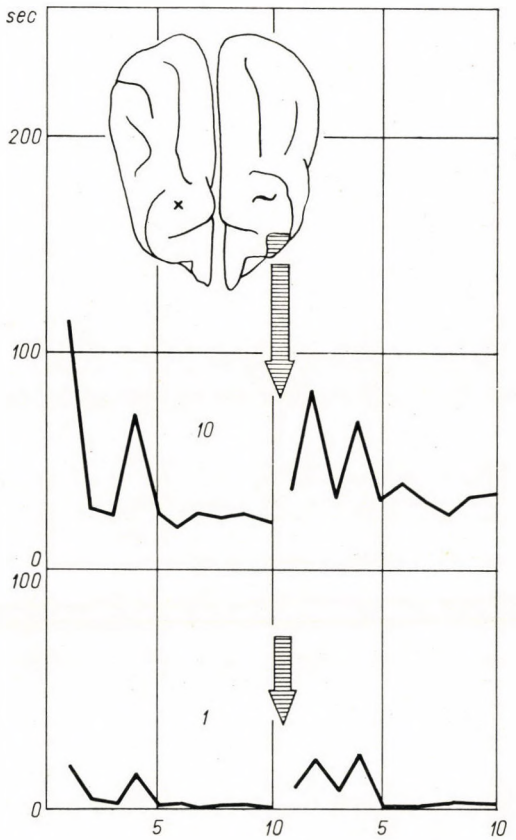

Fig. 4. Effect of unilateral extirpation on the food-acquiring leg reaction. Arrow: extirpation of the lateral third of the anterior sigmoid gyrus on the left side. Signs as in *Fig. 2*

the proreus (frontal) gyrus. In the other dog shown in the *Figure 3* and the film we performed the operations in reversed order. Removal of the proreus (frontal) gyrus caused no substantial change from preoperative activity. However, the food-acquiring leg reaction was totally absent in the 11 experiments following bilateral extirpation of the lateral third of the anterior sigmoid gyrus. It is clearly seen in the film that the animal is lively, mobile, makes repeated attempts at reaching the food with its nose, but does not use its legs for that purpose. It was in the 85th second of the 12th experiment that the dog used its leg for the first time and the tenth leg reaction was performed at the 300th

second in this experiment. In subsequent experiments the attempts made with the nose were abandoned, the times for the first reaching-over and the ten reachings-over returned soon to the preoperative values. The food-acquiring movements of the leg did not become clumsy or slow following extirpation of the lateral third of the anterior sigmoid gyrus, it was only the time elapsed between the first leg reaction and the subsequent leg reactions that were considerably prolonged. We did not combine the extirpation of the medial two-thirds of the anterior sigmoid gyrus with these operations, because the extirpation of adjacent areas would have made it difficult to identify afterwards the area of operation.

The food-acquiring leg reactions suffer also from the unilateral extirpation of the lateral third of the anterior sigmoid gyrus. This was particularly interesting in the case of the animal shown in *Fig. 4*, because this dog performed the reaction both before and after operation invariably with the left leg, ipsilateral to extirpation. Thus, in this case no direct effect on the crossed pathways of the cerebral cortical motor area could be involved.

Thus, following extirpation of the lateral third of the anterior sigmoid gyrus the food-acquiring leg reaction appears later, the interval between the reactions is longer in duration, then in subsequent experiments the picture is gradually normalized. It is interesting to compare this phenomenon with the usually marked and lasting loss of positive motor conditioned reflexes following the same operation. It is only logical to look for a common cause in the background of these two phenomena, because the food-acquiring reaction is fundamentally a natural conditioned motor reflex reaction. Natural conditioned reflexes are more stable than artificial ones and this explains why it is sooner that the former became normal. In the case of behavioural reactions the picture, of course, is more complex; this is indicated by the reappearance of the food-acquiring movements involving the nose at the time the leg reaction is lost.

Finally, wes hould point to the analogy between the disturbances observed in our conditioned reflex experiments and the food-acquiring leg reactions recorded following extirpation of the lateral third of the anterior sigmoid gyrus, and the motor apraxia observed in humans after lesions to the areas 4 and 6. In all three cases the higher positive motor connections built up in the frontal lobe are inhibited, without a direct damage to the structures involved in the performance of movements. It is therefore possible that our experimental models may represent a step forward in the clinical analysis of these motor disturbances.

CHANGES IN ECG, CORTICAL EVOKED POTENTIALS AND STARTLE REACTIONS IN RATS DURING THE ELABORATION OF A CONDITIONED AVOIDANCE REFLEX

By

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In 17 awake, freely moving rats the ECG, the cortical evoked potentials (evoked by acoustic clicks) and the motor activity (especially the startle reaction) were simultaneously registered before and during the elaboration and during the extinction of a conditioned avoidance reflex (the rat had to jump upon a wooden rod to avoid an electric shock from the bottom grid). ECG registration allowed free movements of the animal. As conditioned stimulus, 10 clicks at intervals of 1 sec were employed. Every ten evoked potentials were superponated by means of a two-beam-oscillograph.

The evoked potentials were found to diminish after the first reinforcement by the electric stimulus. They decreased to a minimum and then rose above the initial value. When the first conditioned reflexes appeared there again could be observed a diminution of the evoked potentials below their initial value. Likewise during other motor acts (locomotion within the experimental chamber, searching locomotion, comfort motions) the amplitude of the evoked potentials was diminished. When the rat was hanging on the rod the evoked potentials were smaller than when the animal was staying on the bottom of the chamber. On the background of the initial desynchronization in the ECG, after a few combinations of the acoustic and electrical stimulus there appeared slow rhythmical waves of a theta-like frequency (6—8/sec). During the development of the conditioned reflex, phases of hypersynchronization (6—8/sec) were observed in all cases during the application of the conditioned stimulus, reaching a maximum in amplitude (up to more than 400 μV) and regularity immediately before the conditioned motor act.

At the beginning of the experiments the clicks rarely evoked a startle reaction. These vanished after the first reinforcement, increased again in the following combinations and showed a maximum before the appearance of the first conditioned reflex (startle phase). During the first conditioned reflexes they once more diminished but subsequently again increased. The appearance and the strength of the startle reactions thus went parallel with the changes in

The text will be published in full in the Acta biol. med. germ.

amplitude of the cortical evoked potentials. During the conditioned period the rat frequently jumped immediately after a marked startle reaction (startle jump).

When the conditioned reactions were not reinforced any more (at the beginning of the extinction), the evoked potentials and the startle reactions in the first phase diminished. At this time the animal assumed a rigid, "waiting" posture. During the following unreinforced applications of the conditioned stimulus the evoked potentials and the startle reactions again increased and the animal assumed its normal resting posture. During extinction the synchronized theta-like rhythm in the ECG vanished gradually. Then only after a long extinction period did the evoked potentials diminish below their initial value and the startle reaction became infrequent.

Summing up, it may be concluded that in all situations which have the character of novelty (essential changes of the situation or motor acts that have not yet become automatized) the evoked potentials, especially their positive components, and the startle reactions are diminishing. When the situation is loosing its novelty and the acting stimulus has gained a signalizing meaning *(i.e.* in the initial phase of the conditioned reflex) or the signalizing meaning has already changed *(i.e.* during the extinction), the evoked potentials and the startle reaction increase.

ON THE LOCKING MECHANISM OF THE CONDITIONED MOTOR ESCAPE IN THE RAT

By

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1. The conditioned escape reflex in the rat after its fixation is not extinguishable under normal conditions (conditioned stimulations — defined optic signals; unconditioned stimulations — defined electric current stimulations).

2. The fixation of the conditioned escape reflex in the rat is accelerated if, immediately following the elaboration of the reflex, a series of conditioned stimulations are applied in the same session.

3. If the test was interrupted after the elaboration of the conditioned reflex and continued on the following days with 8 to 10 stimulations daily, its fixation was effected more slowly despite reinforcement prior to the test.

4. If, after the conditioned escape reflex had been elaborated, the conditioned stimulation was administered unreinforced in the same session 100 times per animal in intervals of 50 to 100 sec, the animals always reacted with short periods of latency, *i.e.* with a well fixed conditioned reflex without a phase of fixation instead of the expected extinguishing inhibition.

5. Differentiation stimulations inserted into a series of 100 positive conditioned stimuli had little influence on the conditioned escape reflex. The same was the case when a series of six differentiation stimulations followed a series of positive conditioned stimuli.

6. These reactions occur in case of both forward and backward conditioning.

7. It is assumed that the nociceptive (electric) stimulation has only an initiating effect in elaborating a conditioned reflex and is mainly responsible for a specific state of activity (a biologically negative tonus) of the central nervous system.

8. If, after elaborating the conditioned escape reflex, unconditioned (nociceptive) stimulation is discontinued, a "self-reinforcement" of the conditioned reflex on account of the motor reaction induced by the conditioned stimulation and, thus, a stable dominant connection is brought about between the excited regions of the conditioned stimulation and the excited cerebral motor regions which therefore cannot be extinguished. It must be assumed that the motor reaction has taken over the part of the unconditioned stimulation.

9. The opinion voiced by KNOLL concerning the active focus of the conditioned reflex which cannot be extinguished is compatible with the above observations.

10. The importance of the problem in sports physiology has been discussed.

ELECTROPHYSIOLOGICAL STUDIES OF THE HIPPOCAMPUS IN MAN WITH AVERAGED RESPONSE COMPUTATIONS

By

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1. In patients with severe temporal lobe epilepsy, electrical seizures were found which were confined to one hemisphere and which caused no disturbance in the neocortex (*Fig. 2*).

2. An *Average Response Computer* was used to detect responses evoked in sites within the limbic system by electrical pulses applied to the amygdala and hippocampus, respectively.

3. Stimulation of the baso-lateral nucleus of the amygdala evoked responses in the ipsilateral hippocampus and hippocampal gyrus and, less consistently, in the cortex of the temporal tip. No contralateral responses were found (*Figs 4, 5* and *6*).

4. Stimulation of the hippocampus evoked responses in the ipsilateral hippocampal gyrus and amygdala. No contralateral responses were found (*Fig. 9*).

5. The hippocampus responded to repetitive stimulation by a recruiting response of increasing amplitude and decreasing latency (*Figs 7* and *8*).

6. Evidence having been found that bilateral stimulation of the hippocampus in man can produce a temporary loss of recent memory, a search was made for evidence that sensory evoked stimuli reach the hippocampus.

7. Averaging techniques clearly demonstrated responses of the hippocampus to visual stimuli in man (Fig. *10*).

In the course of certain diagnostic procedures in a small series of patients (nine) suffering from intractable temporal lobe epilepsy, it has been possible to make some observations of physiological interest in addition to those directed towards the primary clinical goals. These clinical goals were two: namely to locate, by recording from electrodes implanted deep in the temporal lobe, regions of maximal spiking activity; and secondly, by stimulating through these electrodes, to define the zones of lowest threshold for seizure discharges. Information derived in these ways was used by the neurosurgeons in deciding laterality for subsequent temporal lobectomy, for the patients in this series were all cases in whom the EEG from scalp recordings gave no lateralizing signs and in whom the clinical seizures were unresponsive to all medicinal therapy. The clinical results obtained in these studies are reported in the publications of my colleagues** [7, 9, 11, 13].

*The work of this investigator is supported by Career Research Award 5—K6—18, 608 and NB 04773 from the National Institutes of Health, USPHS and by ONR Contract 233(69) from the U. S. Office of Naval Research.

**The work of the clinical team (Drs. R. Rand, P. Crandall, C. Markham, R. Walter, W. R. Adey and L. Chapman) is supported by NB 02808 from the National Institutes of Health, USPHS.

This report will be restricted to some of the physiological observations made possible by the rare opportunity to record directly from the human hippocampus, amygdala, hippocampal gyrus and other sites deep within the temporal lobes.

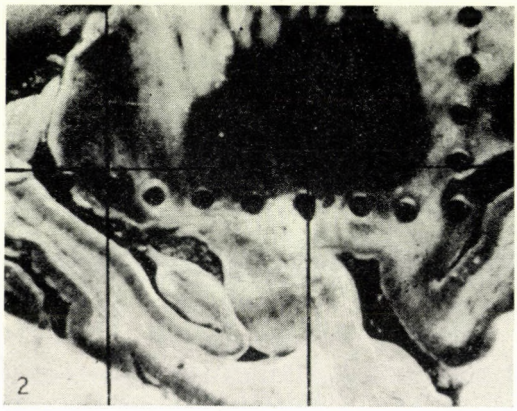

Fig. 1. Section through the temporal lobe of the human brain showing the hippocampus, hippocampal gyrus and amygdala (reproduced from reference [12])

The bipolar electrodes, made of insulated stainless steel, were inserted by the neurosurgeons by the method of TALAIRACH [12], and were left in place from 3 to 6 weeks, thus enabling recordings to be made in the unanesthetized

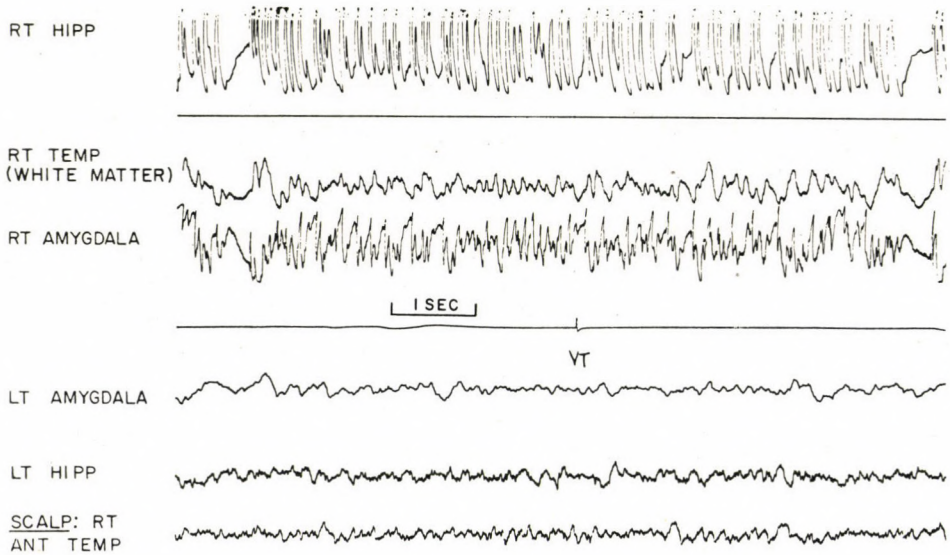

Fig. 2. Recordings from hippocampus and amygdala of both hemispheres in man, showing electrical seizure confined to the right hemisphere only and failing to affect the EEG recorded from the ipsilateral temporal scalp. All scalp recordings remained normal at the time when thees seizure discharges were occurring in depth

state and after the tissues have become stabilized following any initial disturbance caused by the insertion.

In most of the patients in the series so far, the sites chosen for the electrode tips were: the baso-lateral amygdala; 2 positions in the pes hippocampi (hippocampus proper); 3 positions in the hippocampal gyrus (anterior, mid and

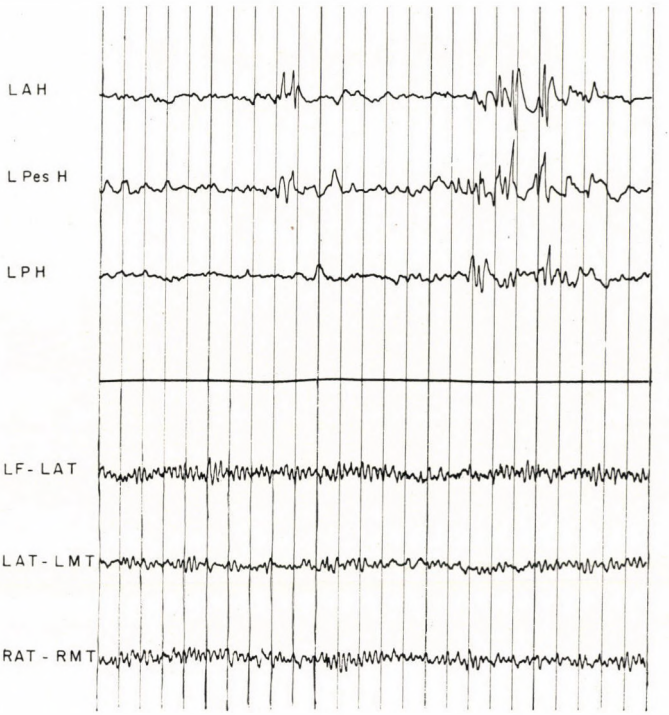

Fig. 3. Spontaneously occurring spikes in the left hippocampus and left hippocampal gyrus of a patient with temporal lobe epilepsy (first 3 channels). Scalp recordings are unaffected (last 3 channels). LAH: anterior position in pes hippocampi. L pes H: mid position in pes hippocampi. LPH: Posterior hippocampal gyrus. LF: Left frontal. LAT: Left anterior temporal. LMT: Left mid temporal. RAT: Right anterior temporal. RMT: Right mid temporal

posterior); and a site within the cortex of the temporal tip. Electrodes were placed in these structures bilaterally (see *Fig. 1*).

In the course of searching, by means of electrical stimulation, for evidence of zones of low threshold for seizure discharge, two striking results were found, one electrophysiological and the other behavioural.

The electrophysiological finding was that electrical seizures of considerable severity could be evoked in hippocampal and amygdaloid structures by stimulation of the ipsilateral hippocampus without any spread either to the ipsilateral temporal scalp electrodes or to the contralateral hippocampus and

amygdala *(Fig. 2)*. This finding led to a study of the connections between these limbic system structures as determinable electrophysiologically in man. The results are described below in Section I. Similarly, spikes of high voltage occurring spontaneously in the hippocampus and amygdala did not disrupt the rhythmic activity recorded from the scalp over the temporal lobe *(Fig. 3* from another patient).

The behavioural finding that emerged from the search for regions of low threshold responses to electrical stimulation, was a striking case of temporary loss of memory induced by simultaneous bilateral (but not by unilateral) stimulation of the pes hippocampi. The memory loss, which was entirely reversible, was only for *recent memory* (over a span of about 3 weeks) without any impairment of long-term memory or of immediate recall. This case has been described in detail in another publication [5]. This is only one more class of observation to add to those derived from neuropathological findings in *Korsakoff*'s disease with its resultant impairment of memory, and from conditioning experiments in animals that implicate the hippocampus as an important link in any circuit subserving memory.

This reasoning suggested the second study to be reported here. If the hippocampus is indeed to be regarded as critical for at least some categories of the memory process in man, it would seem necessary to determine whether impulses initiated in the natural sense receptors can be shown to reach this structure. Below, in Section II, are reported the results of an exploration of the responses evoked in the human limbic system by visual stimuli.

Technique

The technique used for electrical stimulation was as follows: a repetitive train of electrical pulses, each 1/100th msec in duration, of current strengths between 2 and 4 $^1/_2$ mamps was delivered at a rate of 1 per second through each bipolar electrode in turn, the responses from the other electrodes being recorded simultaneously on frequency-modulated magnetic tape, one channel of which recorded a square pulse synchronized with the incidence of each stimulus.

As these recordings from implanted electrodes were being made in unanesthetized and normally behaving man, there was considerable background EEG activity present at all times. This was usually of sufficiently high amplitude to hide from the eye the responses of low amplitude for which search was being made. The magnetic tapes were therefore analysed by the *ARC Computer* [8] at the Massachusetts Institute of Technology for computation of the average response to several stimuli. Details of the use of this computer for detecting low voltage signals in noise of high amplitude have been described by its designer (W. A. CLARK [8]) and in seve ral previous publications by this reporter [1, 2, 3, 4] and will, therefore, not be repeated here

Results

I. *Electrophysiological connections of the hippocampus and amygdala in man*

When electrical pulses were applied to the amygdala of one temporal lobe, responses were recordable from the ipsilateral hippocampus, but not from the contralateral hippocampus or from the contralateral amygdala *(Fig. 4)*. The

region of the amygdaloid complex in which the electrodes were placed in this series was the baso-lateral nucleus. Whether similar results would be obtained from the centro-medial nuclei of the amygdala has not yet been explored.

As responses to stimulation of the amygdala were also recordable from the ipsilateral hippocampal gyrus, it seemed of interest to determine whether their latency would suggest direct transmission from the amygdala or whether

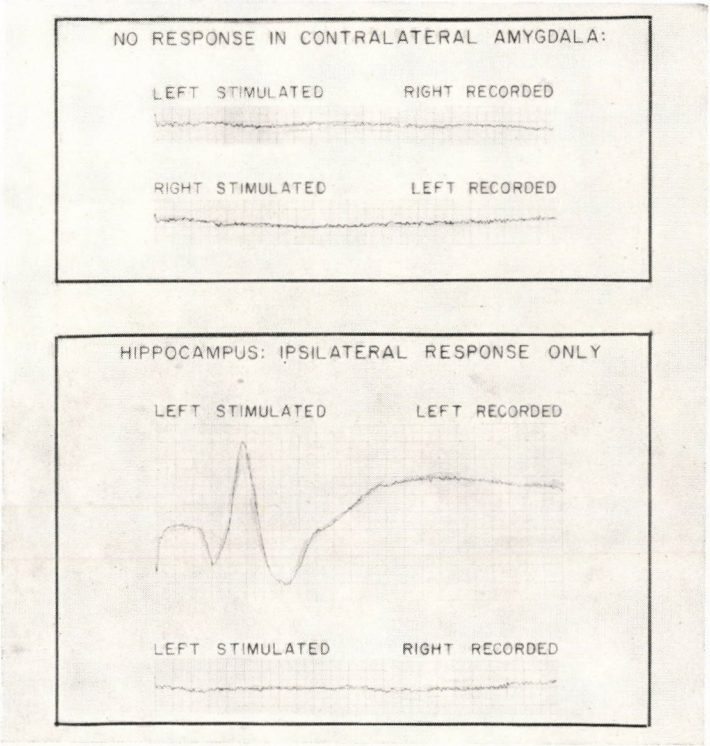

Fig. 4. Unilateral stimulation of the amygdala evokes responses in the ipsilateral hippocampus but not in either the contralateral hippocampus or the contralateral amygdala (reproduced from reference [6])

they reached the gyrus *via* the hippocampus proper. *Fig. 5* shows averages of simultaneously recorded responses from both pes and hippocampal gyrus. The longer latency of response in the latter structure would suggest transmission *via* the pes hippocampi.

Responses to amygdaloid stimulation were less consistently obtained from electrodes in the cortex of the ipsilateral temporal tip. When present, their wave form was suggestive of a polysynaptic route *(Fig. 6)*.

Of the responses described above, those of the ipsilateral pes hippocampi only were of sufficient amplitude to be clearly definable as single evoked poten-

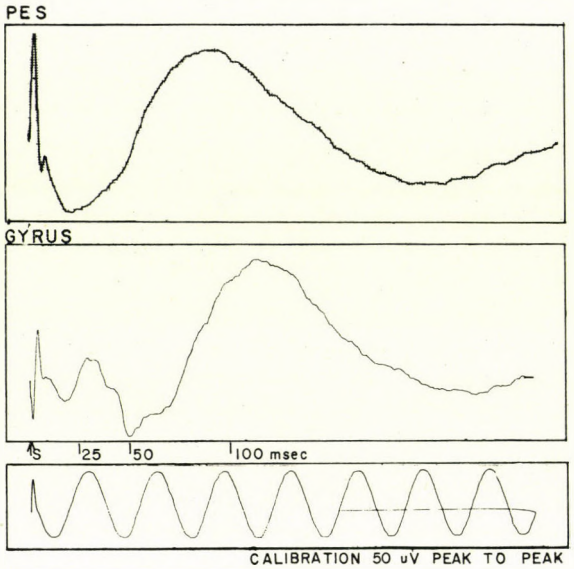

Fig. 5. Average responses to amygdaloid stimulation recorded simultaneously from the pes hippocampi and hippocampal gyrus in man. (The sharp deflections at the left of each record are stimulus artifacts)

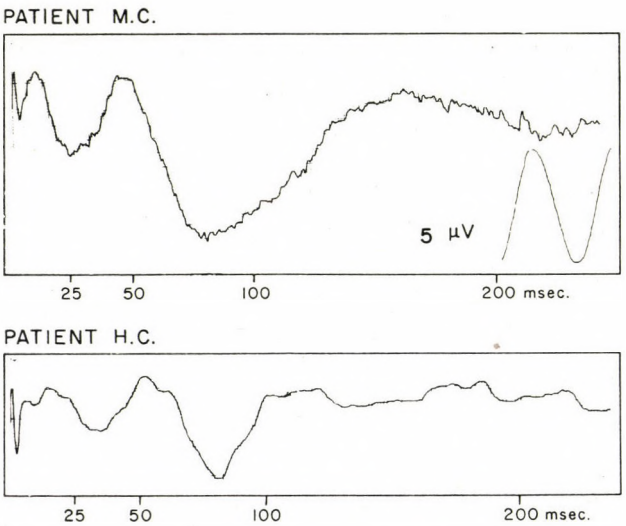

Fig. 6. Average response to amygdaloid stimulation recorded from within the cortex of the ipsilateral temporal tip (reproduced from reference [6])

tials in an inkwritten electroencephalogram. When stimulus rates of 5 to 10 c/s were used, it was seen that these responses showed the phenomenon of recruitment *(Fig. 7)*. An effect similar to this has been found in the cat by GLOOR [10].

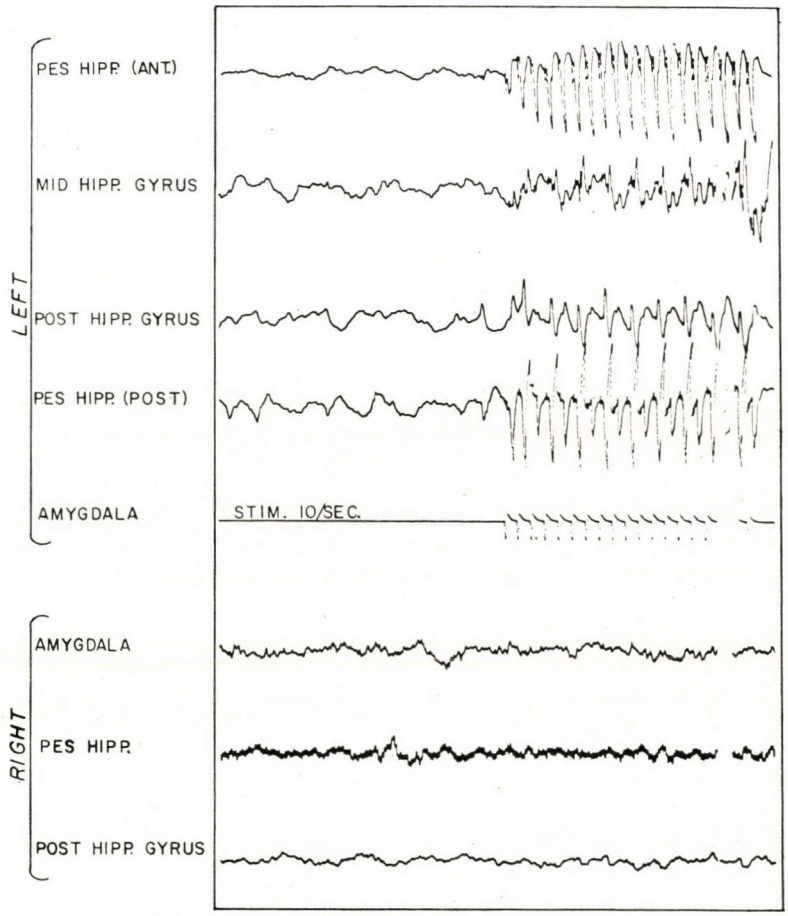

Fig. 7. Recruitment of hippocampal responses following repetitive stimulation of the amygdala in man

When averages of the first 6 responses to a long train of such stimuli are compared with the last 6, not only does the increase in amplitude become striking, but the decrease in latency that accompanies recruitment is clearly demonstrated *(Fig. 8)*.

A similar series of computer analyses were made of responses evoked by stimulation in the pes hippocampi. Responses were readily recordable from the ipsilateral amygdala *(Fig. 9)* but not from the contralateral amygdala nor from the contralateral hippocampus. The latter result was somewhat surprising

in view of the hippocampal commissure, but was consistent with the earlier finding that electrical seizure discharges can take place in the hippocampus without crossing to the other hemisphere *(Fig. 2)*.

When stimulation at 5 to 10 cps was applied to one region in the pes hippocampi and recordings made from a bipolar electrode placed more poste-

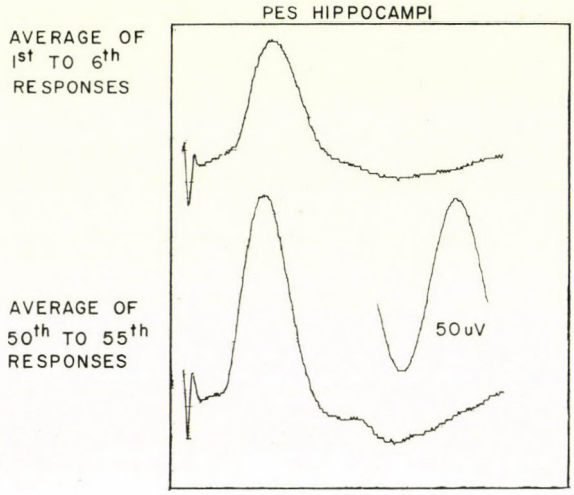

Fig. 8. Averages of the first 6 responses to a train of 55 stimuli compared with the last 6. Note increase in amplitude and decrease in latency. Recordings in pes hippocampi and hippocampal gyrus evoked by stimulation of the ipsilateral amygdala

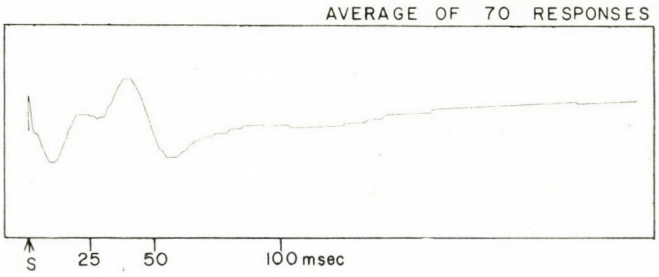

Fig. 9. Average response to stimulation of the pes hippocampi recorded in the ipsilateral amygdala

riorly in the same structure, the responses were of high amplitude and showed recruitment suggesting that the ultimate connection is dendritic in nature.

II. *Visually evoked responses*

A stroboscope flash, repeated approximately once a second, was used as the stimulus, a pulse coincident with the flash being put onto one channel of the magnetic tape for correlation with the recordings from the brain in order to

isolate (by averaging) the potential changes time-locked to the flash. Responses, sensitive to the intensity of the flash, were recordable from the hippocampus but not from the amygdala *(Fig. 10)*.

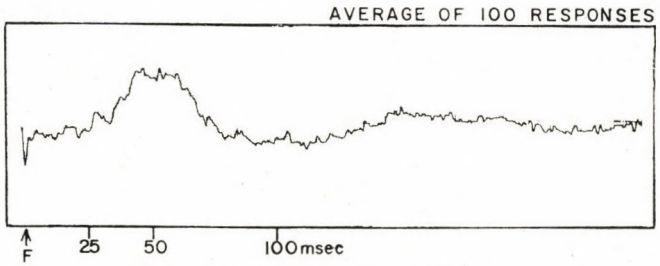

Fig. 10. Average response to light flash recorded from the hippocampus in man

Attempts were made, but with less consistent success, to record hippocampal responses to acoustic clicks; this negative finding may, however, be of no great significance, for clicks of only moderate intensity were used in order not to cause discomfort to the patients.

Conclusions

In this initial study of a small series of cases with temporal lobe epilepsy examined with implanted electrodes, evidence for strong (electrophysiological) connections has been found so far only within the same hemisphere.

The impairment of recent memory by bilateral hippocampal stimulation raises once more the classical question as to whether a stimulating current produces an excess of the normal influence of the structure stimulated or whether it abolishes its role. This question is, of course, not answered by these studies but it can with certainty be said that the electrical stimulus unbalances and distorts the level of activity contributed by the hippocampus to the loop of structures that make up the limbic system.

Any hypothesis that regards the hippocampal formation as playing some role in the functioning of recent memory would necessitate receipt by the limbic system of sensorally evoked responses. The demonstration in these studies of visually evoked responses in the hippocampus of man would lend some of the necessary supportive evidence for such an hypothesis.

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MATHEMATICAL ANALYSIS OF THE ELECTRO-ENCEPHALOGRAMS OF NEUROTIC RATS

By

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Experiments were performed on 25 male albino rats, with chronic subdural electrodes. The EEG was recorded under standard conditions, in stimulus-free environment. Temporary neurosis was induced by conflicting the drinking and defensive unconditioned reflexes. In comparison with the control, the EEG made after the conflict situation showed more marked beta-like activity, high-amplitude spindle bursts and a rapid alternation of relatively synchronized and desynchronized segments. Statistical analysis of the results revealed that

a) the histogram made by the method of Fujimori *et al.* indicated a widening of the frequency spectrum and an increase in the percentage of delta and beta-like components;

b) autocorrelation, carried out manually by the original method of Brazier and Casby, showed in both the normal and the neurotic states light theta-like activity, which became more marked in response to the intervention;

c) analysis of the background activity not containing periodic components by the method of dynamic averages indicated that in neurosis slower waves of higher amplitude were observable;

d) finally, examination by integral approach of the size of the areas delineated by 1 sec portions of the curves according to Drohocki revealed an increased variability of the value of the index.

Our knowledge relative to experimental neurosis has gained little since the fundamental investigations by Pavlov and his associates, first of all Yerofeyeva, Senger-Krestovnikova, Petrova, Ivanov-Smolensky and Asratyan [10, 8]. Particularly scarce is the evidence concerning the subcortical-cortical mechanism of the genesis of experimental neurosis.

According to Simonov [13] and Milutina [9], the course of experimental neurosis is more severe in decorticated animals. Tchukarin [3] observed in dogs displaying nervous disturbances in the early phase synchronized or desynchronized activity, corresponding to the preponderance of inhibitory or excitatory processes. Later, when the cortex had been exhausted, slow waves of variable shape appeared. In the voluminous clinical literature on experimental neurosis, relying upon the evidence presented by Cohn [2], Gastaut *et al.* [7], Roth [11] and others, the appearance of pathological slow waves, disturbances of alpha activity and an increase in the proportion of beta components are mentioned.

We have undertaken to study the intrinsic mechanism of experimental neurosis in rats. In the first step we determined the changes in the EEG record-

ed from subdural electrodes. As it is difficult to judge the conditions from a simple inspection of the curves, the tracings were analysed by mathemathical-statistical methods.

Methods

Twenty-five inbred male albino rats, weighing 150 to 250 g each were used. In each animal three chronic subdural electrodes were placed frontally, and temporo-parietally on both sides. The recordings were made under standard conditions, always in the morning, in a sound-proof, moderately illuminated chamber. After having made four to six control EEGs, neurosis was induced by conflicting the unconditioned drinking and defensive (escape) reflexes. The animals, deprived of water for two days previously, were placed in a special cage, in which they found a dish full of water. The dish and the screen of the cage were connected with the poles of a 40 to 50 V circuit. When trying to drink, the rat closed the circuit by its own body and received a shock. During the conflict situation, intense fear and defensive behaviour were observable. The consequential disturbances in vegetative functions and conditioned reflex activity will be reported elsewhere. For evaluating the results, we compared the EEGs made immediately before and after the conflict, or two days before and one day after the conflict.

Results

Experience has shown that, in accordance with the data in the literature, the resting alert EEG of the rat is characterized by a 5 to 6 Hz, 50 to 200 mV, relatively regular basic activity and a beta-like activity superimposed on it.

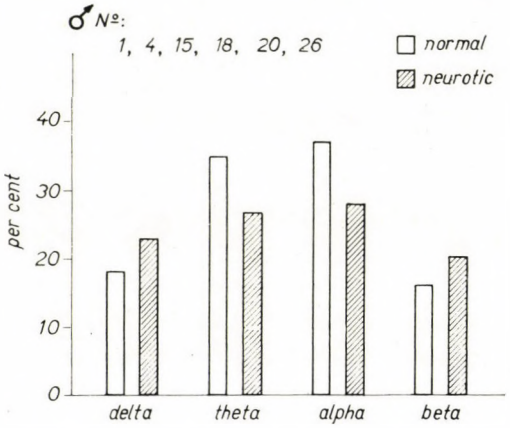

Fig. 1. Six rats in normal and neurotic state. FUJIMORI *et al.*'s histogram method. Percentage distribution of the total amplitudes of different frequency dominions

The EEG of the neurotic animals showed high amplitude spindle bursts, more marked beta-like activity and a rapid alternation of relatively synchronized and desynchronized segments.

1. Using the method of FUJIMORI *et al.*, but without applying the correction suggested by them, we determined the amplitude and period length of the single waves, then grouped them according to wave length and added them up

[6]. This way we obtained a frequency-amplitude spectrum, represented in the form of histogram. In neurosis this was characterized by an increase in the proportion of the slowest and fastest components. As shown in *Fig. 1*, in response to the intervention, the percentage of the delta and beta-like components increased at the expense of the other components.

2. The curves were subjected to autocorrelation analysis, by the method of BRAZIER and CASBY [1]. In the absence of an electronic computer, we carried this out manually. The method makes it possible to analyse the signal-noise

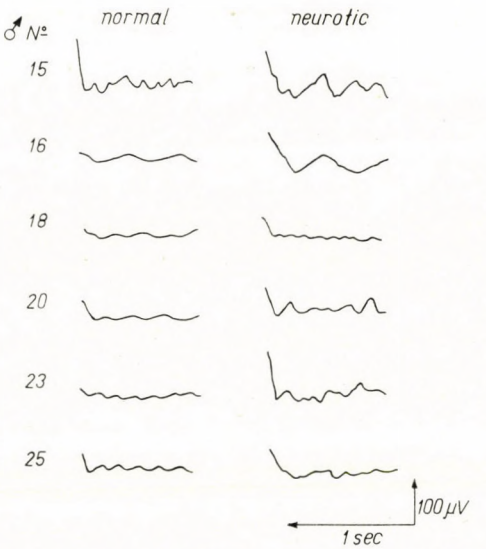

Fig. 2. Six rats in normal and neurotic state. Autocorrelograms of 3-sec segments. Delay from 0 to 1000 msec in 33 msec steps. Breaking up single points in 33 msec steps

ratio, *i.e.* the periodic, rhythmically recurring elements. Three-second segments were analysed in 33 msec steps. As indicated by *Fig. 2*, in most of the rats we found theta, sometimes fast delta-like, periodic activity. The minority of the animals, for example No. 18, showed curves containing practically no components that could have been correlated. In neurosis the autocorrelogram usually contained components of higher amplitude than in the normal state, and the theta-like rhythm became even more marked. The higher initial value, in harmony with the histogram indicated an increased variability of the components.

3. Cross-correlation computations were made between the different portions of the same EEG, by the method of SATO *et al.* [12], using four-second segments, analysed in steps of 33 msec. In neurosis the cross-correlogram showed lower amplitudes than in the normal state, which also suggested an increased variability of the components.

4. We have analysed the curves also by the method of dynamic averages, amply used in statistics, but not employed before in EEG studies. By selecting the proper range, we could study the isolated background activity, without the periodic components, *i.e.* we did just the opposite of what we had done in the

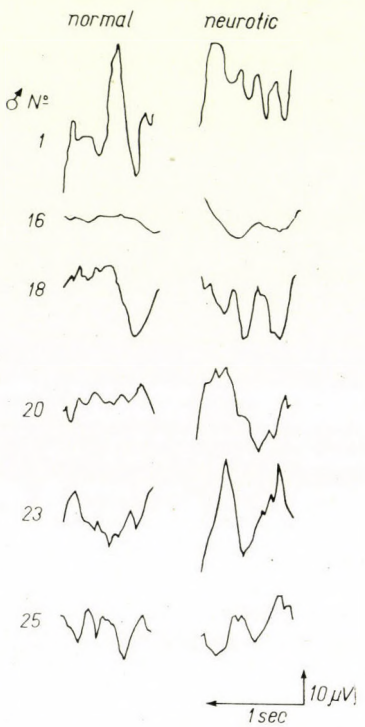

Fig. 3. Six rats in normal and neurotic state. One-sec segments of EEG analysed by the method of dynamic averages. The dynamic averaged interval corresponds to 133, and 166 msec respectively, *i.e.* to the duration of the period of theta-like, rhythmically recurring waves

case of autocorrelation analysis. After the conflict mostly slower waves of higher amplitude appeared in the background activity *(Fig. 3)*.

5. Finally, we examined by integral approach the size of the area under the 1 sec portions of the curve, *i.e.* we measured the total electrical work per unit of time, as suggested by DROHOCKI [4, 5]. To characterize the variation of the results obtained, *i.e.* the variability of electrical activity, we computed the standard deviation of these values. It has been found that variability increased by about 50 per cent in neurosis.

Discussion

From the results the following conclusions have been drawn:

a) the increased frequency of non-periodic elements, the more marked beta-like activity, the periodical desynchronizations observed in the original tracings, indicate an increased strain on the cortex;

b) the appearance of the slow waves may mean that either the cortex has become exhausted, or the subcortical functions have gained preponderance;

c) the broader frequency-spectrum, the higher variability of DROHOCKI's index, the wider range of variation shown by the auto- and cross-correlograms suggest that the intrinsic balance of cortical activity has been upset.

Further investigations are required to determine to what extent the cortex itself, certain subcortical structures and the non-specific activator system are involved in the arisal of this pathological organization of higher nervous activity.

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ACTIVATION DE L'HIPPOCAMPE PAR DES HYPOXIES OXYPRIVES RÉPÉTÉES

Par

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La répétition d'hypoxies oxyprives met en évidence une réactivité particulière de l'hippocampe traduite par des pointes isolées et de rares fuseaux rapides en cours d'hypoxie aiguë et par des décharges organisées en cours d'hypoxie subaiguë, de réanimation et de période post-anoxique.

La sensibilité de l'hippocampe est particulièrement indiquée par la production isolée de décharges hippocampiques en cours d'hypoxie aiguë, et par la prédominance de ces décharges sur les autres paroxysmes convulsifs en cours de réanimation et de période post-anoxique.

La fréquence des lésions hippocampiques dues à l'anoxie est indiquée par de nombreux travaux anatomiques chez l'animal et chez l'homme. D'autre part, les lésions hippocampiques si communes chez les épileptiques, ont été rapportées par SPIELMEYER [6] et son école à une anoxie locale dépendant de la vascularisation atypique du secteur de *Sommer*.

Du point de vue électrophysiologique, peu de travaux ont porté sur les modifications hippocampiques provoquées par l'anoxie. Les études récentes de HUGELIN, BONVALLET et DELL [2], de BAUMGARTNER, CREUTZFELDT et JUNG [1] ont précisé l'activation de la formation réticulaire au cours de l'hipoxie ainsi que son mécanisme rapporté à une origine chémoceptive.

La plupart des recherches électrophysiologiques ont été faites sur des préparations aiguës (encéphale isolé, cerveau isolé, animaux curarisés). Aussi nous a-t-il paru intéressant, pour étudier les réactions de l'hippocampe, d'utiliser des préparations chroniques et de répéter les hypoxies, chez le même animal.

Materiel et méthodes

Trente chats d'âge variable — (jeunes chats de quelques mois, chats adultes) porteur, d'électrodes profondes, implantées dans diverses structures (hippocampe, amygdale, réticulée mésencéphalique et pontique, noyau caudé, cervelet) et d'électrodes corticales (gyrus suprasylvien et ectosylvien) ont été utilisés.

L'anoxie oxyprive a été réalisée par enrichissement en azote et appauvrissement progressif en oxygéne, dans une enceinte transparente (plexiglass). Le comportement de l'animal a pu être ainsi suivi et des films cinématographiques ont été enregistrés.

Durant l'hypoxie des dosages d'O_2 ont été faits dans la cage (Méthode de *Scholander*), le CO_2 dégagé était fixé par la chaux au fur et à mesure de sa production.

Deux types d'anoxie ont été pratiqués: l'un aigu ($O_2 = 4\%$), poursuivi jusqu'au coma et le silence électrique, l'autre subaigu ($O_2 = 8\%$) maintenu pendant plusieurs heures. Lors des

hypoxies aiguës, la période de réanimation (facilitée ou non par des massages respiratoires) a été suivie jusqu'à la répparition de l'activité électrique normale.

Les anoxies ont été systématiquement répétées et certains animaux ont subi jusqu'à 60 anoxies aiguës.

Chez 15 animaux, les cerveaux ont été prélevés pour examen anatomique.

Resultats

Hypoxie aiguë

a) Les différentes périodes de l'hypoxie aiguë : L'hypoxie aiguë (O_2 = = 4%) dont la durée moyenne a été de 6 minutes, entraine chez le chat libre de ses mouvements, des modifications qui évoluent en plusieurs périodes compa-

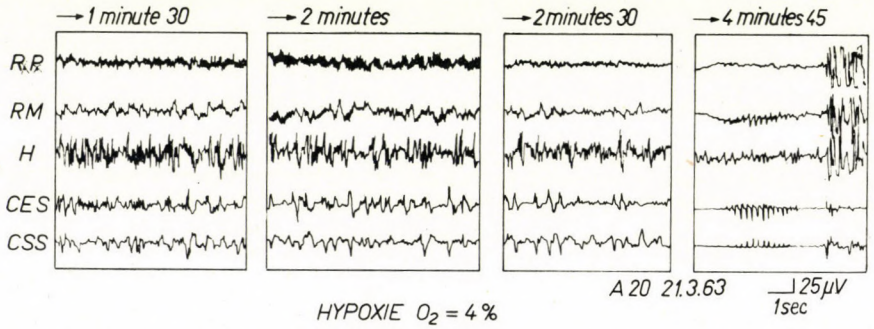

Fig. 1. Hypoxie aiguë. Fuseau réticulaire d'installation progressive et maximum à la deuxième minute d'hypoxie. Rythmes hippocampiques avec pointes isolées, ondes lentes corticales. A 4'45", après, le début de l'hypoxie, persistance d'une activité hippocampique, ondes en «dents de peigne» au cortex et dans la réticulée mésencéphalique. Artéfacts dûs à une convulsion anoxique

(R. P. =réticulée pontique, R. M. = réticulée mésencéphalique, H = hippocampe, C. E. S. = gyrus ectosylvien, C. S. S. = gyrus suprasylvien)

rables, du point de vue électrique, à celles qui ont été décrites sur des préparations aiguës *(Fig. 1)*.

1. *La première période* ou période d'éveil se traduit par une synchronisation hippocampique et une désynchronisation corticale. L'animal est généralement calme, parfois inquiet, rarement agité.

2. *La deuxième période* ou période de dépression correspond à des ondes lentes corticales diffusant plus ou moins aux autres structures. L'animal est inquiet, présente des miaulements plaintifs, est souvent agité; la position debout ou assise n'est pas maintenue et l'animal se couche.

3. *La troisième période* ou période de coma se traduit par un tracé plat, surchargé de courtes périodes d'ondes ralenties (4 à 6 c/s) en «dents de peigne» et qui intéressent diverses structures. C'est au cours de cette période que survient le «spasme anoxique» traduit par une hyperextension du tronc et du cou avec

battements des pattes antérieures, plus rarement des pattes postérieures. Dans nos conditions d'enregistrement, l'expression électrique a été nulle et seuls des artéfacts dûs à l'agitation de l'aminal ont été constatés.

b) Les modifications de l'activité hippocampique: Elles sont de plusieurs types (*Fig. 2*) :

1. *Des pointes* isolées et très amples de 300 à 500 HV surviennent au cours de la période de dépression corticale et persistent avec une amplitude

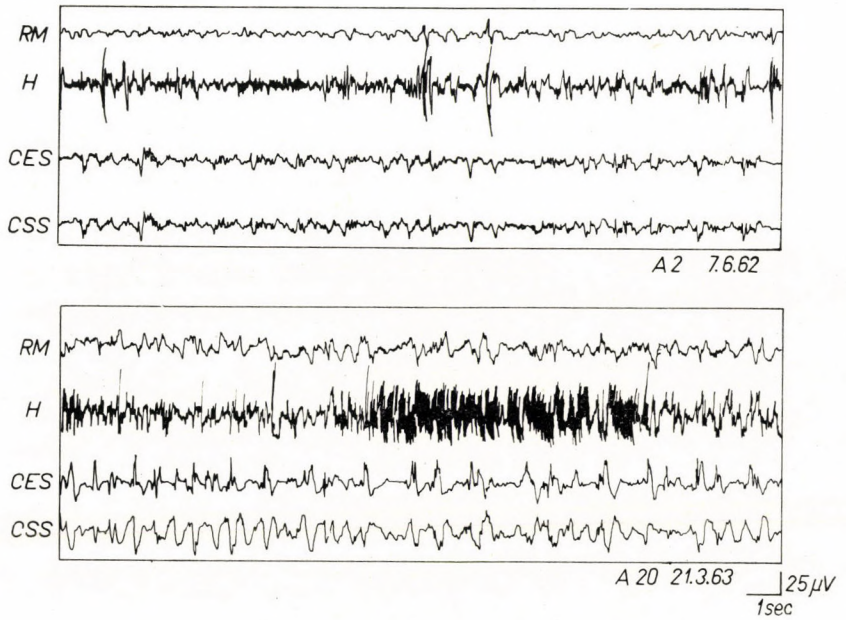

Fig. 2. Hypoxie aiguë
En haut : Pointes hippocampiques isolées et très amples
En bas : Court fuseau hippocampique

moindre jusqu'à la période de coma. Ces pointes apparaissent dès la première hypoxie et sont retrouvées systématiquement par la suite.

2. *Des fuseaux* d'ondes très rapides et de courte durée (5 à 10 sec) surviennent à la fin de la période de dépression corticale et précèdent le «spasme anoxique». Ces fuseaux n'apparaissent qu'après plusieurs hypoxies aiguës. Ils sont rares, inconstants, et ne sont pas retrouvées au cours des hypoxies suivantes.

Ce type d'activation en fuseau intéresse d'autres structures mais avec une différence précise entre la substance réticulaire et les autres formations. Le fuseau réticulaire (ondes rapides de 30 à 40 c/s), d'une durée de 30 à 50 sec survient dès les premières hypoxies et est retrouvé régulièrement par la suite. Ce fuseau débute lors de la période d'ondes lentes corticales et peut persis-

ter jusqu'à la production du «spasme anoxique». Cette activation réticulaire est comparable à celle qui a été constatée sur des préparations aiguës.

Des fuseaux semblables aux fuseaux hippocampiques ont été enregistrés dans le cortex (gyrus ecto-sylvien), le noyau caudé, le cervelet. Les fuseaux apparaissent à la période terminale de l'hypoxie, sont inconstants, de courte durée et strictement limités aux structures intéressées.

Il n'a pas été retenu de relation entre le fuseau réticulaire et les fuseaux des autres formations en particulier hippocampique: ainsi un fuseau rapide hippocampique peut survenir après l'arrêt du fuseau réticulaire.

3. L'activité hippocampique est moins facilement déprimée que l'activité corticale. La disparition des rythmes hyppocampiques lors de la période de coma est plus tardive que celle des rythmes corticaux. D'autre part au moment de la réanimation, l'activité hippocampique réapparait avant l'activité corticale.

Hypoxie subaiguë

La durée de l'hypoxie subaiguë ($O_2 = 8\%$) a varié selon les expériences de 2 à 9 heures.

Les différentes périodes décrites au cours de l'hypoxie aiguë ($O_2 = 4\%$) ne sont pas retrouvées. Du point de vue électrique des périodes de tracé dé-

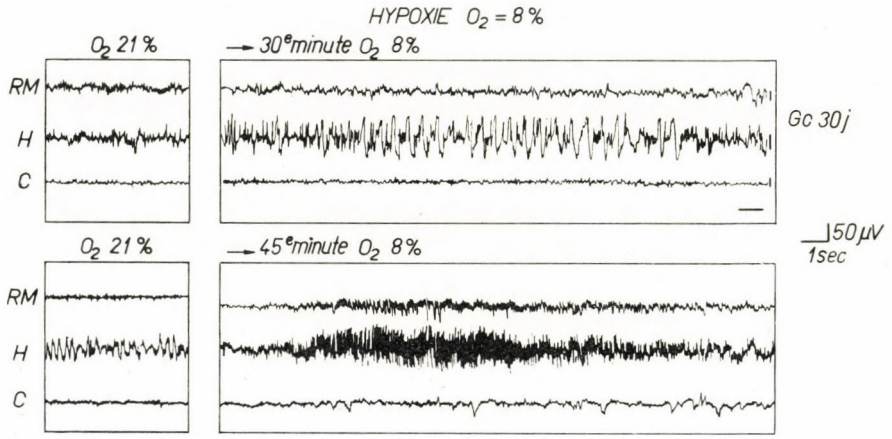

Fig. 3. Hypoxie subaiguë
En haut : Décharge hippocampique isolée
En bas : Fuseau hippocampique propagé à la réticulée mésencéphalique

synchronisé alternent avec des périodes de tracé synchronisé avec ondes lentes. Ces modifications coïncident avec des modifications de l'état vigile de l'animal. Seuls les animaux chez lesquels la teneur en oxygène de l'atmosphère a été abaissée à 4% (atmosphère non renouvelée), ont présenté une période de tracé plat.

Dans ces conditions expérimentales, des *décharges hippocampiques* isolées ou propagées au système limbique ont été les seules manifestations convulsives observées. Elles surviennent après plusieurs hypoxies (3 à 4) et généralement après vingt minutes de séjour en milieu confiné. Elles sont inconstantes et ne sont pas retrouvées au cours des hypoxies suivantes.

Divers types de décharges ont été enregistrés: — décharges électriques hippocampiques à type de pointes ryhtmiques ou de fuseaux d'ondes rapides

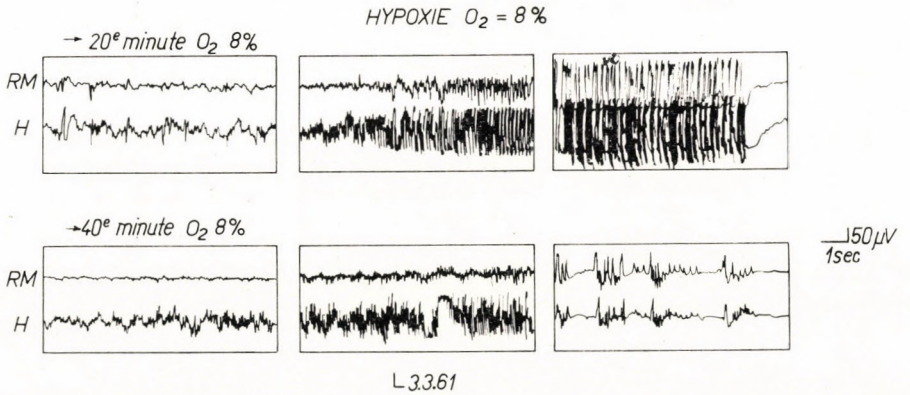

Fig. 4. Hypoxie subaiguë: Décharges tonico-cloniques hippocampiques apparues à la 20ème minute (en haut), et à la 40ème minute (en bas) d'hypoxie

(15 à 20 c/s) pouvant diffuser à l'amygdale et à la formation réticulaire mésencéphalique *(Fig. 3)* — crises tonico-cloniques propagées à la formation réticulaire et au cortex temporal. Les crises sont accompagnées de salivation, mastication ou peuvent être uniquement électriques. Elles peuvent se répéter avec des caractères identiques au cours de la même hypoxie. *(Fig. 4).*

Réanimation et période post-anoxique

En cours de réanimation après hypoxie aiguë et dans la période post-anoxique, surviennent différents paroxysmes convulsifs: myoclonies, crises généralisées isolées ou groupées et évoluant parfois en État de Mal et des crises rhinencéphaliques à point de départ hippocampique. Ces dernières sont plus fréquentes que les autres manifestations convulsives.

Les *crises hippocampiques* sont comparables à celles qui surviennent en cours d'hypoxie subaiguë et correspondent soit à des décharges électriques (pointes rythmiques, fuseaux) soit à des crises tonico-cloniques intéressant le système limbique.

En cours de réanimation, les décharges hippocampiques surviennent après plusieurs hypoxies aiguës et généralement après la dixième minute alors que l'activité électrique est réorganisée *(Fig. 5).*

Dans la période post-anoxique, les décharges hippocampiques apparaissent spontanément. Elles sont facilitées par le sommeil et ont pu être induites en cours de stimulation lumineuse intermittente. Dans certains cas, il n'existe

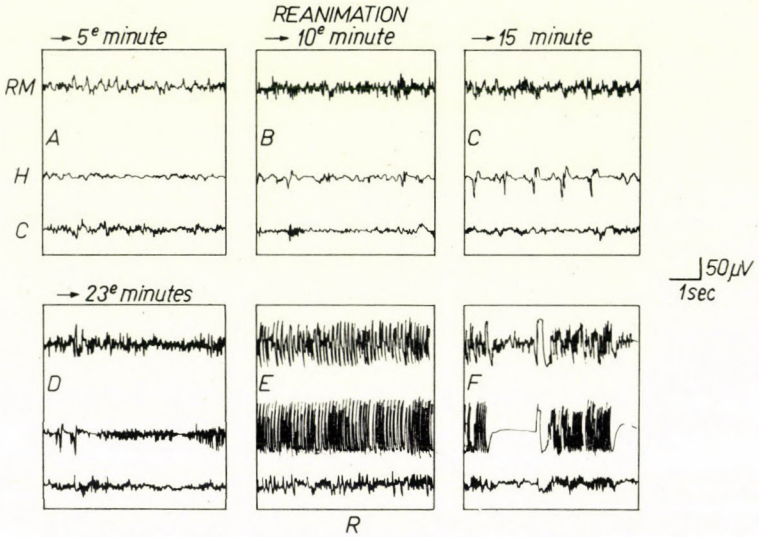

Fig. 5. Réanimation : Installation progressive d'une décharge toni-clonique qui débute par des pointes rythmiques à la 10ème minute pour se préciser à la 23ème minute

pas de concordance entre la décharge électrique et le comportement. D'autre part, certains animaux ont présenté un comportement type KLÜVER et BUCY avec cécité psychique, placidité et hypersexualité.

Discussion

La répétition d'hypoxies oxyprives a mis en évidence chez le chat libre de ses mouvements, une activation de l'hippocampe. Cette activation est différente selon les conditions expérimentales et à ce titre l'activation de l'hypoxie aiguë est à différencier de celle qui se produit en cours d'hypoxie subaiguë, de réanimation ou de période post-anoxique.

En cours d'hypoxie aiguë ($O_2 = 4\%$), l'activation hippocampique se traduit soit par des pointes brèves et amples, soit par un fuseau rapide.

Les pointes hippocampiques constantes dès les premières hypoxies sont comparables à celles décrites lors de la période lente du sommeil du chat [4] et leur apparition peut être favorisée par la dépression corticale qui leur est associée.

Les fuseaux hippocampiques sont rares, inconstants et surviennent à un stade avancé de la période de dépression. Ce type d'activation en fuseaux peut être trouvé au niveau d'autres structures (cervelet, gyrus ectosylvien, noyau

caudé) mais intéresse plus particulièrement la formation réticulaire. La constance et la durée du fuseau réticulaire sont à opposer à la rareté et la brièveté des fuseaux des autres formations. Aussi l'activation réticulaire, dont le mécanisme chémoceptif a été précisé par DELL doit-elle être différenciée de l'activation des autres formations encéphaliques et en particulier de l'hippocampe. L'absence de relation entre les fuseaux des diverses structures est un argument en faveur d'une activation localisée et non propagée.

En cours d'hypoxie subaiguë ($O_2 = 8\%$) de *réanimation* ou de *période post-anoxique*, l'activation de l'hippocampe correspond à des décharges organisées d'expression variable: pointes rythmiques, fuseaux, décharges tonico-cloniques. Les décharges hippocampiques sont les seules manifestations convulsives des hypoxies subaiguës. Elles sont associées à des décharges de type myoclonique ou généralisé en cours de réanimation ou de période post-anoxique.

Ces décharges ne surviennent qu'après plusieurs hypoxies et bien que la répétition des hypoxies soit un facteur essentiel dans leur apparition, leur mécanisme physiopathologique est probablement différent selon les conditions expérimentales: hypoxie subaiguë, réanimation, séquelles post-anoxiques.

La réactivité exquise de l'hippocampe à toute stimulation mécanique, électrique et chimique est un premier facteur à envisager. L'hippocampe est en effet une structure à seuil convulsivant abaissé et dont les décharges sont facilement provoquées chez l'animal et chez l'homme. L'activation provoquée par l'hypoxie peut donc intéresser particulièrement cette structure et les décharges hippocampiques, seule manifestation convulsive de l'hypoxie subaiguë pourraient ainsi être facilitées.

La vascularisation atypique de l'hippocampe, qui pour SPIELMEYER [6], serait à l'origine des lésions anoxiques de la Corne d'Ammon est un deuxième facteur à retenir. Toutefois, dans le sens de cette hypothèse, certaines constatations sont d'interprétation difficile. Il est en effet surprenant que l'hippocampe, structure mal vascularisée, ait une activité qui reste longtemps organisée durant l'hypoxie et soit la première à réapparaître en cours de réanimation. D'autre part les lésions de la Corne D'Ammon sont inconstantes et sur les 15 cerveaux examinés, deux seulement ont présenté des lésions neuronales et prédominant dans les secteurs CA_1 et CA_2.

D'après l'ancienne théorie de la pathoclise de C. et O. VOGT [1922], reprise partiellement par SCHOLTZ [5] il est possible que des facteurs métaboliques favorisent l'apparition des décharges hippocampiques. Cette hypothèse est appuyée par certaines constatations dont celles de MACLARDY concernant les variations du zinc dans le secteur CA_3 au cours des convulsions anoxiques.

Si le mécanisme des décharges hippocampiques provoquées par des anoxies oxyprives répétées reste imprécis et demande des recherches ultérieures, la réactivité particulière de l'hyppocampe à ce type d'anoxie est indiquée par ces résultats.

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THE POSSIBLE ROLE OF THE HIPPOCAMPUS IN THE ORGANIZATION OF THE ORIENTATION REACTION

By

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Behavioural and electrophysiological findings concerning the function of the hippocampus have been presented. It has been established in freely moving cats with chronically implanted electrodes that in contrast to the generally accepted view, hippocampal arousal is characterized by desynchronization, similar to that of the neocortex, and not by theta waves.

Analysing the orientation reaction and its relation to the hippocampal theta activity, it was found that unfamiliar stimuli in familiar environment did not elicit an orientation reaction. Orientation reaction could be elicited only by stimuli having a conditional signal property. The hippocampal theta rhythm was found to be a regular concomitant of the orientation reaction.

An intensification of the orientation reaction was observed after hippocampal lesions. The impairment of conditional reflex performance observed after hippocampal lesions seemed to be related to this factor.

Analysing the effects of hippocampal lesions in a multiple-choice delayed reflex situation it was found that the delayed reflex could not be elaborated in lesioned cats, while the reflex elaborated before the lesion was impaired only temporarily.

The possible function of the hippocampus and the significance of the theta rhythm has been discussed.

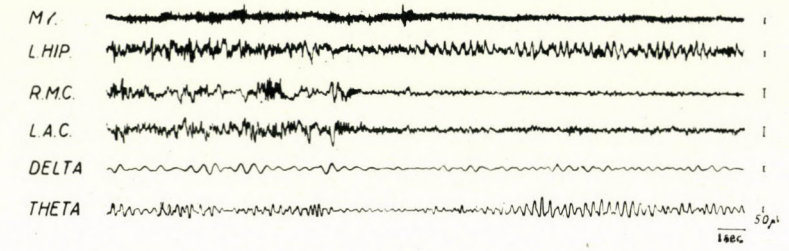
The aspects of hippocampal function in conditioning have been studied in our laboratory for years. In the present paper we wish to report on some new findings in this field.

Although the characteristic hippocampal theta waves had also formerly been recorded [8], GREEN and ARDUINI [7] were the first to subject this peculiar archicortical electrical pattern to a systematic analysis. They found that, in contrast to neocortical desynchronization, arousing stimuli always elicited a synchronous theta rhythm in the hippocampus and termed this phenomenon "hippocampal arousal reaction".

In experiments performed on freely moving cats provided with implanted electrodes it became conspicuous that at awakening from natural sleep this expected reaction consistently failed to appear [9].

In a series of experiments a more systematic control of this unusual finding was made and finally the conclusion has been reached that in the course of awakening from natural sleep a desynchronization, similar or identical to that of the neocortex, was appearing in the hippocampus, too. This reaction could be observed both at spontaneous awakening and at arousal by sound stimuli *(Figs 1 a, 2 a)*. Hippocampal desynchronization was most marked at awaken-

ing from the so-called paradoxical phase of sleep, characterized by a continuous theta activity in the hippocampus [5]. A sudden interruption of this characteristic theta activity was observed at the moment of arousal *(Figs 1 b, 2 b)*. Awakening from natural sleep elicited by high frequency electrical stimulation of the mesencephalic reticular formation was also accompanied by desynchronization in the hippocampus *(Figs 3 a, 3 b)* ; this fact seemed to be of especial importance, since high frequency electrical stimulation of the reticular formation is known to be the most effective way of eliciting hippocampal theta



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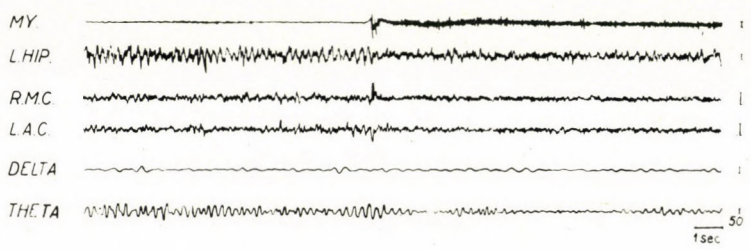
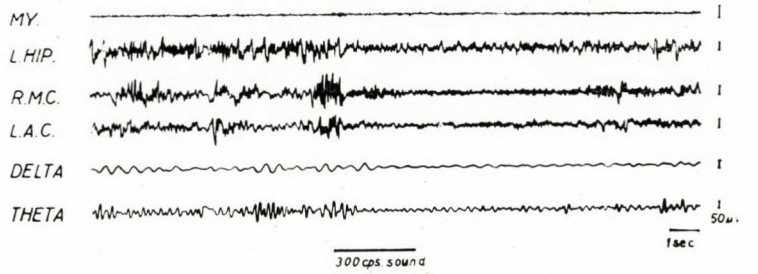


b)

Fig. 1. Spontaneous arousal from deep (synchronized) sleep *(a)*, and from paradoxical (desynchronized) phase of sleep *(b)*.
My = electromyogram of cervical muscles; L. Hip. = left hippocampus; R. M. C. = right sensorimotor cortex; L. A. C. = left auditory cortex; Delta and Theta = delta and theta frequency band of the left hippocampal record filtered by a frequency analyzer

activity in waking animals. The duration of hippocampal desynchronization was found to depend on the strength of somatic reactions accompanying the arousing stimulation. When arousal was seen to be followed by an intensive orientation reaction, desynchronization sometimes appeared only for a short period but never failed to take place. If arousal was not accompanied by orientation, distinct desynchronization appeared in the hippocampus.

From these findings the conclusion has been drawn that *there is no essential difference between the electrical arousal patterns of the hippocampus and that of the neocortex, moreover that hippocampal theta activity is not an inevitable component of the arousal reaction, but belongs to a well-defined behavioural reaction, viz. orientation.*



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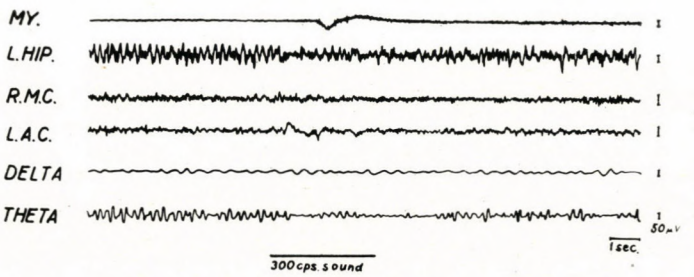

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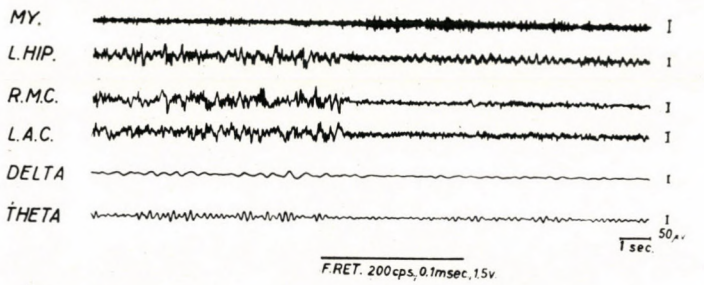
Fig. 2. Arousal from deep sleep *(a)*, and from paradoxical phase of sleep *(b)*, elicited by 300cps tone. Abbreviations as in *Fig. 1*



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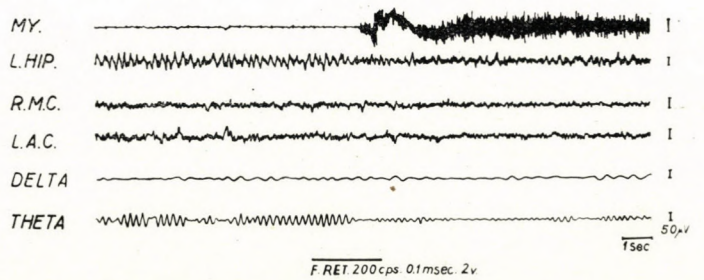

b)

Fig. 3. Arousal from deep sleep *(a)*, and from paradoxical phase of sleep *(b)*, elicited by high frequency electrical stimulation of the mesencephalic reticular formation (200 cps, 0.1 msec, 1.5 V). Abbreviations as in *Fig. 1*

In a second series of experiments the orientation reaction and its relation to hippocampal theta activity were analysed in detail.

According to the classical definition, the orientation reaction is an unconditional reflex elicited by unfamiliar stimuli. The first difficulty in the experiments was the definition of the unfamiliar stimulus. Practically one can never be certain whether a stimulus to be presented in the experimental situation had not met the animal under natural conditions or had not become known by the process of stimulus generalization. This problem — in our opinion — could be settled by considering that stimuli can not be isolated from the environment in which they are presented. Therefore, if an animal is held in a standard environment for a longer period of time and subsequently a supposedly

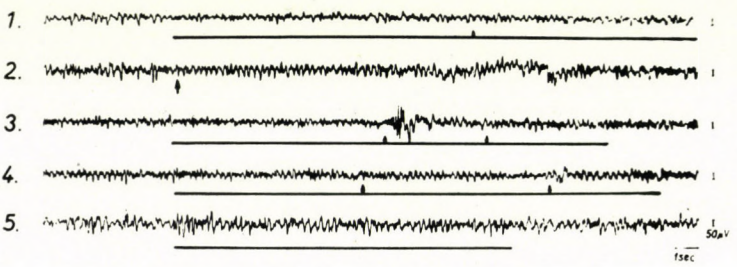

Fig. 4. Hippocampal electrical manifestations to different stimuli applied in the home cage.
Record 1.: Cat 7/1, unknown sound stimulus elicited no somatic reaction, but marked desynchronization in the hippocampus.
Record 2: At the arrow the experimenter entered the home cage; continuous theta activity appeared in the hippocampus.
Record 3: Alimentary signal applied in the home cage elicited desynchronization. At the arrows, the animal rose and watched the door.
Record 4: Effect of avoidance signal in inadequate situation. At the arrows, the animal went back to its resting pillow.
Record 5: In cat 7/4 a new but strong sound stimulus elicited orientation and marked theta activity in the hippocampus

unfamiliar stimulus is presented in the same situation, *i.e.* in a combination never met with before, then that stimulus very probably represents a novelty.

In the present experiments each cat was held in a soundproof chamber ("home cage") for 2 to 4 weeks to ensure a standard environment. It was always the same person who took care of the animals at the same hour every day. The animals soon became accustomed to the "home cage" and followed a normal course of life in every respect. In this way was realized the background for forming a new stimulus-complex by presenting single stimuli. (A familiar situation together with a strange single stimulus must probably act as an unfamiliar stimulus complex.)

This experimental arrangement led to surprising findings. First of all the new stimulus at its first presentation did not elicit the expected tonic orientation reaction; when the cat had been exposed to a hitherto unknown stimulus

in the familiar environment, either no reaction whatever could be observed or although the animal did not seem to take any direct notice of the new stimulus it was urged to perform one of its natural activities, it looked for food at the saucer or went to urinate, *etc.* Simultaneously with these actions marked desynchronizations were recorded in the hippocampus (*Fig. 4*, record 1). In contrast to these manifestations the appearance of the experimenter in the home cage provoked continuous theta activity (*Fig. 4*, record 2). Care was taken in this experiments not to use strong stimuli, considering the well-known fact that a strong stimulus may in itself have a definite conditional signal character. The above described motor reactions and the accompanying hippocampal desynchronization could be observed in 5 out of the 7 animals used in this experiment. To one of the remaining two cats a strong sound stimulus was presented and the expected orientation reaction appeared (*Fig. 4*, record 6). One animal could not become accustomed to its home cage in two weeks and it answered with orientation and escape reactions to the new stimulus. In both cases a continuous theta activity appeared in the hippocampal record.

On the basis of these observations the conclusion seemed warranted that *the strangeness of the stimulus or stimulus-complex does not belong to the essential conditions of the elicitation of an orientation reaction.*

To test the effects of conditional signals in the familiarized environment, alimentary and avoidance conditional reflexes were elaborated in the same animals in different compartments, with the stimuli tested before as new ones. Stimuli now serving as conditional signals were applied again in the home cage. No orientation reaction could be observed in these circumstances, either. The alimentary conditional signals elicited alimentary type reactions, *e.g.* the animal went without hesitation to the feeding place of the home cage; or watched the door through which it usually received the food. A reaction was seldom induced by the avoidance signal; in these few instances the animal retired to its resting place. There was no accentuation in hippocampal theta activity during these reactions (*Fig. 4*, records 3 and 4).

Thus it has been established that if signals elaborated in different special conditional situations are applied in an environment having well established cues, instead of an orientation reaction, habits corresponding to the special requirements of this situation are elicited.

In a next series of experiments the signals of two different and antagonistic conditional situations were interchanged: the alimentary conditional signal was applied in the avoidance situation and the avoidance signal in the alimentary environment.

In many cases a typical orientation reaction appeared in these tests (*Fig. 5*, record 2) but it also happened that the inadequate signal, immediately on its application or after an orientation reaction, elicited the reflex corresponding to the actual experimental situation (*Fig. 5*, records 3 and 5).

The most expressed orientation reactions were obtained in an apparatus used for simultaneous alimentary and avoidance conditioning [2], by reversing the original sites of the conditional signals. Originally the alimentary signal was applied below the feeding device, the avoidance one below the flight place, thus the source of the signals was indicating the direction of the reflex performance. (A detailed description of this method has been presented by GRASTYÁN [6].) Simultaneously with the orientation reaction, a prominent theta activity appeared in the hippocampus in all cases (*Fig. 5*, record 6).

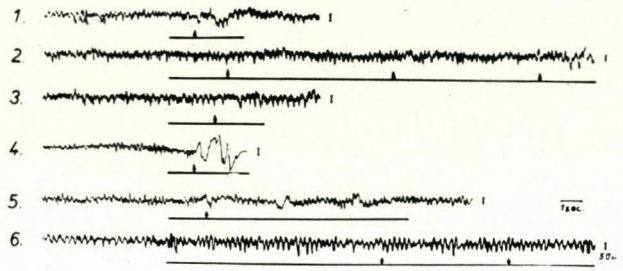

Fig. 5. Hippocampal electrical manifestations on interchanging of the conditional signals.
Record 1: Cat 7/1. Effect of avoidance signal in avoidance conditional reflex apparatus. At the arrow, the animal performed the correct reaction.
Record 2: Effect of alimentary signal in avoidance situation. At the arrows, marked searching movements.
Record 3: Alimentary signal elicited (arrow) avoidance reaction in avoidance conditional reflex apparatus.
Record 4: Alimentary signal in alimentary situation elicited a short latency correct response.
Record 5: Avoidance signal applied in an alimentary reflex situation elicited an alimentary reaction.
Record 6: Cat 6/4. Long lasting orientation reaction elicited by the displaced avoidance signal. At the arrows marked searching movements

On the basis of these observations we feel justified in stating that the orientation reaction consists in a peculiar conditional phenomenon, which appears when different conditional signals are interfering.

It must be emphasized that to produce orientation, the coinciding signals must be motivated approximately at the same level, because if a dominant excitatory process (determined, *e.g.* by the environment) is occurring in the nervous system, even a stimulus having a definite antagonistic character may elicit a reaction corresponding to the dominant process.

Similar observations were made by BERLYNE, emphasizing the importance of the conflict in orientation [1], and by POLEZHAYEV [13] who found that the strength of the orientation is commensurate with the signal meaning of the stimulus which elicits it. In SOKOLOV's opinion [15] orientation appears when a new, unknown stimulus comes into collision with the neuronal pattern of a well-known complex of stimuli. It has been demonstrated in the present experiments that in a familiar environment new stimuli elicit no orientation but a

reaction determined by the environment. In such circumstances the new stimulus is incorporated into the neuronal pattern produced by the signals of the environment. It seems obvious that unfamiliar stimuli cannot have special neuronal patterns, consequently for the production of orientation two conditional signals, both having special neuronal patterns, must interfere.

Hippocampal theta activity has been found [3] to be a regular concomitant of the orientation reaction; consequently the hippocampus must be

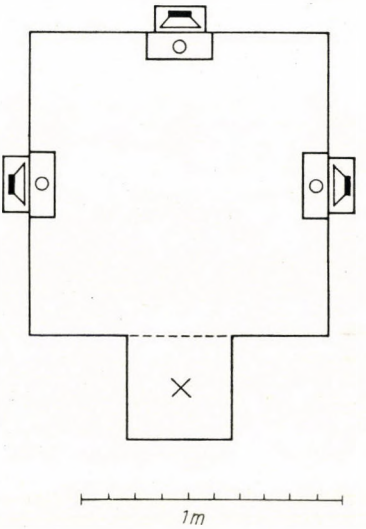

Fig. 6. Apparatus used for the elaboration of the multiple-choice delayed conditional reflex Further details see in text

assumed to play an important role in the organization of orientation. On the basis of histological findings, the structure of the hippocampus is appropriate for the comparison of signals of different modalities and perhaps those being delayed [11]. This assumed function may represent an important phase in the organization of the orientation reaction. The effect of hippocampal lesions seems to support these assumptions.

It is well-known from human pathology that after bilateral hippocampal resection recent memory processes suffer a considerable loss, while memory traces stored before operation remain intact [12, 14].

In order to approach the closer mechanism of this impairment, a study has been made in cats concerning the effect of bilateral hippocampal lesions on different types of conditional reflexes. To produce the lesion, the lateral ventricle was opened through the posterior part of the posterior ectosylvian gyrus and under direct visual control the hippocampus was sucked out. The lesions were never complete but they extended to the greater part of the hippo-

campus [10]. In the control operation the brain tissue covering the lateral ventricles was removed.

There were no essential changes in spontaneous behaviour of the lesioned animals. They had no motor disturbances but were hyperactive both in a strange environment and in the conditional reflex situation.

The effect of the lesions on the learning process was at first studied in a multiple choice delayed reflex situation. The apparatus is shown in *Fig. 6*. On three side-walls of the box feeding devices and loudspeakers are fixed. On the

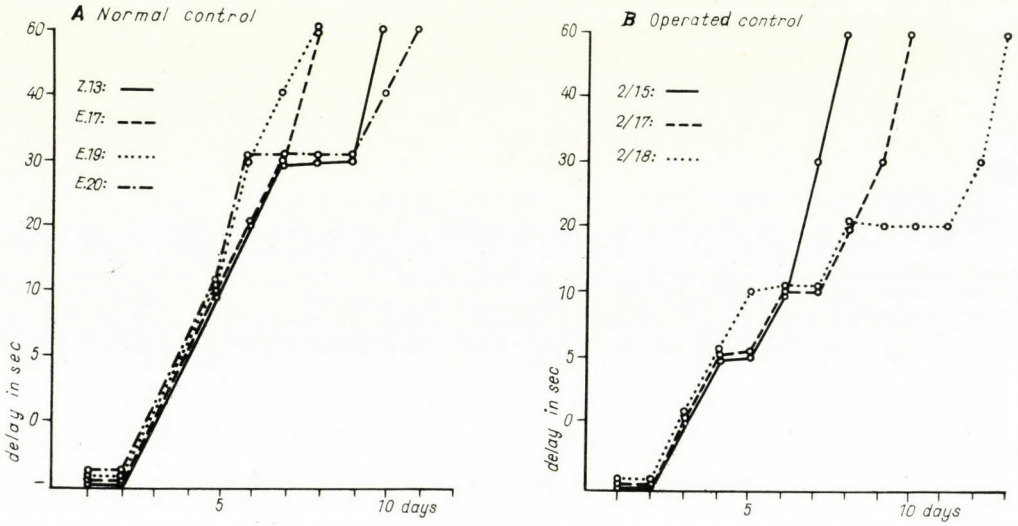

Fig. 7. Elaboration of the delayed reflex in normal and operated control animals. On abscissa: successive days of conditioning, on ordinata: delay in seconds

fourth wall a small box (marked with X) is fixed to restrain the animal during the delay period. The elaboration of the delayed reflex was made in two phases. First the animals learned the acquisition of food at the feeding device signalled by a conditional sound stimulus. In the second phase the animals were restrained in the small box for progressively increasing periods after switching off the conditional signal. A new delay value was only introduced if the performance had reached 90 per cent. The longest delay period was one minute.

The one minute delay period was usually reached in 6 to 12 days by both normal and control animals *(Fig. 7)*. The first phase of the task was easily learned by the animals with hippocampal lesion, but performance declined even at the shortest delay period and the best performance did not surpass a 10 seconds delay *(Fig. 8)*.

The reflex elaborated before lesioning was usually destroyed for some days. Thereafter the achievement improved from day to day and finally it

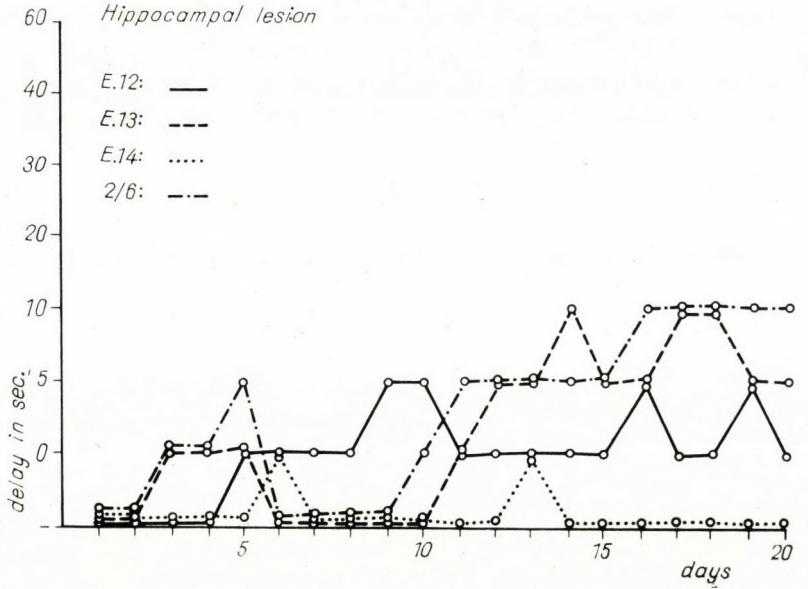

Fig. 8. Elaboration of the delayed reflex in animals with hippocampal lesion. A maximum of 10 sec delay could be attained

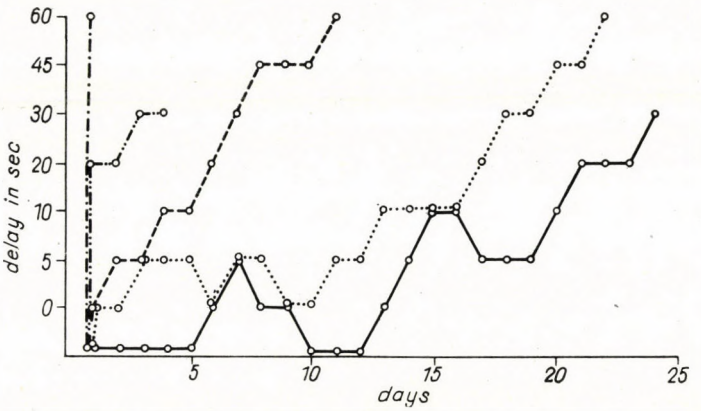

Fig. 9. Re-training after hippocampal lesion. All the animals trained before the lesion attained the level of preoperative performance (60″)

attained the criterional level, though the number of errors was slightly higher than before operation *(Fig. 9)*.

It could undoubtedly be established that retention was less impaired than the learning of a new task after the lesion.

These findings suggested that *the hippocampus plays its role not in the storing of the memory traces but in the dynamics of the learning process, in the selection and shifting of impulses toward the corresponding storage places.*

To check this conclusion we analysed in the above-mentioned double conditioning apparatus the effect of hippocampal lesions on simultaneously elaborated alimentary and avoidance conditional reflexes.

In this dual behavioural background the hippocampal lesion resulted in definite disturbances in the performance of both reflexes. Some insight into the mechanism of these disturbances has been gained by a comparative analysis

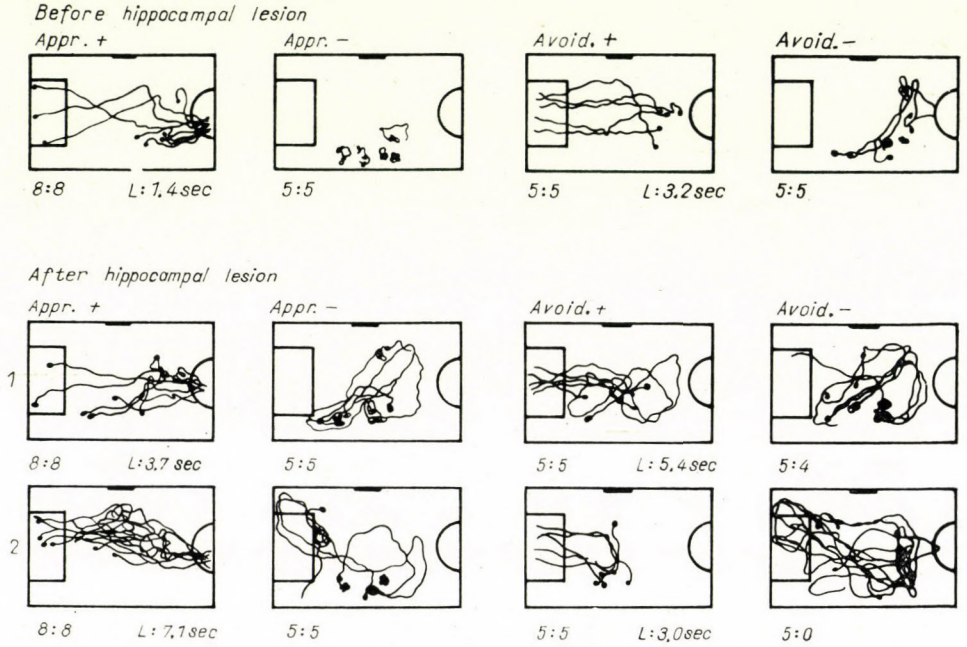

Fig. 10. Movement patterns of cat 8/3 in the dual conditioning box before and during the first and second experimental session after the hippocampal lesion. The feeding device is marked by a semicircle, the flight place by a square in the sketch of the conditioning apparatus. Below, the ratio of reinforcements to correct responses, and the average latency of the reactions are given

of the pre- and postoperative movement patterns. The movements of the animal were registered by a simple method [2]; this consisted in attaching a small lamp to the head of the animal and photographing its light by a camera fixed to the top of the box. Movements in the record are thus represented by continuous lines. The summarized graphs of the conditional reactions of a cat in certain typical experimental sessions before and after operation are shown in *Fig. 10*. Before the lesion the conditional reactions were always accomplished in the possible shortest way. In contrast, after the lesion long-lasting orienting, searching movements consistently preceded the attainment of the conditional goal. The animal was apparently diverted by a series of environmental stimuli which had no influence on it before the lesion. This was expecially noticeable in the

course of incorrect reactions produced to negative signals. The performance improved gradually, but the reflex remained uncertain.

The most characteristic effect of the hippocampal lesion was a hyperactive orientation reaction.

On the basis of these observations the conclusion has been reached that the function of the hippocampus consists in a suitable combination of incoming environmental stimuli with motivational processes taking place simultaneously. The possible mechanisms of this function have been discussed in more detail by E. GRASTYÁN [6].

Acknowledgement

We are indebted to the "Műszeripari Művek", Esztergom, Hungary for the loan of the frequency analyzer.

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RETICULAR CONTROL OF SPLANCHNIC AFFERENTATION

By

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In 37 unanaesthetized cats paralysed with gallamine triethiodide the effect of stimulation of the reticular formation for various lenghts of time on the cerebral cortical evoked potentials of splanchnic and sciatic origin has been investigated. On increasing the non-specific stimulation the evoked potentials of splanchnic origin were found to be gradually blocked after an initial facilitation, whereas the sciatic nerve responses were facilitated in an increasing measure.

Since 1949, when MAGOUN and MORUZZI described the non-specific effect of the brain stem reticular formation on the function of the central nervous system, the question has often been raised how this non-specific system influenced the specific afferent impulsation running along the classical sensory pathways. It has been suggested that the amplitude of the cerebral cortical evoked potential arising in response to short somatic, visual and auditory stimulation decreases, if the brain stem reticular formation is simultaneously stimulated electrically [3, 9, 13, 14]. A decrease of the amplitude of the evoked potential in response to somatic stimulation has been described by HERNANDEZ-PÉON *et al.* [11], who increased the activity of the non-specific brain stem system in the cat by showing the animal a mouse. TROUCHE *et al.* [17] have found the evoked potentials to decrease in those phases of setting up conditioned reflexes, in which, as it is known from other works [7, 8, 10], the increased activity of the brain stem activator system elicits a substantial orientation reaction.

However, the studies mentioned have not been concerned with the interactions between the specific afferentation starting from the internal organs and the reticular formation of the brain stem.

In the present study we have attempted to determine how an increase in the activity of the non-specific brain stem system would influence the cerebral cortical evoked potentials elicited by the stimulation of the splanchnic nerve carrying visceral afferent impulses.

Methods

Acute experiments were made on 37 cats of either sex, anaesthetized with ether-hexobarbital. We exposed the right anterior ectosylvian gyrus from which evoked potentials of the highest amplitude and shortest latency could be recorded in response to splanchnic stimulation

[2]. By means of a stereotaxic apparatus, bipolar deep electrodes were placed in the reticular formation. Under the diaphragm we exposed and fitted with bipolar electrodes the left splanchnic nerve, as well as the sciatic nerve carrying exteroceptive impulses. The latter served for control in the experiments. To control the reticular activator effect, EEG needle electrodes were placed in the frontal, temporal and occipital areas. The general excitatory state of the central nervous system was appraised on the basis of the EEG patterns.

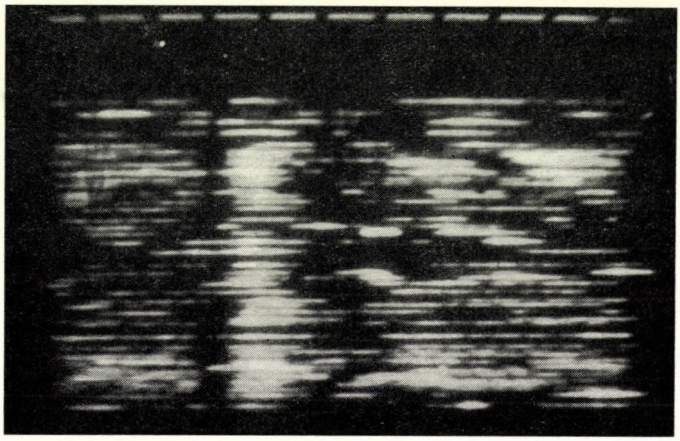

Fig. 1. Appearance of 40 evoked potentials, as recorded by the intensity modulation technique. Splanchnic nerve, 5 V, 0.5 msec, 20 sec intervals.
Light = —, dark = +. Time signal 20 msec

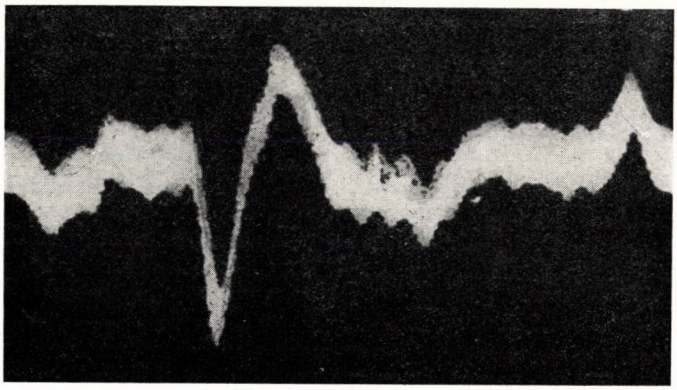

Fig. 2. Densitometric evaluation of *Fig. 1*

The experiments were begun when in cortical activity, the fast activity characteristic of the alert state had gained preponderance, after the effect of anaesthesia has come to an end. Then the animals were paralysed by the administration of gallamine triethiodide and artificial respiration was started. We determined the minimum intensity of stimulation which still increased spontaneous cerebral activity (1.5 to 3.0 V 300 c/s, 1.0 msec). On the basis of the consideration that the mesencephalic activator system is sensitive to the quantity rather than to the quality of the impulses reaching it, we induced various states of non-specific activity by applying the above stimulation for various lengths of time, ranging from 200 to 5000 msec. For this purpose we constructed a special apparatus, which directed the pulses coming from the square pulse generator to the reticular formation

electrode for the desired length of time, then emitted a trigger impulse, initiating thereby the test-stimulus applied to the splanchnic or sciatic nerve (5 to 10 V, 0.5 msec). The same impulse started the ray of the cathode oscillograph. The stimuli were presented at 20 sec intervals. For recording, we used an eight-channel EEG apparatus to determine the level of cerebral electrical activity, and a two-ray cathode-oscillograph for recording the cerebral cortical evoked potentials in response to stimulation of the nerves, from an unipolar lead.

For the sake of a reliable statistical analysis, in the course of recording the intensity modulation technique of KOZHEVNIKOV [12] was applied. The essence of this is that the adequately amplified cerebral cortical activity is applied to the Z axis of the cathode ray, *i.e.* the intensity of the ray running along the screen is modulated. If the ray is caused to divert a little on the Y axis after every run, many responses, in our case 40 stimulations, can be recorded on one square of film *(Fig. 1)*. The film thus obtained was pulled at a given speed in front of an evenly illuminated slit, and the changes in the voltage of the photocell behind it were recorded by means of a direct-writing densitometer. In this way we obtained the mean value of the responses given to 40 stimulations (*Fig. 2*). For evaluating the results, the changes in the latency of the evoked potentials, the amplitude and duration of the positive and negative waves were taken under consideration. Data concerning about 20,000 evoked potentials were analysed during the study.

Results

The results were expressed in the percentage of the evoked potentials recorded at the beginning and end of every experiment, without stimulating the reticular formation. In the graphs the abscissa shows the duration of the stimulation of the reticular formation in msec, and the ordinate the percentage

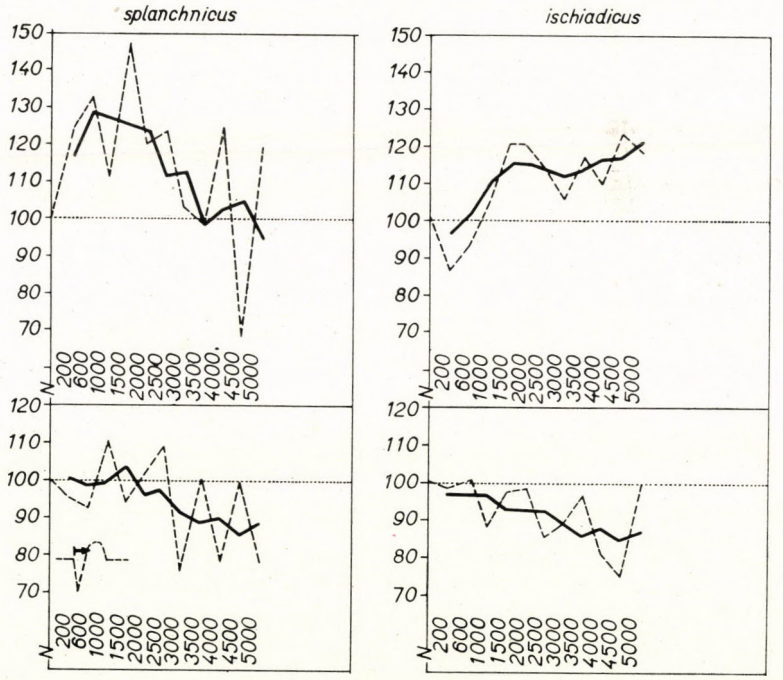

Fig. 3. Positive wave of evoked potentials. Abscissa: duration of stimulation of reticular formation, msec
Ordinate: data expressed in percentage of the normal values (broken line). Solid line: dynamic average

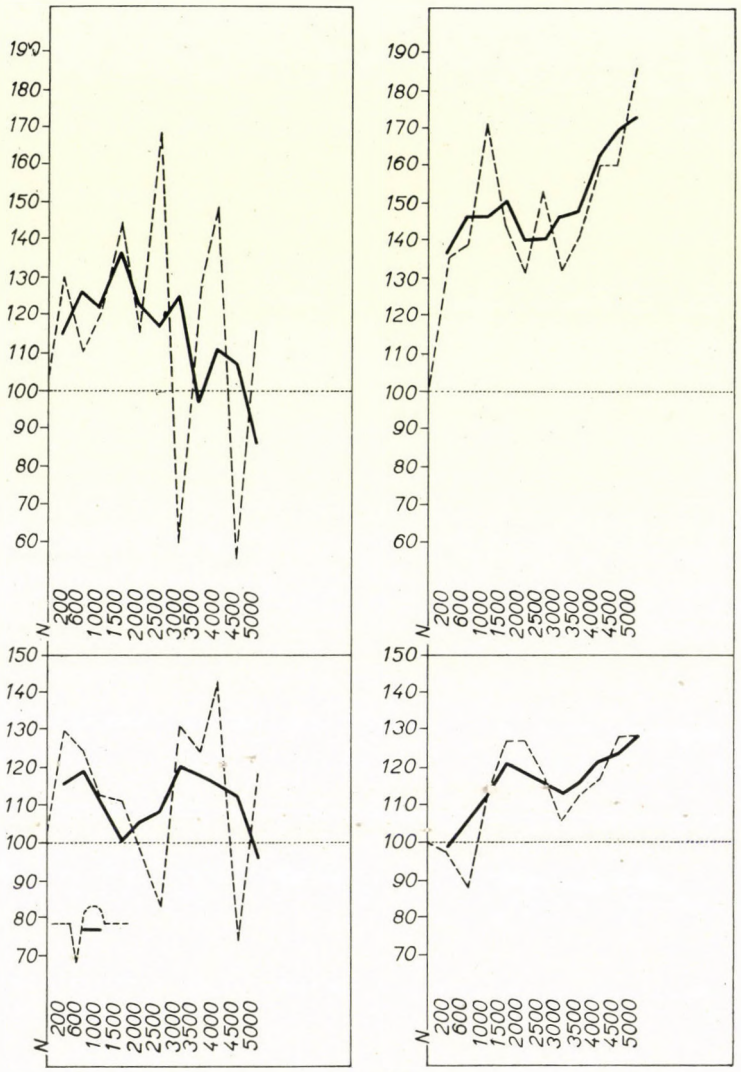

Fig. 4. Negative wave of evoked potentials. Signs as in *Fig. 3*

values. Every single point of the curve represents the mean of at least 160 evoked potentials. To make the tendency of the changes more conspicuous, the curves were subjected to analysis by the method of dynamic averages [16]. No significant change has been recorded in the latency of the responses recorded while altering the measure of non-specific activity.

Fig. 3 shows the data for the positive phase of the evoked potentials. About 20 to 30 per cent of the amplitudes of the positive waves of evoked potentials obtained in response to splanchnic stimulation were facilitated in the course of short (200 to 2500 msec) stimulation of the reticular formation. With

longer stimulation the measure of facilitation tended to decrease. At the same time, on stimulating the sciatic nerve, used as the exteroceptive control, the variations of the single values were much smaller than in the case of the evoked potentials obtained by splanchnic stimulation. The tendency of the change was just the opposite of that outlined above, notably there was a 10 per cent inhibition on stimulating the reticular formation for 200 to 600 msec, and on increasing the duration of stimulation, inhibition gradually turned into 20 per cent facilitation.

In the case of both nerves the duration of the positive wave was reduced in the same measure on increasing the duration of reticular formation stimulation.

Fig. 4 shows the data for the negative waves, in a similar fashion. In the case of splanchnic stimulation there was a conspicuously wide range of variation of the values. The tendency of the change was revealed by the method of dynamic averages. After an initial facilitation there occurred an increasing inhibitory tendency on increasing the duration of stimulation. In the case of the sciatic nerve facilitation was 37 per cent even at 200 msec stimulation of the reticular formation; this increased gradually to reach 88 per cent when the reticular formation was stimulated for 5000 msec.

The negative phase of the response was protracted in the case of both nerves.

Discussion

The effect of stimulation of the reticular formation upon specific afferentation is a controversial problem; some authors reported on a decrease [3, 9, 13, 14, 17], while others on an increase in the amplitude of the evoked potentials [4, 5, 15]. Evaluation must be done with caution, because spontaneous cerebral cortical activity, as the background, may significantly influence the amplitude of the evoked potentials [1]. In our experiments we always determined that minimum intensity of stimulation, which appreciably activated cerebral cortical electrical activity. This threshold-stimulation has made it possible to standardize the stimulations.

First of all, our results show that stimulation of the reticular formation for different periods of time, as a non-specific activation, acts in a different way on the afferent pathways carrying interoceptive and those carrying exteroceptive impulses. While in the case of the splanchnic nerve the amplitude of the responses decreased after an initial facilitation, in the case of the sciatic nerve the measure of facilitation was directly proportional to the duration of stimulation. The fact that at the same time there occurred a decrease in the duration of the wave in the case of both nerves, suggests that while in the case of the splanchnic nerve short stimulation causes first an increase, then a gradual

decrease in the number of cells involved in the response, in the case of the sciatic nerve the number of cells involved in the response increases slightly. It should be borne in mind namely that if the increase of amplitude is associated with a decrease in the duration of the wave, this shows an increased synchronization of the activities of the cells involved in the arisal of the evoked potential. But when the two data change in the same direction, this indicates a change in the number of cells involved in the action.

On the basis of the results it may be stated that the non-specific effect of the activation of the reticular formation, as one of the components of the orientation reaction, is influencing differently the impulses running along the extero- and the interoceptive specific pathways. While the interoceptive signals tend to become inhibited after an initial facilitation, the exteroceptive signals become more and more facilitated. From the biological point of view our data may be interpreted as indicating that the stimulus evoking the orientation reaction and eventually signifying danger, mostly comes from the outer world thus the perception of these increases, while that of the interoceptive signals diminishes.

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SOME ELECTRICAL CORRELATES OF DRIVE PROCESSES ELICITED FROM MEDIAN THALAMIC STRUCTURES

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The role of midline thalamic structures in the conditioning process has been studied with the help of the recruiting potential as indicator in three different ways.

In the first case recruiting responses were elicited in two psychologically opposite situations of avoidance conditioning in order to evaluate the influence of different environmental factors on the potential mechanism.

In the second case low frequency stimulation of the n. centrum medianum, n. reuniens and the n. ventralis anterior was used as a conditional signal and the variations of the morphology of the potentials accompanying the development of conditioning have been analysed.

In the third case stimulations were applied to activate a pre-established avoidance or approach conditional reaction, and the potential configurations at which activation had been reached were compared with potential configurations not reaching the threshold of activation.

The observations suggested that despite their common characteristics different parts of the thalamic diffuse projection system represent different mechanisms. A double functional representation of the region of the centrum medianum and its possible role in the conditional process is discussed.

In an earlier series of experiments [1] it has been established that stimulations in two psychologically different situations of avoidance conditioning resulted in markedly different behavioural effects. Stimulation applied in a moment when the cat was staying on the grid used for applying painful electric shock activated the avoidance reflex without the application of the conditional stimulus. In a sharp contrast to this, stimulations applied on the place of flight elicited a very definite quieting, relaxing effect, during which the cats often assumed the typical posture of normal sleep. Considering that the localization of these effects coincided with structures producing recruiting potential [2], an approach of the significance of the mechanism of this potential in the same conditions as the behavioural effects seemed promising.

Experiments were carried out in 18 cats with implanted stimulating and recording electrodes. Recruiting potentials were elicited from the n. centrum medianum, n. reuniens and the n. ventralis anterior, and recorded from the proreus and suprasylvian gyri. Both mono- and bipolar leads and in most of cases simultaneous EEG and oscilloscopic recording were used. Movements of the animals during conditioning were checked by direct observation and were recorded by the EEG with the aid of a special instrument fixed to the animal's head [3]. After implantation of electrodes in the majority of cases two different

kinds of avoidance conditioning (using electric shocks or cold water [4] as punishment), in the rest both avoidance and approach conditioning was elaborated.

A definite change of amplitude of the recruiting potential could be observed to occur in the two opposite situations of avoidance conditioning, namely a marked decrease of amplitude of the surface negative component when elicited at the site of shocking as compared to those elicited in the flight place *(Fig.1)*.

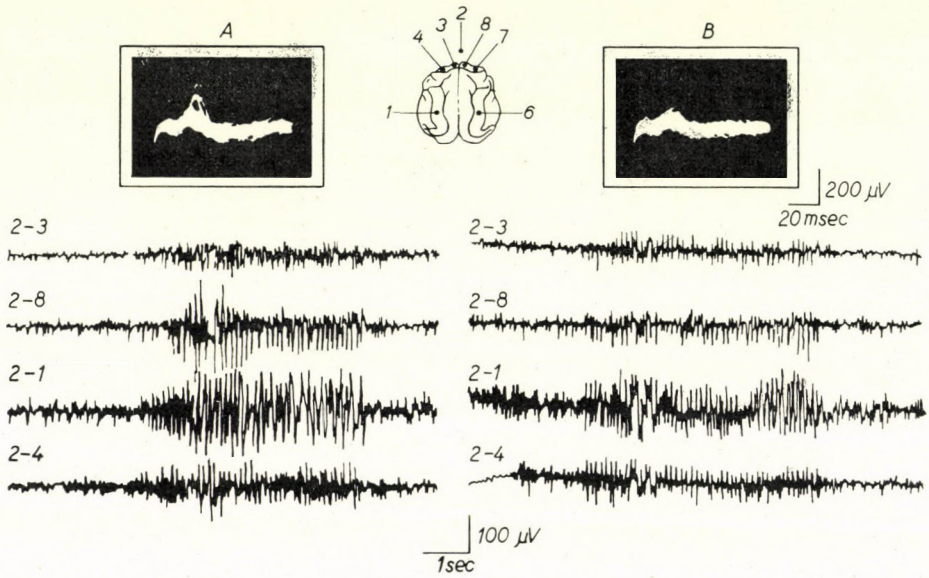

Fig. 1. Oscilloscopic and EEG recordings of the recruiting potentials elicited from the n. reuniens in the case of a stable conditional response

A : On the flight place (on the bench) immediately after the avoidance reaction;
B : On the grid, immediately after removal from the bench, the amplitudes of recruiting potentials are markedly decreased. There are no significant differences in the background electrical activity in the two cases

Application of the conditional signal with the animal in the flight place, or any kind of strong excitement, resulted in a similar effect.

In a second series of experiments it has been studied whether stimulations producing recruiting potentials can serve as conditional signals. A definitely positive answer was obtained in 5 cats by stimulating the n. reuniens. The differences observed during the elaboration of this kind of conditioning as compared to those of the usual conditioning procedure, deserve consideration.

It turned out that more reinforcements were needed to induce the first conditional manifestations in the case of the recruiting potential as a conditional signal than in the usual conditioning process. Moreover, the reflex proved labile even after a high number of reinforcements. An analysis of the recruiting

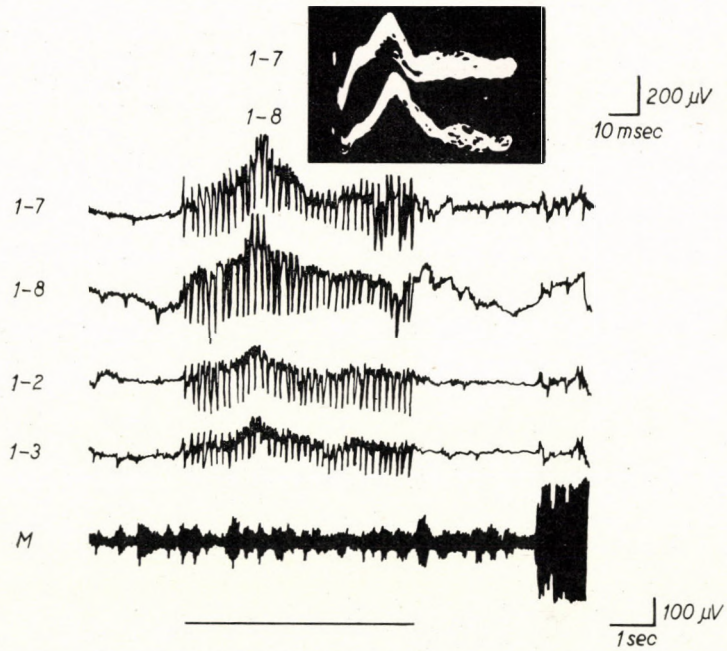

Fig. 2. Stimulation of the n. reuniens elicits typical high amplitude, pure surface negative recruiting potentials. The conditional reaction appears only when stimulation has been terminated. The beginning of the conditional act is shown by the high amplitude oscillations of movement recording (channel marked *M*)

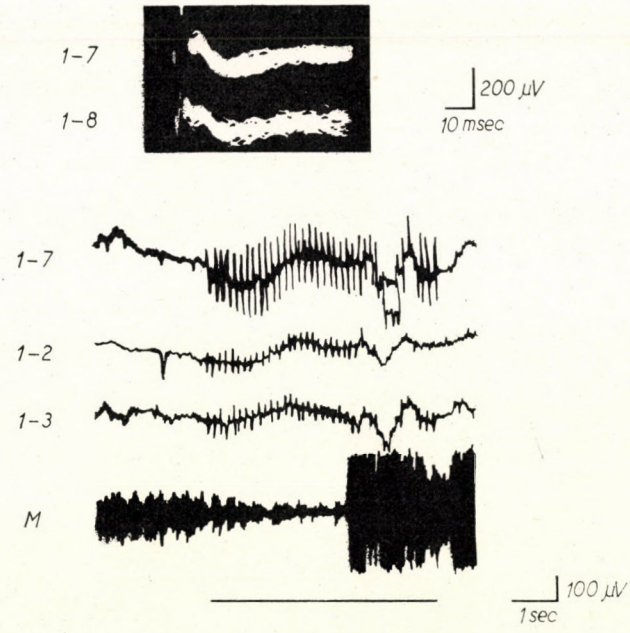

Fig. 3. Stimulation inducing only low amplitude surface positive potentials elicited a short latency conditional reaction during the stimulation

potentials recorded during the conditioning procedure offered some insight into the nature of the happenings. It could be established that under the effect of stimulations producing pure surface negative potentials the avoidance reactions were performed with a constant delay, consistently after the termination of stimulations *(Fig. 2)*. In contrast, stimulations producing less prominent surface negative recruiting potentials preceded by surface positive components,

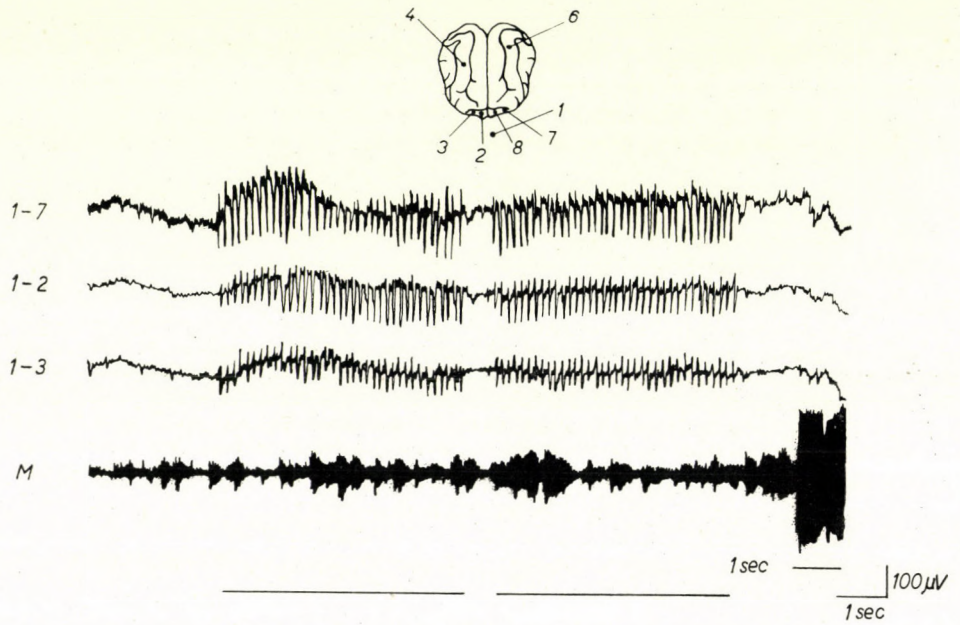

Fig. 4. Repeated stimulation of the same point as in the case of *Fig. 2* suppresses the stable conditional reaction; this appears only after the second train of impulses

elicited short latency conditional responses, consistently appearing during the stimulations *(Fig. 3)*.

These observations suggested that in the case of pure surface negative recruiting potentials it is not the stimulation itself but rather its after-effect which constitutes the real conditioning stimulus. Moreover, from the fact that during this kind of stimulations the animals were generally motionless and the conditional reflex appeared after the stimulations has been terminated, it has been concluded that the recruiting mechanism might reflect an inhibitory mechanism. (A similar conclusion was drawn earlier from a different kind of experiment; GRASTYÁN *et al.* [5]). The assumption could be supported by applying two consecutive stimulations in which the second stimulus train followed the first with the delay of the expected conditional reflex. It was found that the second stimulation completely suppressed the conditional reflex and

appeared after the second stimulation with the same latency as it would have appeared after the first stimulus train *(Fig. 4)*.

In a third series of experiments the correlation between various components of the recruiting potential and the accompanying somatic manifestations have been analysed.

It was found that stimulations of median thalamic structures in the background of pre-established conditional reflexes can activate both the approach and the avoidance reaction. In the case of stimulations producing both activating and relaxing effects depending on the avoidance situation, the electric changes associated with the two effects were characteristically different. Stimu-

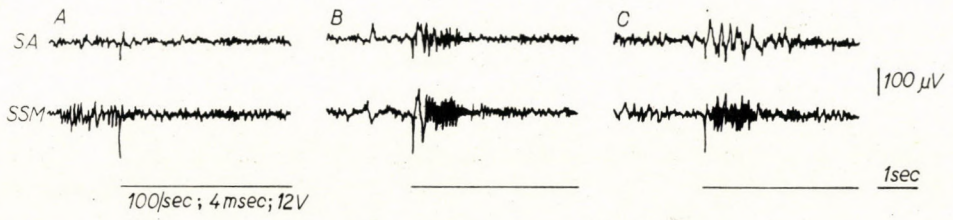

Fig. 5. EEG recordings during the stimulation of the n. centrum medianum producing both activating and relaxing effects depending on the avoidance situation

A : On the grid, stimulation activating the conditional reaction elicited a strong diffuse cortical desynchronization.

B : On the bench, stimulation inducing a marked relaxing effect provoked prompt cortical synchronization.

C : On the bench, continuous stimulation elicited a sleep-like somatic and electric effect

lations inducing activation of the conditional reaction elicited strong diffuse cortical desynchronization while stimulations inducing relaxation even at increased frequencies, provoked prompt synchronization (slow waves, sleep spindles) similar to those appearing in natural rest and sleep *(Fig. 5)*.

In the case of low frequency stimulation, intensities reaching the threshold of activating of the conditional reaction were accompanied by the appearance of a prominent late surface negative component of the potential complex *(Fig. 6)*. This same component could be induced by the simultaneous application of the electrical and conditional stimulus and with stimulations applied during orientation. It was conspicuous in these experiments that stimulations capable of activating conditional reactions always produced polyphasic potentials.

In the cases of stimulations producing pure surface negative potentials, movements appeared only as after-effects. On increasing the intensity of stimulation, finally forced movements appeared also in these cases; these were, however, accompanied by the appearance of a new potential component, as in the above-mentioned cases *(Fig. 7)*.

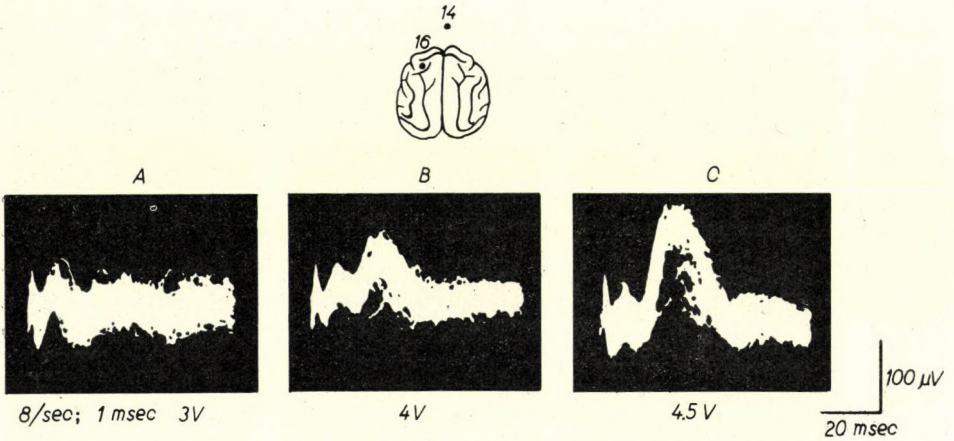

Fig. 6. Oscilloscopic recordings of cortical potentials elicited by stimulation of the n. reuniens with increasing intensities. Intensities reaching the activation threshold of the conditional reaction were accompanied by the appearance of a late surface negative component of the potential complex

A : Stimulation with 3 V produced no somatic effect. *B :* The cat shakes its head. *C :* Stimulation with 4.5 V activated the conditional reaction

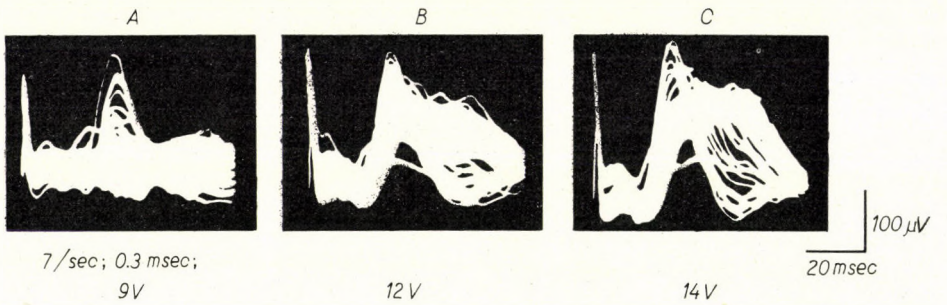

Fig. 7. Oscilloscopic recordings of cortical potentials elicited by stimulation with increasing intensities of the n. centrum medianum.

A : Stimulation producing movements as after-effects induced pure surface negative potentials. *B—C :* Stimulation with higher intensities producing forced movements, elicited a new surface negative potential component

In the course of a systematic analysis of this phenomenon in three animals it was found that at the threshold value of somatic effects it is always a second surface negative potential which characterizes the pattern (Fig. 8).

The assumption that the first surface negative components of the potential complex reflect a general inhibitory, and the second an excitatory mechanism has been suggested by the effects of different parameters. It was found, *e.g.* that by stimulation — in an appropriate conditioned reflex situation — with frequencies of 40—100 cps, causing relaxation it was only the shorter latency first surface negative potential which could be considered to correspond

to this inhibitory mechanism, because the following stimulus fell on the descending limb of this potential, suppressing all later components.

The above observations clearly showed that by the use of recruiting potential as indicator a definite positive correlation can be found between the

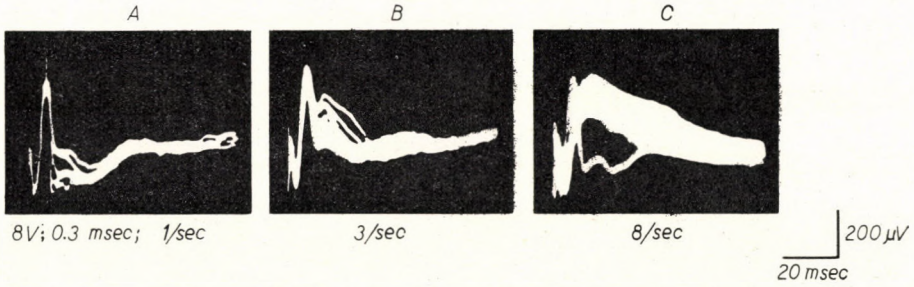

Fig. 8. The intensity of stimulation of the n. ventralis anterior reaching the threshold of somatic effects is accompanied by the appearance of a new late surface negative component of the potential complex.
A : There is no somatic effect. *B—C :* Stimulation produced somatic effects

behavioural and electrical manifestations. The clarification of their more intimate mechanism would require a different approach than that applied in the present study. Still, our finding might provide useful guiding principles in the study of the functional significance of electrical manifestations which are necessarily missing in a pure analytical study.

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DESYNCHRONIZING AND SYNCHRONIZING ELECTRICAL REACTIONS INDUCED BY STIMULATING DORSAL AND VENTRAL LEVELS OF THE AMYGDALOID COMPLEX

By

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Previously it has been shown that fast repetitive stimulation of the amygdaloid complex elicits a low voltage fast cortical activity, similar to the arousal reaction induced by stimulating the brain stem reticular formation.

In the present study it has been observed that within the basolateral amygdala there are two antagonistic systems, one of which desynchronizes while the other synchronizes, the background activity of the neocortex. Experiments were carried out on *encéphale isolé* and *cerveau isolé* cats.

1. Fast repetitive (100—200/sec) stimulation at liminal intensities of dorsal amygdaloid levels (D—3, in particular the central lateral nucleus, A.c.l.) elicits an accelerating-desynchronizing reaction of neocortical electrical activity, mostly recorded from ectosylvian areas. Simultaneously with this cortical response, ample slow and rhythmic waves are generally recorded from the dorsomedian thalamic nucleus. Contrary to the reticulo-cortical desynchronizing effect, which is tonic in type, the response to stimulation of the dorsal amygdala is phasic in nature. The cortical arousal reaction induced by stimulating the dorsal amygdaloid levels persists following complete midbrain transection *(cerveau isolé* preparation).

2. Fast repetitive stimulation of ventral amygdaloid levels (D—6 to —7, in particular the small-celled basal nucleus, A.b.p.) results in a synchronization of neocortical electrical activity in the form of spindles and slow waves. The synchronized responses are more evident in those areas where a desynchronized activity was present prior to stimulation. The synchronizing reaction is not dependent on synchronizing structures from the lower brain stem, since it persists in the *cerveau isolé* preparation.

REMANENCE PHENOMENA IN THE CAT'S CORTICAL SENSORY AREAS

By

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The following variants of remanence phenomena in the auditory and visual cortex have been observed in *encéphale isolé* cats.

1. After protracted acoustic stimulation (0.5—3/sec clicks), spontaneous potentials with no direct connection with an acoustic stimulus appear in various areas of the auditory cortex, exhibiting a morphology identical to that of potentials previously evoked by clicks. This phenomenon of remanence mainly occurs in animals with excessive reactivity, displaying numerous spontaneous arousal reactions and prolonged after-effects following electrical stimulation of unspecific activating systems.

2. After protracted stimulation with rhythmic clicks, fast repetitive stimulation of the reticular formation induces certain potentials independent of any acoustic message and identical to those elicited by clicks in previous stages of the experiment. These potentials elicited by reticular stimulation generally reproduce the rate of clicks currently used during the experiment.

3. After protracted stimulation by rhythmic shocks (1—3/sec) of the lateral geniculate body, spontaneous potentials independent of any geniculate shock are recorded from the visual cortex, reproducing the morphology of geniculo-striate responses, especially of positive-negative waves. These spontaneous potentials develop into a seizure-like pattern. The spontaneous response occurring in the visual area after protracted rhythmic stimulation of the lateral geniculate body persists after extensive isolation of the striate area.

The mechanism of the remanence phenomenon and the role of the reticular formation in evoking spontaneous potentials (reproducing the evoked responses of previous stages) have been discussed.

SLOW SURFACE-NEGATIVE POTENTIALS OF THE CORTEX

By

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In response to a stimulus applied to the surface of the cortex after dendritic potential (DP) — 20 to 30 msec negative potential — slow negativity (SN) of unusually long duration arises (Chang 1951; Goldring and O'Leary 1960) the origin and significance of which has been studied.

Methods

Cats, nembutal, stimulating and recording electrodes Ag—AgCl, D. C. amplifier.

Experimental data

SN arises at greater intensity of stimulation than DP; it can be recorded at a distance of 3 mm; latency of SN is about 15 msec; SN increases during 50—80 msec, reaches 2 mV or more and lasts 300—3000 msec. SN arises in response to repetitive subthreshold (in regard to SN) stimuli with a short (5—20 msec) interval. Thus, SN arises in connection with a significant strengthening or repetition of stimulation. At the stimulation frequency of 10—500 per sec arises a d.c. negative shift. At paired stimuli, SN in response to the second one is depressed while the SN evoked by the first stimulus lasts. Thus SN is similar to electrotonic dorsal root potentials of the spinal cord in form, duration, in relation to paired and tetanic stimulation. On the other hand, if one seeks the analogy among the EEG components, SN can be compared with delta-waves. SN is extremely sensitive to certain drugs. It is weakened or abolished on the local application of strychnine and morphine; it is comparatively resistant to gamma-aminobutyric acid. On the background of SN, DP evoked by stimulation of other points of the cortex is depressed and the primary responses are typically changed; a negative phase is depressed while the positive one is increased and prolonged. The same change in primary responses occurs during natural sleep, at extinction of cortical reflexes and at cathode action.

Hypothesis

SN reflects depolarization of apical dendrites resulting from the activation of glia in their vicinity. On intensive excitation of a complex of cortical neurones, excitation *via* neuroglial connections is transmitted to oligodendrocytes. Then their long-lasting depolarization for some seconds occurs (HILD and TASAKI 1962) with a release of chemical substances in the glia-neuronal space and depolarization of apical dendrites. Thus, SN does not seem to be a direct postsynaptic process. Supposed glia-dendritic mechanism may form the basis of a certain kind of cortical inhibition.

EXCITABILITY AND PROJECTIONS OF SENSORY PATHWAYS TO THE HYPOTHALAMUS

By

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Electrophysiological investigations have demonstrated that the stimulation of the sciatic nerve evokes short latency (7—10 msec) responses in the posterolateral hypothalamus. Such responses propagate in oligosynaptic pathways through the medial lemniscus. Their latency and resistance to anaesthesia simulate properties of responses recorded in the medial lemniscus, upon peripheral stimulation. Their neuronal recovery was, however, found to be prolonged due to an inhibitory effect of the midbrain reticular formation, as lesions in this area shortened the neuronal recovery and introduced recovery peaks in the recovery cycle. In addition to these oligosynaptic projections, there extend also multisynaptic pathways through the midbrain reticular formation to the anterior and medial hypothalamus, as lesions in the former area either abolish or reduce the amplitude and prolong the latency of the long latency (30—40 msec) responses usually recorded in the rostral hypothalamus. Such long latency responses in animals with reticular formation lesions also show a delayed neuronal recovery and a much greater sensitivity to anaesthesia, as compared to normal animals. This would suggest the involvement of more complex pathways with additional synapses in the propagation of these responses in the lesioned animals.

In addition to the oligo- and polysynaptic somatosensory projections to the hypothalamus, there exist visual projections as well. Photic stimuli evoke short latency (8—10 msec) responses in the anterior hypothalamus and preoptic area, while in the caudal hypothalamus long latency potentials (30—40 msec) are recorded. The short latency of the former responses and their resistance to anaesthesia and anoxia, would suggest the existence of direct sensory projections from the optic pathways to the hypothalamus.

The described somatosensory and visual projections play an important role in the behavioural, endocrine and visceral regulation of the hypothalamus.

PRESYNAPTIC INHIBITION IN THE CENTRAL NERVOUS SYSTEM

By

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Frank and Fuortes (1957) and Frank (1959) first clearly showed that there were two distinct types of inhibition. Subsequent investigation by our research groups at *Canberra* has revealed that in the spinal cord a large proportion of the inhibitions is effected by the presynaptic type of inhibition, in which depolarization of excitatory presynaptic fibres depresses their synaptic action. In fact it has now been shown that virtually all medullated primary afferent fibres in the spinal cord are depolarized by conditioning volleys with a consequent depression of their synaptic efficacy.

The contrast between the presynaptic and postsynaptic inhibitory mechanism is illustrated in *Fig. 1*. Postsynaptic inhibitory action is shown on the right side by a special synaptic knob which liberates a transmitter that causes an ionic flux tending to hyperpolarize the subsynaptic membrane and so produces the inhibitory postsynaptic potential (IPSP). As illustrated in the specimen records of IPSP—EPSP interaction, the action of excitatory synapses is counteracted by an antagonistic action on the postsynaptic membrane; hence the generation of an impulse can be inhibited as shown by the records in the lower row. Presynaptic inhibitory action is illustrated in relation to a special type of synaptic knob that is shown superimposed on a large synaptic knob that is associated with monosynaptic activation of motoneurones. Such superimposed synaptic knobs have now been recognized in many locations in the nervous system by Gray (1962, 1963), Kidd (1962) and by Szentágothai (1963). When this presynaptic inhibitory ending is activated, there is a depolarization of the Ia afferent fibre as revealed by subtracting the extracellular potential change from the intracellular in the specimen records of the primary afferent depolarization for 1, 2 or 4 volleys. This depolarization of the monosynaptic E fibre results in a diminution of its spike potential and a consequent depression of its synaptic efficacy, as revealed by the depressed monosynaptic EPSPs, which may fail to evoke reflex discharges as shown in the specimen records labelled presynaptic inhibitory depression. As a further consequence, an inhibition of the monosynaptic reflex spike discharge will be recorded in the ventral root, as shown in the specimen records below.

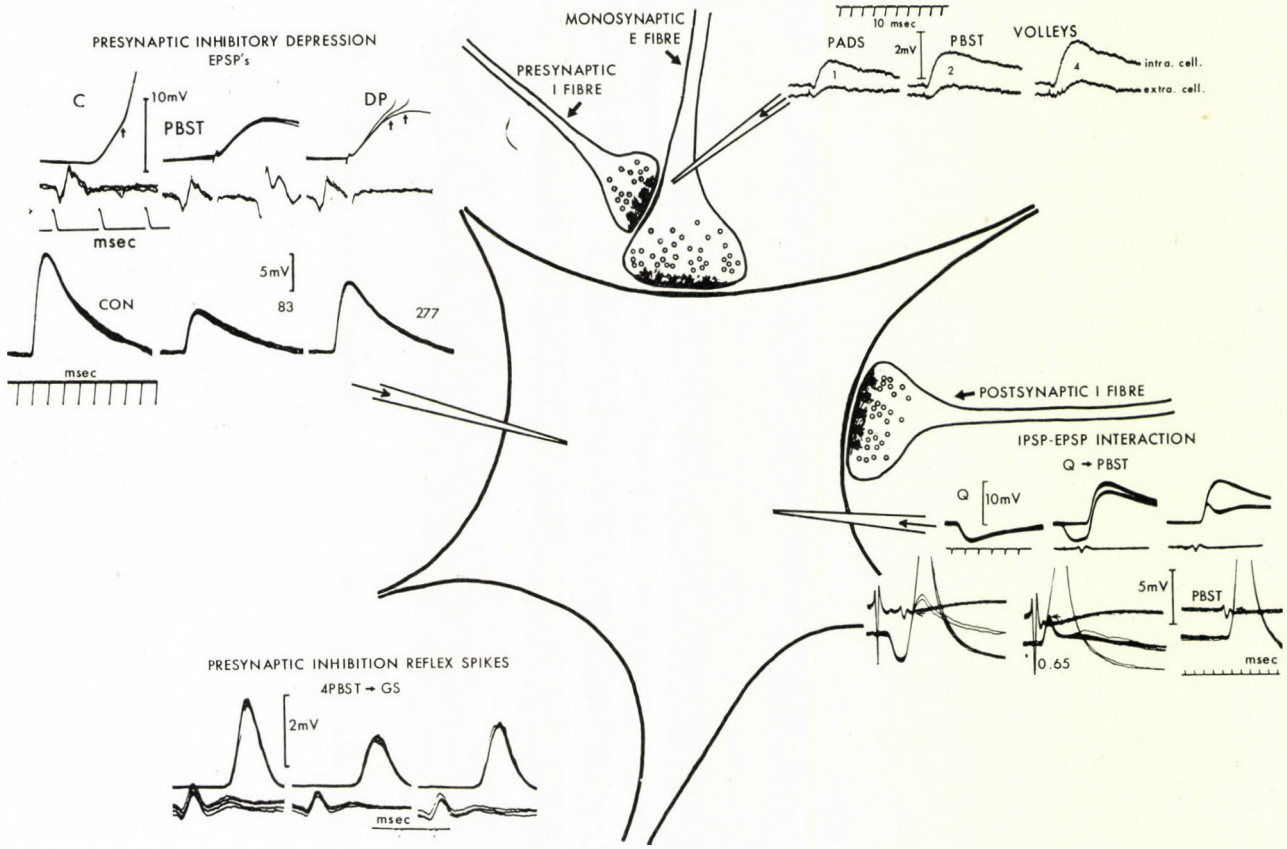

Fig. 1. Diagram of presynaptic and postsynaptic inhibition on a motoneurone. A presynaptic inhibitory knob is shown making contact with a large excitatory synaptic knob at the top of the figure and to the right there is shown postsynaptic inhibitory knob. Various specimen records are shown in appropriate places as described in the text

Integrative functions of presynaptic inhibition

A comprehensive investigation of the presynaptic inhibitory action on a wide variety of afferent fibres of limb nerves has shown that three major types can be distinguished in relation to the identity of the recipient afferent fibres, but no topographical pattern has been discerned. For example the Group Ib fibres of a muscle receive presynaptic inhibitory action from the Group I afferent fibres of all muscles of that limb regardless of such functional relationships as synergism or antagonism. Special significance therefore attaches to the subdivision into the three categories, defined by the recipient afferent fibre; the Ia type, the Ib type; and the flexor reflex afferent type, which has been chiefly investigated in relation to the large cutaneous afferent fibres.

Presynaptic inhibition on Ia afferent fibres

By several different experimental procedures it has been shown that volleys in Group Ia and Ib afferent fibres of flexor muscles depolarize the Group Ia fibres of both extensor and flexor muscles, and hence exert a presynaptic inhibitory action. This depolarization is directly observed by intracellular recording from the afferent fibres in the dorsal part of the spinal cord as illustrated in *Fig. 2*, where a Group Ia afferent fibre from quadriceps muscle is seen to be depolarized by Group I volleys from the knee flexor muscles, posterior biceps — semitendinosus (PBST in *J* to *M*) and the pretibial flexors (PDP in *C*), but virtually not at all by volleys in nerves of the various extensor muscles (*D*, *E*, *F*) or by cutaneous volleys (*G*, *H*, *I*). By a finer discrimination it can be shown as in *Fig. 3* that both the Ia and Ib afferent impulses from flexor muscles are effective in depolarizing Group Ia fibres from any type of muscle. It should be noted that the knee extensor muscle, quadriceps, is a partial exception to the rule of exclusive action by flexor afferents in that both Ia and Ib quadriceps volleys exert a small depolarizing action in Ia afferent fibres.

The depolarization of primary afferent fibres can be demonstrated very readily by the increased excitability of the fibres as tested by the application by brief electrical pulses (*cf.* WALL, 1958). In this way it has been shown that the depolarization of Group Ia fibres is greatest close to their terminals in the ventral horn, but there is also evidence for a focus of depolarization in the intermediate nucleus (ECCLES, SCHMIDT and WILLIS, 1963) and electrotonic spread of the depolarization out along the dorsal roots gives the dorsal root potential. Other evidence for Group Ia depolarization is provided by the potential field that it produces in the cord, with maximum negativity in the ventral horn and positivity in the dorsal columns, and by the generation of impulse discharges, the dorsal root reflex.

These various procedures show that the presynaptic depolarization has a characteristic slow time course: latency about 4 msec, summit at about 20 msec, and a total duration of at least 300 msec. The briefer duration of the dorsal root reflex is attributed to accommodation. Several afferent volleys in

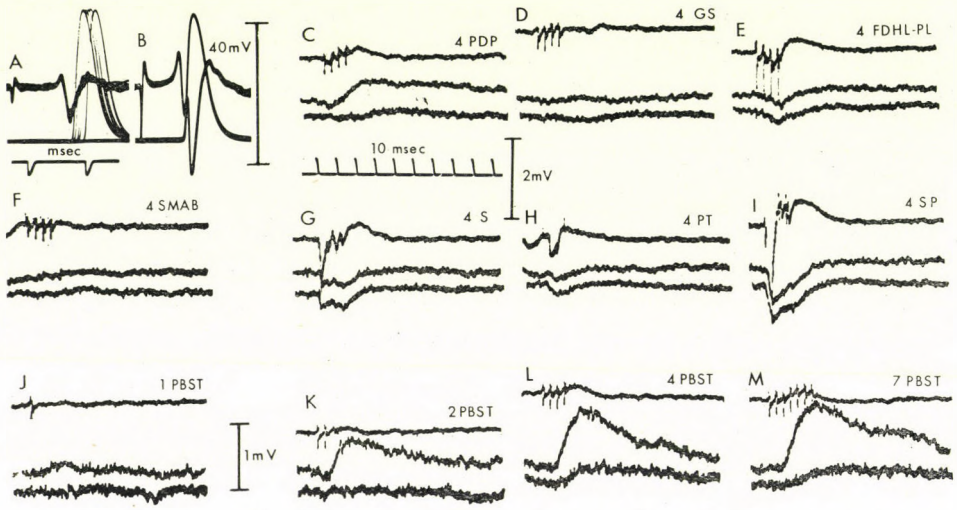

Fig. 2. Intracellular recording of primary afferent depolarization. As shown by the spike responses to a quadriceps afferent volley in *A*, *B* (lower traces) the microelectrode was inserted into a quadriceps afferent fibre at the upper L_6 segmental level. The threshold stimulus strength for this fibre excited a rather large Ia-afferent volley with no sign of a Ib component, as may be seen by comparing the upper traces of *A* and *B*, which are recorded from L_6 dorsal root by a surface electrode. With *B* the quadriceps nerve stimulus was supramaximal for Group I. With all other records *(C—M)* the upper trace was recorded in the same way as that of *A* and *B*, and signalled the afferent volleys, but the time constant of the amplifier was too brief to record P waves such as those of *Figs 1, 2*. The second trace in these records is the intracellular potential recorded with an amplifier having a time constant of 1 sec, while the lowest trace gives the potential produced by an identical series of nerve volleys, but recorded after withdrawal of the microelectrode to a just extracellular position. Any change of potential across the fibre would be registered as the difference between the intracellular and extracellular traces when the initial parts of their traces were superimposed. Note that depolarizations of the fibre occurred in *C*, *K*, *L*, *M*; but that there was virtually no membrane change in all other records. Four volleys at 280/sec in the nerves indicated by symbols were employed in *C — I*, and with *J — M* various numbers of PBST volleys at the same frequency were employed. Note higher amplification for *J — M* and same time scale for *C — M*. Muscle afferent volleys were maximum for Group I, while cutaneous volleys were generated by stimuli three to four times threshold (ECCLES, MAGNI and WILLIS, 1962)

quick succession considerably increase the depolarization, and potentials in excess of 1 mV are regularly observed in the fibres in the cord dorsum. Presumably the depolarizations are several times larger in the fibre terminals in the ventral horn.

In every respect this presynaptic depolarization accounts for the depression for the monosynaptic EPSP which is observed after conditioning by Group I volleys from flexor muscles *(Fig. 4A, B)* (FRANK and FOURTES,

1957; ECCLES, ECCLES and MAGNI, 1961), and also for the depression of monosynaptic testing reflex *(Fig. 4C)* (ECCLES, SCHMIDT and WILLIS, 1962). The responses of both flexor and extensor motoneurones are equally depressed, which corresponds to the similar depolarizations of the

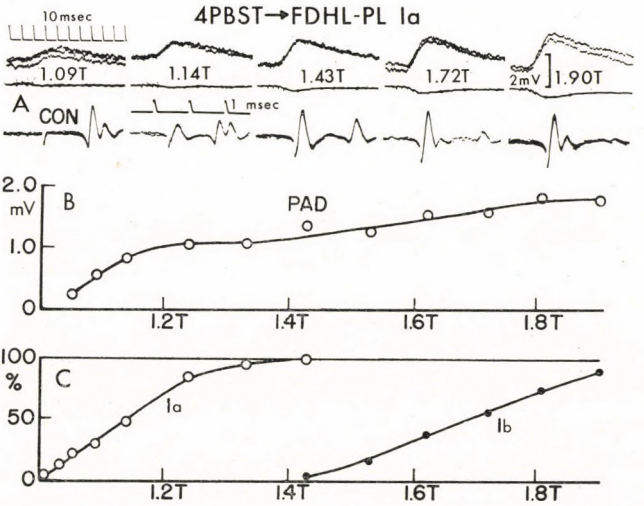

Fig. 3. Primary afferent depolarization (PAD) by Group Ia and Ib afferent volleys of PBST nerve. In upper row of *A* are specimen records of PADs recorded by a microelectrode in a FDHL-PL Group Ia fibre (membrane potential, −64 mV) and of P waves of the cord dorsum (middle row) evoked by 4PBST volleys at the indicated strengths relative to threshold. In the lowest row of *A* are specimen records at fast speed from the cord dorsum showing testing of the Ia Ib composition of the afferent volleys by the double stimulus technique, the results being plotted in *C* as percentages of maximum. The strengths of the first stimuli are shown above relative to threshold with the exception of the control spike potential (CON) which is for a maximum Group I volley. In *B* the sizes of the PADs are plotted against stimulus strengths. Note that the specimen records at 1.43T were at maximum for Group Ia and barely above threshold for Ib, while 1.14T and 1.72T gave approximately 50% excitation of Group Ia and Ib fibres, respectively. Voltage scale in *A* is for PAD records only, while the slow time scale is for both the PAD and the P waves. (ECCLES SCHMIDT and WILLIS, 1963b)

Group Ia afferent fibres. It now remains to discuss the possible physiological meaning of this presynaptic inhibitory action.

When employing the usual procedures of testing by a monosynaptic reflex, presynaptic inhibition appears to be more potent than post-synaptic inhibition, even large monosynaptic reflexes being virtually suppressed *(cf. Fig. 4C)*; hence it would be expected that presynaptic inhibition would play an important role in the control of the reflex movements. For example, under certain circumstances presynaptic inhibition provides the negative sign in a homeostatic system. Thus in *Fig. 5* stretch of flexor muscles by a powerful extensor contraction would depress the activation of extensor motoneurones by means of the presynaptic inhibitory action on the group Ia

afferent fibres of the extensors, *i.e.* on the gamma-loop system of the extensors. The weaker flexor muscles have access to a system which reduces the excitation of extensor motoneurones; and the flexor muscles would exercise a particularly effective influence of this kind when they were actively contracting against

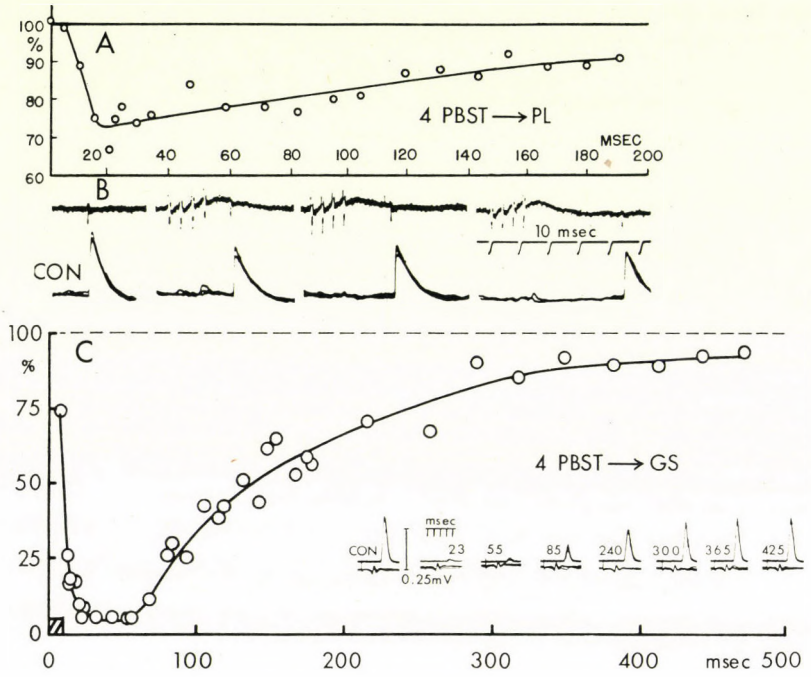

Fig. 4. A : Time course of depression of the EPSP produced in a plantaris motoneurone by monosynaptic action of a Group I afferent volley in plantar nerve. The conditioning was produced by four PBST volleys, maximal for Group I, and the abscissae show intervals between the first of the conditioning volleys and the testing plantaris volley. Specimen records composed of super-imposed tracings are shown in *B*, the ordinates of the plotted curve being the sizes of the testing EPSPs expressed as percentages of the control (CON). *C :* Time course of depression of the monosynaptic reflex discharge evoked by a maximum Group I gastrocnemius volley. The conditioning was produced as in *A* by four PBST volleys, and the abscissae show the intervals between the first of the conditioning volleys and the testing gastrocnemius volley. Specimen records are shown in the inset with testing intervals given in msec, CON being the control reflex spike (ECCLES, 1963)

the more powerful extensors. Possibly this action is of importance in terminating the extensor phase of the step. The duration of the presynaptic inhibition corresponds well to the duration of the extensor quiescence in a step. However, this presynaptic inhibitory action would also be equally exerted on the monosynaptic excitation of flexor motoneurones. Evidently the presynaptic inhibition that is initiated by Group I impulses from flexor muscles has a more complex functional meaning that still eludes our understanding.

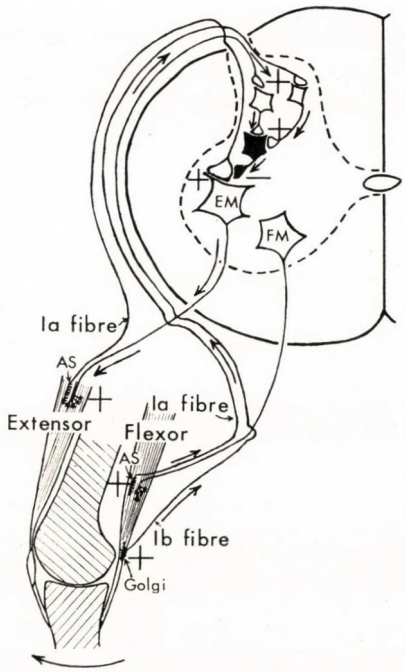

Fig. 5. Diagram showing how a contracting extensor muscle extends joint and strongly stretches a flexor muscle, particularly if it is contracting. The resulting discharge up the Ia and Ib fibres from the flexor excites an interneuronal pathway that leads to presynaptic inhibition of the Ia synapses on the extensor motoneurone, so introducing the — sign in the circuit that began with powerful extensor activation

Presynaptic inhibition on Ib afferent fibres

The same methods of investigation have shown that Ib afferent fibres from muscle are just as effectively depolarized by a presynaptic inhibitory action, but they have a distinctive receptive field (ECCLES, SCHMIDT and WILLIS, 1963a). For example the intracellular recording from the Ib fibre in *Fig. 6* shows that this fibre is depolarized by Group I volleys from extensor muscles even more effectively than by flexor Group I volleys. In addition cutaneous volleys exert a considerable depolarization in *Fig. 6*, but this does not occur with some Ib fibres.

In *Fig. 7* the depolarization of the Ib primary afferent fibres was tested by the resulting increase in excitability; Ia afferent impulses were entirely without effect on Ib fibres *(Fig. 7C, D)*, which contrasts with their considerable depolarizing power on Ia fibres *(cf. Fig. 3)*. Group II and III volleys from muscles also have an appreciable depolarizing influence on Ib fibres.

In summary it can be stated that Ib afferent fibres receive by far the largest part of their presynaptic inhibition from Ib impulses of either flexor or extensor muscles. The probable pathways are shown diagrammatically

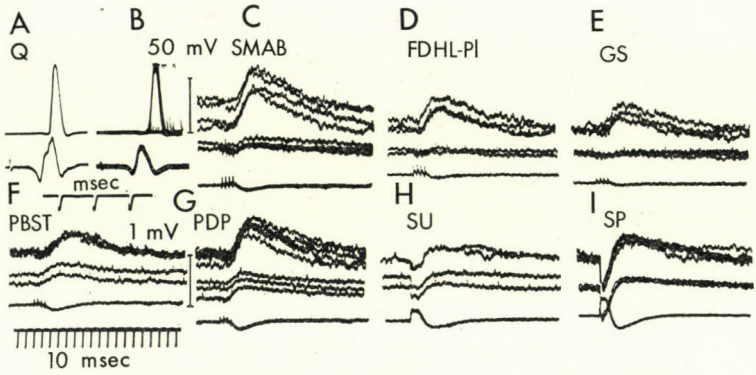

Fig. 6. Intracellular records from a Q Group Ib fibre. The Q spike potential is shown after a stimulus maximal for Group I in *A* and at threshold for the impaled fibre in *B*. Slow depolarizing potentials are recorded in *C*—*I* as described for *Fig. 9*. The four muscle afferent volleys (220/sec) in *C*—*G* were at a strength maximum for Group I. The single cutaneous volleys were at a strength 4 times threshold. Note separate time and potential scales for the spike potential in *A* and *B* and for the slow potentials in *C*—*I* (ECCLES, SCHMIDT and WILLIS, 1963a)

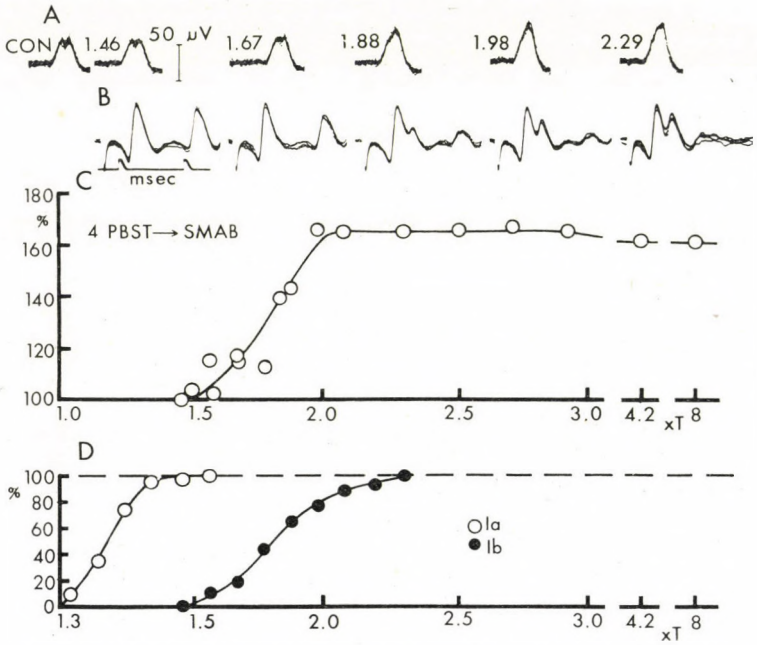

Fig. 7. Afferent fibres responsible for increasing the excitability of Group Ib fibres of an extensor nerve. In *A* is shown the Group Ib antidromic spike potential (CON) recorded in the SMAB nerve after the centrally evoked Group I volley had collided with a Group Ia hamstring volley. This Ib spike was conditioned at an interval of 36 msec by 4 PBST volleys (300/sec) produced by strengths of stimulation indicated relative to the threshold strength in the specimen records of *A*. The amplitude of the conditioned spike relative to the control size is plotted in *C* against the strengths of the stimuli setting up the conditioning volleys. A double stimulus series was employed to determine the Ia-Ib composition of the PBST volleys. Specimen records of this series are given in *B* and the series is plotted in *D* (ECCLES, SCHMIDT and WILLIS, 1963a)

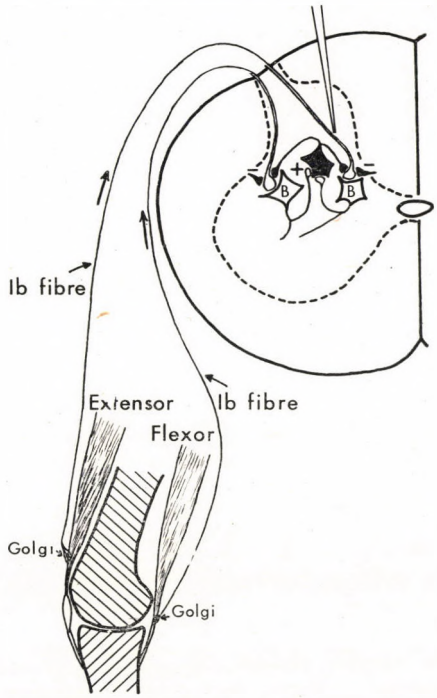

Fig. 8. Diagram of probable pathways for presynaptic inhibition by Group Ib impulses. Ib fibres from both flexor and extensor muscles are seen to end on interneurones whose axon collaterals converge onto a presynaptic inhibitory neurone that sends its axonal terminals to the synapses of Ib primary afferent fibres

in *Fig. 8*, where Ib fibres from the *Golgi* tendon organs of both flexor and extensor muscles are seen to act *via* interneuronal pathways in order to depolarize Ib afferent fibres and so depress their synaptic excitatory power. This influence thus exerts a negative feed-back control on the central actions mediated by Ib afferent fibres.

Presynaptic inhibitory action on cutaneous afferent fibres

Investigations have been restricted to the large cutaneous afferent fibres, the alpha group of HUNT and MCINTYRE (1960). Intracellular recording from such fibres reveals that they receive powerful depolarizing influences from all cutaneous nerves of the same limb (SU, SP and PT in *Fig. 9*). The Group I volleys from all of the four muscles (second row in *Fig. 9*) also produced an appreciable depolarization. When the stimuli were increased so as to excite all of Group II and most of the Group III fibres (third row), there was a considerably larger depolarization in every instance.

Fig. 10 illustrates an experimental test in which the excitability of cutaneous fibres was employed as a measure of the depolarizing influence exerted on

them by Group I muscle impulses over the whole threshold range. The calibration series in row *A* was employed in assessing the increased excitability produced by 4 conditioning PBST volleys (row *B*) set up by the indicated stimulus strengths. Depolarization of the cutaneous fibres was produced only when the PBST stimuli were above 1.6T (curve *D*), and comparison with the Ia Ib

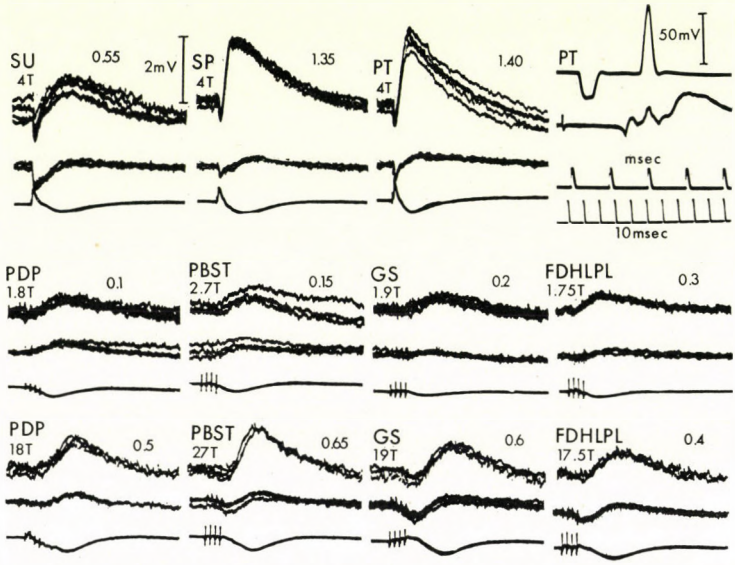

Fig. 9. Intracellular recording of the primary afferent depolarization (PAD) of a fibre of the PT nerve. The SU record in the upper right hand corner shows in the upper trace the intracellular spike and in the lower the cord dorsum potential. The spike was photographed at the end of the experimental series when the resting potential had somewhat declined from the initial value of −50 mV. All other records were at a higher amplification and at the much slower sweep speed in order to display the depolarizations produced by afferent volleys in various cutaneous and muscle nerves of the hind limb, as indicated by the symbols. The upper traces are the intracellular records, depolarization being upwards. The middle traces are the field potentials similarly recorded, but with the microelectrode just outside the fibre; and the lower traces are the cord dorsum potentials, but with upward deflexion negative. All records are formed by the superposition of several, usually four, traces. Subtraction of the extracellular fields from the intracellular potentials gives the PADs, which are shown in mV for each record. The cutaneous nerves in the first row have been stimulated with single shocks of 4 times threshold strength. All other nerves were stimulated with 4 shocks at 300/sec and the stimulus strength is indicated on each record relative to the threshold (T). The 1 mV calibration is for the intracellus lar and extracellular records. The 10 msec timer is for all except the spike record, which wa- recorded at the faster sweep speed (ECCLES, SCHMIDT and WILLIS, 1963c)

compositions plotted in *E* (from the series partly illustrated in *C*) shows a very good correspondence between the size of the Ib volleys in PBST nerve and the depolarization of the cutaneous fibres, while even a maximal Ia volley was without effect.

In the plotted curves of *Fig. 11*, the time course of the depolarization of cutaneous fibres has the characteristic long duration of presynaptic inhibition (*cf. Fig. 4)* for the four different types of afferent input indicated by the sym-

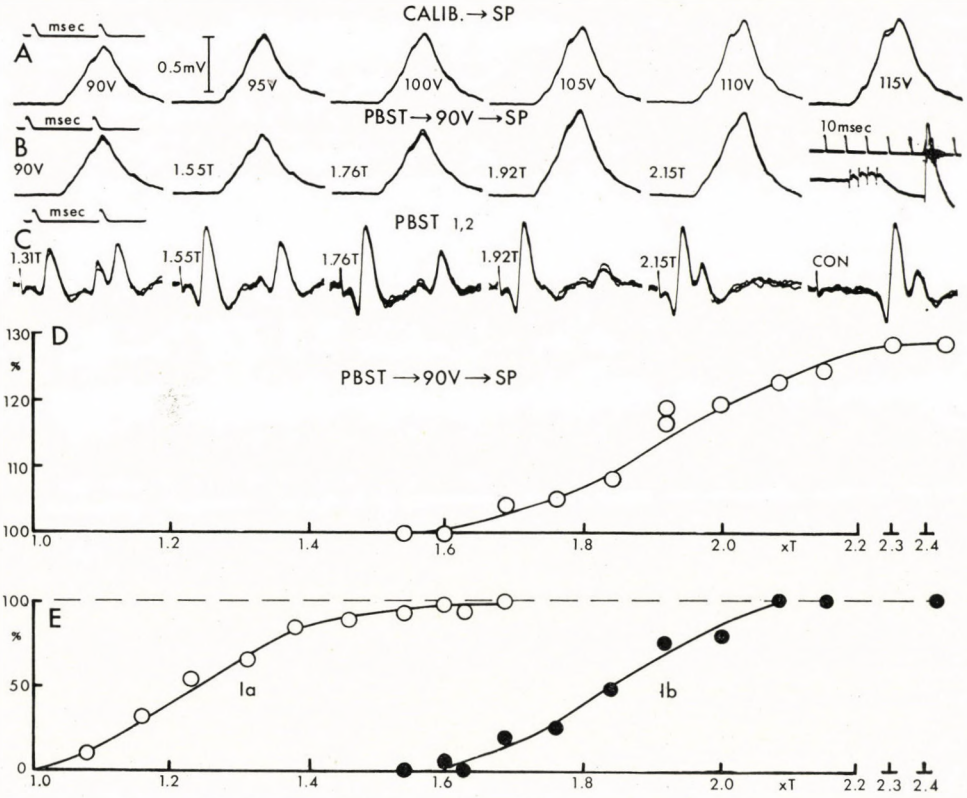

Fig. 10. Excitability changes produced in SP fibres by Group Ia and Ib afferent volleys from PBST nerve. The excitability changes were measured relative to a calibration series of antidromically conducted spikes in SP nerve that were produced by current pulses driven by a range of voltages as illustrated in the specimen series (CALIB) in the upper row *(A)*. In the series of *B* the pulse voltage was constant at 90 V and was preceded at a fixed interval of 35 msec by 4 afferent volleys in PBST nerve at 220/sec, as is indicated by the record at slow sweep to the extreme right of *B*. The stimulus strength relative to the threshold strength for each of the conditioning series of PBST volleys is indicated. In the specimen records in *C* the double stimulus technique was used to evaluate the Ia-Ib composition of the fourth volley of the tetanic train in the PBST nerve for the stimulus strengths indicated relative to threshold. These spike potentials were recorded at the entry of L_7 dorsal root into the cord, the spike to be left being for the last of the train of four volleys, that to the right being the testing volley, which alone is shown as CON. The percentage Ia and Ib compositions of the PBST afferent volleys at each stimulus strengths (relative to the PBST threshold) that was used in plotting in *D* the percentage increases in excitability of SP afferent fibres that were derived from records such as those of *B*. Voltage calibrations in *A* are for *A* and *B* only. Same time scale for *A*, *B* and *C* except for extreme right of *B*. Note broken abscissal scales to right of *D* and *E* (Eccles, Schmidt and Willis, 1963c)

bols. This was also illustrated by the intracellular records of *Fig. 9*. It is also seen in *Fig. 11* that Groups I, II and III of muscle each have a presynaptic inhibitory effect on cutaneous afferent fibres, though at a level much below that exerted by a cutaneous volley.

Fig. 12 shows diagrammatically the postulated pathways for the presynaptic inhibitory influences on cutaneous primary afferent fibres. The princi-

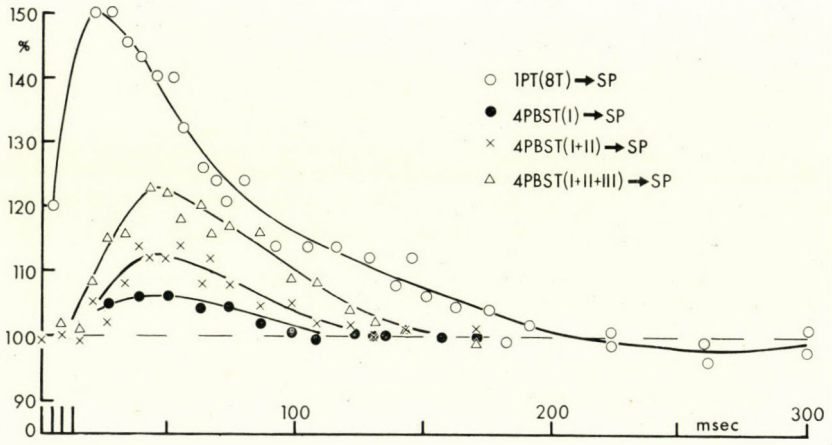

Fig. 11. Excitability changes produced by muscle (PBST) and cutaneous (PT) volleys in cutaneous (SP) fibres. A microelectrode filled with 4M NaCl (resistance 1.5 m) was inserted from the cord dorsum at L_7 segmental level to a depth of 1.5 mm. The excitability was tested by single pulses of 0.2 msec duration applied by a *Grass Stimulator*, the electrode being negative to the indifferent electrode. Note that each of the three curves for PBST was determined for four volleys at 220/sec at the times indicated by the short vertical lines for the abscissa (ECCLES, SCHMIDT and WILLIS, 1963c)

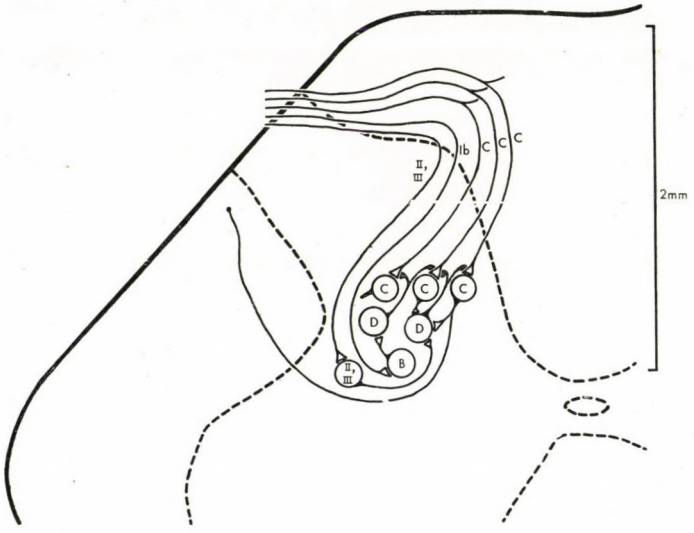

Fig. 12. Diagram illustrating the pathways for presynaptic inhibitory actions on cutaneous primary afferent fibres. Three cutaneous afferent fibres *(C)* and single Ib, II and III muscle afferent fibres are shown with monosynaptic endings on the interneurones and also the secondary interneurones *(D)* that are postulated on the presynaptic inhibitory pathways. A separate pathway is shown for Ib presynaptic inhibition. The locus of the presynaptic depolarization is shown at a depth of 1.5 to 1.75 mm (ECCLES, SCHMIDT and WILLIS, 1963c)

pal action is by cutaneous volleys, but the pathways for the actions by Ib, II and III muscle afferent fibres are also shown. As with Ib presynaptic inhibition, there is the basic performance of a negative feed-back system: cutaneous impulses produce presynaptic depolarization of cutaneous fibres including those conveying the impulses, with the consequence that the effectiveness of later impulses in these fibres is depressed. In this way the presynaptic inhibition

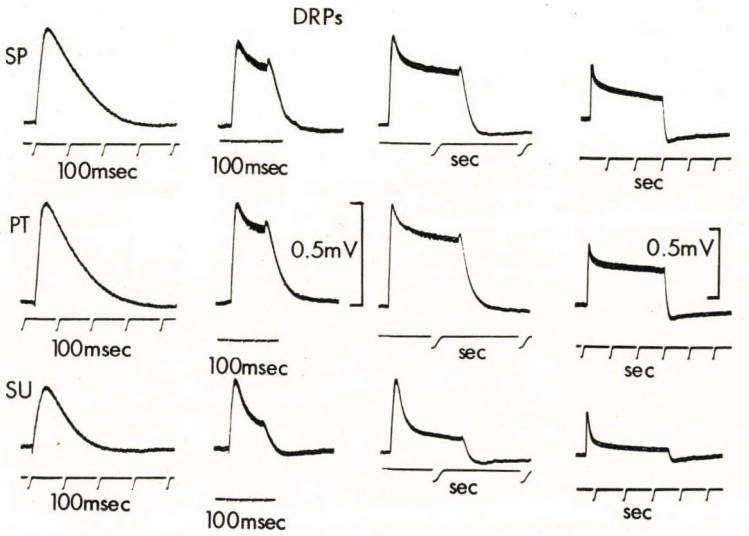

Fig. 13. Dorsal root potentials produced by single and by prolonged repetitive stimulation of cutaneous nerves. The first responses in rows *A*, *B* and *C* were produced by a single stimulus of 4T strength to the indicated nerves. All other responses are produced by repetitive stimulation (4T strength at 220/sec) at the indicated durations. Note the changes in sweep speed. DC amplification throughout,but there is a lower amplification for the records on the extreme right. (ECCLES, SCHMIDT and WILLIS, 1963c)

resulting from a continuously acting input will automatically turn itself down to some steady level. The effectiveness of this negative feed-back control is demonstrated by the dorsal root potentials during a prolonged repetitive stimulation. For example in *Fig. 13* an early maximum declines to reach in 0.5 to 0.8 sec a steady state at rather less than half the maximum, which is then maintained for many seconds. There is a similar initial decline to a steady state for the dorsal root potentials produced by repetitive volleys from a flexor muscle and which would be largely effective on Group Ia and Ib fibres.

The presynaptic inhibitory action on cutaneous afferent fibres has been demonstrated by the depression of the discharges evoked by afferent volleys in cutaneous fibres; the flexor reflex exhibit the prolonged depression characteristic of presynaptic inhibition; there is a similar prolonged inhibition of the discharges evoked in the ipsilateral tract in the dorso-lateral column *(Fig. 14)*.

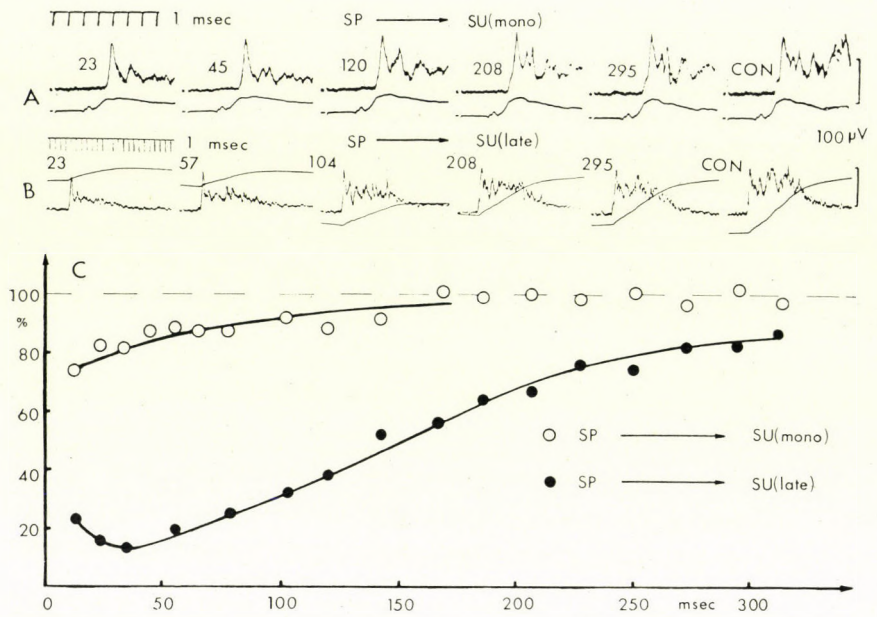

Fig. 14. Inhibition of discharges recorded from the ipsilateral cutaneous tract that was isolated in the upper lumbar segments and mounted on electrodes for monophasic recording. The action potentials were evoked by a single SU stimulus (4 times threshold) and were conditioned by another single stimulus (4 times threshold) in SP. The specimen records in *A* show in the upper line the early tract discharges in the lower one the cord dorsum potentials. Each record consists of three superimposed sweeps. The numbers indicate the interval in msec between the two volleys, and CON is the control record. *B* shows a similar series recorded with a slower sweep speed, and with integrated traces so that the amplitude of the late discharges can be measured. Potential scales are for the monophasic records only. In *C* (open circles) the depression of the monosynaptic discharge *(i.e.* the amplitude of the first spike in *A)* is plotted in per cent of the mean control value against the interval between the two volleys in msec. The filled circles show the depression of the late discharges as given by the integrated records of *B*. These were measured after the time of the monophasic spike in order to reject its contribution to the integrated response, *i.e.* measurements were from 1.3 msec to 19 msec after the onset of the monophasic discharge (ECCLES, KOSTYUK and SCHMIDT, 1962)

Presynaptic inhibitory action on Group II and III muscle fibres

In preliminary investigations on group II fibres from muscle it was found that their presynaptic depolarization corresponded closely to that of cutaneous fibres. There are indications also of a similar correspondence with Group III fibres from muscle. We have seen above that both of these types of afferents are effective in depolarizing cutaneous fibres. There is much other evidence of similar central actions by these high threshold muscle afferents and by cutaneous afferents; hence collectively they have been termed the flexor reflex afferents (FRA) (HOLMQVIST, LUNDBERG and OSCARSSON, 1956, 1960). It has further been postulated that the FRA afferents from a single system in producing and receiving presynaptic inhibitory action.

The three systems of presynaptic inhibition

In *Fig. 15* there are shown diagrammatically the pathways responsible for the three types of presynaptic inhibition as specified by the recipient fibres, Ia, Ib and cutaneous. The thicknesses of the pathways are proportional to the mean depolarizations produced in the intracellular records from almost one

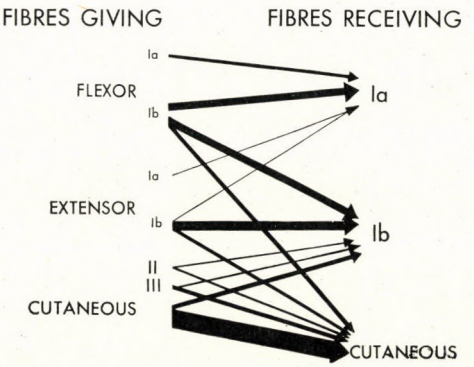

Fig. 15. Diagram showing types of afferent fibres depolarizing Group I fibres of muscle and alpha cutaneous fibres. The types of afferent fibres which produce the depolarizations are listed to the left. The widths of the arrows from each of these types to each of the three recipient types give approximate measures of the amount of depolarization (PAD) that are produced, being averages of measurements on a large number of fibres. (ECCLES, SCHMIDT and WILLIS, 1963c)

hundred primary afferent fibres. This diagram serves to summarize the distinctive characters of the pathways concerned in the three types of inhibition. It also illustrates the negative feed-back character of presynaptic inhibition, particularly the Ib and cutaneous types, where the dominant action was from Ib onto Ib and from cutaneous onto cutaneous.

Presynaptic inhibitory action from higher centres onto the spinal cord

It has been shown (ANDERSEN, ECCLES, and SEARS, 1962; CARPENTER, LUNDBERG and NORSELL, 1962) that the stimulation of the sensori-motor region of the cerebral cortex produces a depolarization of primary afferent fibres (Ib and cutaneous) and an associated presynaptic inhibition, which is as large and prolonged as that produced by afferent volleys from the limb nerves *(Fig. 16)*. The descending pathway is in the pyramidal tract. Apparently the descending fibres relay synaptically on interneurones that are on the presynaptic inhibitory pathway for the afferent fibres from the limbs.

Stimulation of the cerebral cortex fails to have any presynaptic inhibitory action on Ia afferent fibres. However, a very effective action is produced by stimulation in the region of the dorsal longitudinal tract in the medulla

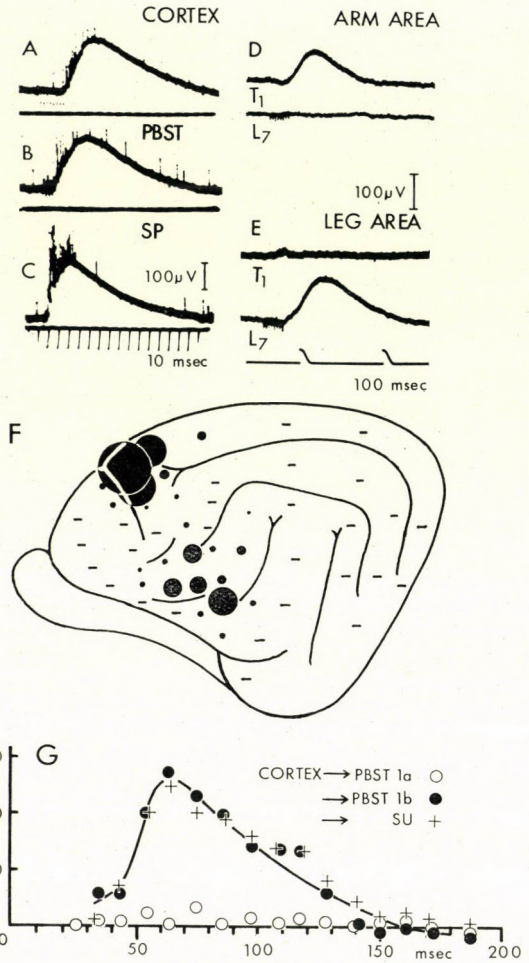

Fig. 16. *A*—*C* show dorsal root potentials led from a L_7 dorsal rootlet which was isolated except for its cord entry and recorded by two electrodes, one about 1 mm from the cord, the other on the distal cut end. There was brief repetitive stimulation of the contralateral cortex, *A*, and of posterior biceps semitendinosus nerve, *B* (PBST), as described in text; in *C* a single volley in the superficial peroneal nerve. *D* gives dorsal root potentials produced by repetitive stimulation of contralateral cortical arm area with similar recording from dorsal rootlets of first thoracic (T_1) and L_7 segments, while in *E* the contralateral cortical leg area was stimulated. In *F* the sizes of the dorsal root potentials produced in a L_7 dorsal root of the contralateral side are shown by sizes of filled circles for stimulating sites over the convex surface of the cortex, zero actions being shown by — signs. *G* shows that the time courses of excitability changes produced by cortical stimulation in two types of fibre (●, + points) resemble the dorsal root potential in *A*. There was no action on the third fibre type (○ points). Percentages are measured as described in *Fig. 10*; time is measured from the first stimulus of the conditioning tetanus (ANDERSON, ECCLES and SOARE, 1962)

and lower pons (CARPENTER, ENGBERG and LUNDBERG, 1962), the descending pathway being in the ventral quadrant of the spinal cord. Brain-stem stimulation was found also to effect depolarization of Ib and cutaneous afferent fibres.

Conclusions

It can be concluded that presynaptic inhibition is employed in a wide variety of systems, both those operating entirely within the spinal cord, where it often has negative feed-back character, and those responsible for influences from higher centres. It has been suggested that the descending inhibitory influences reported by HAGBARTH and KERR (1954) and also by HOLMQVIST and LUNDBERG (1959, 1961) are mediated to a large extent by presynaptic inhibitory mechanisms.

When attempting to understand the physiological significance of the negative feed-back provided by presynaptic inhibition, it is helpful to consider the operational conditions that normally obtain with all the diversity of afferent input into the spinal cord. Hitherto it has been assumed that all of this sensory information is processed in the spinal cord during transmission through interneuronal pathways which offer opportunities for inhibitory action at each synaptic relay. This inhibition would be exerted by a specific postsynaptic process as illustrated in *Fig. 1*. Rejection of sensory input could occur only after it had excited interneurones. Presynaptic inhibition provides a mechanism for suppressing the sensory input before it has exerted any synaptic action. In this way a powerful afferent input can suppress all trivial inputs before they have an effective action on the central nervous system, which, as a consequence, is "cleared" for the "urgent" reflex actions set in train by the powerful input. It will be objected that the powerful input will also itself be subjected to the diffuse depressant action of the presynaptic inhibition which it generates; but if it is sufficiently powerful, the depression will be negligible, as is illustrated for example with the monosynaptic tract discharges in *Fig. 14*. In general terms it can be stated that presynaptic inhibition provides the first stage in what we may term "perceptual attention", whereby powerful sensory inputs with an implication of urgency can suppress all concurrent trivial inputs into the central nervous system. Presynaptic inhibition probably also is responsible for contrast phenomena.

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CENTRAL NERVOUS MECHANISM IN THE ADAPTATION TO THE BODY TEMPERATURE-LOWERING EFFECT OF HISTAMINE

By

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According to data in the literature, lowering of body temperature in response to histamine is progressively diminishing and after the 3rd—4th dose the drug has no such effect. At the same time the similar activity of other substances persists unchanged.

An enhanced elimination of histamine, as a possible explanation of this "tachyphylactic" phenomenon, could not been verified experimentally (histaminase, antihistaminase determination).

Studying in rats the body-temperature-lowering effect of repeated subcutaneous injections of histamine it has been found that saline injected after the third, almost ineffective histamine dose suspended the adaptation to histamine; subsequent administration of histamine again caused a marked lowering of body temperature.

The phenomenon points to the role of the central nervous system in the development of rapid adaptation to histamine which may involve a mechanism similar to habituation or conditioned reflexes.

VEGETATIVE AND EEG RESPONSES ELABORATED TO THE EFFECT OF HYPOXIA

By

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In different animal species the conditioned vegetative and EEG responses elaborated to indifferent (optic + acoustic) stimuli coupled with inhalation of air with 6 to 10 per cent oxygen have been studied by recording oxygen consumption, body temperature, respiration, and electrical activity of the neocortex and of different subcortical structures. The early signs of the vegetative conditioned response and the bioelectrical manifestations associated with it have been analysed.

The vegetative responses were found to be identical with or reciprocal to the effect of the unconditioned, hypoxic, stimulus. The EEG patterns indicated the conditioned character of both types of vegetative response.

THE INFLUENCE OF PITUITARY-ADRENOCORTICAL FUNCTION ON THE AVOIDING CONDITIONED REFLEX ACTIVITY IN RATS

By

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Abundant data have been accumulated concerning the complex functional unity of the living organism with its biosphere, and showing that all changes in the environment result in biological responses. Such reactions may be characterized as common to the strain or to the large classes of living beings, but there are many reactions which display individual varieties as due to acquired and inherited ontogenetical and phylogenetical experiences. The environmental changes lead to adaptational processes manifesting themselves at both the neural and the humoral level. At the neural level a wide scale of behavioural changes produced by external stimuli may be observed, such as attentive, orienting, emotional reactions accompanied by the development of conditioned reflexes as well as a habituation to neutral signals. The changes in humoral regulation elicited by environmental stimuli, especially the alterations in pituitary-adrenal function, may have an important role in specific and non-specific functional adaptation.

Reiss was the first to report that the administration of corticosterone was followed by increased learning ability in the rat [1]. Mirsky, Miller and Stein could increase the acquisition of avoiding conditioned reflex activity in monkeys by the administration of ACTH [2]. According to our previous observation, that the alimentary conditioned reflex performance was inhibited by the simultaneous application of a conditioned stimulus with a noxious stimulus (painful electric shocks to the legs), a relation has been revealed between the intensity of reflex inhibition and the reactivity of the pituitary-adrenal system in the rat [3]. In experiments on dogs in alimentary conditioned reflex situation, it was found that following inhibition of the conditioned responses by the simultaneous administration of a noxious stimulus with the test stimulus the ratio of hydrocortisone to corticosterone in adrenal venous blood was significantly increased in the resting period in those dogs which displayed a longer inhibitory period [4]. In the Pavlovian terminology, the prolonged inhibitory period following the breaking of a conditioned reflex by an external noxious signal should be due to higher intensity of internal inhibitory processes. It was found, further, that hydrocortisone treatment resulted in a lengthening of

the inhibitory period, a fact indicative of the direct involvement of adrenocortical steroids in these events. The above mentioned experiments and the neuropathological observations of subjects treated with ACTH or corticosteroids may confirm the important role of adrenocortical hormones in wide scale of brain functions.

The present report deals with the influence of the pituitary-adrenal system on both the internal inhibitory processes, as classified by the Pavlovian terminology, and the motivational phenomena accompanied with conditioned reflex behaviour.

The avoiding conditioned reflex was established in a jumping box, using a buzzer as the conditioned signal and an electric shock (50 V) given through the grill on the floor of the box as the unconditioned stimulus. The male albino rats used in the experiment could avoid the shock by jumping onto a 10 cm high bench. During the sessions consisting of 12 trials the conditioned reflex activity as well as the number of intersignal reactions was recorded. The intersignal reaction as spontaneous goal-directed motor activity corresponding to a sign of motivation plays an important role in the establishment of conditioned reflexes.* The number of intersignal responses was high in the early period of conditioning; their appearance was the first sign in the establishment of temporary connections. During the stabilization of the conditioned response the number of spontaneous motor reactions decreased significantly but never disappeared completely. According to our opinion, such a decrease in spontaneous motor activity is a function of the discriminative internal inhibition and corresponds to one form of the Pavlovian internal inhibitory processes.

Changes of adrenocortical function were induced by ACTH administration as well as by bilateral adrenalectomy.

In the first series, the effect of adrenal hyperfunction was studied by administering 2 IU/100 g body weight of ACTH two hours before the conditioning. The effect of ACTH depended on the phases of the training period. On administering ACTH on the second day of conditioning, conditioned reflex activity was increased and the intersignal reactions disappeared in those rats which had given a positive response in one-third of the trials on the previous day. In the next conditioning session, the reflex activity and the number of intertrial reactions corresponded to those of the control, saline treated animals *(Fig. 1)*.

There were no observable changes either in the speed of acquisition of conditioned reflex activity or in the number of spontaneous goal-directed reactions in the early phase of training after ACTH treatment before the conditioning procedure was begun.

* See the paper of E. Endrőczi in this issue.

ACTH administration following the stabilization of conditioned reflex activity failed to influence the reflex performance, but a decrease or full disappearance of spontaneous intersignal reactions could be observed *(Fig. 2)*.

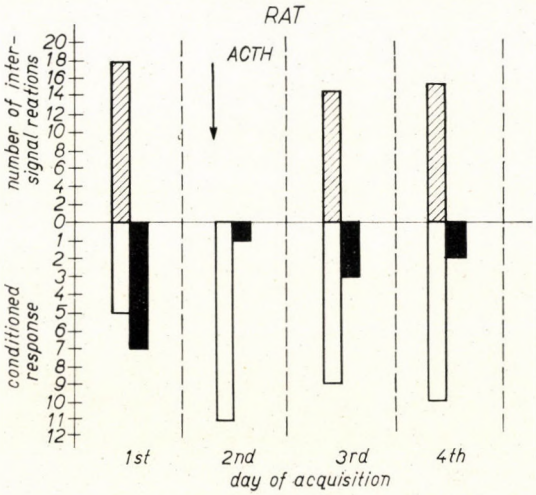

Fig. 1. Effect of ACTH on conditioned reflex performance as well as on spontaneous goal-directed motor activity in the early phase of conditioning. The white columns correspond to the positive responses, the black ones to the negative responses to the conditioned stimuli

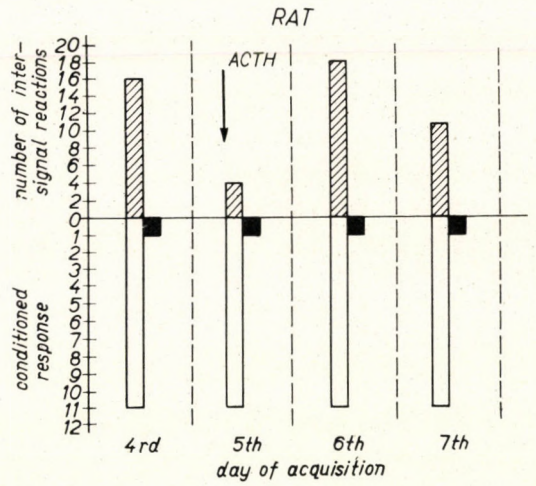

Fig. 2. Effect of ACTH administration on conditioned responses and intersignal reactions in the phase of stabilization of the conditioned reflex

These findings are in good agreement with the observations of REISS, MIRSKY, MILLER and STEIN [1, 2] concerning the effect of adrenal hyperfunction on conditioned reflex performance, furthermore demonstrate the decreas-

ing effect of ACTH on the number of spontaneous intersignal reactions. The inhibition of spontaneous goal-directed behaviour corresponding to a decrease in motivation, might have been the result of a higher intensity of the internal inhibitory processes enhanced by ACTH treatment. This relationship between adrenocortical function and motivation was then clearly demonstrated by experiments in adrenalectomized rats.

No significant alterations could be observed in the forming of temporary connections or in the intensity of spontaneous intersignal activity in rats adrenalectomized and maintained by DOCA treatment before conditioning. These findings lead to the conclusion that the retarded learning of the avoiding conditioned reflex by hypophysectomized rats observed by APPLEZWEIG and BAUDRY [5] could not be due to adrenal insufficiency.

There were, however, no changes in the conditioned reflex performance of rats which were adrenalectomized after the conditioned response had stabilized, an increase occurred in the number of spontaneous intersignal reactions. This high number of the intersignal reactions persisted even when after the administration of 150—200 trials conditioned reflex activity was highly stabilized. Adrenalectomy in a later period of stabilized conditioned reflex activity resulted in the reappearance of spontaneous intertrial motor responses which had been suppressed during the earlier phase of the training period. In the sham-operated controls a decrease in the number of goal-directed motor reactions could be observed due to the stress-induced endogenous ACTH release *(Fig. 3)*.

These results also indicate that the discriminative internal inhibition developed during the stabilization of the temporary connections brings about a decrease in motivation; these phenomena are in a close relationship with adrenocortical function. In the absence of pituitary-adrenocortical activation, internal inhibition is weakened, as shown by the high score of intertrial reactions.

In the second series of experiments the effect of ACTH on the extinction of alimentary conditioned reflex was studied in cats. The methodical details of the experiment have been described previously [6]. These investigations revealed that in the absence of a differential signal in the stereotype, a few per cent of spontaneous goal-directed motor activity persisted even at the highly stabilized stage of conditioning. During extinction this resting level of spontaneous goal-directed motor activity disappeared earlier than the inhibition of conditioned reflex activity had been developed.

After the administration of ACTH of 10 IU/kg on the 3rd day of the extinction, a temporary inhibition of both the conditioned response and the spontaneous goal-directed motor activity was observed. On the 5th day of extinction, when the ACTH effect was eliminated, the extinction curve was again normal *(Fig. 4)*.

According to the Pavlovian concept, the extinction of a conditioned reflex is considered a result of the internal inhibitory processes. Our findings have revealed that administration of ACTH in the period of extinction increases the intensity of internal inhibition and thus causes a rapid decrease of both the conditioned responses and the spontaneous goal-directed activities.

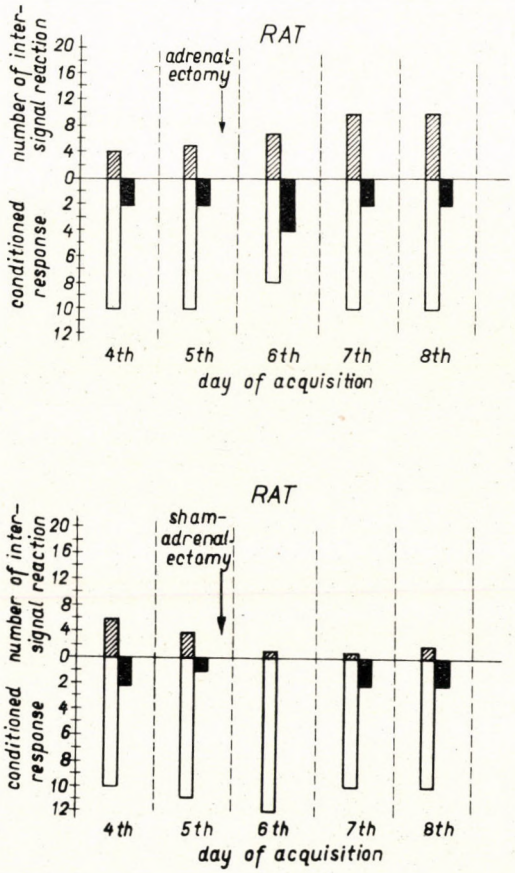

Fig. 3. Changes in conditioned reflex performance and in the number of intersignal reactions following adrenalectomy and sham-adrenalectomy

The different forms of internal inhibitory processes related with discrimination and extinction were influenced by adrenocortical activity. Thus hyperfunction caused a decrease in motivation during the formation of the conditioned reflex or enhanced extinction, while in the absence of corticosteroid secretion a weakness of the internal inhibitory processes was apparent.

The foregoing data raise the question of the relationships existing between pituitary—adrenal function and the individual differe nces in adaptation caused

by environmental stimuli. Our previous studies have revealed wide individual variations in the rate of resting adrenocortical secretion; this may be considered to depend on genetic factors [7]. In cats with high or low resting adrenocortical secretion within the normal range, a low or high number of intersignal reactions were observed in an alimentary conditioned reflex situation [6]. In an avoiding conditioned reflex situation, on the other hand, it was observed that the performance of conditioned reflex in rats having higher resting corticosterone output was more intensive, than in those with low adrenocortical secretion [8]. The quality of adrenocortical secretion also has a role in this kind of

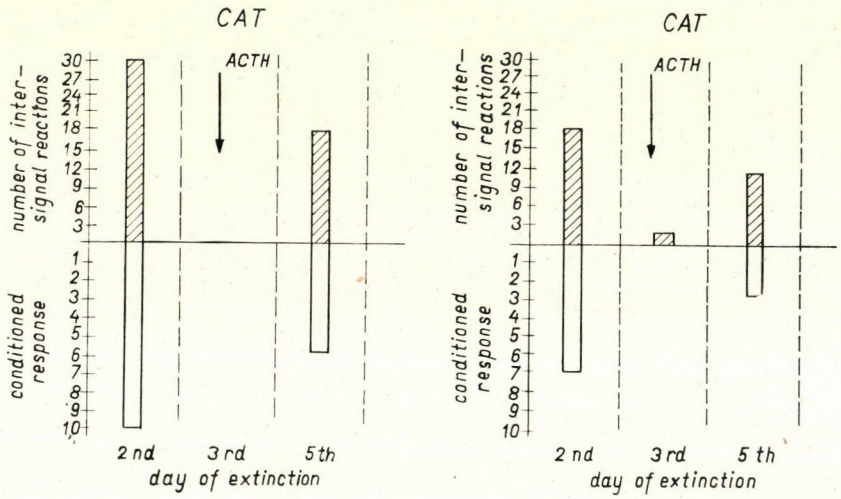

Fig. 4. Effect of ACTH administration on the extinction of alimentary conditioned reflex in the cat

adaptation, especially the ratio of hydrocortisone to corticosterone, or the appearance of substances normally not present in adrenal venous blood. Thus, dogs having a higher ratio of hydrocortisone to corticosterone in their resting adrenocortical secretion showed a longer inhibitory period following the breaking of the alimentary conditioned reflex by the application of the conditioned signal together with a painful stimulus [4]. A higher secretion rate accompanied with an absolute increase in the secretion of polar corticosteroids could be observed after chlorpromazine treatment [7]. WOODBURY found that the excitability of the central nervous system could be increased by treatment with polar corticosteroids, such as hydrocortisone; the less polar substances, like corticosterone, had no effect or counteracted the effect of the polar derivatives [9]. According to these observations, the mechanism of pituitary—adrenal action upon the conditioned reflex behaviour of rats is questionable, corticosterone being the main product of the rat adrenal and hydrocortisone

is not secreted in the lack of certain enzymatic activity. To explain the problem, one might assume the role of polar corticosteroids, or that in the rat corticosterone can simulate the action of polar corticosteroids unlike to other species.

The participation of the pituitary-adrenal system in the mechanism of neurohumoral adaptation manifests itself through a negative feed-back effect. This assumption in supported by our previous findings indicating that the archicortex, paleocortex, hypothalamus and mesencephalon participate in the integration of pituitary-adrenal function as well as in the internal inhibitory processes and motivation [6, 7].

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EFFECTS OF THE MESENCEPHALIC RETICULAR FORMATION ON SOME VEGETATIVE REFLEXES

By

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The effect of electrical stimulation of the mesencephalic reticular formation on some sympathetic and parasympathetic vegetative reflexes has been studied in acute experiments in cats and dogs under intravenous chloralose anaesthesia, in certain cases with d-tubocurarine or Flaxedil.

In a first series of experiments in 15 cats and 5 dogs the effect of liminar and supraliminar stimulation of the mesencephalic reticular formation, by means of monophasic rectangular currents, was studied on the carotid sinus pressor reflex induced by bilateral occlusion of the common carotid for 15—20 sec. Reticular stimulation was performed using stereotaxically implanted silver bipolar electrodes 0.2—0.4 mm in diameter. Histologic examination showed that most of the stimulated points were in the midbrain pontile oral reticular nucleus.

In most instances, liminar and supraliminar stimulation of the mesencephalic reticular formation, together with bilateral occlusion of the common carotids, resulted in an increase of the carotid sinus pressor reflex. In rare cases, liminar stimulation of the mesencephalic reticular formation had no effect or diminished the amplitude of the carotid sinus pressor reflex.

In a second series of experiments carried out in 25 dogs, the effect of liminar and supraliminar electrical stimulation of the mesencephalic reticular formation was investigated on the vesico-constrictor reflex induced by stimulation with monophasic rectangular pulses of the central end of the transected pelvic nerve. Bladder contractions were recorded on a kymograph by means of a hydroaeric system; blood pressure was recorded in the right femoral artery. In the course of the experiments two dogs were decerebrated by brainstem transection rostral to the anterior colliculi.

A total of 37 points was explored in the midbrain reticular formation. In 32 cases stimulation of the mesencephalic reticular formation elicited inhibition or occlusion of the vesico-constrictor reflex induced by stimulation of the central end of the pelvic nerve. This effect of the reticular formation persisted after decerebration. In 5 cases, the mesencephalic reticular formation facilitated the vesico-constrictor reflex. Reticular stimulation occasionally influenced

the vesical and tensional components of the pelvic reflex in a different manner. Following concomitant stimulation of the mesencephalic reticular formation and of the central end of the pelvic nerve, phasic long-lasting changes (increase or decrease) of the excitability of the vesico-constrictor reflex arch occurred.

A comparison of the two series of experiments shows that stimulation of the mesencephalic reticular formation mostly facilitates the carotid sinus pressor reflex and inhibits the vesico-constrictor pelvic reflex.

The functional significance of this finding has been discussed.

EFFECT ON THE NERVOUS SYSTEM OF THE COMPONENTS OF A CARDIOPATHOGENIC DIET

By

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The components of the complex diet S_{65} bringing about a state of excitement and increased irritability of the nervous system and inducing finally an infarctoid cardiopathy are less detrimental when administered one by one. The diet S_{65} was found to cause considerably more serious lesions in nervous function than the total of the effects caused by the components themselves. Consequently, the damaging effects are synergized, the components of the diet mutually potentiate and strengthen their effects.

At the last meeting of the *Physiological Society* we have shown that the cardiopathogenic diet, marked S_{65} caused a gradually increasing state of excitement in the central nervous system 2 or 3 weeks before lesions could be found in the myocardium. This state was assumed to have a role in the development of the heart lesion.

In the present experiments we have studied the effect of the various components of the fat and salt rich diet S_{65} on the functional changes of the nervous system.

In the experiments 44 male white rats were used. Of these, 17 animals were fed a diet rich in salt, 15 animals a diet rich in fat, while 12 animals were given a normal diet and served for control. The salt diet contained Na, Ca, Cl and phosphate in excess and was poor in K and Mg. The fat diet contained overdoses of cholesterol and Vitamin D_2.

Central nervous function was examined by recording the EEG and conditional reflex responses. For the EEG examinations two frontal and two occipital electrodes had been built in. Records were taken after healing of the wounds from freely moving animals, registering both the spontaneous electric activity and the cortical response to 0.4 V square-wave stimuli.* The cortical response was induced on the basis of changes in temporal desynchronization. The EEG was received in all animals before and repeatedly during the whole course of the experiment.

In the conditioned reflex experiments a temporary relation was established by combining sound stimuli and electric shock. Changes in breathing caused

*The examination of the frequency of the spontaneous electric activity was carried out by means of an exact mathematical method, *Fourier* analysis, done by electronic computer *Ural II*.

by the whining called forth by the stimulus were taken as responses. At the beginning of the experiment 40 positive responses to 40 stimuli were considered as 100 per cent and further values were related to this figure. Reinforcement was done every day. After the experiments the heart of the animals was examined histologically.

Results

Spontaneous cortical activity of animals on a normal diet and in a state of repose was about 5 c/s around 80 μV. In the control group these values remained practically constant throughout the experiment, without significant

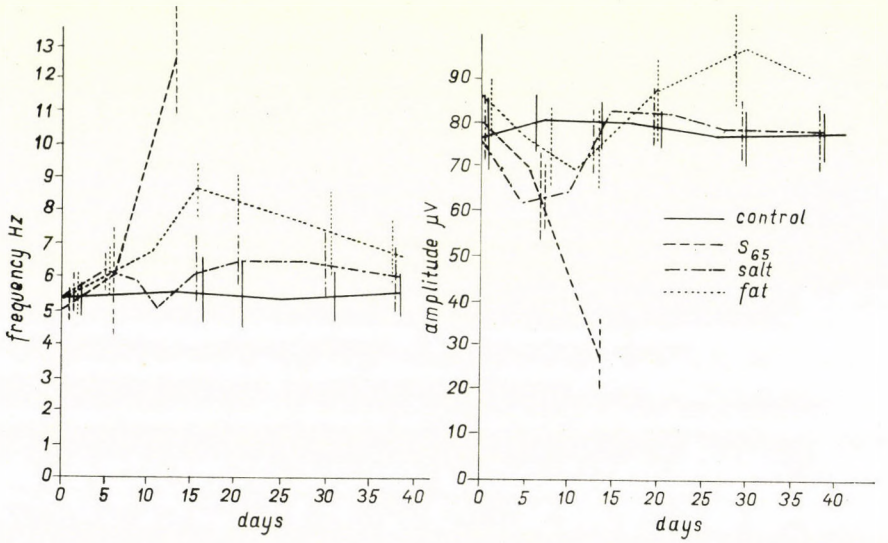

Fig. 1

scattering. In contrast, in our previous experiments the cardiopathogenic diet S_{65} induced a rapid increase in frequency at decreasing amplitude; these values were 13 c/s and 30 μV, respectively, on the 14th day.

In the group fed the fat diet, frequency increased slowly; it was 9 c/s on the 18th day and then decreased to its initial value. The amplitude was slightly decreasing till the 12th day when it was about 70 μV, then it slowly rose. Scattering was more expressed in this group than in the former one.

In the group kept on the salt diet, the changes observed were insignificant; the values did not surpass the limits of normal, with a scattering corresponding to that in the control group *(Fig. 1)*.

Taking the time of desynchronization in response to the applied stimuli preceding the experimental diet as 100 per cent, upon the effect of the full S_{65} diet it became 308 per cent as early as the 11th day.

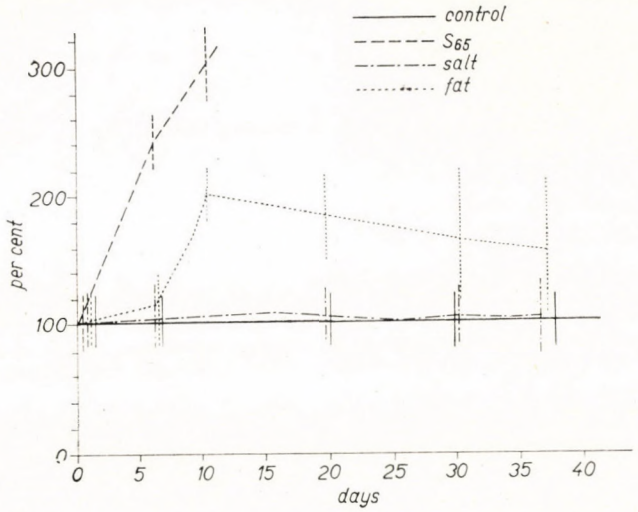

Fig. 2

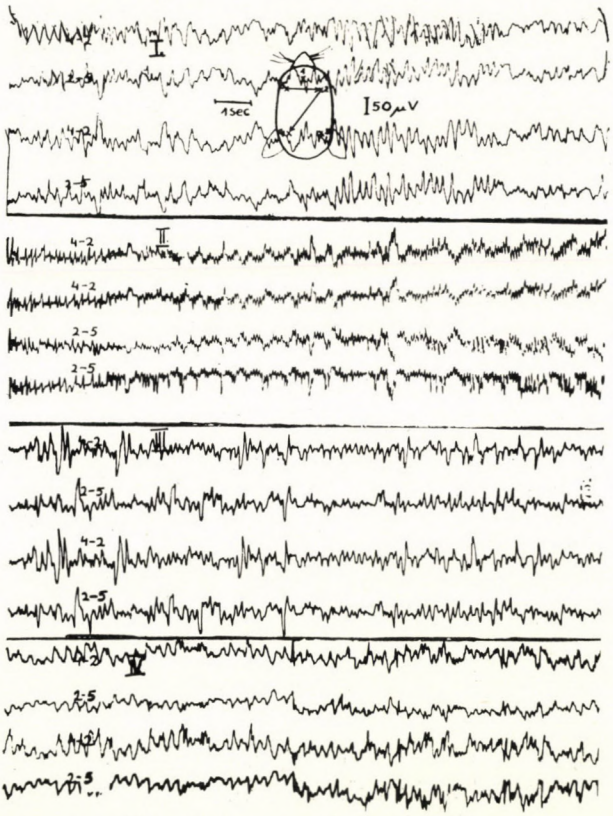

Fig. 3. Spontaneous electric activity

I. At the beginning of the experiment: normal;
II. Upon the effect of diet S_{65}: increased frequency and decreased amplitude on the 14th day;
III. Upon fat load: slight deviation on the 40th day;
IV. Upon salt load: practically no change on the 40th day

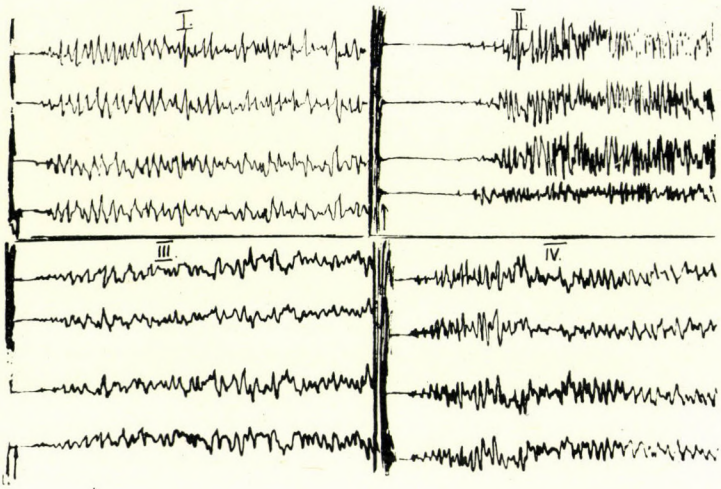

Fig. 4. Response to square wave electric stimulation
I. Slight desynchronization at the beginning of the experiment;
II. Upon the effect of diet S_{65}, considerable elongation by the 14th day;
III. Upon the effect of the fat diet, hardly any change by the 40th day;
IV. Upon the effect of the salt diet, practically no change by the 40th day

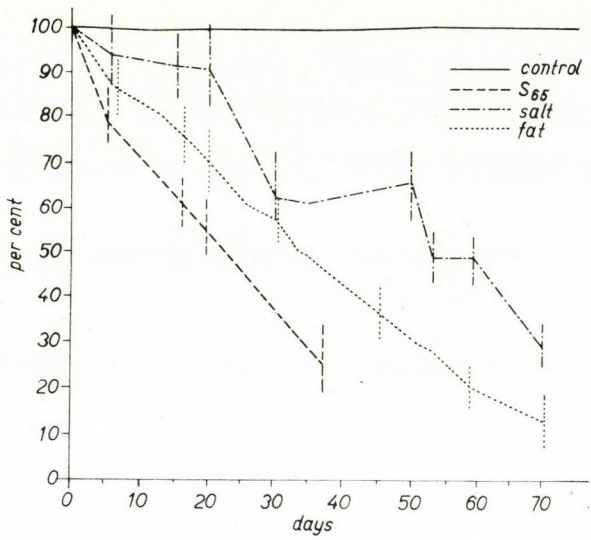

Fig. 5

In the group kept on the fat diet it was 200 per cent on the 12th day; subsequently it decreased and on the 25th day it was 170 per cent. Scattering of the values was greater than in the control group. The salt diet caused insignificant changes in desynchronization; they practically corresponded to the values in the control group and showed a similar scatter *(Fig. 2)*.

The following EEG records serve for illustrating those reported above *(Fig. 3)*.

In the conditioned reflex experiments upon the effect of diet S_{65} the number of reflex responses had decreased by 75 per cent by the 35th day. The fat diet induced a decrease of 50 per cent. The salt diet one of 40 per cent by the 35th day and 25 per cent on the 70th day *(Fig. 5)*.

As regards weight, the animals fed the diet S_{65} or the fat diet showed some loss in weight by the end of the experiment. The salt diet caused a considerable loss in weight.

As revealed by the histological examinations, all the animals kept on diet S_{65} showed parenchymal degeneration of the heart muscle. In the group kept on the fat diet the myocardium was normal or showed at most hyperaemia. In animals fed the salt diet the myocardium showed no damage.

As shown by our results, diet S_{65} caused early lesions in the central nervous system. The lesions became gradually more conspicuous. The single components of that diet induced symptoms later and in a slight degree.

The changes caused by the fat diet were easy to recognize though of minor importance.

The salt diet in itself had not brought about any changes in nervous function that could have been estimated by the applied methods. Some fine functional or structural changes were presumably induced, since excess of salt has been found to potentiate the effect of fats in the cardiopathogenic diet S_{65}.

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Biochemia

STUDIES ON THE KINETICS OF NAD-DECOMPOSITION

By

T. GÁNTI and J. FODOR

YEAST FACTORY, BUDAPEST

(Received May 18, 1963)

An attempt has been made to establish the optimum conditions of nicotine amide adenine dinucleotide (NAD) extraction from yeast, taking into account the kinetics of extraction and decay of the compound.

The results supported the findings of LOWRY *et al.* (1961), as regards the rate of decomposition of NAD. The *Arrhenius*-diagram, plotted on the basis of computed *k*-values (constant of the decomposition rate of NAD) has a straight line. Gradient of temperature, energy of activation and frequency-factor calculated from the measured data, were within the expected range. The constant of the decomposition rate of NAD in pure aqueous solution showed a deviation from the rate in the extracted yeast. The characteristic constants were: Temperature gradient 2.5/10° C; activation energy 26.98 kcal/moles; frequency factor 0.59×10^{16}; optimum temperature 80° C; duration of extraction 5 minutes.

The processes recommended for the preparation of nicotine amide-adenine dinucleotid (NAD) are based on the extraction of the compound from the cell by heat treatment. The different processes show considerable divergencies in the degree of temperature, or in the length of time of heat treatment; at 90—92° C for 8—15 minutes (LE PAGE 1949); at 90° C for 4—7 minutes, and subsequently at 94° C for 1—2 minutes (KORNBERG and SPRICE 1953); at 90° C for 5 minutes (NEILANDS and AKESON 1951); at 85° C for 10 minutes (SHIN *et al.* 1960); at 82° C for 10 minutes (OKUNUKI *et al.* 1955); at 80° C for 5 minutes (TADOKORO and TAKASUGI 1939) is the yeast treated. Our own observations, as well as data in the literature (LOWRY *et al.* 1961; RAUEN 1956) show that decomposition is not negligible at the temperature of extraction. In view of this, it has been attempted to establish the optimum conditions, taking into account the kinetics of the extraction and decay of NAD.

Material and Methods

In the experiments, fresh pressed yeast from the 2nd plant of the Yeast Factory, Budapest (Budapesti Élesztőgyár) was used. The NAD used was produced from pressed yeast according to KORNBERG and SPRICE (1953), with some modifications. The ADH used was a product of REANAL, Budapest.

Measurement of NAD-content : 4.4 ml buffer (4.5 g of $Na_2H_2P_2O_7$. 10 H_2O + 3 ml alcohol, made up to 100 ml with distilled water; pH 9.3) is added to 0.5 ml of material. The photometer is set to zero at 340 mμ in a 1 cm thick cuvette. 0.1 ml crystalline alcohol dehydrogenase-enzym 10 mg/ml is added, then 3 minutes later the extinction of the solution is read. Extinction of 0.01 = 10.0454 μg NAD.

Determination of decomposition rate of NAD, with aqueous NAD-solution: 0.4 g NAD (with 76 per cent NAD content) is made up to 100 ml with distilled water, then placed in a water bath kept at the desired temperature with an accuracy of $\pm$ 0.05° C with the aid of an ultrathermostat. As zero point of time, the twentieth minute from the placing into the water bath is chosen since, according to preliminary experiments, the sample takes up the desired temperature in 20 minutes. The decay is a monomolecular reaction and therefore the test may be begun at any time.

NAD-extraction of yeast: 500 g of yeast is suspended in 500 ml water, and this is poured into 25 ml beakers. The suspensions are heated to the desired temperature in 5 minutes, and kept at that degree for 5 minutes, and then rapidly cooled to 0° C. The samples are then centrifuged and 3 g of basic lead acetate (freshly suspended in cold water) is added to each supernatant at pH 7.4. They are centrifuged, the lead is eliminated by H_2S, separated by centrifugation and the pure supernatant is estimated for NAD-content. The extractions were performed at 50, 60, 70, 80, 90 and 100° C.

Decomposition in extraction fluid: 100 g yeast was suspended in 100 ml of water, and warmed to 85° C, and 95° C, respectively, for 5 minutes. Subsequently samples were taken after 5, 10, 15, 20 and 30 minutes. The samples were cooled to 0° C, and the NAD-content of the supernatant was measured spectrophotometrically as above, with an "UVIPHOT" apparatus, at 340 mμ.

Results

Data in the literature, as well as our own results, show that the decomposition of NAD increases towards the alkaline range, and slowly increases towards the acid range. Its own pH is about 4.3 in aqueous solution. *Fig. 1*

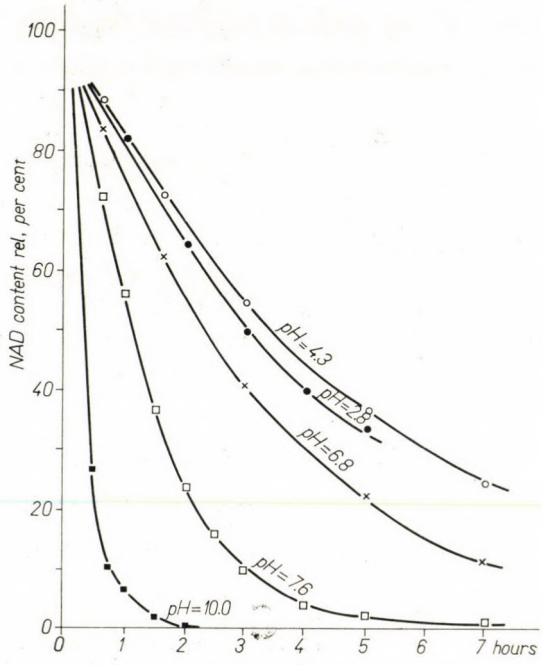

Fig. 1. Decrease of NAD concentration at different pH-values and 76° C

shows the decrease of NAD-concentration at various pH-values, at 76° C. Considering that the preparation of NAD occurs in the acid range, the present paper does not contain results other than its own pH-range and in addition a more acid value, pH 2.8.

The required pH-values were set with acetic acid at 25° C, neglecting

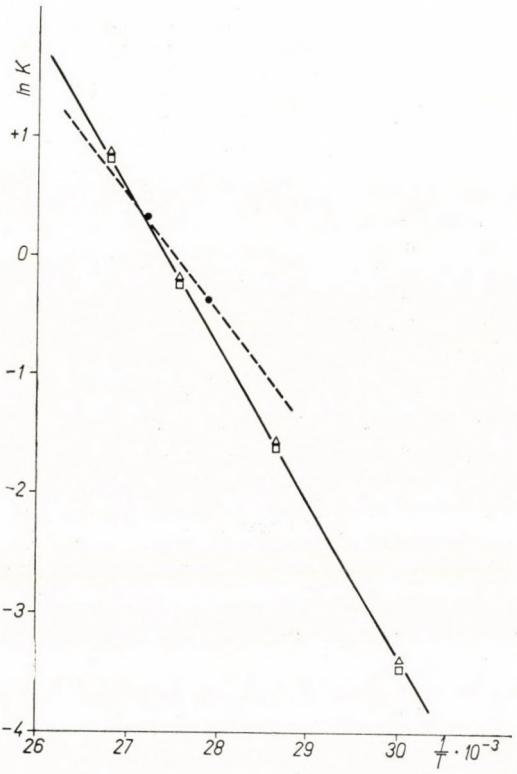

Fig. 2. Arrhenius-curve of NAD decomposition. The continuous line pertains to the pure aqueous solution, the dotted line to the extraction fluid. The triangles show the results at pH 2.8, the quadrangles those at pH 4.3

subsequent changes caused by temperature variations. The first part of the measurements concerns the kinetics of NAD-decomposition in pure aqueous solution. For calculating the rate-constant, the relationships concerning the reactions of first order were suitable, provided that NAD-decomposition is a monomolecular reaction. This assumption was verified by computation. NAD-decomposition proved to be monomolecular at various temperatures and pH-values.

Table I shows the values of the rate-constant (k-values) plotted against temperature, at two selected pH-values. The data of *Table I*, in connection with

Table I

k-values of decomposition, as the function of temperature and pH

temperature		k_{hours}^{-1}	
°K	°C	pH = 2.8	pH = 4.3
333	60	0.034*	0.032*
349	76	0.220	0.200
358	85	—	0.708**
363	90	0.783	0.719
368	95	—	1.395**
373	100	2.400*	2.300*

* Literary data (LOWRY *et al.* 1961)
** Estimated in natural media

$(1/T - \ln k)$ (*Arrhenius*-curve), are shown in *Fig. 2*. It may be seen that the measured data were correct. The results obtained may be brought into harmony with the values of LOWRY *et al.* (1961), achieved at other temperatures.

The temperature gradient of the rate constant obtained by a trial and error method amounted to 2.5/10° C, computed from the *Arrhenius*-curve, at about 80° C.

The equation of the *Arrhenius*-curve, as is well-known, permits the calculation of activation energy and frequency factor of the decomposition reaction.

The *Arrhenius* equation is as follows:

$$\ln k = -\frac{H}{R} \cdot \frac{1}{T} + \ln A \qquad \text{(a)}$$

where:

H = energy of activation, kcal/moles
R = 0.001987 kcal/moles
T = absolute temperature, °K
A = frequency factor, hours^{-1}
k = rate constant of decomposition, hours^{-1}.

The equation yields a straight line, the tangent of the inclination angle being $-H/R$, the abscissa $\frac{1}{T}$, the ordinate $\ln k$, the point of intersection of the ordinate axis, $\ln A$.

The energy of activation, according to the equation of a straight line across two given points is:

$$H = -0.001987 \frac{\ln k_2 - \ln k_1}{1/T_2 - 1/T_1}. \qquad \text{(b)}$$

Table II contains H values, obtained on basis of formula (b). With the transformation of formula (a), the frequency factor A may be computed from

$$A = k \cdot e^{H/RT} \qquad \text{(c)}$$

the results of which are shown in *Table III*.

Table II

Activation energy of NAD-decomposition

Temperature		H kcal/moles	
T_2	T_1	pH = 2.8	pH = 4.3
349	333	27.1	26.8
363	349	23.2	23.1
373	363	30.2	31.2
373	333	26.5	26.8
Mean:		26.76	26.98

Table III

Frequency factor of NAD decomposition

Temperature, °K (T)	$A_{hours^{-1}}$	
	pH = 2.8	pH = 4.3
333	1.20×10^{16}	0.60×10^{16}
349	1.28×10^{16}	0.57×10^{16}
363	1.02×10^{16}	0.61×10^{16}
Mean:	1.17×10^{16}	0.59×10^{16}

In the second part of the measurements, the accompanying materials and contaminations left behind, during the preparation of NAD were examined as to whether they exert an influence on the kinetics of the NAD-decomposition.

The measurements of the rate of decomposition were performed with purified 35, 50 and 75 per cent NAD. As revealed by the results, the order and the rate constant were the same as before. The products of decomposition did not influence the rate constants.

The above measurements were supplemented with several other examinations to clear up the influence of additional substances on the rate of decomposition, and it was found that the organic materials left behind during preparation had no influence whatever on the order of reaction and on the k-values.

Measurements in natural medium were limited by the fact that the concentration of NAD in the extraction fluid is varying along the lower limit of the measurement range.

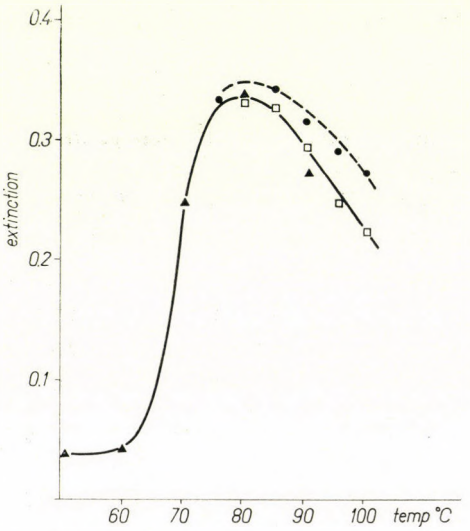

Fig. 3. Extraction curve of NAD. The continuous line shows the NAD-content of two series of extraction fluid, in per cent, after 5 minutes heat treatment. The dotted line represents the theoretical curve of extraction without decomposition, calculated on the basis of the data obtained in pure aqueous solution. Were the rate-constant identical in pure aqueous solution and the extraction fluid, then the dotted curve became asymptotically horizontal

Consequently, individual measurements have shown a wide scattering, and the *Arrhenius*-curve plotted on the basis of their mean values differed from that obtained in pure NAD-solution. This is indicated by the straight dotted line in *Fig. 3.* The curve and the rate of extraction display that the decomposition of NAD is probably faster in a natural medium than in a pure aqueous one, or in the presence of the above-mentioned accompanying materials.

Measurements were also performed to study the variations of the active compound content during extraction. The results obtained in intervals of 5 minutes are shown in *Fig. 3.*

Discussion

The results have substantiated the data provided by LOWRY *et al.* (1961) as to the rate of NAD-decomposition. The accuracy of the data was checked and the *Arrhenius* diagram was plotted; this turned out to be straight line.

The temperature gradient is 2.5/10° C, and therefore it is expedient to perform extraction — in order to avoid decomposition of the active compound — at the lowest possible temperature.

The greatest quantity of active compound was extracted at 80° C by 5 minutes heat treatment. Considering that, as computed by means of the decomposition rate coefficient, decomposition at 80° C during 5 minutes is negligible, it can be assumed that the active compound content extracted at 80° C approaches the maximum.

During the preparation of NAD there are residual contaminations which do not influence the decomposition rate of NAD, but this probably is not true for the extraction fluid (see *Arrhenius* curve). The experimental curve shown in *Fig. 3* is the algebraic sum of extraction rate and decomposition rate, which act simultaneously. If the quantity of NAD decomposed during 5 minutes at various temperatures is calculated on the basis of the separately obtained rate constants of decomposition, and these amounts are added to the individual points of the curve, the deducted curve of the rate of extraction in pure form is arrived at, whose trend should become horizontal after completing the extraction. However, it shows a continuing descending tendency (dotted line in *Fig. 3)*. It is therefore assumed that the rate of NAD decomposition is greater in the extractional fluid, than in pure aqueous medium, or with the above discussed accompanying substances. Thus a temperature of 80° C should be chosen and extraction for 5 minutes will suffice.

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TRYPTIC HYDROLYSIS OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

By

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A trypsin-resistant "core"-fraction has been isolated from the tryptic hydrolysate of denatured glyceraldehyde-3-phosphate dehydrogenase. Four peptides could be separated by means of gel-filtration and micropreparative paper chromatography. It has been established that the large peptides are homologues and contain the whole active site of the enzyme. The possibility of the employment of the "core"-fraction for analytic purposes is raised.

The sequence-analysis of proteins, using the methodical facilities now available, is carried out by studying the molecule fragments produced by different hydrolytic procedures and a mosaic-like summing up of the results thus obtained. In the case of a high molecular weight object the application of the "traditional" hydrolytic methods results in a great number of fragments the mosaic-like summing up of which cannot, or only at the expense of extreme difficulties, be accomplished. It follows that in the case of high molecular weight objects the adoption of specific hydrolytic methods producing only a few large fragments, in addition to the "traditional" procedures, is of utmost importance.

In the course of the tryptic hydrolysis of glyceraldehyde-3-phosphate dehydrogenase (GAPD), (D-glyceraldehyde-3-phosphate: NAD oxidoreductase, phosphorylating, 1.2.1.12.), the formation of a trypsin-resistant "core"-fraction has been observed, consisting of large peptides (Dévényi *et al.* 1963). The aim of the present work has been to examine whether this "core" is well defined, can be obtained reproducibly and whether it contains the essential portions of the molecule. Should these conditions prevail, this fraction may then be used for the partial structural analysis of GAPD, as a large sub-fraction.

Materials and Methods

GAPD. Four times recrystallized swine muscle enzyme was used throughout (Elődi and Szörényi 1954).

Trypsin. Novo Industri A/S "Tripure Novo" preparation (Copenhagen, Denmark). In order to remove chymotryptic contaminations, the enzyme was incubated before use in 1/16 N hydrochloric acid at 37°C for 10 hours, at 5 mg per ml concentration (Redfield and Anfinsen 1956).

Chymotrypsin. "Reanal" preparation, three times recrystallized.

Subtilisin. "Serva" preparation, crystalline.

Pepsin. "Light" preparation, 1 : 10,000.

Paper electrophoretic apparatus. For micropreparative fractionation and fingerprinting an apparatus constructed in our institute was used, at 25—30 V per cm voltage gradient (DÉVÉNYI *et al.* 1964).

Identification of amino acids. The amino acids were identified by means of "double-buffered" paper electrophoresis (DÉVÉNYI 1964) combined with chromatography in butanol. Solvent: butanol—acetic acid—water, 120 : 30 : 50.

Fingerprinting. Whatman 3 and Whatman 3 MM papers, 40×40 cm in size were used for electrophoresis in pyridine—acetic acid buffer, pH 5.0 and 6.5, respectively, at 27 V per cm voltage gradient. At right angles to the direction of the electrophoresis ascending chromatography in a butanol system was performed.

Results

The preparation of trypsin-resistant "core" from heat-denatured GAPD

An about 2 per cent GAPD solution, dialysed salt-free, was denatured in a boiling water bath for 10 minutes, under vigorous stirring. The precipitated protein was centrifuged and washed with distilled water. The precipitate was

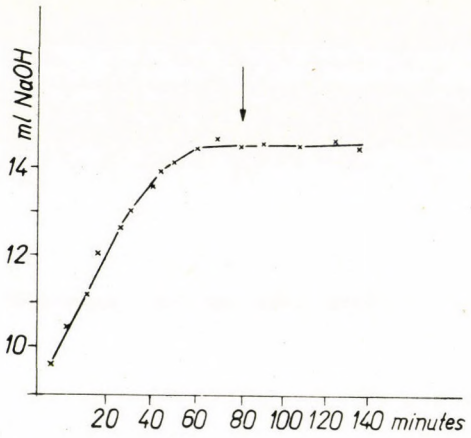

Fig. 1. Tryptic hydrolysis of denatured GAPD. Substrate concentration, 1 per cent; hydrolysis with 1/30 weight of trypsin, at pH 7.8, 25°C. At the time indicated further 1/60 weight of trypsin was added to the reaction mixture

then dehydrated with acetone and dried in air. The dried protein thus obtained was digested by trypsin in a *Radiometer* TTT1 type autotitrator *(Fig. 1).*

Hydrolysis was stopped by heat-denaturation and the mixture containing the precipitate was filtered. About 8 to 10 per cent of the starting material precipitates from the solution. To the supernatant trichloroacetic acid was added up to 3.3 per cent end concentration. The thick precipitate formed was centrifuged and washed with 3.3 per cent trichloroacetic acid, acetone-ether, and finally with ether. The precipitate forms a gel with acetone therefore ether should be applied already at the beginning of washing.

The fractionation pattern of the precipitate formed with trichloroacetic acid is shown in *Table I.*

Table I

Preparation and purification of the tryptic "core"-fraction of GAPD

GAPD

↓ 1. Heat-denaturation
↓ 2. Dehydration with acetone

Denatured GAPD

↓ Tryptic hydrolysis

Crude hydrolysate (90 to 92 per cent)

↓ 3.3 per cent trichloroacetic acid end concentration

Trichloroacetic acid-soluble peptides

Crude "core"-fraction

↓ 1. Oxidation
↓ 2. Gel-filtration

Oxidized low molecular weight peptides

Oxidized high molecular weight peptides (Tc)
↓ Chromatography in butanol

	R_f
Tc-A	0.45
Tc-B	0.33
Tc-C	0.23
Tc-D	0

The precipitate formed with trichloroacetic acid was oxidized with performic acid (performic acid—hydrogen peroxyde, 8 : 2, preincubated at 4° C for 30 minutes). 1 g of the trichloroacetic acid-precipitate was dissolved in 5 ml of 80 per cent formic acid, then 10 ml of performic acid was added. It was allowed to stand at room temperature 30 minutes and then dried *in vacuo*. The oxidized product can readily be solved in dilute ammonia, giving a dark brown colour. 100 mg of the oxidized material was dissolved in a small amount of 1 per cent ammonia solution, and gel-filtered on a 0.9 cm × 50 cm *Sephadex* G-25 column. The column was developed with 0.01 per cent ammonia. The oxidized product could be separated into two fractions, a fast moving one of high molecular weight and a slow moving one of low molecular weight. Both fractions can easily be recognized by their brown colour; this simplifies the collection of fractions. The high molecular weight fraction is precipitated at 3.3 per cent trichloroacetic acid end concentration, while the low molecular weight

fraction is not. Only the high molecular weight fraction (fraction Tc) has been used in the further experiments.

Fraction Tc could be separated into 4 components by ascending chromatography in a butanol system *(Fig. 2)*.

The most rapidly moving component was peptide Tc-A, the R_f value of which was about 0.45. The paper chromatographic method applied in the present investigations is simpler than the cellulose powder-column chromato-

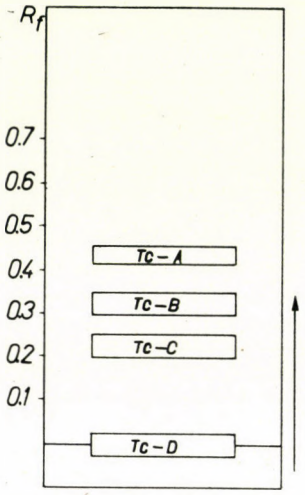

Fig. 2. Paper-chromatographic separation of fraction Tc. Ascending chromatography in butanol—water—glacial acetic acid, 120 : 50 : 30

graphy used in our earlier work (DÉVÉNYI *et al.* 1963). A disadvantage of the method is, on the other hand, the poor colour reaction with ninhydrin of the large peptides encountered in fraction Tc. At higher concentrations, however, the "tailing" phenomenon takes place, hindering the good resolution.

Maintaining the standard conditions of denaturation of GAPD, fraction Tc can well-reproducibly be obtained. Varying the conditions of denaturation, however, the composition of fraction Tc changes as well. If, for example, dehydration by acetone is omitted, fraction Tc-A is absent in the butanol-chromatogram, or if GAPD is oxidized by performic acid prior to tryptic hydrolysis, practically no "core"-formation can be observed.

The control of trypsin-resistance of the precipitate formed with trichloroacetic acid

There is a possibility that the crude "core"-fraction may consist fragments of protein, unsplit after an incomplete enzymatic hydrolysis though not precipitating on boiling, *i.e.* the fraction is not partially trypsin-resistant.

In order to decide the question the precipitate formed with trichloroacetic acid was incubated with trypsin under the experimental conditions described above *(Fig. 3)*.

As it can be seen in *Fig. 3*, no alkali consumption occurred on the addition of trypsin. On the other hand, the addition of chymotrypsin (*A*) or subtilisin (*B*) evoked considerable consumption of alkali.

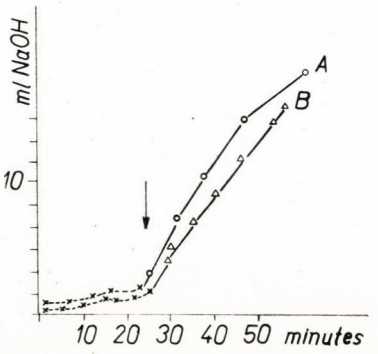

Fig. 3. Trypsin-resistance of the crude "core"-fraction. At 0 time 1/30 weight of trypsin was added to the suspension at pH 7.8, 25°C. At the time indicated by the arrow chymotrypsin (*A*) and subtilisin (*B*), respectively, were added to the reaction mixture

Tryptic hydrolysis of peptides of fraction Tc

The trypsin resistance of the "core"-fraction is lost due to the performic acid oxidation applied in the course of purification. As a consequence the Tc-peptides become digestible by trypsin. It has been examined whether the peptides obtained from their tryptic hydrolysate are identical with or differ from those found in the trichloroacetic acid-soluble fraction of the tryptic hydrolysate of GAPD *(cf. Table I)*.

60 mg of fraction Tc was fractionated by ascending chromatography in butanol system. The four peptide-zones were eluted and digested by trypsin in 0.05 M Tris buffer, pH 7.9, at 37° C for 3 hours. The hydrolysates were concentrated and fingerprinted. Since on the tryptic fingerprints of the Tc-peptides predominantly neutral peptides are to be found, the performic acid-oxidized neutral peptides of the tryptic hydrolysate of GAPD were used for comparison. The neutral fraction was isolated by micropreparative electrophoresis from the trichloroacetic acid-supernatant of the tryptic hydrolysate of GAPD, then oxidized with performic acid and fractionated by ascending chromatography in the butanol system. The R_f value of peptides thus obtained are shown in *Table II*, column GAPD-Ox-N. The neutral subfractions of the tryptic fingerprints of the Tc-peptides are also presented in *Table II*.

It follows from the R_f values of *Table II* that the tryptic subfractions of the Tc-peptides are present among the components of the tryptic hydrolysate

Table II

R_f values in butanol of the neutral components of the Tc-peptides hydrolysed with trypsin

GAPD-Ox-N*	Neutral peptides of tryptic hydrolysed Tc-peptides			
	Tc-A	Tc-B	Tc-C	Tc-D
0,43	0.40		0.44	0.43
0.38	0.37		0.37	0.39
0.33		0.32		0.33
0.29	0.28	0.28	0.29	0.27
0.20		0.20	0.25	0.19
0.12				

* GAPD-Ox-N = Neutral peptides, after performic acid oxidation, of the trichloroacetic acid-soluble fraction of the tryptic hydrolysate of GAPD *(cf. Table I)*.

of GAPD. It appears also from the *Table*, since relatively many identical peptides are present in the hydrolysates of the Tc-peptides, that these large fragments are homologues. To verify this concept, analysis of the neutral subfractions is insufficient; the investigations should be extended onto the basic and acidic components also.

The peptic hydrolysis of Tc-peptides

The peptides obtained by chromatography in butanol of 60 mg of fraction Tc were eluted, then digested by 1/30 weight of pepsin in 0.02 N hydrochloric acid at 37° C for four hours. The hydrolysate was neutralized, dried *in vacuo* and fingerprinted. The tracings of the fingerprints are shown in *Fig. 4*.

It is to be seen from the tracings in *Fig. 4* that the homologous character of the Tc-peptides is much more pronounced on the basis of peptic subfractions than on that of the R_f values of the neutral tryptic peptides. This is only obvious since pepsin is a non-specific proteinase and thus produces more and smaller subfractions than trypsin. This fact accounts for the far greater number of peptides which strongly underlines the homologous character.

The most pronounced connection occurs between peptides Tc-A and Tc-B, as in the peptic hydrolysate of fragment Tc-B all of the peptic subfractions comprised by peptide Tc-A can be found *(Fig. 4 A* and *B)*. On this basis, peptide Tc-B can be described as a derivative of component Tc-A.

Fraction Tc	Symbols of peptic peptides
Tc-A	1/b, 1, 1/a, 2, 5, 6, S/1
Tc-B	1/b, 1, 1/a, 2, 5, 6, S/1, S/5, S/7, S/8, B/1a, *B*/1, B/4b, B/5, B/5a
Tc-B	Tc-A, S/5, S/7, S/8, B/1a, B/1, B/4b, B/5, B/5a

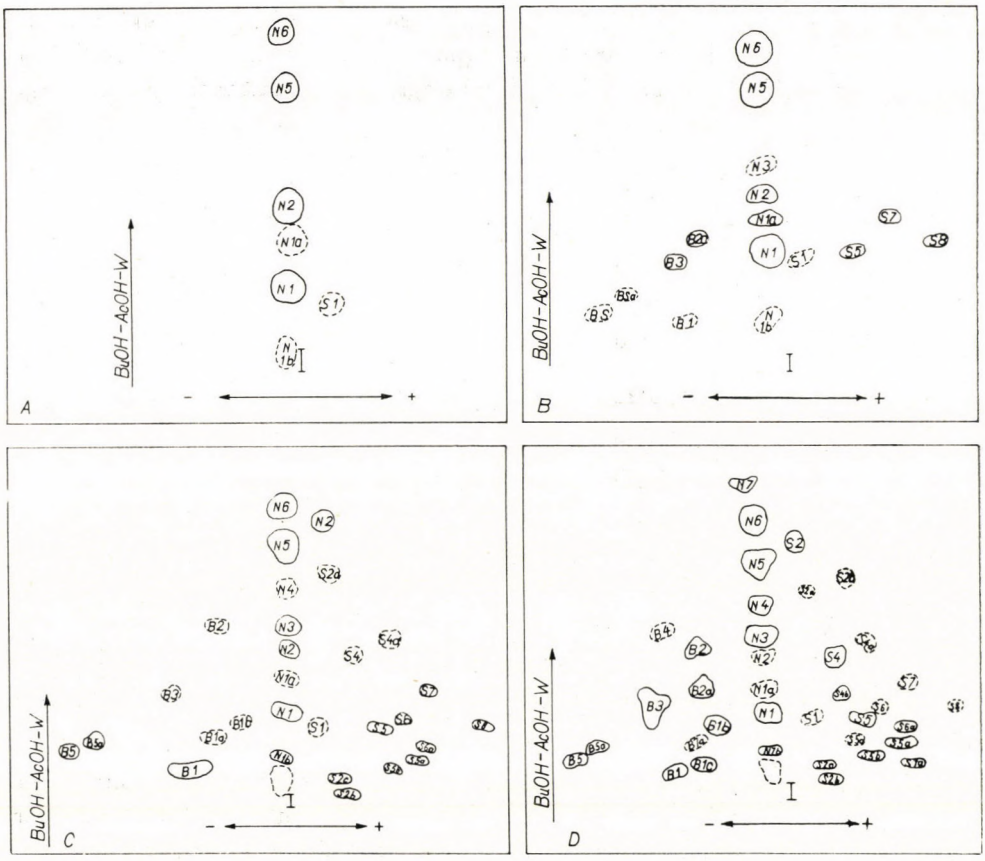

Fig. 4. Peptic fingerprints of the Tc-peptides. *A* = Tc—A, *B* = Tc—B, *C* = Tc—C, *D* = Tc—D

Demonstration of the active centre of GAPD in the Tc-peptides

In the study of large subfractions it is of interest which part of the molecule is represented by the polypeptide-portion examined, that is whether they contain the active site(s) of the enzyme (HARRIS *et al.* 1963; CUNNINGHAM and SCHEPMAN 1963; SEGAL and GOLD 1963), or do not participate directly in the enzymatic function. To decide the question, the peptic hydrolysate of the relatively stable ^{14}C-acetyl-GAPD, formed during the hydrolysis p-nitrophenyl-^{14}C-acetate, has been compared with the peptic hydrolysate of the Tc-peptides.

The peptic hydrolysate of the ^{14}C-acetyl-GAPD has been fractionated by micropreparative electrophoresis at pH 5, at 1 mg per ml concentration. *(Fig. 5, strip A).* The strongly labelled zone p3 was then eluted, dried, oxidized with performic acid and fractionated by eletrophoresis at pH 5. As a result of oxidation the labelled zone p3 separates into three zones *(Fig. 5, strip B).*

The main bulk of zone p3 becomes neutral on oxidation (p3a). In addition, two slightly acid zones are formed (p3c and p3b). The neutral zone p3a was further purified by ascending chromatography in butanol system *(Fig. 5, strip C)*. The zone separated in butanol into two components, zone p3a-1 with a low R_f value ($R_f = 0.05$) and a minor component, p3a-2, of a higher R_f value. The qualitative amino acid composition of the HCl-hydrolysates of the eluted neutral and acidic zones is shown in *Table III*.

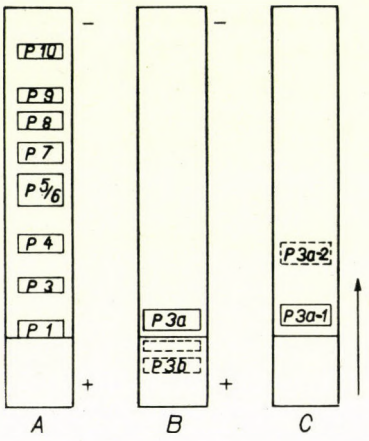

Fig. 5. Fractionation of the peptic hydrolysate of ^{14}C-acetyl-GAPD
A = electrophoresis of the crude peptic hydrolysate, in buffer pH 5
B = electrophoresis of the labelled fraction p3 after performic acid oxidation, in buffer pH 5
C = ascending chromatography in butanol system of zone p3a which is neutral at pH 5

Table III

Amino acid composition of peptic peptides of GAPD treated with p-nitrophenyl-^{14}C-acetate

p3a-1 (N1b)	p3a-2	p3b	The active site of GAPD (HARRIS *et al.*)	
$CySO_3H$		$CySO_3H$	$CySO_3H$	1
Glu				
Asp	Asp	Asp	Asp	2
Ser		Ser	Ser	2
Thr	Thr	Thr	Thr	2
Ala		Ala	Ala	1
Val			Val	1
Leu/Ile			Ile	1
Lys			Lys	1

It should be noted that a component with an R_f value identical with that of peptide p3a-1 has also been isolated from the peptic fingerprints of the Tc-peptides (N1b), the amino acid composition of which entirely agreed with that of the former.

It can be seen from *Table III* that the qualitative amino acid composition of peptide p3a-1 differs only in its Glu content from that of the active site described by HARRIS *et al.* This fragment, since it comes from the labelled zone of the peptic hydrolysate of ^{14}C-acetyl-GAPD, represents the active centre of the enzyme and therefore it is to be found in every Tc-peptide (component N1b).

Discussion

The trypsin-resistance of some peptide linkages may be traced back to several factors. It may be due to the presence of certain side chains which render the —CO—NH— bonds, located at the lysine and arginine residues, inaccessible to trypsin. An example of this is furnished by the N-terminal Lys. Glu . . . fragment of ribonuclease, or by the C-terminal . . . Lys. Asp. OH peptide of MS hormone, *etc.* In these cases the resistance is caused by the side chain of the residue in the immediate vicinity of the peptide bond in question. An indirect type of resistance can be observed in the course of the enzymatic hydrolysis of native proteins. It is known that the overwhelming majority of proteins cannot or can only very slowly be digested in the native state by proteolytic enzymes. The contrasting exceptions to this, like fumarase (INAGAKI *et al.* 1958), are extremely rare. The resistance due to the native conformation can partly or completely be abolished by the disintegration of the steric structure, *i.e.* by denaturation.

The partial resistance found in the case of heat-denatured GAPD is probably caused by the interaction of the reactive SH-groups of the enzyme. This is supported by the fact that oxidation with performic acid prior to tryptic digestion prevents the formation of the "core". Consequently, during tryptic hydrolysis of GAPD a so-called steric "core" is formed.

Based upon the tryptic and peptic subfractionation of the Tc-peptides, it may be assumed that these large peptides are homologues, among which peptide Tc-A is of the simplest structure, as shown by the peptic hydrolysis. The analyses carried out so far suggest that this peptide and the somewhat greater peptide Tc-B may be regarded as large subfractions of GAPD and can be used for the determination of the peptic peptide-sequence of the enzyme.

In the use of high molecular weight subfractions it is far from being indifferent which portion of the protein is represented by them. Obviously, from a structural point of view the active site of the enzyme is of the greatest interest. The structure of the active centre of GAPD is known from the work of HARRIS

et al. (1963) and others (CUNNINGHAM and SCHEPMAN 1963; SEGAL and GOLD 1963):

$$\text{Lys.Ile.Val.Ser.Asp}\overset{x}{\text{N}}\text{H}_2\text{.Ala.Ser.Cys.Thr.Thr.Asp}\overset{x}{\text{N}}\text{H}_2\text{.Cys.Leu.Ala.Pro.Leu.Ala.Lys}$$

where the Cys residue marked with x binds the substrate in the course of enzyme action. Taking into account the amino acid composition of peptide p3a-1, and that of the two smaller peptic fragments, it is very probable that peptide N1b, which can be found in every Tc-peptide, represents the active centre of GAPD. The difference in glutamic acid content cannot be interpreted unequivocally for the present. The question could be decided only by quantitative analyses which are in progress in our laboratory.

It follows from all this that under certain circumstances, the "core" formed during the tryptic hydrolysis of GAPD, can be applied as a large subfraction of the enzyme and may be used for studying the region near the active centre as well as the intramolecular position of the active site.

Acknowledgements

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PHYSICO-CHEMICAL AND IMMUNOLOGICAL PROPERTIES OF PURIFIED PATHOLOGICAL MACROGLOBULIN

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Macroglobulin has been isolated from the serum of a patient suffering from *Waldenström*'s macroglobulinaemia. The isolated macroglobulin did not prove homogeneous at ultracentrifugation. It consisted to 85 per cent of a component with a sedimentation coefficient of 18 S and a molecular weight of 860,000, and another component, the quantity of which amounted to 15 per cent, with a sedimentation coefficient of 27 S and a molecular weight of 1,300,000. On electrophoresis, the macroglobulin behaved as a homogeneous, monodisperse system in a veronal buffer of 0.1 μ and pH 8.6.

Under the effect of 2-mercaptoethanol, used at an end concentration of 0.15 M, the macroglobulin was fragmented into a dissociation unit having a sedimentation coefficient of 6.27 S and a molecular weight of 153,000. Since the dissociation of the macroglobulin could be demonstrated by viscosimetry, it is pointed out that macroglobulins might be estimated also by a simple method. 2-mercaptoethanol caused a profound alteration in the absorption spectrum of the macroglobulin.

Immunological experiments revealed that the isolated macroglobulin was related to gamma globulin, and that the two macroglobulin components different in molecular weight were different also serologically.

The clinical signs of the syndrome termed WALDENSTRÖM's macroglobulinaemia (1944) or essential hyperglobulinaemia have been discussed by a number of authors (JIM and STEINKAMP 1956; MACKAY *et al.* 1956, 1957; WALDENSTRÖM 1952, 1958), but its diagnosis must be confirmed by ultracentrifugal analysis (WALDENSTRÖM 1952). The cause of this is partly that, apart from their sedimentation behaviour, the pathological macroglobulins may vary to a great extent in physico-chemical properties and are mostly heterogeneous at ultracentrifugation. On the basis of sedimentation coefficients of the components, molecular aggregation may be concluded upon. As indicated by dissociation experiments with mercaptoethanol (DEUTSCH and MORTON 1958), the subunit of the molecular aggregate is a protein similar to a gamma-globulin having a sedimentation coefficient of 6 to 7 S. Although a relationship exists between the pathological macroglobulins and gamma-globulin on the basis of common antigenic groups (KUNKEL 1960), it is not yet decided whether these macroglobulins are simple polymers of the normal gamma-globulin (DEUTSCH and MORTON 1958), or should be considered individually as specific proteins on grounds of their physico-chemical (PUTNAM 1959) and serological (HABICH 1953) properties. According to the data in the literature it is also unclear (KORNGOLD and VAN LEEUVEN 1957; MORTON and DEUTSCH 1958) whether the macro-

globulins displaying different sedimentation coefficients are different also immunologically. In view of these unsolved problems, studies of pathological macroglobulins are still in the foreground of interest.

In this paper we present results on the physico-chemical and immunological properties of the macroglobulin isolated by us.

Materials and Methods

Isolation and purification of macroglobulin. The serum of a patient suffering from *Waldenström*'s macroglobulinaemia, which was extremely viscous and gave a positive *Sia* test (SIA and WU 1921), was diluted 1 : 10 with distilled water (DEUTSCH and MORTON 1958) and centrifuged. The precipitated macroglobulin was dissolved in 0.15 M NaCl solution, reprecipitated with distilled water, centrifuged again, and so forth. This procedure was repeated five times.

Physico-chemical methods. The sedimentation studies were carried out in a *Phywe Type U-77* ultracentrifuge, using a 0.15 M NaCl solution of pH 7 as the solvent throughout. The sedimentation coefficients were extrapolated to 20°C, water as solvent and infinite dilution ($s^{\circ}_{20,w}$).

Electrophoretic homogeneity was studied in an *Antweiler* microelectrophoresis apparatus, using a veronal buffer of 0.1 ionic strength and pH 8.6.

The diffusion constant was determined in a diffusiometer adapted to the *Phywe* ultracentrifuge.

Viscosity was measured with an *Ostwald*-type viscosimeter, at 20°C. Stability of temperature was ensured by the use of a *KT-Colora* ultrathermostat.

Molecular weight was calculated by SVEDBERG's equation (SVEDBERG and PEDERSEN 1940), the frictional ratio was computed by *Perrin*'s formula.

Partial specific volume was measured by means of a 5 ml picnometer. The absorption spectrum was studied in an *Unicam Sp.* 500 spectrophotometer.

Protein was determined by the *Kjeldahl* method.

2-mercaptoethanol (2-MCE) (*Light*) was used in the experiments.

Immunological methods. Rabbits weighing 2 to 3 kg were immunized for 3 weeks with a total of 250 mg of purified macroglobulin. The immune serum obtained by exsanguinating the animals was inactivated at 56° C for 30 minutes.

Agar-gel diffusion was studied on OUCHTERLONY plates (1949), containing 2 per cent agar in 0.15 M NaCl solution.

For immunoelectrophoresis, plates prepared with 2 per cent agar in veronal-Na HCl buffer of pH 8.2, μ 0.05 were used (HIRSCHFELD 1961), and run at 200 V and 18 to 20 mA.

Quantitative precipitation was estimated in 0.15 M NaCl solution, with an end volume of 4 ml (HEIDELBERGER and KENDALL 1929, 1935; KABAT and MAYER 1948). The N content of the precipitate was calculated after solution in 0.1 M NaOH from the appropriate calibration curve on the basis of the spectrophotometric extinction at 280 mμ.

Results

The electrophoretic and sedimentation diagrams of the whole macroglobulinaemic serum (*A*, *C*), and the protein components not precipitated from it by tenfold dilution with water (*B*, *D*) are shown in *Fig. 1*. The results obtained by evaluating these findings are summarized in *Table I*. According to the electrophoretic components the serum was hypergammaglobulinaemic, the gammaglobulin amounting to 55.4 per cent was quantitatively precipitated on diluting the serum 1 in 10 with distilled water *(Fig. 1, B)*. According to the sedimentation diagram, 56 to 57 per cent of the proteins of the whole serum settled as

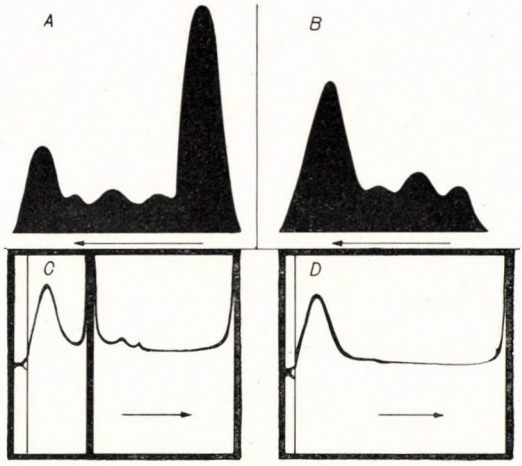

Fig. 1. Electrophoretic and ultracentrifugal diagrams of macroglobulinaemic serum *A :* electrophoretic diagram of whole macroglobulinaemic serum. *B :* electrophoretic diagram of protein components not precipitated from the macroglobulinaemic serum on tenfold dilution with distilled water. The arrows indicate the direction of running. Ionic strength: 0.1; mA: 1.5; V: 90. *C* and *D* are the sedimentation diagrams corresponding to *A* and *B*, respectively. *C*, at 45,000 r. p. m.; recorded 20 minutes after acceleration. Protein concentration 10 mg/ml. *D*, at 39,000 r. p. m., recorded 36 minutes after acceleration. Protein concentration 6 mg/ml

macroglobulin *(Fig. 1, C)*. The three different components, 18 S, 26 S and 32 S, sedimenting as macroglobulin, were, however, almost completely precipitated on diluting the serum with distilled water, and they were absent from

Table I

Electrophoretic and ultracentrifugal analysis of macroglobulinaemic serum

Electrophoretic components	Relative %	g protein / 100 ml serum	Supernatant of tenfold dilution with distilled water: Rel. %	Supernatant of tenfold dilution with distilled water: g protein / 100 ml serum	Ultracentrifugal components	Relative %
gamma-globulin	55.4	5.120	—	—	globulins	
beta-globulin	6.4	0.593	13.1	0.550	7 S	?
alpha$_2$-globulin	8.0	0.740	19.0	0.800	18 S	48.7
alpha$_1$-globulin	4.4	0.407	10.7	0.448	26 S	6.8
albumin	25.8	2.390	57.2	2.402	32 S	2.5
					albumin	42.0
electrophoresis →		9.250		4.200		
↑						
total protein						
↓						
Kjeldahl →		9.30		4.140		

the sedimentation diagram *(Fig. 1, D)*. Thus, the fraction behaving as gammaglobulin in electrophoresis was a macroglobulin showing 3 different sedimentation coefficients at ultracentrifugation. The data in *Table I* also indicate that the protein concentration of the macroglobulinaemic serum was much higher

Fig. 2. Electrophoretic appearance of macroglobulin. Ionic strength: 0.1; mA: 1.5; V: 90, veronal buffer, pH 8.6

(9.25 to 9.30 g per 100 ml) than the normal average of 7 g per 100 ml. The macroglobulin concentration was 5.05 to 5.15 g per 100 ml serum, 16 to 17 times the normal 0.3 g/100 ml (COOPER 1960).

Physico-chemical properties of the purified macroglobulin

The macroglobulin purified five times consisted of two components on ultracentrifugation. The sedimentation coefficient of the component making up 85 per cent was 18.0 S, and that of component amounting to 15 per cent was 27 S *(Fig. 5, A)*. The 32 S component occurring in whole serum was not present in the purified macroglobulin. As 9 days had elapsed between the first analysis of the macroglobulinaemic serum *(Fig. 1, C)* and the isolation of macroglobulin, we assumed that the disappearance of the 32 S component was due to dilution resulting from transfusion, and to prednisolone treatment. Changes in the protein spectrum in response to cortisone have been described in the literature (PUTNAM 1959). In fact, the serum used for the isolation of macroglobulin *(Fig. 4, A)* did not later contain the 32 S component.

The diffusion constant of our macroglobulin preparations was $D_{20,w}$ 1.94×10^{-7} cm²/sec, their partial specific volume, $V = 0.737$ cm³/g. As determined by *Svedberg*'s equation (SVEDBERG and PEDERSEN 1940), the molecular weight of the 18 S macroglobulin was 860,000, that of the 27 S component, 1,300,000.

On electrophoresis the purified macroglobulin behaved as a monodisperse system *(Fig. 2)*, and appeared as a homogeneous symmetrical compo-

nent. Similar results have been reported by other authors (DEUTSCH and MORTON 1958; PUTNAM 1959; CAPUTO and APPELLA 1960; KALDOR *et al.* 1962).

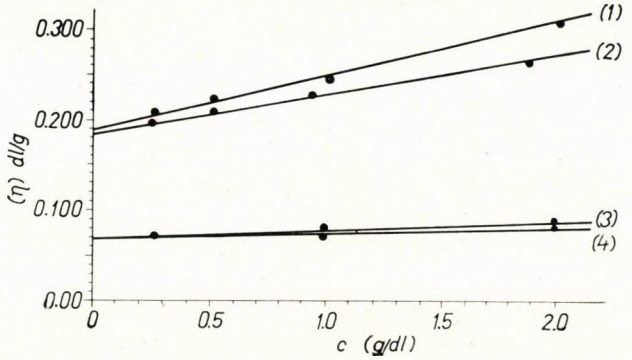

Fig. 3. Intrinsic viscosity (η) of macroglobulin (*1*), macroglobulinaemic serum (*2*), hypergammaglobulinaemic serum (*3*) and normal serum (*4*). Ordinate: intrinsic viscosity, abscissa: protein concentration, g/100 ml

The intrinsic viscosity at 20° C was 0.19 dl/g *(Fig. 3)*. To facilitate comparison, we determined the intrinsic viscosity of a macroglobulinaemic, a hypergammaglobulinaemic and of a normal serum. The results were: macroglobulinaemic serum, 0.185 dl/g; hypergammaglobulinaemic serum with 32 per cent gamma-globulin, 0.068 dl/g; normal serum, 0.068 dl/g.

The absorption maximum of the purified macroglobulin was at 279—280 mμ, the minimum was at 250 mμ *(Fig. 7)*.

Effect of treatment with 2-mercaptoethanol

As macroglobulins were shown (DEUTSCH and MORTON 1958) to dissociate with SH compounds, we examined the effect of 2-MCE. In the presence of 2-MCE, components 18 S and 26—27 S were found to dissociate *(Fig. 4, B)*. In the sedimentation diagram instead of the components 18 S and 26—27 S a 6.2 to 6.5 S component appeared *(Fig. 4, A)*, making up 56 per cent, *i.e.* a quantity equal to that of the macroglobulin contained in the whole serum. The quantity of the other component in the sedimentation diagram amounted to 44 per cent and had a sedimentation coefficient of 4.3 to 4.5 S.

The five times purified macroglobulin *(Fig. 5, A)* dissociated completely in response to 2-MCE *(Fig. 5, B)*. The sedimentation coefficient of the dissociation unit was 6.27 S; the diffusion constant, 3.85×10^{-7} cm^2/sec. On the basis of sedimentation and diffusion, the molecular weight was $M_{s\,D} = 153{,}000$, the frictional ratio, $f/f_0 = 1.54$.

Thus, 2-MCE was able to dissociate both the isolated macroglobulin and that present in serum, as shown by a decrease of the sedimentation coeffi-

cient and the molecular weight. Relative viscosity of both the isolated macroglobulin and the macroglobulinaemic serum also decreased by 15 to 20 per cent *(Fig. 6)*. On the other hand, 2-MCE, used in the same concentration, caused a slight increase of the viscosity of normal and hypergammaglobulinaemic

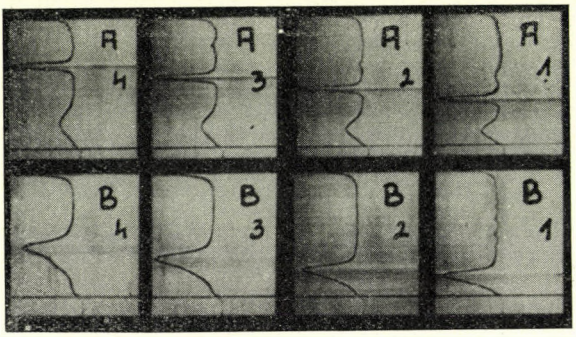

Fig. 4. A : Sedimentation diagram of whole macroglobulinaemic serum. 39,000 r. p. m. Protein concentration 8 mg/ml. The first recording was made 36 minutes after acceleration, the subsequent ones at 10-minute intervals, from right to left. *B :* Sedimentation diagram of whole macroglobulinaemic serum in the presence of 0.1 M 2-MCE. 46,000 r. p. m. Protein concentration, 10 mg/ml. The first recording was made 30 minutes after acceleration, the subsequent ones were made at 20-minute intervals

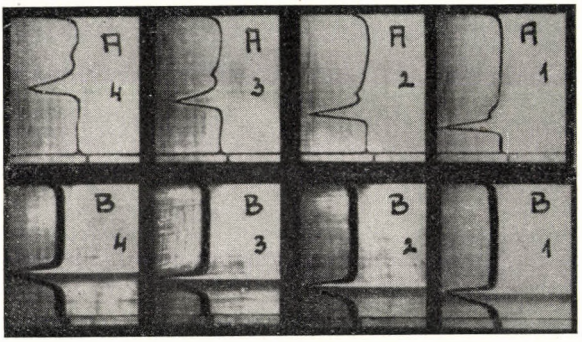

Fig. 5. A: Sedimentation diagram of macroglobulin. 39,000 r. p. m. Protein concentration, 4 mg/ml. The first recording was made 15 minutes after acceleration, the subsequent ones at 10-minute intervals, from left to right; *B :* Sedimentation diagram of macroglobulin, in the presence of 0.15 M 2-MCE. 46,000 r. p. m. Protein concentration, 10 mg/ml. The first recording was made 30 minutes after acceleration, the subsequent ones at 10-minute intervals, from left to right

serum. It appears that while in the ultracentrifuge the macroglobulin of normal serum demonstrably dissociates in response to 2-MCE, this change is not manifest in the η_{rel} value, obviously owing to the low concentration of macroglobulin in normal serum. The 15 to 20 per cent decrease of the viscosity of the macroglobulinaemic serum obtained in response to treatment with 2-MCE

offers a possibility to demonstrate the increased quantities of macroglobulin by a simple method.

The sites of maximum and minimum absorption did not change under the effect of 2-MCE *(Fig. 7)*, but, at the same protein concentration, extinction was lower at every wave length examined than that of the native macroglobulin. This phenomenon may be correlated with a reduction of the covalent disulphide bridges playing a role in the stabilization of the secondary structure,

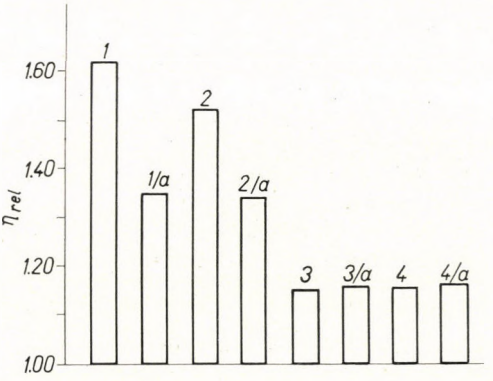

Fig. 6. Changes of relative viscosity, η_{rel}, under the effect of 2-MCE
1. macroglobulin. *1/a* macroglobulin in the presence of 0.15 M 2-MCE.
2. macroglobulinaemic serum. *2/a* macroglobulinaemic serum in the presence of 0.1 M 2-MCE
3. normal serum. *3/a* normal serum in the presence of 0.1 M 2-MCE.
4. hypergammaglobulinaemic serum. *4/a* hypergammaglobulinaemic serum in the presence of 0.1 M 2-MCE. Protein concentration, 20 mg/ml in every case

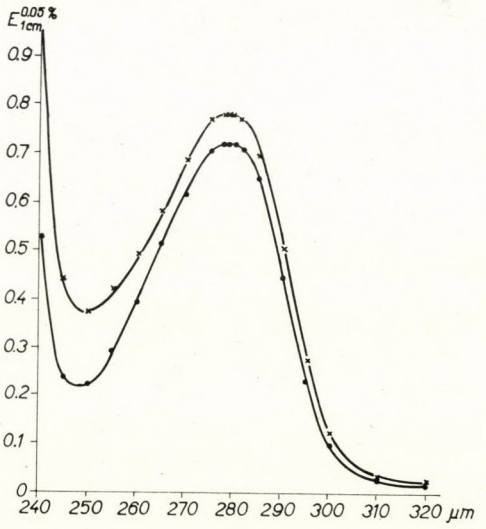

Fig. 7. Absorption spectrum of macroglobulin x-x-x-x
Absorption spectrum of macroglobulin in the presence of 0.006 M 2-MCE — · — · —

inasmuch as a change takes place in the state of the chromophores as a result of this reduction. As it was seen in connection with the measurements of molecular weight, the reduction of the disulphide linkages goes together with a fragmentation of macroglobulin, thus, the effect on the absorption spectrum "resembles" the effect observed on breaking up the primary structure (for example by tryptic digestion). All these indicate that in the case of macroglobulins we may deal with a peculiar manifestation of aggregation. The decrease of absorption under the effect of 2-MCE also indicates that this treatment is sufficiently gentle to cause no rupture of other linkages taking part in the secondary structure.

Immunological properties

Quantitative precipitation tests with rabbit immune serum *(Fig. 8)* showed that 1 ml of immune serum contained 270 μg N specific antibody.

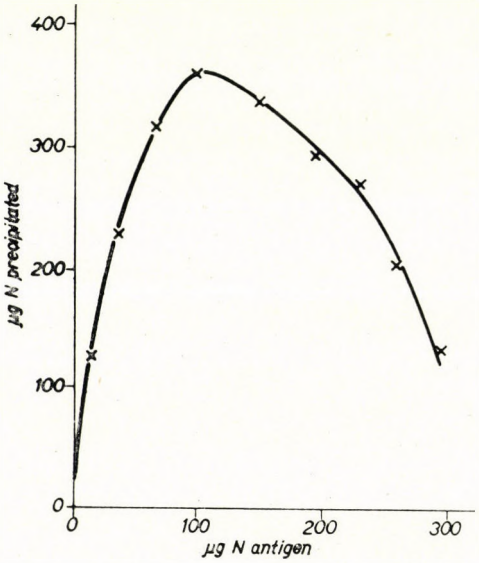

Fig. 8. Quantitative precipitation of macroglobulin by antibody produced in rabbit

On agar gel diffusion *(Fig. 9)*, the different concentrations of macroglobulin antigen yielded two precipitation bands of different intensity. The reaction was weaker with anti-human gamma globulin.

At agar gel immunoelectrophoresis the macroglobulin antigen yielded two precipitation bands. Although the macroglobulin component of higher sedimentation coefficient (27 S) was not isolated, the double bands obtained on the *Ouchterlony* plate and on immunoelectrophoresis suggested that the components of different sedimentation coefficients (18 S, 27 S) were different in immunological specificity. KORNGOLD and VAN LEEUVEN (1957) reported on

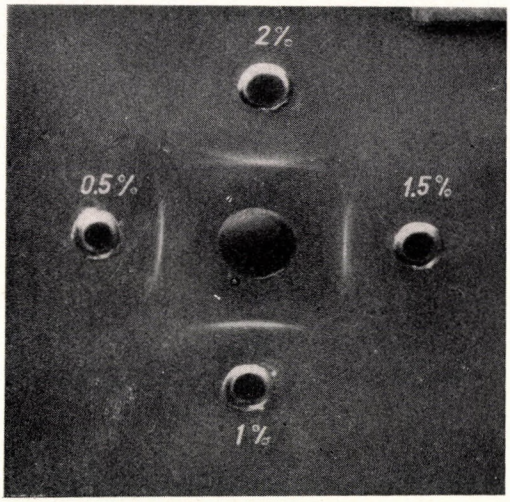

Fig. 9. Immunological reaction of macroglobulin in agar gel with antibody produced in rabbit. 2%, 1.5% *etc.* stand for macroglobulin concentration in g per 100 ml

similar observations, while MORTON and DEUTSCH (1958) denied there being a difference in immunological specificity between the two components.

Discussion

The ultracentrifugal and electrophoretic properties of the macroglobulin isolated by us from the serum of a patient with macroglobulinaemia, as well as the principal physico-chemical characters of the dissociation unit obtained by the use of 2-MCE were similar to those reported in the literature (DEUTSCH and MORTON 1958; PUTNAM 1959; CAPUTO and APPELLA 1960). Although the sedimentation coefficients (18 to 19 S, 26 to 27 S) obtained by us now and earlier (HAJDU *et al.* 1963) as well as the molecular weight of nearly 160,000 of the dissociation unit seem to support the aggregation theory (DEUTSCH and MORTON 1958), the differences in solubility in distilled water, *etc.*, indicate, among others, that the aggregation of normal gamma globulin does not satisfactorily explain the formation and accumulation of macroglobulins. This is supported by the fact that in the case of macroglobulins of different origin new immunological determinants appear alongside the antigenic groups common with those of gamma globulin. On the basis of all these it may be surmised that the synthesis of gamma globulin, pathological globulins and macroglobulins starts from a common protein. From this common protein the widest variety of globulins may be produced, depending on the damage of the enzymes involved in further synthesis. This might explain why the macroglobulins appear to be

individual proteins (HABICH 1953; PUTNAM 1959) and, although not physiological products, they are related to the normal components.

Acknowledgement

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Physiologia

THE EFFECT OF MESENCEPHALIC LESIONS AND STIMULATION ON PITUITARY-THYROID FUNCTION

By

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In experiments on male albino rats it has been shown that bilateral electrocoagulation in the mesencephalic reticular formation results in a considerable increase in thyroid ^{131}I uptake.

On stimulation of the same *loci* with chronic deep electrodes, the uptake of ^{131}I decreases considerably. The decrease in the uptake of ^{131}I resulting from stimulation could be observed in adrenalectomized as well as cortisone-treated animals.

After stimulation, the rate of thyroidal ^{131}I release also diminishes.

The central nervous system is well-known to play an important part in the control of pituitary-thyroid function. While there are several data to show that this control is exercised through the hypothalamus (GREER 1952; GREER and ERWIN 1956; GANONG *et al.* 1955; BOGDANOVE 1957; D'ANGELO 1958; HARRIS and WOODS 1958; KOVÁCS *et al.* 1959, 1960; KOVÁCS and VÉRTES 1963; AVERILL *et al.* 1961; SHIZUME *et al.* 1962a; D'ANGELO and SNYDER 1963), information is scarce concerning the pertaining role of other nervous structures (MESS 1958; YAMADA 1961; KNIGGE 1961; LUPULESCU *et al.* 1962; SHIZUME *et al.* 1962b).

On the basis of experiments with lesioning as well as stimulation several authors have hinted at the importance of the mesencephalon in the control of pituitary-adrenocortical function (NEWMAN *et al.* 1958; ENDRŐCZI and LISSÁK 1960; SLUSHER and HYDE 1961; TAYLOR and FARRELL 1962).

Several data in the literature and our own earlier studies (HARRIS and WOODS 1958; KOVÁCS *et al.* 1959, 1960; BROWN-GRANT 1956) have pointed to the close functional relationship between the pituitary-adrenocortical and the pituitary-thyroid systems during the hypothalamic control of the activities of these two endocrine systems; therefore, in the present experiments the role of the mesencephalic reticular formation in pituitary-thyroid function has been studied.

Methods

The examinations were carried out on male albino rats of 150 to 200 g body weight. The experiments had the double aim of studying the effects on pituitary-thyroid function of electrocoagulation placed in the mesencephalic reticular formation and of stimulation of the same region with chronic deep electrodes.

Electrocoagulation was performed bilaterally with a *Horsley-Clark* stereotaxic apparatus under pentobarbital anaesthesia with 4 mA for 6 sec. Sham-operated animals were used as controls. The working up of the animals began at the end of the second postoperative week, when they were given 5 μC $Na^{131}I$ intraperitoneally. Then the uptake of ^{131}I by their thyroid glands was determined, with the *in vivo* method described elsewhere (KOVÁCS *et al.* 1960) 1, 3, 6, 9 and 24 hrs after the injection of ^{131}I.

In the stimulation experiments bipolar electrodes partly of stainless steel and partly of silver, insulated with varnish or glass, respectively, were implanted under pentobarbital anaesthesia in the corresponding territory of the brain. The electrodes were fixed to the skull with dental cement (Adhesor II). The leads of the individual electrodes were soldered to a miniature plexiglass socket and after proper insulation the socket was fastened to the skull with Renit. These types of electrode may be used for several months.

After the first postoperative week the animals in which thyroid function had been determined with the test for ^{131}I uptake were stimulated with a square impulse generator (0.5—1.5 V, 3 msec pulses, 15 or 50 cps, 30 sec on-off periods for 10 min). During stimulation the poles were changed every half minute. After stimulation 1 to 5 μC $Na^{131}I$ was injected intraperitoneally and 2 hours later the thyroidal ^{131}I uptake was determined *in vivo*.

Some groups of animals bearing electrodes (experimental animals as well as controls) were subjected to adrenalectomy or cortisone treatment (5 mg Adreson daily for 5 days). One week after adrenalectomy or at the end of cortisone treatment, the animals were stimulated, injected with ^{131}I and the uptake of ^{131}I by their thyroid glands was determined as described above.

In the cases where the rate of thyroidal ^{131}I release was used as the indicator of thyroid function, the animals were injected also with 5 μC of $Na^{131}I$ intraperitoneally. Forty-eight hours later thyroid ^{131}I uptake was determined and, taking the values thus obtained to be 100 per cent, the release curve was plotted on the basis of 2 measurements daily and the effect of stimulation on the curve was observed. In these cases the same stimulation parameters were used as before.

After the experiments all the animals were killed with an overdose of pentobarbital, their brain was removed, fixed in formol and the site of the lesions or of the electrodes was identified in paraffin-embedded sections stained with cresyl-violet. The thyroid glands were also removed and weighed with a torsion balance, with a precision of 0.2 mg.

Results

Bilateral electrocoagulation in the mesencephalic reticular formation induced a strong increase in thyroidal ^{131}I uptake. The increase of uptake was particularly marked during the first 6 hours following the injection of ^{131}I. As a result of the increased iodine turnover in the thyroid gland of the lesioned animals, measurement at later points of time showed considerably less difference *(Fig. 1)*. No difference was noted in thyroid weight.

The lesions were placed bilaterally, somewhat laterally to the central grey matter and a little dorsally to the red nucleus. In the fronto-occipital plane they extended from the posterior level of the mamillary body to the posterior level of the interpeduncular nucleus.

Stimulation of the mesencephalic reticular formation with electrodes placed as described before and at 50 or 15 cps was equally followed by a considerable decrease in ^{131}I uptake *(Figs 2, 3)*. This decrease in response to stimulation occurred also in adrenalectomized or cortisone-treated animals *(Figs 4, 5)*.

Fig. 6 shows the effect of stimulation on the thyroidal ^{131}I release rate. As seen, in response to stimulation there was a considerable decrease in ^{131}I release.

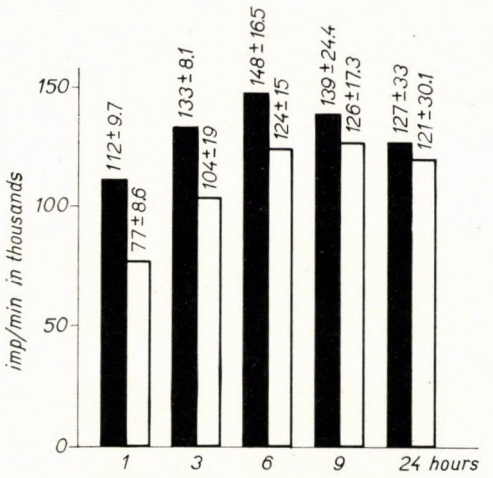

Fig. 1. Effect of bilateral electrocoagulation in the mesencephalic reticular formation on thyroidal ^{131}I uptake
Black columns: 12 lesioned animals
White columns: 8 control animals

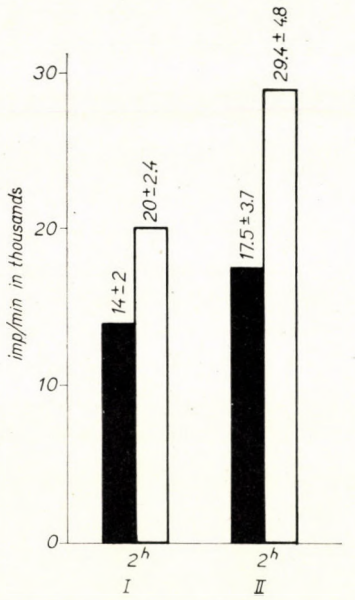

Fig. 2. Effect of stimulation of the mesencephalic reticular formation on thyroidal ^{131}I uptake (Stimulation: 0.5—1.5 V, 3 msec pulses, 50 cps)
Black columns: 2 groups of stimulated animals
White columns: 2 groups of control animals
I.: $t = 5.8$ DF: 17 $p < 0.01$
II.: $t = 4.88$ DF: 11 $p < 0.01$

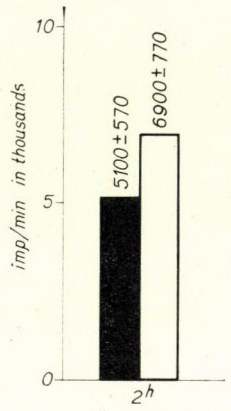

Fig. 3. Effect of stimulation of the mesencephalic reticular formation on thyroidal ^{131}I uptake
(Stimulation: 0.5—1.5 V, 3 msec pulses, 15 cps)
Black column: stimulated animals
White column: control animals $t = 4.4$ DF: 9 $p < 0.01$

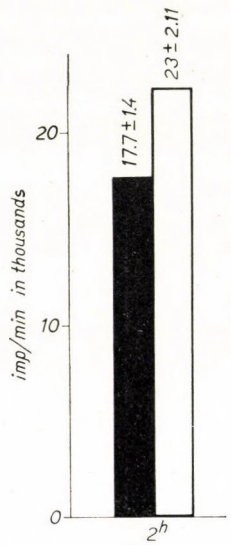

Fig. 4. Effect of stimulation of the mesencephalic reticular formation on thyroidal ^{131}I uptake in adrenalectomized animals
(Stimulation, as in *Fig. 2*)
Black column: stimulated animals
White column: control animals $t = 4.41$ DF: 7 $p < 0.01$

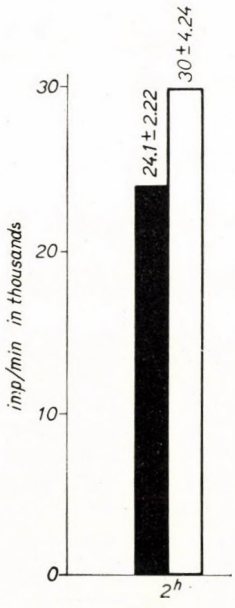

Fig. 5. Effect of stimulation of the mesencephalic reticular formation on thyroidal ^{131}I uptake in cortisone-treated animals

(Stimulation: as in *Fig. 2*)
Black column: stimulated animals
White column: control animals $t = 2.56$ DF: 9 $p < 0.05$

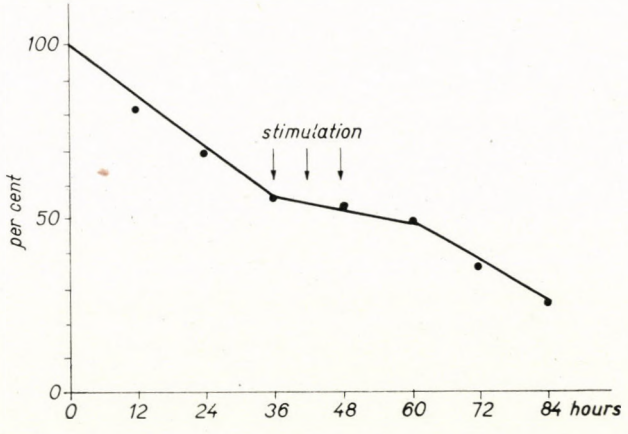

Fig. 6. Effect of stimulation of the mesencephalic reticular formation on the rate of thyroidal ^{131}I release

Discussion

Our results indicate that a lesion placed in the mesencephalic reticular formation or its electrical stimulation exerts a considerable influence on pituitary-thyroid function. On the basis of our experiments this effect seems to be inhibitory.

As to the underlying mechanism, two possibilities suggest themselves. One of them is that it is the increased ACTH secretion occurring in response to stimulation which results in a decrease of pituitary-thyroid function. This assumption is contradicted by the fact that in the present experiments inhibition of pituitary-thyroid function resulted in response to stimulation also in adrenalectomized or cortisone-treated animals.

The other possibility is that the nerve fibres coming from, or passing through, the mesencephalic reticular formation transmit a special stimulus towards the hypothalamus, influencing pituitary-thyroid function through the hypothalamic structure which controls TSH secretion.

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STUDIES ON THE ROLE OF THE MESENCEPHALIC RETICULAR FORMATION IN THE MOTIVATION AND AVOIDING CONDITIONED REFLEX PROCESSES FOLLOWING THE MESENCEPHALIC AND SYSTEMIC ADMINISTRATION OF CHLORPROMAZINE

By

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The effects of chlorpromazine, injected subcutaneously or into the mesencephalic reticular formation in rats during the elaboration and stabilization of an avoiding conditioned reflex, have been studied by analysing the performance of the reflex and the spontaneous intersignal reactions.

In response to the injection of 5 to 50 μg of chlorpromazine into the mesencephalic reticular formation the number of spontaneous intersignal reactions decreased significantly, without any change in the performance of the conditioned reflex.

In response to the systemic administration of 0.1 mg/100 g body weight of chlorpromazine, only the number of the intersignal reactions decreases, while doses of 0.2 to 1.0 mg/100 g body weight inhibit also the performance of the conditioned reflex and even the motor reactions to the unconditioned stimulus.

The results are indicative of an involvement of the mesencephalic reticular formation in the organization of the spontaneous goal-directed motor reactions.

In the course of studies of the organization of behavioural processes by either the Pavlovian conditioned reflex method or the free operant technique in recent years the various CNS* stimulants and tranquillizers have been used more and more often, beside the stimulation and ablation, as well as electroencephalographic examinations. Changes in behaviour or in the EEG caused by drugs acting on the CNS, including chlorpromazine, supply information as to the physiological organization of behavioural processes and the biochemical background of the mechanism of organization (Knoll 1962).

In the present experiments we have analysed in the rat the changes ensuing upon the injection of chlorpromazine into the mesencephalon, first of all into the mesencephalic reticular formation, comparing them with the changes induced by the systemic administration of the drug.

Methods

Male albino rats, weighing 150 to 200 g, fed a standard diet and allowed water *ad libitum*, were used.

In one group of rats, under hexobarbital anaesthesia a chronic micro-cannula was placed into the mesencephalon by means of a stereotaxic apparatus. The cannula consisted of a glass capillary 0.3 to 0.8 mm in diameter and a thin polyethylene tube attached to it. The cannula was fixed to the top of the skull by means of dental cement. The drug was dissolved in 0.001

* CNS = central nervous system

ml of physiological NaCl solution and injected intracerebrally by a microinjector. The training to set up the avoidance conditioned reflex was begun 4 to 6 days after operation. The animals were kept in separate cages, housing one animal each.

Reflex conditioning. The avoiding conditioned reflex was set up in the apparatus described in detail in a previous paper (BOHUS *et al.* 1963). In this the electric shock serving as the unconditioned stimulus came from the metal grid applied on the floor of the cage. To escape from the shock, the animals had to jump onto a 10 cm high bench.

As the conditioned stimulus, we presented a buzzing sound for 10 seconds, during the last two seconds of which we gave two short 50 V shocks. The number of trials was 12 daily, the duration of the interval between two trials was 60 sec. In the course of the elaboration and stabilization of the conditioned reflex we used the reinforcing unconditioned stimulus only when the animal have failed to jump on the bench in response to the conditioned stimulus. During conditioning the avoiding conditioned reflex performance and the number of the spontaneous intersignal reactions was recorded.

Mode of chlorpromazine administration. Chlorpromazine was administered in different stages of the acquisition and stabilization of the conditioned reflex, not sooner than during the third sessions, 120 minutes before the beginning of conditioning.

The intramesencephalic doses of chlorpromazine (Largactil, Specia) were 5.0, 25.0 and 50.0 μg; the subcutaneous doses were 0.05, 0.1, 0.2, 0.5 and 1.0 mg per 100 g body weight.

After the experiments had been completed, the brain of the cannulated animals was removed, fixed in formalin and examined for cannula placement by the histological method of GUZMAN-FLORES *et al.* (1958).

Results

General characteristics of the avoiding conditioned reflex activity and of the spontaneous intersignal reactions

The conditioned reflex was acquired by 16 to 22 trials. After the temporary connection had become stable, during the 20 days of the experimental period (240 trials), the reflex was performed in the same way by the individual rats.

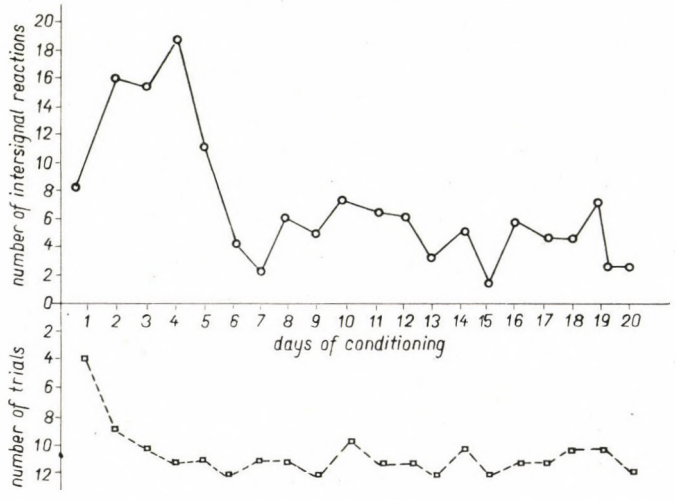

Fig. 1. General characteristics of the performance of the avoiding conditioned reflex and of the spontaneous intersignal reactions during the elaboration and stabilization of the conditioned reflex. o: spontaneous intersignal reactions; □: positive responses to the conditioned stimulus

Spontaneous intersignal reactions had appeared before the conditioned reflex; their number was high in the early phase of the development of the temporary connection, then a significant decrease resulted following the stabilization of the reflex, after about 50 to 70 trials. With minor oscillations, this reduced number of intersignal reactions persisted in the course of further training *(Fig. 1)*.

The effect of intramesencephalic chlorpromazine administration on conditioned reflex activity and spontaneous intersignal behaviour

In response to the injection of 5 to 50 μg chlorpromazine into the mesencephalic reticular formation the number of spontaneous goal-directed intersignal reactions significantly decreased in the period of development and stabilization of the conditioned reflex, without any alteration in avoiding conditioned reflex activity. The dose had no influence on this effect of chlorpromazine.

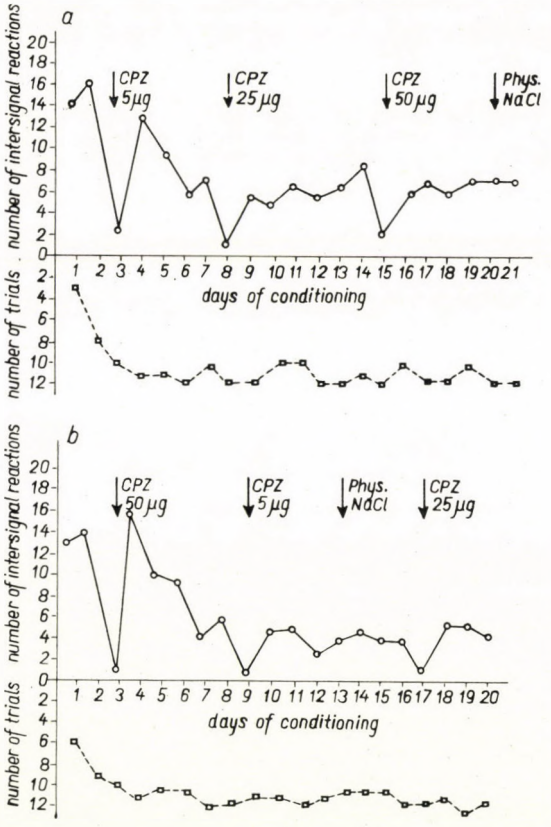

Fig. 2. Effect of chlorpromazine (CPZ), injected into the mesencephalic reticular formation, on spontaneous intersignal motor activity and avoiding conditioned reflex performance. (*a* and *b* represent observations made in two different animals)

The physiological NaCl solution used in the control experiments produced no change either in reflex activity or in the number of intersignal reactions *(Figs 2a, 2b)*.

After the administration of chlorpromazine into the mesencephalic reticular formation, the behaviour of the animals changed characteristically during the interval between the presentations of the conditioned signals. While in the periods without treatment the animals were "waiting" for the signal, usually sitting in the same spot of the floor of the conditioning apparatus or performed spontaneous intersignal motor reactions, *i.e.* a goal-directed movement (jumping on the bench to avoid being shocked), during the intervals between the trial after the administration of chlorpromazine orientative, washing automatisms could be observed, which appeared when the animal was first placed into the experimental situation, but disappeared completely parallel with the development and stabilization of the conditioned reflex. At the same time, as it has already been mentioned, the number of the spontaneous goal-directed reactions was significantly reduced.

If the cannula had been placed into other mesencephalic structures, the injection of chlorpromazine produced no change either in reflex performance or in intersignal activity.

Effect of subcutaneously injected chlorpromazine on conditioned reflex activity and spontaneous goal-directed behaviour

The changes produced by the subcutaneous injection of chlorpromazine prior to conditioning depended to a great extent on the dose injected. A dose of 0.05 mg/100 g body weight produced no change either in the performance of the conditioned reflex, or in the number of intersignal reactions. Following the injection of the 0.1 mg/100 g dose, the number of intertrial reactions decreased significantly (without any change in reflex activity), then returned again to the corresponding level on the next day *(Fig. 3)*.

In response to the subcutaneous injection of 0.2 mg/100 g body weight of chlorpromazine the spontaneous intersignal activity disappeared, the performance of the reflex in response to the presentation of the conditioned stimulus was impaired, but the motor reaction was performed by every animal without exception on the presentation of the reinforcing unconditioned stimulus. After a dose of 0.5 mg/100 g body weight some animals did not perform the motor reaction in response to the unconditioned stimulus, either, and this was still more obvious following treatment with 1.0 mg/100 g, when the animals showed only minimal signs of having noticed the electric shock *(Fig. 4)*.

The orientation and washing automatisms observed following the mesencephalic administration of chlorpromazine appeared also in response to the subcutaneous injection of 0.1 mg/100 g of chlorpromazine; animals treated with 0.2 mg/100 g were apparently somnolent.

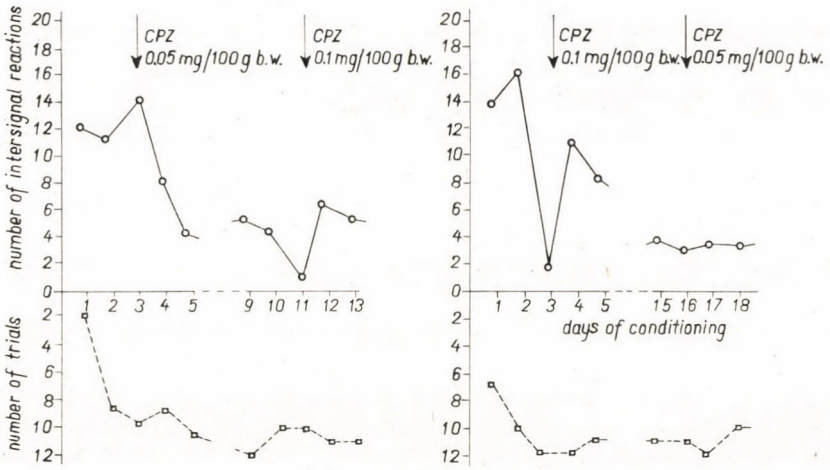

Fig. 3. Effect of a low subcutaneous dose of chlorpromazine (CPZ) on spontaneous goal-directed motor reactions and on the performance of the conditioned reflex

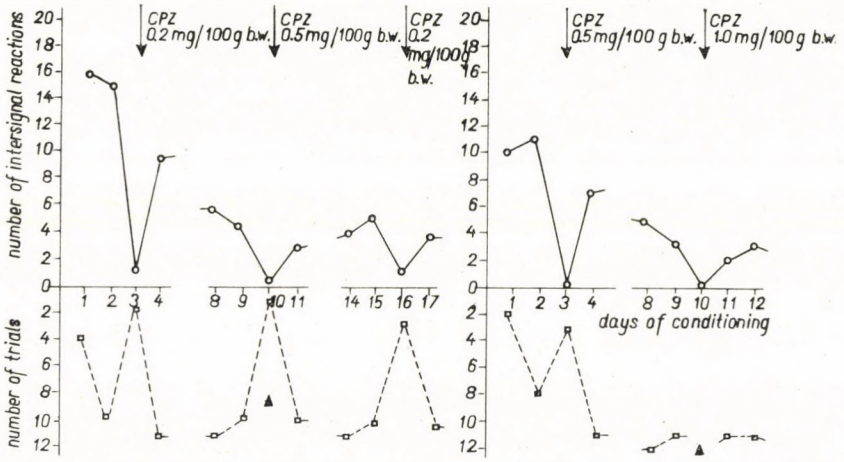

Fig. 4. Effect of a high subcutaneous dose of chlorpromazine on conditioned reflex performance and intersignal reactions. ▲: no defensive motor reaction to either the conditioned or the unconditioned stimulus

Discussion

The disturbance of the avoiding conditioned reflex activity, which resulted in our experiments from the subcutaneous administration of 0.2 mg/100 g body weight of chlorpromazine, has been observed also by ADER and CLINK (1957), DENENBERG *et al.* (1959), as well as IRWIN (1960). In response to the 0.5 mg/100 g dose, only the reaction to the conditioned stimulus was inhibited in some animals, while others failed to respond to the unconditioned stimulus,

too, by trying to escape. The individual differences in the responses, noticed also by IRWIN (1960) are due apparently to individual differences in the sensitivity to chlorpromazine.

A dose of 0.1 mg injected subcutaneously caused no change in the performance of the conditioned reflex, but the number of intersignal reactions was significantly reduced. In our earlier experiments involving cats in alimentary conditioned reflex (ENDRŐCZI and LISSÁK 1962) and rats in avoiding conditioned reflex situations (BOHUS and ENDRŐCZI 1964) it was shown that the intersignal reaction as spontaneous goal-directed motor activity belong to the early signs of the conditioned reflex and may be considered to represent a manifestation of motivation. As the conditioned reflex is becoming stabilized, the decrease in spontaneous intertrial activity may be brought into correlation with the discriminative function, and may be considered to be a result of the internal inhibition phenomena of the Pavlovian terminology, notably a result of the discriminative internal inhibition. On this basis the reduction in the number of intersignal reactions following the subcutaneous injection of a low dose of chlorpromazine may be explained by assuming that the drug promotes the processes of internal inhibition. This hypothesis is supported also by the observations, according to which chlorpromazine enhances the intensity of the internal inhibition processes, in the Pavlovian terminology (BOLONDINSKY 1962).

The changes in conditioned reflex performance and intersignal activity following the systemic administration of chlorpromazine, a drug acting centrally, has made it possible to study the mechanism of the organization of the conditioned reflex activity and of the motivation phenomena by administering the drug topically in the brain. On the basis of electroencephalographic studies LONGO, BERGER and BOVET (1954), HIEBEL, BONVALLET and DELL (1954), ANOKHIN (1957), as well as MARTIN, DEMAAR and UNNA (1958) stated that the principal site of action of chlorpromazine was the reticular formation. Following the local administration of chlorpromazine, VOLKOVA and KHANANASVILI (1962) have observed that in the cat the reticular formation was the subcortical structure most sensitive to the drug. In the light of these data it could be surmised that in the changes of behaviour following the systemic administration of chlorpromazine the reticular formation would play a significant role.

There are often contradictions concerning the role of the reticular formation in the development of conditioned reflex processes. So for example YOSHII *et al.* (1958; cited by JOHN *et al.* 1961), GASTAUT (1958) and ROITBAK (1958) claim that the reticular formation plays a central role in the elaboration of the conditioned reflex, while CHOW and RONDELL (1960), as well as KREINDLER *et al.* (1959) found that the conditioned reflex was unimpaired, and avoiding conditioned reflex learning and visual discrimination were normal after the removal in several steps of, or lesion to, the reticular formation. In our

earlier experiments we could observe in the dog a facilitation of the conditioned reflex inhibited by a noxious, painful stimulus, in response to stimulation of this area (ENDRŐCZI *et al.* 1959), while in the cat low-intensity electrical stimulation of the reticular formation resulted in an inhibition of the spontaneous goal-directed intertrial reactions in the alimentary conditioned reflex situation (ENDRŐCZI and LISSÁK 1962).

In the present experiments the chlorpromazine injected locally into the mesencephalic reticular formation reduced, independently of the dose, the number of spontaneous intersignal motor reactions during the development and stabilization of the temporary connection, leaving the performance of the avoiding conditioned reflex unaffected. These observations tend to indicate that the mesencephalic reticular formation would play a role first of all in the organization of the motivated (goal-directed) behaviour. At the same time, the lack of a disturbance in conditioned reflex activity suggests that the inhibition of the conditioned reflex performance by a high dose of chlorpromazine is realized through other subcortical, eventually cortical, structures.

By injecting chlorpromazine locally, no precise information could be obtained as to the mechanism of the inhibition of spontaneous goal-directed motor activity in the reticular formation. According to our earlier observations and to the data of other authors, the decrease in the number, or the total disappearance, of intersignal reactions may be due both to an inhibition and a facilitation of the function of the reticular formation. According to KILLAM (1962), chlorpromazine inhibits the function of the reticular formation, or inhibits the responses to certain incoming informations, exerting a sort of filtering effect in the reticular formation. YOSHII *et al.* (1958, cited by JOHN *et al.* 1961), GASTAUT (1958), GRASTYÁN *et al.* (1959), have arrived at the conclusion that the internal inhibition, to which the inhibition of the intersignal activity is due, results from an inhibition of the reticular activation system. On the other hand, RINALDI and HIMWICH (1955) found that, as opposed to a low dose of it, a high dose of chlorpromazine was stimulating the activity of the reticular formation. Our observations, too, indicate that the chlorpromazine injected locally into the mesencephalic reticular formation activates the pituitary-adrenocortical system (BOHUS and ENDRŐCZI 1964b), which activation resulted from the stimulation of this structure (ENDRŐCZI and LISSÁK 1963).

The present investigations, pointing to the involvement of the mesencephalic reticular formation in the organization of the spontaneous goal-directed motor reactions, make it likely that the topical administration of drugs acting on the CNS into the proper areas of the central nervous system may be used with success in studies of the organization of behavioural processes, because such drugs alter only temporarily the function of the affected structures.

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SPLANCHNICOTOMY AFFORDS PROTECTION AGAINST ACUTE RENAL FAILURE IN DOGS

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A syndrome analogous to human acute renal failure has been induced in dogs by unilateral nephrectomy and the temporary clamping of the artery of the remaining kidney. The same intervention was not followed by renal failure and all pathological changes disappeared after 14 days if the animals were splanchnicotomized on the left side at the time of right nephrectomy. The difference in the length of survival between the two groups was significant statistically. Neurogenic factors are suggested to play a significant role in the genesis of acute renal failure.

The term "acute renal failure" was applied by SMITH (1951, 1958) to conditions in which, under the effect of some noxious influence, subjects with previously intact kidney develop oliguria and uraemia in a few days. The syndrome is often fatal. Specific nephrotoxic agents, precipitation of blood pigments, allergic states, shock and dehydration have been described as pathogenic factors.

HAMILTON *et al.* (1948) as also PHILLIPS and HAMILTON (1948), by removing the right kidney and clamping the left renal artery for a few hours, succeeded in inducing in dogs a condition similar to human acute renal failure. If clamping was released after 2 hours, the animals survived and the level of nonprotein nitrogen (NPN) as also the clearance values became normal in 2 to 3 weeks. Clamping for 4 to 6 hours resulted in death in 4—8 days with symptoms of acute renal failure. It was demonstrated by OLIVER *et al.* (1951) that the histological changes in the kidney of such animals were analogous to those described in cases of human acute renal failure.

Earlier investigations (BÁLINT *et al.* 1960) showed that the course of acute renal failure induced in dogs by compression of the renal artery depended on the nature and depth of the anaesthesia under which the injury was inflicted. While most of the deeply anaesthetized animals survived, the majority of animals which had been operated upon under superficial, ether anaesthesia succumbed to the injury. Apart from the grave clinical picture in the latter category, necropsy revealed ischaemia of the entire renal cortex, whereas only slight clinical symptoms and comparatively insignificant histological changes were observed in animals that had been subjected to clamping of the renal artery under deep chloralose anaesthesia. These observations seemed to justify

the assumption of a decisive role of central nervous impulses in the genesis of acute renal failure. Further support of that view was yielded by experiments in which the elimination of the central nervous apparatus by the division of the spinal cord at the height of the fourth thoracic vertebra prevented the development of renal failure. In the present study, impulses coming from the central nervous system were blocked by splanchnicotomy.

Material and Method

A total of 11 adult dogs of both sexes was nephrectomized from the right paracostal approach and two weeks later, after pretreatment with 0.01 g/kg morphine and subsequent superficial ether anaesthesia the hilum of the left kidney was exposed and the renal artery clamped. Two hours later the compression was released, and the wound was closed.

Further 10 dogs were treated similarly, with the difference that simultaneously with the right nephrectomy from abdominal approach, the splanchnic nerve was divided on the left side below its emergence from the diaphragm, above the suprarenal gland.

Subsequently the blood NPN was estimated every 2nd or 3rd day for 14 days. The surviving animals were sacrificed on the 14th day. All the dogs were dissected, and the stump of the splanchnic nerve was examined in order to verify its transection. The results were evaluated statistically by means of the χ^2 test and *Student*'s "t"-test (FISCHER 1946).

Results

Table I shows the results arranged according to the length of survival. Animals alive on the 14th postoperative day were regarded as survivors. Two animals survived, and 9 animals died between the 3rd and 9th day in the morphin-ether anaesthetized (control) group, whereas only a single dog died on the 5th day and the rest survived in the splanchnicotomized group. The difference in the length of survival between the two categories was significant statistically ($p<0.01$).

Table I

Nature of intervention	Survival 14 days	Spontaneous death	Total
Clamping of renal artery	2	9	11
Splanchnicotomy + clamping of renal artery	9	1	10

$p < 0.01$

Fig. 1 illustrates the changes in NPN. The number of days following clamping of the renal artery is plotted on the abscissa, NPN values are indicated on the ordinate. The controls exhibited high values and with two exceptions died before the 14th day (dotted line). After an initial rise, the NPN

level diminished in the splanchnicotomized group and approached the normal value between the 10th and 14th day. Only a single animal of this group died on the 5th day with a NPN value of 500 mg per 100 ml (solid line).

The kidney of animals that had succumbed to the injury presented the following picture. The major part, and in some cases the whole, of the renal

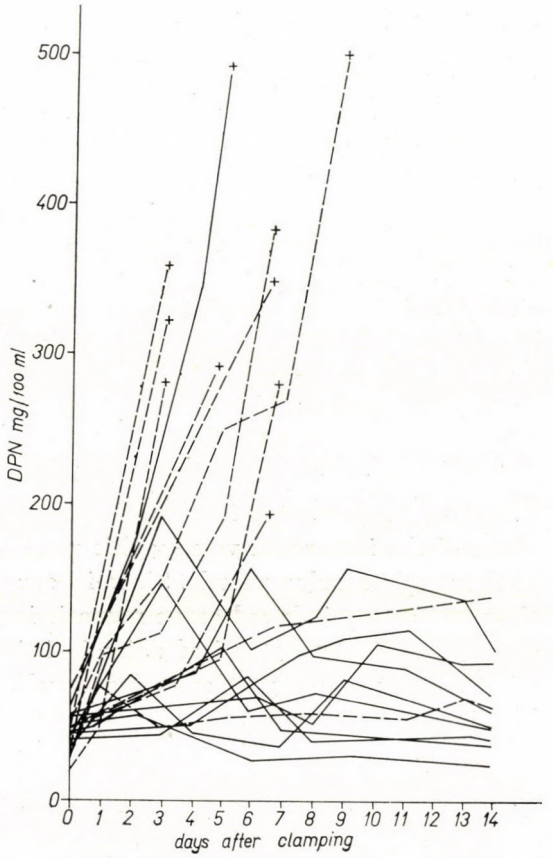

Fig. 1

surface was severely necrotized. The cortical substance appeared yellowish on the cut surface, and haemorrhagic spots were observed in the medulla. Histological examination revealed tubular damage, disrupted basement membranes and relatively intact glomeruli.

In the splanchnicotomized dogs irregularly scattered scarred spots of necrosis 2 to 2½ cm in diameter were found on at most one third of the renal surface. The cortex appeared to be somewhat enlarged at the expense of the medulla, and even the parts underneath the necrosed surface had a normal consistency and a normal colour.

Discussion

The data listed in *Table I* and *Fig. 1* make it evident that splanchnicotomy preceding the compression of the renal artery prevented the development of acute renal failure, and that the consecutive functional disturbance was only temporary. Histological changes were restricted to part of the kidney in the splanchnicotomized animals; the major part of the cortex remained intact and continued to function.

Relying on the evidence of clearance tests, numerous authors (BULL *et al.* 1950; LAUSON *et al.* 1949, *etc.)* have pointed to renal ischaemia as the cause of human acute renal failure. Other authors (MUNCK 1958; BRUNE *et al.* 1955), however, could show by means of the gas-inhalation method in humans, and we ourselves by direct measurement of the renal blood flow in animals, that a considerable (about 30 to 60 per cent of the original) volume of blood was flowing through the kidney during acute renal failure. The low inulin extraction points to considerably reduced filtration, the low paraaminohippuric acid extraction to an impairment of tubular function. To spastic condition of the afferent arterioles has been inferred from the fact that the reduction of the glomerular filtration rate exceeded that of the renal blood flow (BÁLINT *et al.* 1961; LAUSON *et al.* 1949; SELKURT 1945, 1946).

According to FINCKH (1962), except in the initial phase there occurs a disturbance in the tone of the intrarenal vessels; this would manifest itself with a constriction of the preglomerular vessels. Since in our present experiments, clamping of the renal artery induced grave necrosis in the innervated kidneys and failed to do so in splanchnicotomized animals, it is safe to assume that noxious impulses increase the tone of preglomerular vessels *via* the splanchnic nerve. This is why injury of the denervated kidney is seldom followed by vasoconstriction and consequential necrosis. These results are in harmony with our earlier experiments (BÁLINT *et al.* 1960) in which deep anaesthesia or division of the spinal cord at the height of the fourth thoracic vertebra prevented the development of acute renal failure. All these observations unanimously point to the role of neurogenic factors in the pathogenesis of renal failure.

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PREVENTION OF EXPERIMENTAL RENAL FAILURE BY CHLORPROMAZINE

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Temporary ligation of the renal artery causes acute uraemia, and it has been possible to prevent its development in dogs by the administration of chlorpromazine. The difference in the length of survival between the untreated and the treated animals was statistically significant. Histological changes in the kidneys were slighter in the surviving animals. Since chlorpromazine inhibits the autonomic subcortical centres, the fact that it prevents acute renal failure points to the pathogenic role of the autonomic nervous system and of the increase in sympathetic tone.

"Acute renal failure" is the term applied by SMITH (1958) to the frequently fatal syndrome characterized by the sudden onset of oliguria and uraemia. PHILLIPS and HAMILTON (1948) induced a similar syndrome in dogs by a temporary clamping of the renal artery. It was demonstrated by OLIVER (1951) that the histological changes in the kidney of such animals were analogous to those observed in cases of human acute renal failure.

In an earlier study (BÁLINT *et al.* 1960) we clamped the renal artery of dogs according to HAMILTON *et al.* (1948) under superficial ether anaesthesia after morphine pretreatment. The animals died with symptoms of grave uraemia, necropsy revealed widespread renal cortical necrosis. On the other hand, no uraemia developed and the animals survived the ligation if it had been performed under deep and prolonged chloralose anaesthesia. This striking difference in survival pointed to the involvement of the central nervous apparatus. Therefore, in a third group, the kidney was practically denervated by dividing the splanchnic nerve a week before inducing renal ischaemia. Acute renal failure failed to develop also in this group (FEKETE *et al.* 1963).

Treatment with lytic cocktail and/or chlorpromazine is an efficacious form of sympathicolytic therapy (VÉGHELYI *et al.* 1961). According to FOURNEL (1952), HERSHEY and GUCCIONE (1955) chlorpromazine prevents the circulatory disturbance in shock and has been found by FOURNEL that it acted as a sympatholytic agent, similar to dibenzylamine in this respect. We attempted to use sympatholytical chlorpromazin prevention of uraemia by the help of deconnection of the central nervous system

Material and Method

Adult dogs of both sexes were used in the present study. The right kidney was removed from the paracostal approach. Two weeks later, after pretreatment with 0.01 g/kg morphine and under superficial ether anaesthesia, the left renal hilum was exposed and the renal artery clamped for 2 hours. Subsequently the artery was released and the wound was closed.

The first (control) group consisted of 11 dogs. It was mentioned in our previous work (BÁLINT *et al.* 1960): these animals received no treatment after the operation. The second group contained likewise 11 dogs. These animals received slow intravenous infusion of 1 ml lytic cocktail (composed of 1 mg chlorpromazine, 1 mg promethazine and 2 mg pethidine) pro kg body weight after the closing of the wound, and during the next 48 hours 5 intramuscular injections of 1 ml cocktail, each. Rectal temperature was recorded serially and blood non-protein nitrogen was determined according to CLEGHORN and JENDRASSIK (1934) every other day.

The χ^2 test was used for statistical evaluation of survival times. Survival was considered definite if the animal was alive on the 14th day after the intervention. The surviving animals were killed on the 14th day. The kidneys of all animals were removed immediately after death, embedded in paraffin and the sections were stained with haematoxylin-eosin.

Results

Results, grouped according to length of survival, are listed in *Table I.* Nine members of the first group died between the 3rd and 9th day after the intervention, and 2 animals were killed on the 14th day, whereas only one animal died spontaneously and 10 were killed on the 14th day in the second group. The difference in the length of survival between the two groups was significant statistically ($p<0.01$).

Table I

Survival of control (morphine + ether) and test animals (morphine + ether + lytic cocktail)
$p < 0.01$

Treatment	Survival (days)	Spontaneous death	No. of cases
Morphine + ether	2	9	11
Morphine + ether + lytic cocktail	10	1	11

Changes in the NPN level are illustrated in *Fig. 1.* The number of days after compression of the renal artery is indicated on the abscissa, the NPN values are indicated on the ordinate. Values found in the controls are shown by a dotted line.

The NPN value varied between 300 and 500 mg per 100 ml in the control animals which died between the 3rd and 9th day after the ligation. NPN values found in the test animals are indicated by a solid line; these values had approached the normal level by the 14th day.

The kidneys of animals died before the 14th day presented the following picture. The major part, and in certain cases the whole, of the surface displayed

grave necrosis. The cortical substance on the cut surface had a yellowish colour and a blurred structure; there were haemorrhagic spots 2.0 to 2.5 cm in diameter in the medullary substance. The glomeruli showed, as a rule, no grave histological changes; haemorrhage and eosinophile exudate were in some cases found in *Bowman*'s capsule. The cytoplasmic structure of the cells was disso-

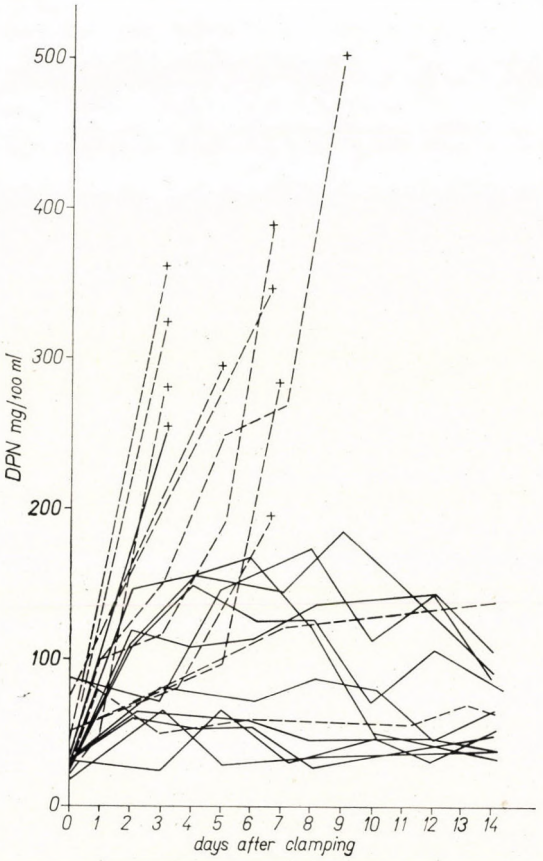

Fig. 1. Non-protein nitrogen values in animals subjected to clamping of the renal artery under ether anaesthesia after morphine pretreatment (dotted line). Non-protein nitrogen values in animals subjected to clamping of the renal artery under ether anaesthesia after morphine pretreatment, and subsequently treated with lytic cocktail (solid line)

ciated and the nuclei failed to stain. The tubules were filled partly with homogeneous and partly with granular material. A typical example of this group is shown in *Fig. 2.*

The surface of the kidney of the test animals had a normal colour in some members of this group, and was paler than normal in others. The structure had disappeared over an area that covered from 1/3 to 2/3 of the cortex. Tubules in the intact area stained normally, while in the necrosed area they showed

no nuclear staining and revealed signs of structural disintegration. Tubules in this area were filled with calcified clumps and a homogeneous eosinophile mass. A typical example of this group is shown in *Fig. 3.*

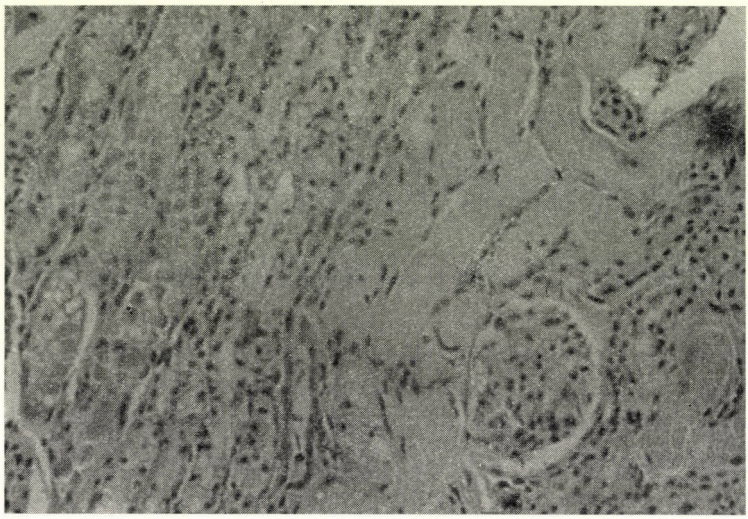

Fig. 2. Histological picture of kidney. The animal was subjected to compression of the renal artery under morphine-ether anaesthesia and died of acute renal failure. Note comparatively unimpaired glomeruli. No epithelium is visible in the convoluted tubules which are filled with pale-red granulated cytoplasm. Haematoxylin-eosin

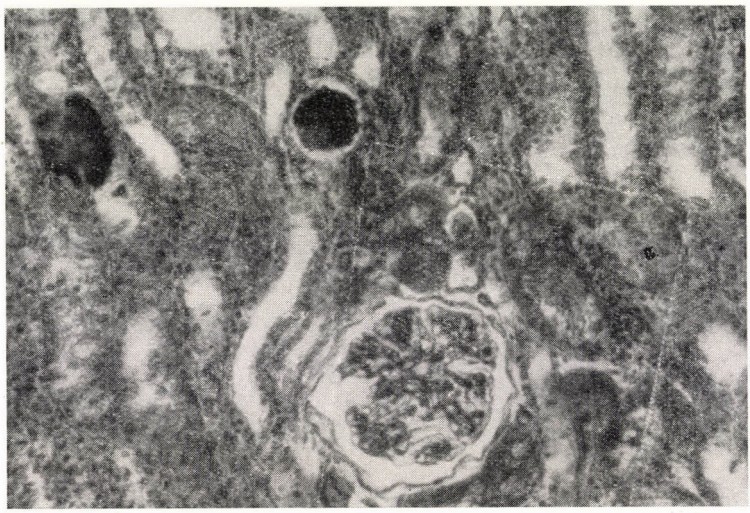

Fig. 3. Histological picture of kidney of animal subjected to clamping of renal artery under morphine-ether anaesthesia and treated with lytic cocktail. Nuclear staining in the tubular cells is mostly preserved; the epithelium shows signs of disintegration; the lumen has disappeared. Note darkly staining calcification in 2 tubules. Haematoxylin-eosin

Discussion

The findings have made it evident that the lytic cocktail inhibits the development of experimentally induced acute renal failure. The NPN level became normal after 14 days in most cases and, in contrast to the untreated controls, the treated animals survived the clamping of the renal artery.

Opinions vary regarding the mechanism through which chlorpromazine inhibits the development of renal failure. Small doses of the drug reduce the synthesis of adrenaline and reverse its effect (KOPERA 1955). Chlorpromazine has according to HEIMAN and WITT (1955), a hypnotic effect, and after its administration the EEG is similar to that of natural sleep. Several authors regard the brain stem reticular formation as the drug's point of attack. WASE *et al.* (1956), experimenting with labelled chlorpromazine in rats, found the isotope accumulated in the hypothalamus. DECSI and MÉHES (1958) suggest that chlorpromazine reduces oxidative phosphorylation in the brain, especially in the hypothalamus. The drug is known to diminish metabolism and to paralyse thermoregulation. No change of temperature was observed in the present experiments. Promethazine and pethidine, the other ingredients of the lytic cocktail, act merely as adjuvants of chlorpromazine, their antihistaminic and hypnotic effect is negligible.

According to FINCKH (1962), acute renal failure is caused by tonicity changes of the intrarenal vessels due to vasoconstriction. Both the present and our earlier investigations point to the increased sympathetic tone as a pathogenic factor of acute renal failure. Chlorpromazine is inhibiting the increase of sympathetic tone, and it seems that any treatment exerting such an effect is reducing the possibility of the syndrome's development.

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CHANGES IN RENAL FUNCTION AFTER PROCAINE TREATMENT IN ACUTE RENAL FAILURE

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A condition similar to human acute renal failure has been induced in dogs by temporary compression of the renal artery in superficial morphine-ether anaesthesia. The sequelae were oliguria and azotaemia, with death in a few days. Most of the animals survived if they had been subjected to perirenal procaine infiltration after releasing the renal artery. This treatment relieved the anuria, increased renal blood flow and improved the extraction of PAH. Procaine being a vasodilator substance it has been concluded that anuria and azotaemia following renal ischaemia are due to a constriction of renal vessels.

The syndrome of acute renal failure, experimentally induced in dogs, has been discussed in detail in several previous papers (BÁLINT *et al.* 1954, 1960, 1961). It was found that the removal of a kidney and compression for 2 hours of the artery of the remaining kidney led to death in a few days when the arterial clamping was performed under superficial ether anaesthesia after morphine pretreatment. The animals died with symptoms of anuria and azotaemia; *post-mortem* examination revealed in the kidney histological changes similar to those associated with human acute renal failure. The animals survived if compression of the renal artery had been performed in deep chloralose anaesthesia (BÁLINT *et al.* 1960) or if, before the intervention, the splanchnic nerve had been divided (FEKETE *et al.* 1964), or the kidney denervated pharmacologically (TARABA 1964). It would, thus, seem that neurogenic factors were involved in the mechanism of the syndrome. The oliguric and azotaemic state was transient in the animals mentioned above, their kidney showed slighter changes, and survival was significantly longer, than in the previous group.

Factors releasing shock or inducing acute renal failure are well known to give rise to oligaemia, a diminution of cardiac output and renal ischaemia (BRULL *et al.* 1950; PHILLIPS *et al.* 1946). LAUSON *et al.* (1944) found that the rate of glomerular filtration (GFR) and effective plasma flow (EPF) were diminished in proportion to the intensity of shock. By the use of up-to-date procedures it was revealed that, instead of the assumed complete renal ischaemia, the decrease in renal blood flow (RBF) was not more than 40 to 60 per cent in animals (BÁLINT *et al.* 1961; SELKURT 1945) and also in humans (MUNCK 1958; REUBI *et al.* 1962; SHALDON *et al.* 1962). The comparatively unimpaired RBF was associated with a disproportionately reduced GFR, a sign pointing to

a spasm of the afferent vessels. Since glomerular filtration amounts in these conditions to a fraction of the normal, the development of oliguria and azotaemia seems to be unavoidable.

Procaine, as an agent of denervation, is well known. OSSIPOV (1962), for example, introduced a 0.25 per cent solution of procaine parenterally with satisfactory results in cases of transfusion shock. FRIIS (1949) recommended intravenous procaine for the treatment of anuria caused by sulphathiazole, while LANGERON *et al.* (1946) used it successfully for the purposes of tissue infiltration.

The present study was designed to establish the effect of procaine on the experimentally induced anuric state in dogs.

Material and Method

Dogs of both sexes, with body weights from 12 to 20 kg, were nephrectomized from the right paracostal approach. After 2 weeks, members of the control group (11 animals) were pretreated with 0.001 g/kg morphine; the hilum of the remaining left kidney was exposed with utmost precaution and the renal artery clamped under superficial ether anaesthesia. The artery was released, renal circulation restored and the wound closed after 2 hours of compression. The test animals (14 dogs) were treated similarly but with the difference that, after the release of the renal artery, the fibrous capsule of the kidney and the perihilar connective tissue were infiltrated with 70 to 90 ml of 0.5 per cent procaine. Blood non-protein nitrogen (NPN) was determined according to CLEGHORN and JENDRASSIK (1934) every second day.

A total of 9 acute experiments were carried out on the surviving animals. Under chloralose anaesthesia the left renal vein was connected to the left jugular vein by means of a polyethylene T cannula and renal blood flow (RBF_{dir}) was estimated. For the details of this procedure see BÁLINT and FEKETE (1960). Arterial pressure was determined by a mercury manometer. Blood samples obtained simultaneously from the femoral artery and the renal vein were used for determining the extraction of inulin (E_{in}) and paraaminohippuric acid (E_{PAH}). We computed the so-called direct clearance (C_{in}) by means of the formula $C = RPF \cdot E$; the product of the directly measured RPF and the extraction of a given substance gives the clearance of that substance. Urine was collected by means of an ureteric catheter. Statistical evaluation was performed with the χ^2 test and *Student*'s t-test.

Results

Table I shows results according to survival. The criterion of survival was that the animal was alive on the 14th day following compression of the renal artery. It has been reported (BÁLINT *et al.* 1960) that from the 11 animals sub-

Table I

Intervention	Total	Death	Survival
Compression of renal artery under morphine-ether anaesthesia	11	9	2
The same plus perirenal procaine infiltration	14	4	10

$p < 0.01$

jected to a clamping of the renal artery under morphine-ether anaesthesia 2 animals survived and 9 animals died between the 3rd and 9th day. Of the 14 animals in the present experimental group, 10 survived and 4 died. The difference in the time of survival between the two groups was significant statistically ($p<0.01$).

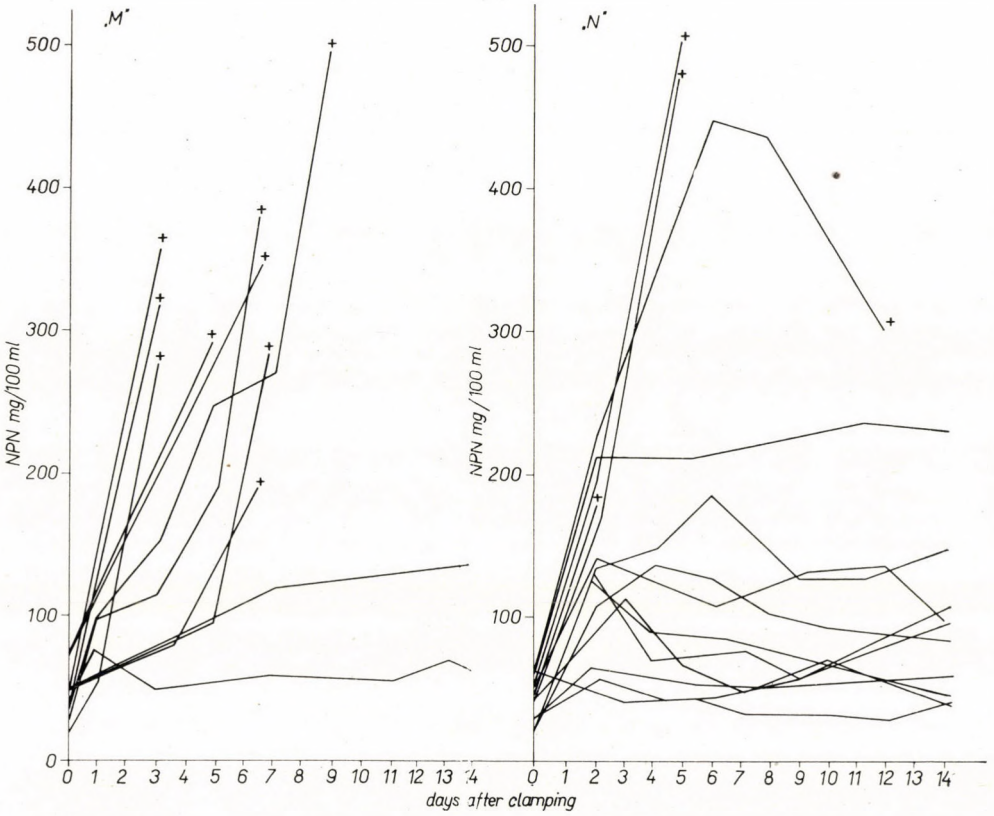

Fig. 1

Fig. 1 illustrates the behaviour of NPN up to the 14th day after renal clamping. The controls with two exceptions exhibited high values before death (250 to 400 mg per 100 ml). After an initial rise, NPN diminished in the test group and approached the normal value between the 10th and 14th day. (The four animals of this group which died had a high NPN level.)

Table II shows the results of the acute experiments with the means and scatterings. Data obtained from the surviving animals were compared (i) with those of the gravely azotaemic controls (*i.e.* the animals in which the renal artery had been clamped in superficial morphine-ether anaesthesia without subsequent procaine treatment; the NPN level reached 225 mg per 100 ml in

Table II

	After compression of renal artery		Controls (after removal of right kidney)
	untreated	treated with procaine	
n	18	9	8
Arterial pressure mm Hg	105 ± 3	101 ± 5	104 ± 5
RBF, ml/100 g	229 ± 24	290 ± 37	457 ± 29
$R_{ren \cdot kg}$	4.65 ± 1.33	232 ± 0.23	1.38 ± 0.26
E_{PAH}	0.08 ± 0.02	0.30 ± 0.05	0.68 ± 0.03
E_{in}	0.05 ± 0.01	0.05 ± 0.02	0.19 ± 0.03
C_{in}	7 ± 3	11 ± 4	57 ± 9
V ml/min	0.08 ± 0.02	0.34 ± 0.15	1.01 ± 0.25

Values of RBF, V ml/min and C_{in} are referred to 100 g kidney weight.
$R_{ren \cdot kg}$ = relative resistance per 1 kg kidney weight. Quotient of arterial pressure and RBF per sec, multiplied by 0.1

these animals); (ii) with the data obtained from unilaterally nephrectomized but otherwise normal animals. This second control group included those animals of our preceding study (BÁLINT *et al.* 1961) on which the acute experiment had been carried out without previous compression of the artery of the remaining kidney.

It is evident from *Table II* that arterial pressure was comparable in all groups. In acute renal failure, the RBF per 100 g kidney weight (*i.e.* about 50 per cent of the control value) increased by about 30 per cent on a single treatment with procaine. Relative resistance per 1 kg kidney weight in the azotaemic animals was significantly higher than in the controls, and decreased considerably under the effect of procaine. E_{PAH}, which registers tubular function, significantly increased in the animals treated with procaine ($p>0.01$), although it still remained significantly lower than in the controls ($p>0.001$). E_{in} had the same value in treated and non-treated animals; since in the former there was a 30 per cent rise in RPF, the amount of filtrate increased. The improvement of haemodynamic conditions manifested itself most clearly in diuresis; oliguria ceased in the test animals. The value of V/min was 0.34 ± 0.15 ml/min against 0.08 ± 0.02 in the controls ($p<0.02$).

Discussion

Nerve endings are paralysed and vasoconstriction is inhibited by procaine; the drug, therefore, is widely employed as a sympathicolytic agent.

The so-called self-regulation of the kidney is suspended by procaine (WAUGH and SHANKS 1960); vasoconstriction is partly or completely inhibited, and the renal vessels behave almost like elastic tubes (BRULL *et al.* 1955).

Since renal ischaemia of 2 hours duration was induced in both the experimental and the control animals, differences in survival and renal function must have been due to the procaine treatment. Procaine infiltration prevented the trauma from becoming fatal in most cases. Changes in renal haemodynamics were considerably less significant in the procaine-treated animals than in the untreated controls. This phenomenon was presumably due to that ischaemia which otherwise follows the compression of the renal artery is less severe if the tone of the afferent vessels is lowered by procaine. Procaine infiltration thus lessens post-traumatic ischaemia and vasoconstriction, promotes glomerular filtration and diuresis.

The improvement of renal function and the survival of animals after treatment with procaine justify the conclusion that post-traumatic anuria is due to vasoconstriction.

FINCKH *et al.* (1962) have demonstrated that the development of acute renal failure in humans is due to disturbances of circulation, intrarenal circulation in particular. RBF remains essentially satisfactory, while filtration is significantly decreased with the result that the renal tissues are gravely injured. Unchanged RBF with reduced filtration points to a disturbance in the tonicity of afferent and efferent arterioles, in other words a constriction of the praeglomerular vessels and a lack of the usual balanced tone in the postglomerular vessels. A lesion of the glomerular membrane might also occur. According to our results, the deconnection of the sympathetic nervous system prevents the disturbance of arteriolar tonicity.

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THE EFFECT OF COMPOUNDS INHIBITING CARBOHYDRATE METABOLISM ON THE DEXTRAN ANAPHYLACTOID INFLAMMATION

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The effect on dextran anaphylactoid inflammation of certain compounds, known to block glucose metabolism, has been investigated. Malonic acid, maleic acid, arsenate and fluoride, administered in non-toxic doses, intensively inhibited the development of dextran oedema.

The inhibitory effect of malonic acid could be suspended with fumaric acid and succinic acid. The above metabolic poisons block intensively the oedema-enhancing action of insulin.

The potential role in the antiphlogistic effect of the inhibition of glucose metabolism is discussed.

Many reports and observations have pointed to the close correlation between antiphlogistic effect and the effect on carbohydrate metabolism. This is illustrated by the example of the natural and synthetic glucocorticoids, whose action on carbohydrate metabolism is an indicator and permanent feature of the antiphlogistic effect. On the other hand, in certain pathological conditions involving disorders of carbohydrate metabolism (diabetes mellitus, *Gierke*'s disease) the course of the inflammatory reactions is different from the usual.

GOTH *et al.* (1957) and ADAMKIEWICZ (1959) have reported that dextran does not produce anaphylactoid oedema in animals with alloxan diabetes. At the same time, hypoglycaemic agents (insulin, antidiabetic sulfonamides) potentiate the tendency to oedema formation (ADAMKIEWICZ *et al.* 1960; ADAMKIEWICZ and LANGLOIS 1960; ADAMKIEWICZ and ADAMKIEWICZ 1960). The above authors also succeeded in preventing the development of dextran oedema by glucose infusion.

In our investigations concerning the correlation between the antiphlogistic effect and the effect on carbohydrate metabolism a significant step has been achieved by the discovery of a new, highly effective compound, Gy-97 (GÖRÖG *et al.* 1963), which is an antiphlogistic agent and has at the same time significant effect on carbohydrate metabolism. On the basis of the above data and some studies concerning the effect of antiphlogistic agents on carbohydrate metabolism we have arrived at the conclusion that the antiphlogistic effect and the inhibitory action on carbohydrate metabolism were correlated with

each other. Starting out from this assumption, we have investigated the effect of agents known to poison glucose oxidation on the dextran anaphylactoid inflammation model.

Materials and Methods

Organic dicarbonic acids: sodium succinate, sodium malonate, sodium maleinate (BDH). We used Na_2HAsO_4 as arsenate, NaF as fluoride.

Anaphylactoid oedema was induced by injecting dextran (*Fluka*), 1 ml of a 6 per cent solution in physiologic NaCl solution per 100 g body weight intraperitoneally into male *Wistar* rats weighing 120 to 180 g. The degree of generalized oedema was assessed by measuring the volume of the hind leg.

In the insulin experiments dextran was administered intravenously, half an hour after the subcutaneous injection of 4 U/100 g of insulin. The intravenously injected 300 μg dose of dextran by itself, without insulin, produced neither oedema, nor hyperaemia in the extremities. In these experiments we assessed the extent of the fast-developing oedema by inspection, instead of by volumetric measurement. According to GÖZSY and KÁTÓ (1960) we inspected the four limbs and the nose and graded the inflammatory reaction 0 to 100 per cent. The mean of the 5 values thus obtained showed the intensity of the inflammatory reaction in the given animal.

Results

In the first step we examined the effect of compounds wellknown to poison glucose oxidation. As the data in *Table I* indicate, maleic acid, malonic acid, arsenate and fluoride inhibited the development of dextran anaphylactoid oedema at concentrations much lower than the toxic ones. In the case of high doses of arsenate the oedema appeared, but much later and with much less intensity than in the controls. In the case of the other inhibitors no oedema appeared.

Table I

Compound	Dose mg/kg subcutaneously	Number of experiments	Oedema μl
Control	—	25	438 ± 12.4
Malonate	5	8	195 ± 8.2
	25	10	103 ± 6.6
	100	10	46 ± 4.4
Maleinate	100	10	71 ± 6.8
	300	10	33 ± 2.4
Arsenate	2	7	240 ± 7.6
	6.6	10	97 ± 5.9
	20	10	61 ± 5.7
Fluoride	20	10	10 ± 2.1

We have conducted experiments to determine how fumaric acid and succinic acid — members of the citric acid cycle — would influence the anti-oedema effect of malonic acid. The data in *Table II* show that, when administered together with malonic acid, fumarate almost completely blocked the anti-oedema effect of the latter. In the presence of fumarate, malonic acid blocked the development of oedema in doses about triple the originally effective one. Succinate acted three times less potently than fumarate in this respect.

Table II

Malonate mg/kg, subcutaneously	Fumarate mg/kg intraperitoneally	Succinate mg/kg, intraperitoneally	Number of experiments	Inhibition of dextran oedema, per cent
100	—	—	15	85 ± 5.3
100	100	—	10	8 ± 1.1
300	100	—	9	83 ± 4.7
100	—	100	10	34 ± 3.6
100	—	300	10	3 ± 0.2

As the data in *Table III* indicate, in insulin-treated animals the subminimal dextran dose produces significant generalized oedema in 7 to 15 minutes after the injection. Insulin does not potentiate oedema formation in animals pre-treated with the glucose metabolism poisoning agents tested.

Table III

Compounds	Dose, mg/kg, intraperitoneally	Number of experiments	Intensity of inflammatory reaction		
			15 minutes	30 minutes	60 minutes
Control	—	16	65	75	72
Malonate	100	10	10	0	0
Maleinate	100	10	25	10	12
Arsenate	5	8	14	0	0
Fluoride	10	10	10	10	0

Discussion

Several inflammation models are used extensively in the search for new antiphlogistic compounds. Although they bear resemblance to it, they are not identical with the arthritides occurring in human pathology. Moreover, there are great variations in the extent to which the different phlogistic agents can be inhibited. In the present experiments we have succeeded in inhibiting

the development of dextran anaphylactoid inflammation by the use of compounds acting as metabolic poisons. Our choice has been justified by the fact that most of the data relative to the correlation between glucose metabolism and experimental inflammation are based upon the influence on anaphylactoid inflammation. In the mechanism of anaphylactoid inflammation, histamine liberation has a significant role to play, like in practically every type of experimental inflammation. Studies *in vitro* on histamine liberation in anaphylactoid reactions have revealed that general metabolic poisons (KCN, uranyl nitrate), as well as compounds specifically poisoning glucose oxidation (monoiodoacetate malonic acid) block that process requiring much energy (MOUSSATCHÉ and PROWOST-DANON 1958; CHAKRAVARTY 1960). Within aerobic oxidation, the citric acid cycle has a significant role in the liberation of histamine (MOUSSATCHÉ and PROWOST-DANON 1958).

The above hypotheses have been supported by our present results, notably that the effect of malonic acid inhibiting anaphylactoid oedema can be suspended by succinic acid and fumaric acid. It has been shown namely that in alloxan diabetes the citric acid cycle is blocked, on the one hand, and that insulin enhances glucose oxidation mainly by stimulating the citric acid cycle, on the other.

It has been demonstrated by direct tests (RÉDEI *et al.* 1961) and confirmed by our present results that insulin does not potentiate dextran oedema by increasing the entrance of dextran, a glucose polymer, into the cell. The decisive factor in the oedema-potentiating effect of insulin seems to be the increase of glucose oxidation. Thyroxine and TSH enhance oedema formation in a similar way (UNGAR *et al.* 1951).

Several hypotheses may be put forward to explain how the inhibition of glucose oxidation by the known antiphlogistic agents and other compounds inhibiting experimental inflammations is correlated with the antiphlogistic activity. The inhibition of histamine liberation is merely one of them, and although the role of histamine in the inflammatory process is subject to debate, it undoubtedly plays a role in experimental inflammations.

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Pharmacologia

ON THE ADRENERGIC BETA RECEPTORS OF THE NICTITATING MEMBRANE

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Studying the adrenergic beta receptors of the cat's nictitating membrane, l- and d,l-isoprenaline have been shown to cause relaxation of the contraction induced by cervical sympathetic stimulation, adrenaline infusion, amphetamine, ergotamine and tolazoline. Dichloroisoproterenol antagonized the above effects.

As early as 1906, DALE (1906) distinguished between excitatory and inhibitory adrenergic receptors. On the basis of their relative reactivity to a series of sympathetic amines, AHLQUIST (1948, 1962) classified the adrenergic receptors into alpha and beta types. According to this, the adrenergic effects causing vasoconstriction, contraction of the nictitating membrane and uterus, mydriasis, relaxation of the bowels, *i.e.* exerting an excitatory action except on intestinal function, would be the result of alpha receptor excitation, whereas the adrenergic effects producing vasodilatation, bronchial dilatation, relaxation of the uterus and stimulation of the heart, *i.e.* those of inhibitory nature except for the action on the heart, would arise as a result of beta receptor excitation. The single organs, and even the single parts of the vascular area involved, give decisively alpha or beta type responses (*e.g.* the kidney shows responses mainly of the alpha type, the heart mainly of the beta type).

The adrenergic mediator substances differ in their actions on the alpha and beta receptors and in their affinity to the latter. Noradrenaline is a potent alpha-type agent, isoprenaline is a potent beta-type one, while adrenaline has mixed effects.

According to AHLQUIST's classification (1948, 1962), the nictitating membrane is an organ possessing alpha receptors: it responds by contraction to most of the sympathetic amines and to many other substances. In some cases, however, it could be substantiated that the nictitating membrane contained not only alpha receptors. It has been shown that adrenaline caused relaxation of the nictitating membrane in response to ergotoxin (ROSENBLUETH 1932; ACHESON 1940), ergotamine (BACQ 1934) and cocaine (RYALL 1961). One of us (GYÖRGY 1957) has observed the same effect after treatment with tolazoline and F-933.

On the basis of theoretical considerations, isoprenaline, active preponderantly on the beta adrenergic receptors, would be more likely to exert an effect of similar nature. KONZETT (1941) claimed that this compound had no effect on the nictitating membrane, but LISSÁK *et al.* (1944, 1946) observed that isoprenaline exerted an inhibitory, relaxant effect on the innervated and denervated nictitating membrane of the cat, after treatment with phenylephrine. THOMPSON (1958) found that isoprenaline relaxed the lysergic acid diethylamide-induced contraction of the isolated nictitating membrane of the cat. WALZ and MAENGWYN-DAVIES (1960) observed such an effect of isoprenaline on the dog's nictitating membrane. SCHMITT and SCHMITT (1960) produced by the use of isoprenaline a typical beta excitation, *i.e.* a relaxation inhibited by dichloroisoproterenol (DCI) during the lasting contraction of the nictitating membrane induced by continuous stimulation of the cervical sympathetic nerve.

In general, d,l-isoprenaline, the racemic variant of the compound, has been used in the investigations. LUDUENA (1962) has shown that the active and specific beta stimulator compound is l-isoprenaline, the d-form had beside a weak beta-stimulant effect also an alpha-blocking activity. For this reason the results obtained by the use of d,l-isoprenaline should be revised, because in some effects the alpha blocking activity of d-isoprenaline may also play a role.

In our experiments we wished to investigate

1. under what conditions are the beta receptors presumably present in the nictitating membrane demonstrable, after what types of contraction does isoprenaline, or eventually adrenaline, cause relaxation of the nictitating membrane?

2. how specific are these effects, *i.e.* are they inhibited, and to what extent, by DCI, a specific beta antagonist?

3. is there any significant difference between d,l- and l-isoprenaline in their actions on the nictitating membrane?

Methods

A total of 53 cats of either sex anaesthetized with chloralose + urethane (50 and 400 mg/kg, respectively), or decapitated by the intracysternal administration of 20 per cent $MgSO_4$ under superficial ether narcosis were used. Blood pressure was recorded from the cannulated common carotid by means of a mercury manometer. The contractions of the nictitating membrane were recorded kymographically (isotonic lever; transmission 1 : 12). The test compounds were injected into the right femoral vein, in some experiments adrenaline was infused into the left femoral vein. At the beginning of the experiment 5 mg/kg heparin was injected. The cervical sympathetic fibre was stimulated preganglionarly (1 to 4 Volts, 4 to 8 c/s, 0.15 to 0.3 msec).

The compounds used were d,l-isoprenaline HCl (Propylon, EGyT), l-isoprenaline bitartrate (Isolevin, Cilag), l-adrenaline HCl (Richter), amphetamine sulphate (Aktedron, Chinoin), ergotamine tartarate (Ergam, Richter), tolazoline HCl (Tolazolin, EGyT), dichloroisoproterenol (DCI) (Boehringer), heparin (Richter) and cocaine HCl.

The solutions of adrenaline and isoprenaline were prepared freshly and stabilized with ascorbic acid, 0.1 per cent.

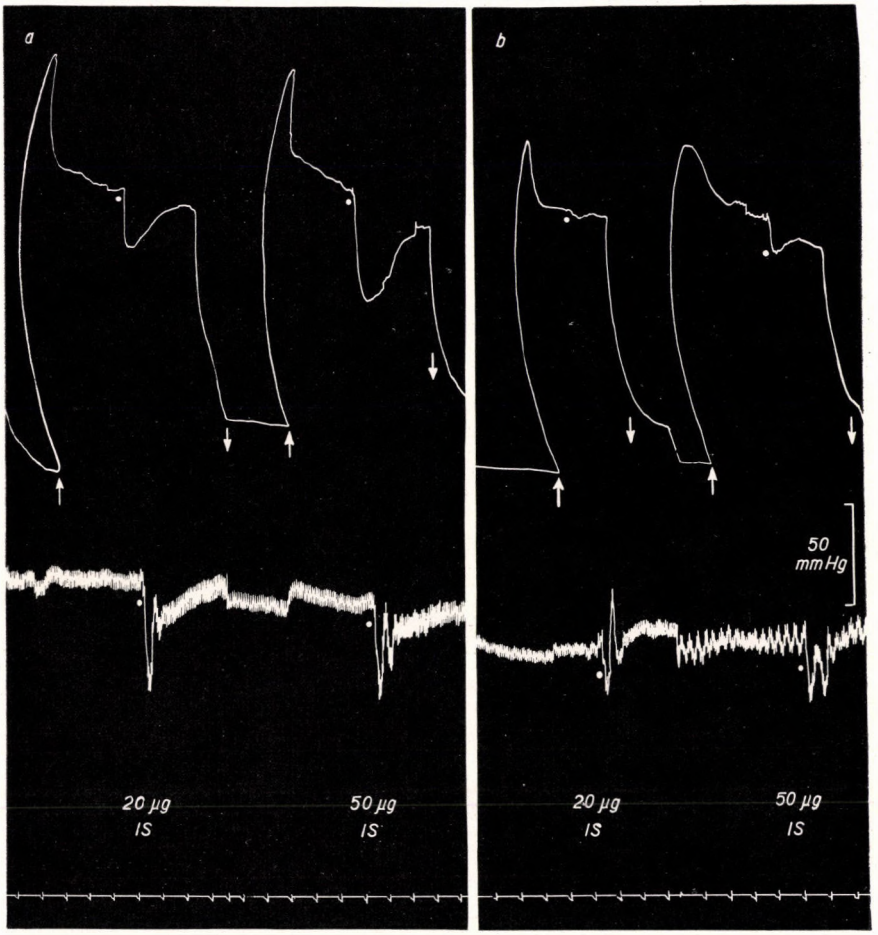

Fig. 1. Cat, 3500 g. Chloralose-urethane anaesthesia. Signs, in downward order: Nictitating membrane, blood pressure, time signal on the 0 line in minutes. (↑) stimulation (1.2 V, 8.2 Hz, 0.29 msec), (↓) stimulation discontinued. IS = d,l-isoprenaline. The interval between *a* and *b* is 8′, during which time the animal received 1 mg/kg DCI

Results

In anaesthetized and decapitated cats we examined the effects of l- and d,l-isoprenaline, as well as of l-adrenaline on the tone of the nictitating membrane during preganglionic cervical sympathetic stimulation and adrenaline infusion, as well as after one injection of amphetamine, ergotamine or tolazoline. (Isoprenaline had no effect on the nictitating membrane of non-pretreated animals.)

1. Preganglionic stimulation of the cervical sympathetic nerve. Continuous preganglionic stimulation of the cervical sympathetic nerve caused lasting contraction of the nictitating membrane, relaxed temporarily by 5 to 50 µg/kg doses of both l-and d,l-isoprenaline. This effect was inhibited by 1 mg/kg

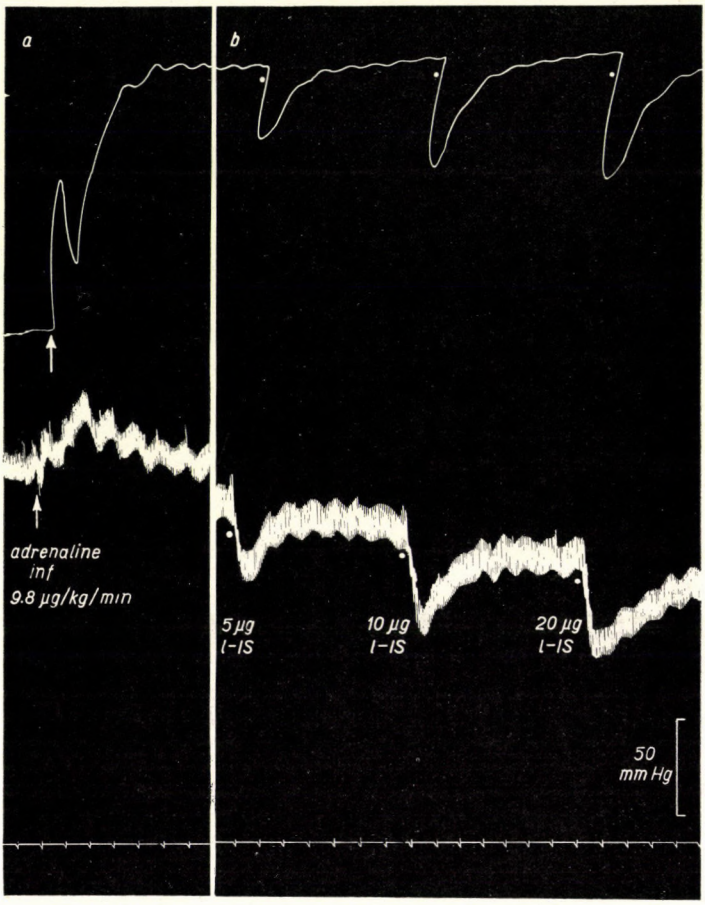

Fig. 2. Cat, 3000 g. Chloralose-urethane anaesthesia. Signs, in downward order: Nictitating membrane, blood pressure, time signal on 0 line in minutes. From (↑) adrenaline infusion (9.8 µg/kg/min) till the end of experiment. Duration of interval between *a* and *b*, 11′. 1—IS = d,l-isoprenaline

DCI intravenously *(Fig. 1)*. Adrenaline, in doses of 5 to 20 µg/kg, was ineffective, or caused further contraction.

2. *Adrenaline infusion.* Adrenaline was infused at a rate of 10 µg/kg/minute. The contraction of the nictitating membrane caused by this treatment was relaxed by l- and d,l-isoprenaline, in doses of 5 to 20 µg/kg. This effect, too, was inhibited by DCI *(Figs 2* and *3)*.

3. *Amphetamine, ergotamine, tolazoline.* In intravenous doses of 1 to 2.5, 0.2 and 1 to 3 mg/kg, amphetamine, ergotamine and tolazoline, respectively, caused lasting contraction of the nictitating membrane. The subsequent injections of l- and d,l-isoprenaline caused relaxation, inhibited by DCI *(Figs 4, 5, 6)*. In doses of 5 to 20 µg/kg, adrenaline either evoked further contraction, or was ineffective, but never caused relaxation.

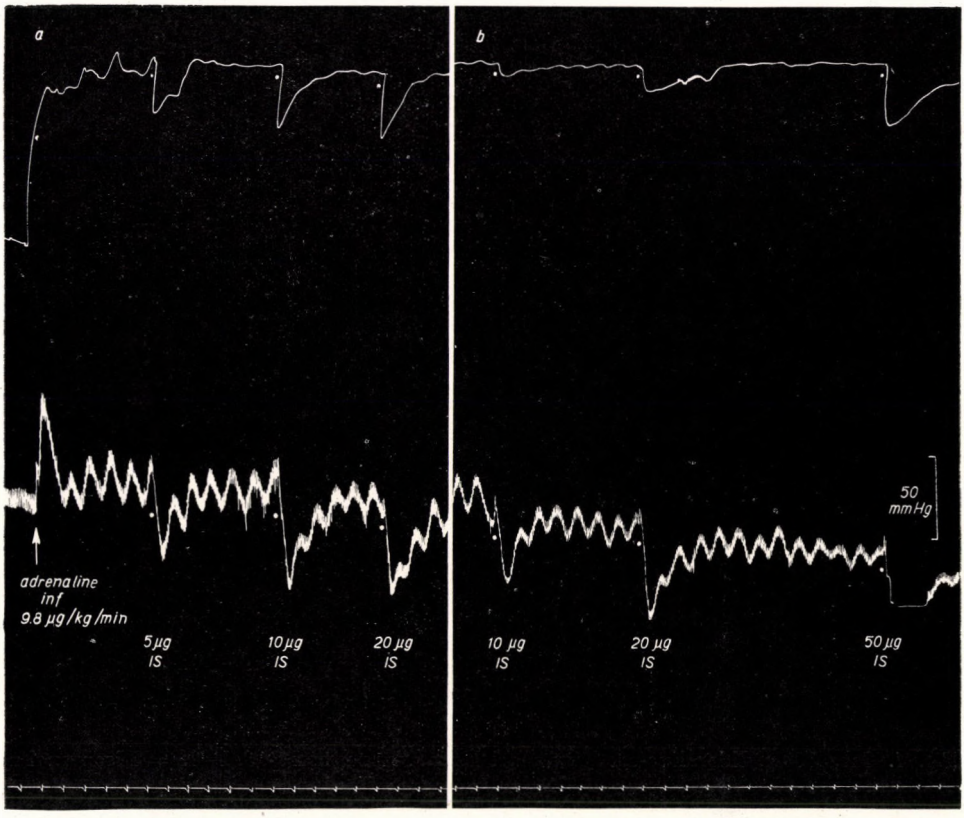

Fig. 3. Cat, 3000 g. Chloralose-urethane anaesthesia. Signs, in downward order: Nictitating membrane, blood pressure, time signal on 0 line in minutes. From (↑) adrenaline infusion (9.8 µg/kg/min) till the end of experiment. IS = d,l-soprenaline. During the 13′ interval between *a* and *b* the animal received 500 µg/kg DCI

Discussion

We have found that in anaesthetized and decapitated cats isoprenaline invariably relaxed the contraction of the nictitating membrane induced by preganglionic stimulation of the cervical sympathetic nerve and by various drugs. That this inhibiting effect was a result of beta receptor excitation, is proved by the fact that it was inhibited by DCI. The isoprenaline dose-response curves plotted before and after DCI ran parallel, the antagonism appears to be competitive.

Our results are in total agreement with those obtained by Lissák *et al.* (1944, 1946), Thompson (1958), Walz and Maengwyn-Davies (1960), as well as Schmitt and Schmitt (1960). It seems therefore unquestionable that the nictitating membrane of the cat contains both alpha and beta receptors.

The effects of l- and d,l-isoprenaline are qualitatively identical, the weak alpha-blocking activity (Luduena 1962) of the dextro-rotatory variant

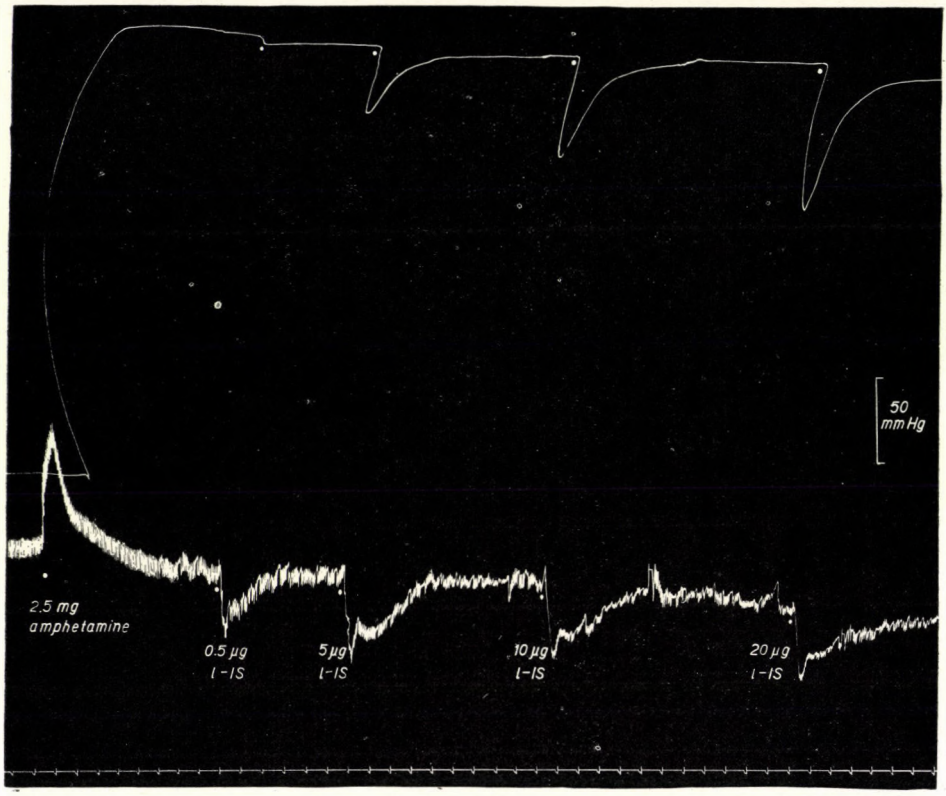

Fig. 4. Cat, 3000 g. Chloralose-urethane anaesthesia. Signs as in *Fig. 3.* 1—IS = 1-isoprenaline

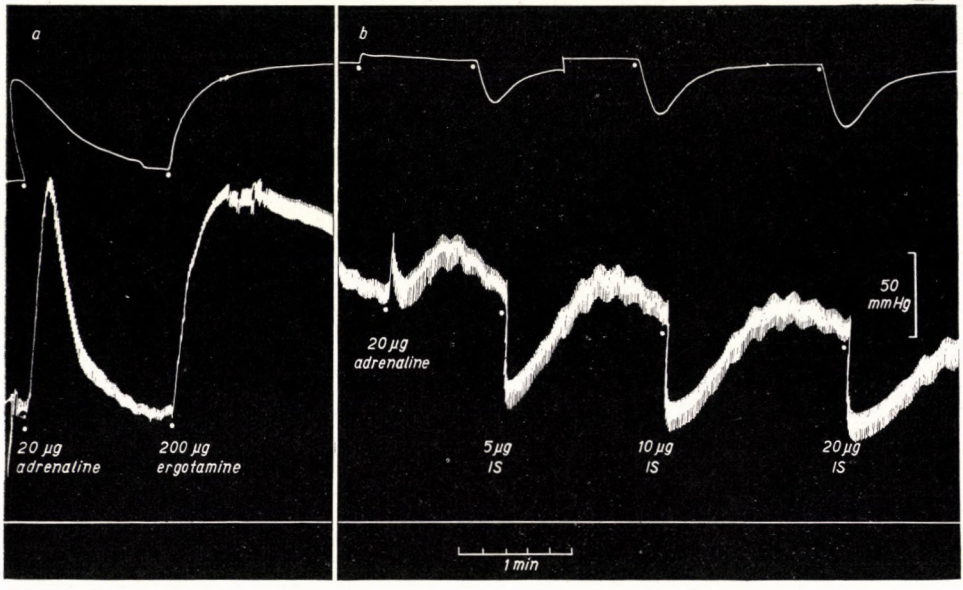

Fig. 5. Cat, 3400 g, decapitated. Signs in downward order: Nictitating membrane, blood pressure, 0 line, time signal. IS = d,l-isoprenaline. Duration of interval between *a* and *b*, 14′

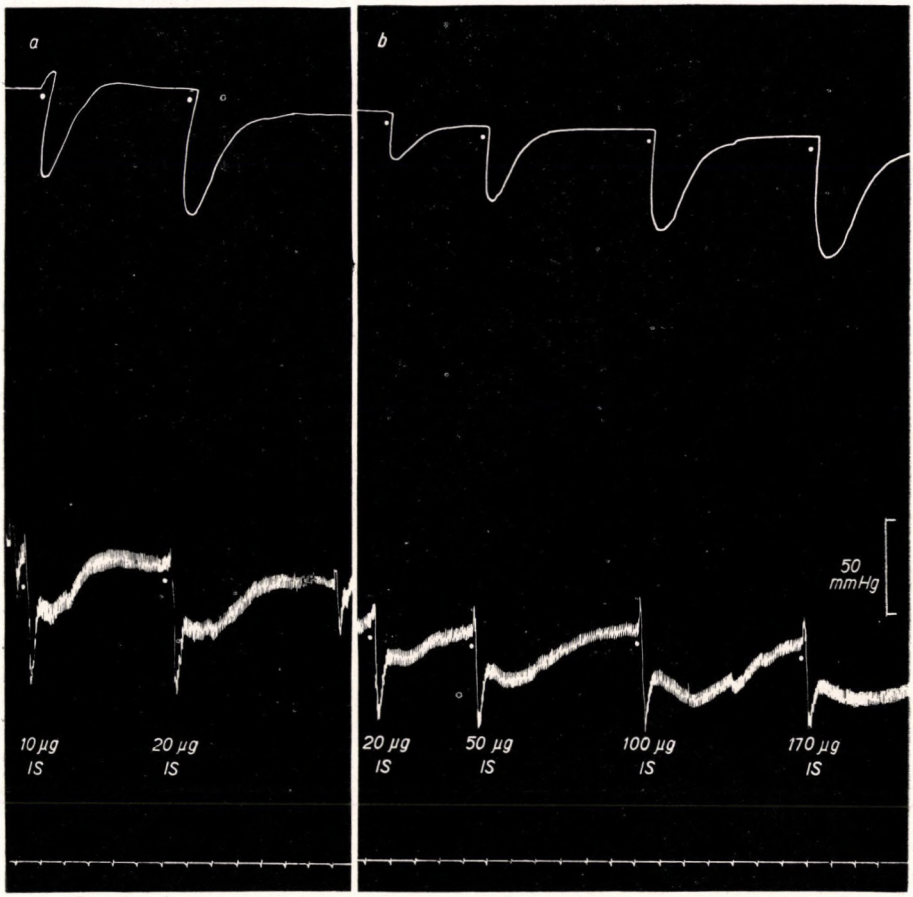

Fig. 6. Cat, 3200 g. Chloralose-urethane anaesthesia. Signs, in downward order: Nictitating membrane, blood pressure, time signal on 0 line in minutes. IS = d,l-isoprenaline. The animal was treated previous'y with 1.5 mg/kg tolazoline, and during the interval of 17′ between *a* and *b*, with 500 µg/kg DCI

seems to play no role in the relaxation of the nictitating membrane caused by the racemic variant, because DCI inhibits the action of the latter, too.

Under the conditions employed, isoprenaline relaxed the nictitating membrane in every experiment, its effect was constant. As opposed to this, adrenaline never caused any significant relaxation of the nictitating membrane, and therefore we do not think it can be used for the demonstration of the presence of beta receptors in that organ. Adrenaline caused relaxation only after excessively high doses of ergotamine (Bacq 1934) or ergotoxin (Rosenblueth 1932; Acheson 1940), and eventually after tolazoline plus some other alpha blocking agent (György 1957), or cocaine. According to Ryall (1961) in the latter case noradrenaline, too, is effective, and the ganglionic blocking activity of the compounds is responsible for the relaxing effect.

The relaxing effect of isoprenaline observed in our experiments cannot be ascribed to a ganglionic blocking action, partly because ganglionic blocking agents do not influence the actions of adrenaline, ergotamine and tolazoline, on the one hand, and because isoprenaline itself has no ganglionic blocking effect, on the other (BÜLBRING 1944). The results obtained by LISSÁK *et al.* (1944, 1946) that isoprenaline relaxes the denervated nictitating membrane, also indicate that the drug does not act by blocking ganglia.

Apart from their theoretical significance, our results are of a certain practical importance in that they represent a simple, readily reproducible method for studies of the beta adrenergic stimulants and inhibitors.

Acknowledgements

We are indebted to Miss E. Seress and Miss A. Varga for valuable technical assistance

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Note added in proof: In the same time as our paper has been submitted, was published the work of C. B. SMITH (J. Pharmacol. exp. Ther. **1963**, 142:163—170) dealing with the same topic.

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THE EFFECT OF VAGAL STIMULATION AND ACETYLCHOLINE ON THE SUSCEPTIBILITY TO FIBRILLATION OF THE MAMMALIAN HEART AT DIFFERENT BODY TEMPERATURES*

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The effect of stimulation of the right peripheral vagal stump as well as that of acetylcholine injection or infusion on fibrillation thresholds of the auricles and ventricles has been studied in the anaesthetized cat, further in the isolated *Langendorff* heart of cats at different body and perfusion fluid temperature. The lowering of fibrillation thresholds by vagal stimulation or acetylcholine was more expressed at lower body temperatures, *i.e.* hypothermia increased myocardial sensitivity to vagal influence. In addition the appearance of arrhythmia and ventricular fibrillation after vagal stimulation, acetylcholine infusion or injection was more frequent at lower than at normal body temperatures. All this is only true for the arrhythmogenic and fibrillatory vagal effects, since the intensity of the negative chronotropic action of vagal stimulation and of acetylcholine injection is definitely diminished by hypothermia. Possible explanations of this discrepancy as well as of the mechanism of the increasing fibrillatory effect of acetylcholine and vagal stimulation in hypothermia are discussed.

In an earlier paper it has been shown on the cat heart *in situ* that enhanced susceptibility to fibrillation in hypothermia was mainly due to the hypothermic increase of the central vagal influence on the heart (SZEKERES *et al.* 1961). The role in this phenomenon of possible changes in the sensitivity of the myocardium to vagal influences during hypothermia has not been ruled out and thus the aim of the present study was to elucidate how far was affected by hypothermia under the influence of stimulation of the peripheral vagi or of acetylcholine administration the tendency of the heart to fibrillation. The question is all the more interesting since according to MONTGOMERY *et al.* (1954) intracoronary infusion of acetylcholine and prostigmine prevents ventricular fibrillation due to surgical manipulation in the hypothermic dog, a finding which could not be confirmed by SHUMACKER *et al.* (1956). Nevertheless both groups of authors reported on a lower incidence of hypothermic fibrillation in the dog if the right vagus was stimulated.

* This paper is dedicated to Professor Helena RAŠKOVÁ on the occasion of her 50th anniversary.

Methods

Forty five cats of both sexes with an average weight of 3 kg were used. The animals were anaesthetized with 1.5 ml/kg of a solution containing 4 per cent diallylbarbiturate and 16 per cent ethylurethane, administered intraperitoneally. Artificial respiration was maintained throughout the experiment. The thorax was opened by midsternal approach, two pairs of silver electrodes were introduced through small apertures made in the pericardium and stitched to the ventral surface of the right ventricle and auricle, respectively. The electrocardiogram was recorded by an oscillograph.

Susceptibility to fibrillation was determined by the fibrillation threshold, *i.e.* by the minimum current required for the induction of fibrillation by electrical stimulation. Fibrillation in both auricles and ventricles was induced by rectangular pulses of 1 msec duration and 20 c/sec frequency. The strength of current passing through the heart could be read directly in mA from a specially built oscilloscope. In experiments where the effect of vagal stimulation was studied both vagi were cut in the neck and the right peripheral vagal stump was stimulated by rectangular suprathreshold pulses of 1 msec duration and 20/sec frequency for 10 seconds. Details of the method have been given in a previous paper (SZEKERES *et al.* 1961). In other experiments performed on animals with intact vagi, 5.0 μg/kg acetylcholine (acetylcholinebromide, *Hoffmann-La Roche)* was injected intravenously, or 1 : 100,000 acetylcholine solution was administered in intravenous infusion at a rate of 1 ml/kg/min. Cooling was performed by placing ice bags into the abdominal cavity. The temperature of the blood in the heart was measured by a thermocouple introduced into the right atrium.

In experiments on isolated hearts the *Langendorff* cat heart preparation was used with stimulating electrodes attached to the right auricle and ventricle. The heart was perfused with *Locke* solution of 38° and 26° C temperature as described elsewhere (SZEKERES and LÉNÁRD 1960). Acetylcholine was used in an endconcentration of 10^{-7}.

For statistical analysis of the experimental data *Student*'s "t" method was used.

Results

Suprathreshold stimulation of the right peripheral vagal stump at body temperature reduced the auricular fibrillation threshold by about 20 per cent.

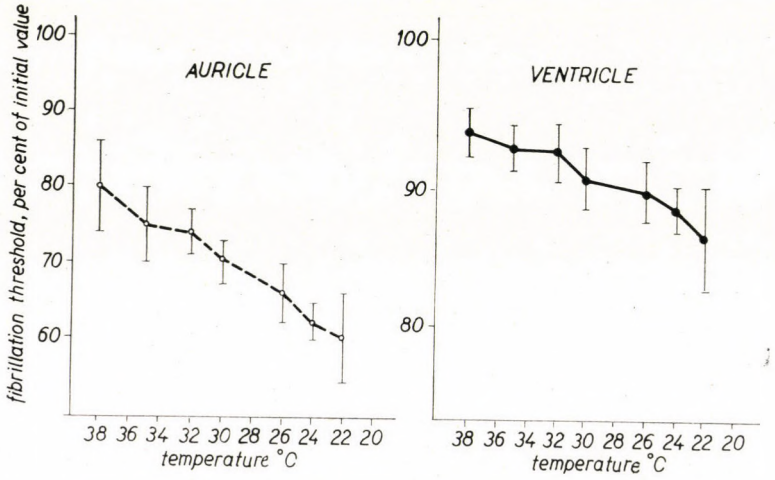

Fig. 1. Influence of vagal stimulation on auricular and ventricular fibrillation thresholds of the cat heart *in situ* at different body temperatures. (Average of 10 experiments)
Ordinate: fibrillation threshold in per cent of prestimulation value at the given temperature.
Abscissa: body temperature, °C
Solid line: ventricular fibrillation threshold
Dotted line: auricular fibrillation threshold

If the animal was gradually cooled, then on vagal stimulation with the same parameters the decrease of the fibrillation thresholds became more and more expressed at lower temperature ranges. At 22° C body temperature it reached about 40 per cent of the initial prestimulation value at that same temperature. Similar but less pronounced changes could be observed in the ventricles *(Fig. 1)*. In spite of the smaller extent of changes seen in the ventricles the difference between the fibrillation threshold-reducing effect of vagal stimulation at normal body temperature and in hypothermia of 22°—26° C was statistically significant for the ventricles and even more for the auricles *(Table I)*.

Table I

Statistical analysis of the effect of vagal stimulation on auricular and ventricular fibrillation thresholds of normothermic and hypothermic vagotomized anaesthetized cats (n = 10)

Heart temp. °C	Fibrillation threshold in per cent of prestimulation value at given temperatures*			P	Average percentual decrease of fibrillation threshold due to vagal stimulation	Difference between normo- and hypothermic decrease %	P
	Value before stimulation	Vagus stimulation	Difference				
AURICLE							
38±0.5	100.0 (0.384 mA)	80.1±6.3 (0.308± 0.024 mA)	—19.9±4.2 (0.076± 0.016 mA)	>0.02 <0.05	19.9±4.2	+90.4	<0.01
22—26	100.0 (0.620 mA)	62.1±4.0 (0.385± 0.025 mA)	—37.9±2.0 (0.235± 0.012 mA)	<0.01	37.9±2.0		
VENTRICLE							
38±0.5	100.0 (0.581 mA)	93.6±1.5 (0.544± 0.009 mA)	—6.4±1.2 (0.037± 0.008 mA)	>0.02 <0.05	6.4±1.2	+75.0	>0.02 <0.05
22—26	100.0 (0.982 mA)	89.1±3.8 (0.875± 0.037 mA)	—10.9±3.6 (0.107± 0.035 mA)	>0.02 <0.05	10.9±3.6		

* Absolute values in parentheses

The striking increase of the prestimulation values expressed in mA at lower temperature ranges as seen in *Table I* for the vagotomized heart *in situ* and in *Table III* for the isolated *(Langendorff)* heart on the one hand and the hypothermic decrease of the same values for the heart *in situ* of the "intact" anaesthetized cat *(Table II)* — on the other hand represent additional data

to our previous statement (SZEKERES *et al.* 1961) concerning the role of nervous and especially vagus control in the hypothermic increase of susceptibility to fibrillation of the cat heart *in situ*.

Table II

Statistical analysis of the effect of acetylcholine infusion (1 : 100,000 at a rate of 1 ml/kg/min) on auricular and ventricular fibrillation thresholds of normothermic and hypothermic anaesthetized cats (n = 10)

Heart temp. °C	Fibrillation threshold per cent of preinfusion value at given temperature*			P	Average percentile decrease of fibrillation threshold due to acetylcholine infusion	Difference between normo-and hypothermic decrease %	P
	Value before acetylcholine	Acetylcholine infusion	Difference				
AURICLE							
38±0.5	100.0 (0.422 mA)	75.0±5.1 (0.317± 0.022 mA)	—25.0±5.0 (0.105± 0.021 mA)	>0.01 <0.02	25.0±5.0	+88.4	>0.01 <0.02
22—26	100.0 (0.269 mA)	52.9±5.8 (0.142± 0.016 mA)	—47.1±5.3 (0.127± 0.014 mA)	<0.01	47.1±5.3		
VENTRICLE							
38±0.5	100.0 (0.612 mA)	91.2±4.1 (0.558± 0.025 mA)	—9.8±4.0 (0.054± 0.024 mA)	>0.02 <0.05	9.8±4.0	+80.6	>0.02 <0.05
22—26	100.0 (0.350 mA)	82.3±3.0 (0.288± 0.010 mA)	—17.7±2.8 (0.062± 0.098 mA)	>0.01 <0.02	17.7±2.8		

* Absolute values in parentheses

Intravenous infusion of 1 : 100,000 acetylcholine solution, administered at a rate of 1 ml/kg/min, exerted the same temperature-dependent effect as vagal stimulation on the fibrillation thresholds of both auricles and ventricles *(Fig. 2)*. Again, the difference between acetylcholine effect at body temperature and that in hypothermia is statistically significant *(Table II)*.

Since the latter experiments were performed in animals with intact nervous connections to the heart and the possibility of a nervous or hormonal interaction was not excluded, it seemed reasonable to investigate in the isolated heart the response to acetylcholine at different temperatures of the perfusion fluid. The results summarized in *Table III*, show that the isolated *Langendorff* heart behaved just as did the heart *in situ* with its nervous connections intact,

i.e. the reduction of auricular and ventricular fibrillation thresholds by acetylcholine was significantly stronger in hypothermia (26° C) than at normal body temperature (38° C).

Thus it seems to be well established that the peripheral *i.e.* myocardial sensitivity to vagal arrhythmogenic and fibrillatory influences increases in hypothermia.

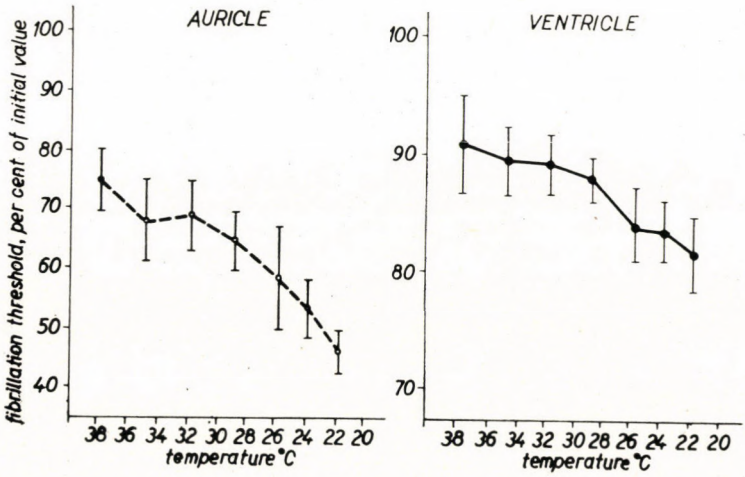

Fig. 2. Influence of acetylcholine infusion (1 : 100,000 at a rate of 1 ml/kg/min) on auricular and ventricular fibrillation thresholds of the cat heart *in situ* at different body temperatures. (Average of 10 experiments) Signs as in *Fig. 1*

This conception is further supported by the observation that the appearance of "spontaneous" (without electrical stimulation) arrhythmias and ventricular fibrillation after vagal stimulation, injection of acetylcholine (0.5—5.0 μg/kg) and infusion of acetylcholine (1 : 100,000—1 ml/kg/min) is more frequent at low than at normal body temperature *(Table IV)*.

The question arises whether the above demonstrated increase of the vagal and acetylcholine effects in hypothermia is a general rule, valid for all fundamental functional qualities of the heart, or is limited to the genesis of arrhythmias and fibrillation, these being resultants of changes in several fundamental cardiac parameters such as conductivity, rhythmicity, refractoriness and excitability. This question is all the more justified since in an earlier paper (Szekeres and Lénárd 1959) we could show that chronotropic and inotropic cardiac responses to adrenaline were not enhanced, but markedly diminished in hypothermia. On the other hand Cookson and DiPalma (1955) observed a high incidence of ventricular fibrillation in hypothermia on the administration of 10 μg of adrenaline, which usually is not harmful in the normothermic animal.

As disturbances of cardiac impulse generation may serve as basis for cardiac arrhythmias and fibrillation, the chronotropic response to vagal stimu-

Table III

Effect of acetylcholine (10^{-7}) perfusion on auricular and ventricular fibrillation thresholds of the isolated Langendorff cat heart at different temperatures (n = 10)

Temp. of perfusion fluid °C	Fibrillation threshold in per cent of preinfusion value at given temperature*			p	Average percentile decrease of fibrillation threshold due to acetylcholine infusion	Difference between normo- and hypothermic decrease %	p
	Value before acetylcholine	Acetylcholine infusion	Difference				
AURICLE							
38	100.0 (0.570 mA)	74.6±7.2 (0.425± 0.041 mA)	—25.4±6.3 (0.145± 0.036 mA)	>0.01 <0.02	25.4±6.3	+180.3	>0001 <0.01
26	100.0 (1.130 mA)	28.8±18.9 (0.325± 0.214 mA)	—71.2±16.4 (0.805± 0.185 mA)	<0.01	71.2±16.4		
VENTRICLE							
38	100.0 (0.752 mA)	81.7±4.2 (0.614± 0.032 mA)	—18.3±3.8 (0.138± 0.029 mA)	>0.01 <0.02	18.3±3.8	+155.7	<0001 >0.01
26	100.0 (1.350 mA)	53.2±17.4 (0.718± 0.235 mA)	—46.8±13.8 (0.632± 0.186 mA)	>0.01 <0.02	46.8±13.8		

* Absolute values in parentheses

Table IV

Appearance of "spontaneous" (without electrical stimulation) arrhythmias and ventricular fibrillation after vagal stimulation, acetylcholine injection and infusion in the cat heart in situ at different temperature ranges

Heart temp. °C	Vagal stimulation			Acetylcholine injection			Acetylcholine infusion 1 : 100 000 1 ml/kg/min intravenously		
	No. of animals	No. of arrhythmias	No. of ventricular fibrillation	No. of animals	No. of arrhythmias	No. of ventricular fibrillation	No. of animals	No. of arrhythmias	No. of ventricular fibrillation
26—38	31	3	1	30	1	1	16	4	∅
20—26	31	10	4	29	9	2	14	5	4

lation and acetylcholine injection has been investigated at different body temperatures. The results are summarized in *Table V*. Intensity of the chronotropic action of acetylcholine as well as that of vagal stimulation diminished at lower body temperatures, similarly to adrenaline in our afore-mentioned findings.

Consequently, in hypothermia not all cardiac functions are changed in the same direction by vagal stimulation and acetylcholine and so it still remains to be cleared which other fundamental functions of the heart undergo changes in hypothermia to cause the increased tendency to fibrillation under the influence of vagal stimulation and acetylcholine.

Table V

Effect of stimulation of the right peripheral vagal stump as well as that of injection of 5 μg/kg acetylcholine on the heart rate in anaesthetized cats at different temperatures

Heart temp. °C	Heart rate					
	Vagal stimulation (n = 10)			5 μg/kg acetylcholine (n = 10)		
	heart rate/min		percentile decrease of heart rate	heart rate/min		percentile decrease of heart rate
	initial value	vagal stimulation		initial value	acetylcholine	
38±0.5	191.1±11.0	90.2±37.2	47.4	181.2±13.2	130.4±22.1	72.0
34±0.5	155.3±30.2	70.1±16.4	45.7	171.3±15.4	119.5±18.1	69.7
30±0.5	100.2±14.8	40.2± 9.3	40.1	111.2±16.9	72.4± 9.5	65.1
26±0.5	67.2± 8.1	22.4± 4.1	33.3	83.1± 9.2	50.1± 5.3	60.3
22±0.5	56.1± 3.2	15.1± 4.7	26.9	69.1± 6.8	37.7± 6.2	54.6

Discussion

The data presented above clearly show that the decrease of the auricular and ventricular fibrillation threshold due to stimulation of the peripheral vagal stump or to acetylcholine infusion is more expressed at lower than at normal temperatures. Thus, in addition to the increased central vagal effect in hypothermia (SZEKERES *et al.* 1961) an increase in the sensitivity of the cooled myocardium to vagal influence may be responsible for the enhanced susceptibility to fibrillation of the hypothermic heart. This observation seems to be at variance with the data of MONTGOMERY *et al.* (1954) and SHUMACKER *et al.* (1956) who found a decreased incidence of ventricular fibrillation in the hypothermic dog, if the right vagus was stimulated. No satisfactory explanation is given for this discrepancy and interpretation of the findings is all the more difficult since in the same experiments of SHUMACKER bilateral vagotomy prevented hypothermic ventricular fibrillation.

On the other hand, the intensity of the negative chronotropic effect of vagal stimulation or acetylcholine infusion is diminished in hypothermia, as it is evident from our present experiments. Similar conclusions have been

drawn for acetylcholine and vagal stimulation by RINDANI and MERCHANT (1957) from experiments on isolated frog and dog hearts, for vagal stimulation by ANAND (1952) working on the isolated frog heart, by BIEWALD and RATHS (1959) working on the hamster heart *in situ*, and by COOKSON and DIPALMA (1955) on the dog heart *in situ*.

In contrast to these findings JOURDAN and MARDUEL (1944) claim that chronotropic vagal action is increased in hypothermia. They based this assumption on the prolonged duration of cardiac standstill evoked by strong vagal stimulation but there was no evidence of an increase in the intensity of negative chronotropic action.

It is difficult to explain this difference between fibrillatory and chronotropic cardiac responses to vagal stimulation and acetylcholine in hypothermia, considering that one of the main factors influencing susceptibility to fibrillation is the functional state of the higher and lower pacemakers. In earlier experiments we could show (SZEKERES and LÉNÁRD 1958) that in the isolated heart the sinoatrial pacemaker is much more sensitive to hypothermia than the atrioventricular and ventricular pacemakers. Thus a hypothermic shift of the pacemaker from the sinus to lower centres is made possible, as it has indeed been observed by WILLARD and HORVATH (1959) in anaesthetized rats, COVINO and BEAVERS (1958) in the isolated rabbit heart, by MALMÉJAC and NEVERRE (1956) in the dog heart-lung preparation, by SIEMS *et al.* (1955) as well as by GROSSE-BROCKHOFF and SCHOEDEL (1942a) in the intact anaesthetized dog.

The loss of sinus dominance and the concomitant increase in ectopic activity facilitates the appearance of ectopic foci and thereby that of arrhythmia and fibrillation. The diminished responsiveness of the sinoatrial pacemaker in hypothermia may account for the hypothermic decrease of the chronotropic vagus effect, independently from the fact that we do not actually know whether the first phenomenon is the result of metabolic or of permeability changes in the sinoatrial pacemaker.

In spite of the diminished chronotropic vagal and acetylcholine action in hypothermia, it seems that acetylcholine increases the tendency to pacemaker-shifting towards lower centres, as GROSSE-BROCKHOFF and SCHOEDEL (1942b) observed in hypothermic anaesthetized dogs.

As to the other fundamental features of cardiac function involved in the pathomechanism of arrhythmias and fibrillation, diastolic excitability seems not to be substantially affected by hypothermia (ANGELAKOS *et al.* 1957; COVINO and BEAVERS 1957) or by vagal stimulation (HOFFMAN *et al.* 1952).

On the other hand, absolute and relative refractory periods are definitely prolonged by cooling, as has been shown by HUIZING *et al.* (1955) in the isolated rabbit heart, ANGELAKOS *et al.* (1957), as well as by COVINO and D'AMATO (1962) in the dog heart *in situ*. However, vagal stimulation itself shortens the absolute refractory periods in the auricle of the dog heart *in situ* (HOFFMANN

et al. 1952) and acetylcholine acts in the same way in isolated auricles and papillary muscles of cats (DiPalma and Mascatello 1951). It is therefore possible that the beneficial antifibrillatory effect of the prolongation of refractory periods in hypothermia is counteracted by the vagal influence.

Conduction time is considerably prolonged in hypothermia, as shown by Sommerwille (1959) in man, by Covino and D'Amato (1962) in anaesthetized dogs. This fact alone is sufficient to establish favourable conditions for fibrillation, especially if we take into consideration the findings of Covino and D'Amato (1962) according to which conduction time is more prolonged in hypothermia than the refractory period, thus the ratio conduction time/refractory period increases. Although conduction time is somewhat shortened by acetylcholine in isolated auricles (Vaughan Williams 1959) and by vagal stimulation of the auricle *in situ* of the anaesthetized dog (Hoffmann *et al.* 1952), no convincing data are available as to the significance of this finding in the total effect.

On the basis of the experimental and literary data presented, the enhanced hypothermic sensitivity of the heart to peripheral vagal influence, manifesting itself with a more expressed reduction of the fibrillation thresholds, may be partly due to an increase of the ratio conduction time/refractory period and partly to a shift of the pacemaker to lower autonomic centres; — both changes being the result of an interaction of hypothermia and vagal effect. To prove this conception, further experimental analysis of the details is necessary.

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N-(ω-AMINOALKYL)-PHTHALIMIDE DERIVATIVES, A NEW GROUP WITH ANTIFIBRILLATORY ACTION

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Starting from the procaine amide structure a new group of drugs possessing antifibrillatory activity, the alkylamine substituted phthalimide derivatives have been developed. Leaving the phthalimide radical unchanged, the effect of alterations in the tertiary amine group as well as in the length of the alkyl chain on antifibrillatory activity of these derivatives has been analysed. Substitution in the tertiary amine group by diethylamine, dimethylamine or a morpholino group proved unsuccessful, whereas substitution by a piperidine group resulted in marked antifibrillatory activity increasing with the length of the alkyl chain. Toxicity and the blood pressure depressing effect increased as well. Substitution by a piperazine ring markedly increased antifibrillatory activity, but also toxicity. The N-methyl piperidine compound containing four alkyl-groups in the chain proved to be 1.7 times as potent in auricular and 2.5 times as potent in ventricular fibrillation as quinidine, at the same time its toxicity was only 1.6 times higher than that of quinidine.

Owing to the considerable development in the last few years in the field of pathophysiology and clinical diagnosis of heart rhythm disturbances, more and more attention is paid to substances capable of preventing or abolishing cardiac arrhythmias and fibrillation. A systematic synthesis of antiarrhythmic and antifibrillatory drugs is, however, rendered difficult by the fact that most of the hitherto produced substances with antiarrhythmic properties are different in chemical structure, thus a clear demonstration of structure-activity relationship is not easy.

For a planned synthesis of new drugs the most expedient way seems to start from the structure of a highly effective, well-known and well-proved drug as model. Among the anti-arrhythmic drugs used in clinical therapy quinidine has still preserved its dominant position in spite of the increasing number of new highly effective compounds. However, owing to its complicated structure we decided to use the more simple but in clinical therapy similarly popular procaine amide as a model compound. Thus a new group of drugs possessing antifibrillatory activity, namely the alkylamine substituted phthalimide derivatives have been developed (HIDEG and HANKOVSZKY 1963). The parallelism of this group with procaine amide is best demonstrated by the structure of N-(ω-diethyl-amino-ethyl)-phthalimide *(Fig. 1)*, which differs from procaine amide mainly by the absence of the primary amine group bound to the aromatic ring of the procaine amide; moreover, instead of an amide it

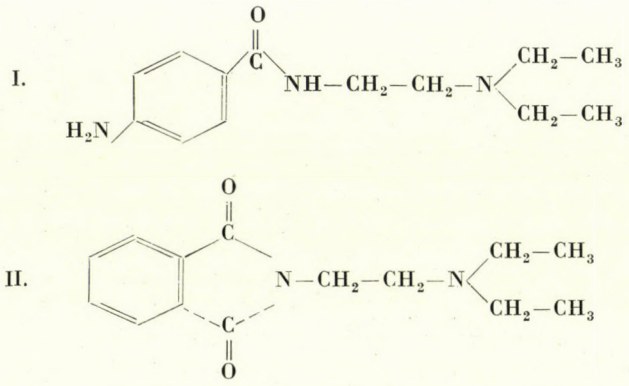


Fig. 1. I. Procaine amide. II. N-(ω-diethyl-aminoethyl)-phthalimide

contains an imide-bond, thus a new five-membered carbonic ring is composed. In the present work, the phthalimide radical was left unchanged, and the effect of changes in the tertiary amine group as well as in the length of the alkyl chain on antifibrillatory activity of these derivatives has been analysed.

No pharmacological data concerning the antifibrillatory action of phthalimide derivatives have been found by us. The only available papers on phthalimide derivatives containing some pharmacological reference (DONAHOE and SEIWALD 1957; DONAHOE *et al.* 1961) deal with the synthesis of quaternary N-(ω-piperidino-alkyl)-phthalimides in order to get effective muscle paralysing agents. But according to the brief pharmacological documentation of these papers, the compounds hitherto synthetized seem to possess a poor muscle relaxing effect.

Methods

Experiments were performed in 33 cats of both sexes with an average weight of 3 kg. The animals were anaesthetized with 1.5 ml/kg of a solution of 4 per cent diallylbarbiturate and 16 per cent ethylurethane, administered intraperitoneally. To determine antifibrillatory activity, the fibrillation threshold method, *i.e.* estimation of the minimal strength of current (rectangular impulses of 20 c/s frequency and 1 msec duration) necessary to induce fibrillation of the ventricles and auricles was used. The procedure has been described in detail in an earlier paper (SZEKERES *et al.* 1961). The dose enhancing the fibrillation threshold to double the initial value was used to compare the antifibrillatory potency of different substances. The antifibrillatory activity relative to quinidine has been expressed for each drug, quinidine being taken for 1. Quinidine has been used instead of procaine amide as a basis for comparison, since the latter compound is much less stable and less effective than quinidine. (Dose of quinidine enhancing fibrillation threshold to double the initial value, *i.e.* diminishing susceptibility to fibrillation to 50 per cent of initial is for the auricles: ED_{50} and 19/20 confidence limits = 4.2 (2.4—7.35) mg/kg; and for the ventricles: ED_{50} and 19/20 confidence limits = 7.8 (4.6—13.3) mg/kg.)

The hypotensive effect was expressed by the dose lowering blood pressure to 50 per cent of the initial value and hypotensive activity related to that of quinidine, the latter being taken for 1. Intraperitoneal toxicity in mice as well as intravenous toxicity in rats was determined according to LICHTFIELD and WILCOXON (1949), and values related to quinidine (quini-

dine = 1) have been calculated. (Quinidine rat i. v. LD_{50} and 19/20 confidence limits = 95.2 (77.8—118) mg/kg.)

Finally the relative therapeutic indices (that of quinidine being = 1):

$$\frac{\text{Relative antifibrillatory activity}}{\text{Relative toxicity}}$$ have also been computed.

Results

The effect of different phthalimide derivatives possessing 1, 2, or 3 alkyl groups in the chain and different aliphatic or alicyclic tertiary amine groups at the site of the tertiary amine group of the procaine amide (R) — on relative intraperitoneal mouse and intravenous rat toxicity, on relative auricular and ventricular antifibrillatory potency, as well as on relative hypotensive activity, is summarized in *Table I.*

If not more than one alkyl group is present in the chain, then only the compound with a piperidine group as substituent shows a slight antifibrillatory and a more expressed hypotensive action. The morpholino group has a stronger hypotensive action without any antifibrillatory effect. If the chain contains two alkyl groups so besides the piperidine derivative also the N-methyl piperazine-, the oxyethyl-piperazine- as well as the piperazine derivative exhibited some antifibrillatory activity.

Of the compounds possessing 3 alkyl groups in the chain the piperidine derivative proved to be the most active, the N-methyl piperazine and the oxyethyl piperazine compounds were less active against auricular fibrillation than with a two-membered methylene chain, whereas the ventricular activity of the former considerably increased while that of the latter remained practically unchanged.

Morpholino- and pyridine-derivatives were equally ineffective at all investigated chain lengths.

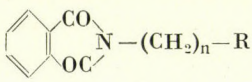
As on the basis of the above results it was obvious that mainly the derivative containing a piperidine group exhibited a consistent antifibrillatory effect at different chain lengths, it seemed worth while to synthetize its homologues with 4 and 5 alkyl groups in the chain, to study the effect of chain length on antifibrillatory activity as well as on toxicity. The results are summarized in *Fig. 2.* As seen, both the auricular and the ventricular antifibrillatory activities increased parallel with the chain length, but toxicity also increased. The relative auricular therapeutic index did not change with 3, 4 or 5 alkyl groups in the chain, whereas the ventricular relative therapeutic index showed a marked decrease if the chain contained 5 alkyl groups.

Accordingly, in further experiments we started from the longest chain possessing still optimal effects, *i.e.* from that containing 4 alkyl groups, and, leaving the chain length unaltered, we analysed the influence of changing the

Table I

The effect of increasing the length of the methylene chain as well as of various substitutions of the tertiary amine group on intraperitoneal and intravenous toxicity and auricular and ventricular antifibrillatory activity of phthalimide derivatives

R = number of alkyl groups in the chain
n = substitution of the tertiary amine group



	R	Rel. toxicity Quinidine = 1		Rel. antifibr. activity Quinidine = 1		Rel. hypotensive activity Quinidine = 1
		mouse i. p.	rat i. v.	auricle	ventricle	
1		—	—	0.12	0.07	1.22
		—	—	∅	∅	2.26
		—	—	∅	∅	∅
2		0.40	1.00	0.06	0.15	∅
		—	—	∅	∅	∅
		—	—	∅	∅	—
		1.38	—	0.42	0.20	0.90
		0.56	—	0.32	0.14	0.59
		—	—	∅	∅	—
		0.68	—	0.34	0.96	∅
3		1.12	1.21	0.73	0.86	0.84
		—	—	∅	∅	∅
		—	—	∅	∅	0.90
		1.16	—	0.18	0.50	0.57
		0.56	—	0.04	0.16	∅

tertiary amine group on toxicity, fibrillation threshold and therapeutic index.

The effects of these changes on toxicity as well as on auricular and ventricular antifibrillatory activity are shown in *Fig. 3*. The N-methylpiperazine derivative showed a slight antifibrillatory activity only, but a 1.7 times higher toxicity as that of quinidine. Substitution with N-methyl-benzylamine

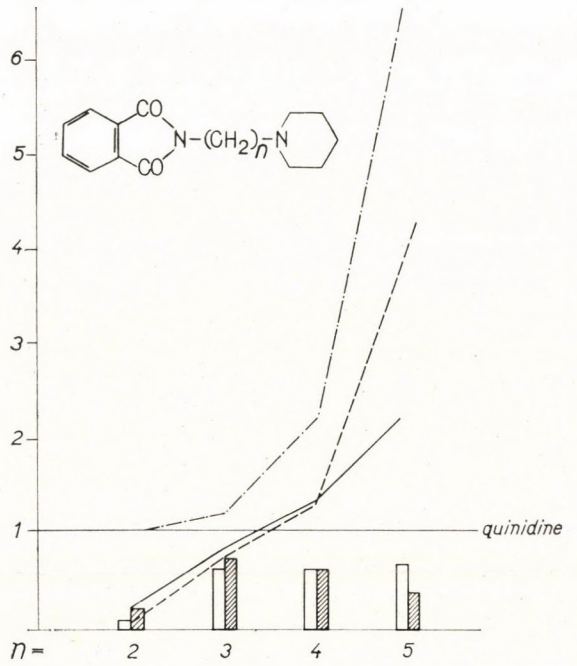


Fig. 2. The effect of increasing the length of the methylene chain in phthalimide derivatives on auricular and ventricular antifibrillatory activity, toxicity and auricular and ventricular therapeutic indices. Ordinate: values of auricular and ventricular antifibrillatory activity, toxicity, auricular and ventricular therapeutic indices, as related to quinidine

——— Relative ventricular antifibrillatory activity
- - - - - Relative auricular antifibrillatory activity
—.—.— Relative toxicity(rat, intravenously)
□auricular ▨ventricular } relative therapeutic index $\frac{\text{(rel. activity)}}{\text{(rel. toxicity)}}$
n = number of alkyl-groups in the chain

did not change substantially the auricular antifibrillatory activity, but enhanced the latter of the ventricles to 0.6 of that of quinidine. However, toxicity became nearly 2.4 times as high as that of quinidine. The pyrrolidine derivative seemed to have a more favourable effect; it exerted about half the antifibrillatory activity of quinidine on the auricles and 0.8 of that on the ventricles, and its toxicity was also somewhat less than that of the former compound. With the piperidine derivative both auricular and ventricular antifibrillatory activity

surpassed that of quinidine by about 30 per cent, but toxicity was 2.2 times that of quinidine. If the piperidine ring contained an additional methyl group at the 4′ site, a considerable increase of both, and especially that of the ventricular antifibrillatory activities was observed, but at the same time toxicity increased to four times that of quinidine.

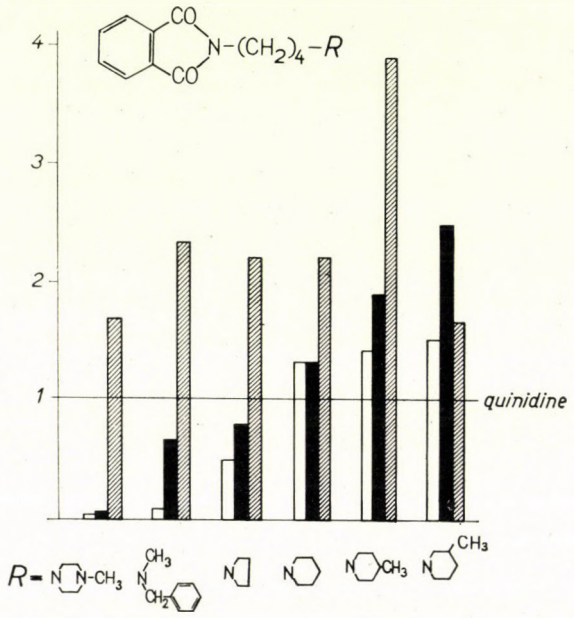


Fig. 3. The effect of various substitutions of the tertiary amine group at R in phthalimide derivatives (the chain length remaining constant) on auricular and ventricular antifibrillatory activity and intravenous toxicity.
Ordinate: values related to quinidine of auricular and ventricular antifibrillatory activity and intravenous toxicity.
□ Relative auricular antifibrillatory activity
■ Relative ventricular antifibrillatory activity
▨ Relative toxicity (rat, intravenously)
R = substitution of tertiary amine group

A surprisingly advantageous change was brought about by binding the additional methyl-group to piperidine at carbon atom 3′ instead of 4′. Then the antifibrillatory activity of the auricles was about 1.7 times, that of the ventricles more than 2.5 times as high as that of quinidine, and simultaneously toxicity was considerably reduced, to a value of about 1.6 times that of quinidine. The relative therapeutic indices of the above compounds are shown in *Fig. 4.* As seen, there was a striking difference between the 3′methyl-piperidine-derivative and all the others, the former possessing an equal auricular, but 1.5 times better ventricular therapeutic index than quinidine.

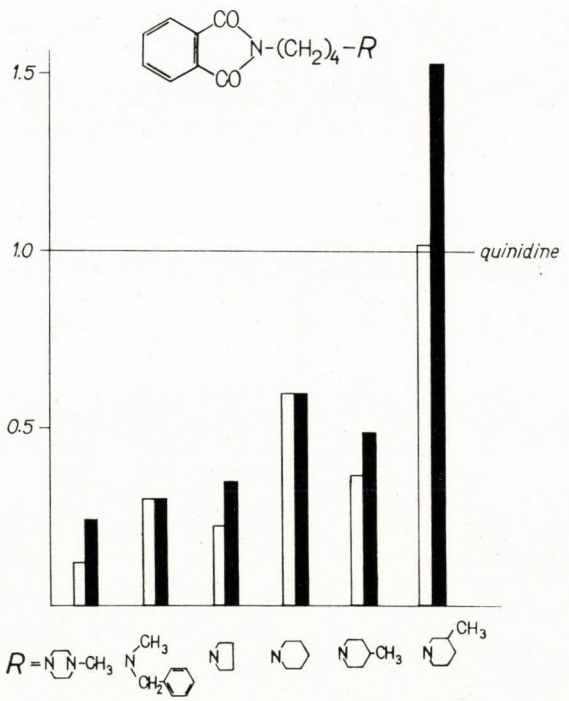


Fig. 4. The effect of various substitutions of the tertiary amine group at R in phthalimide derivatives (the length of the methylene chain remaining constant, n = 4) on auricular and ventricular relative therapeutic indices.

Ordinate: values related to quinidine of auricular and ventricular therapeutic indices

□auricular
■ventricular
} relative therapeutic index $\frac{\text{(rel. activity)}}{\text{(rel. toxicity)}}$

R = substitution of tertiary amine group

Discussion

As already mentioned, a systematic synthesis of effective antifibrillatory drugs is rendered difficult by the fact that very little is known about the relationship of chemical structure to antifibrillatory activity. A number of substances with entirely different chemical structure are known to possess antifibrillatory activity and it is extremely difficult to attribute this action to one or the other basic structure common to all antifibrillatory substances. This is obvious if we consider that antifibrillatory drugs do not uniformly act on the heart, since fibrillation itself is the resultant of changes in various basic parameters of cardiac function, such as refractoriness, excitability, rhythmicity and conductivity. So diverse drugs may abolish or prevent arrhythmia and fibrillation by influencing different cardiac parameters. It is also known that these drugs are not equally active in auricular and ventricular fibrillation, quinidine for instance acts rather in atrial fibrillation and flutter, whereas

procaine amide is more effective in rhythmic disturbances of ventricular origin. Thus it seemed to be more expedient to search for a relationship between structure and ability of the drug to change one or several basic functional cardiac parameters, resulting in an inhibition of arrhythmia and fibrillation. In this connection, auricular and ventricular antifibrillatory activity should be considered separately. Unfortunately in that direction a systematic gathering of data has not even begun. In spite of this, from the practical point of view it seemed sufficient to start from the structure of some well proved and active drug as model. Thus quinidine and other related antimalarial drugs have extensively been studied in the normal (BURNO *et al.* 1954; HESS and SCHMIDT 1959) and hypothermic animal (ANGELAKOS and HEGNAUER 1957). Alpha-fagarine-like compounds have likewise been synthetized and pharmacologically analysed in series (DIPALMA *et al.* 1950), furthermore antihistamines (DIPALMA and SCHULTS 1950; MCCAWLEY *et al.* 1951) and local anaesthetics (CARDEN and STEINHAUS 1956; DIPALMA and SCHULTS 1950; LANZONI and CLARK 1955).

From the above experiments some general conclusions can be drawn as follows.

1. The phthalimide derivatives just as procaine amide seem to be more active against ventricular than against auricular fibrillation.

2. It is clearly visible from *Table I* that conversion of the tertiary amine at R to a quaternary one results in the complete loss of antifibrillatory activity. This is in good agreement with the observations of DIPALMA *et al.* (1950) on alpha-fagarine-like compounds.

3. Increasing the length of the methylene chain in the phthalimide series increased antifibrillatory potency and toxicity at the same time. Similar conclusions have been drawn by DIPALMA *et al.* (1950) on the basis of their work on alpha-fagarine-like compounds and by DAWES (1946) when testing synthetic quinidine substitutes for antifibrillatory activity.

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РЕЗЮМЕ

ИССЛЕДОВАНИЯ В СВЯЗИ С РАСПАДОМ NAD

Т. ГАНТИ и Й. ФОДОР

Измерения при различных температурах подтвердили данные Лаури и сотрудников относительно скорости распада никотинамид-аденин-динуклеотид (НАД). Из полученных данных кривая Аррениуса получается прямой. Вычисленные из данных измерений градиент температуры, постоянная действия и энергия активации оказываются в реально ожидаемом диапазоне. Постоянная скоростъ распада, полученная из чистого водного раствора НАД не одинакова с постоянной величиной скорости распада, полученной в соке вытяжки, в которой измеряется более высокая величина скорости распада. Оптимальная температура экстракции 80° С, время экстракции 5 минут, в течение более длительного времени распад слишком продвигается, а экстракция не улучшается. Главные характерные постоянные: температурный градиент 2,5/10° С, энергия активирования 26,98 кал/моль; постоянная действия при pH = 4,3 : $0{,}59 \cdot 10^{-16}$.

ТРИПСИНОВЫЙ ГИДРОЛИЗ ДЕГИДРОГЕНАЗЫ ГЛИЦЕРАЛЬДЕГИД-3-ФОСФАТА

Т. ДЕВЕНЬИ, М. ШАЙГО, Э. ХОРВАТ, Б. СЁРЕНЬИ и Л. ПОЛЬГАР

Из трипсинового гидролизата дегидрогеназы денатурированного глицеральдегид-3-фосфата авторы изолировали «core»-фракцию, резистентную в отношении трипсина. Фильтрацией геля и микропрепаративной бумажной хроматографией удалось выделить четыре пептида. Авторы установили, что пептиды с большим числом членов гомологичны и содержат активную энзимную область. Выдвигается возможность применения «core»-фракции для аналитических целей.

ФИЗИКО-ХИМИЧЕСКИЕ И ИММУНОЛОГИЧЕСКИЕ СВОЙСТВА ОЧИЩЕННОГО ПАТОЛОГИЧЕСКОГО МАКРОГЛОБУЛИНА

М. САБОЛЬЧ, Л. ОРОС и Я. ХАНКИШШ

Авторы изолировали макроглобулин из сыворотки больного с макроглобулинемией Вальденштрёма. Изолированный макроглобулин в ультрацентрифуге не оказался однородным. Присутствующий в бóльшем количестве (85%) компонент имеет коэффициент осаждения 18 S и молекулярный вес в 860 000. Другой компонент (15%) имеет коэффициент осаждения в 27 S и молекулярный вес в 1 300 000. При электрофореза макроглобулин показал при pH = 8,6, в буферном растворе 0,1 μ — поведение однородной, монодисперсной системы.

После применения 2-меркаптоэтанола в концентрации 0,15 М макроглобулин фрагментировался на диссоциационную единицу с коэффициентом осаждения в 6,27 S и с молекулярным весом в 153 000. Диссоциацию макроглобулина удалось выявить также при помощи вискозиметрии. На основании результатов вискозиметрии авторы указывают на возможность выявления макроглобулинов при помощи более простых методов исследования. 2-меркаптоэтанол в значительной мере изменил спектр светопоглощения макроглобулина.

В иммунологических экспериментах было установлено, что изолированный макроглобулин находится в родстве с гаммаглобулином и что два компонента макроглобулина с различным молекулярным весом серологически также различны.

ДЕЙСТВИЕ ПОВРЕЖДЕНИЯ И РАЗДРАЖЕНИЯ СРЕДНЕГО МОЗГА НА ФУНКЦИЮ СИСТЕМЫ ГИПОФИЗ-ЩИТОВИДНАЯ ЖЕЛЕЗА

Ш. КОВАЧ, Ж. ВЕРТЕШ, А. ШАНДОР и М. ВЕРТЕШ

В экспериментах на белых крысах-самцах было установлено, что билатеральная электрокоагуляция, проведенная в сетчатой формации среднего мозга, обуславливает значительное повышение поглощения J^{131} щитовидной железой. При действии раздражения постоянными электродами, помещенными в этой же области, поглощение J^{131} в значительной мере снижается. Наблюдаемой после раздражения снижение поглощения J^{131} проявляется также у животных с удаленными надпочечниками или лечившихся кортизоном. При измерении размера отдачи J^{131} щитовидной железой после раздражения также наблюдалось снижение активности щитовидной железы, то есть уплощение кривой выделения радиоактивного иода.

ИССЛЕДОВАНИЕ РОЛИ РЕТИКУЛЯРНОЙ ФОРМАЦИИ СРЕДНЕГО МОЗГА В ПРОЦЕССАХ МОТИВАЦИИ И ОБОРОНИТЕЛЬНЫХ УСЛОВНЫХ РЕФЛЕКСОВ ПОСЛЕ ВВЕДЕНИЯ ХЛОРПРОМАЗИНА В СРЕДНИЙ МОЗГ ИЛИ ВО ВСЮ СИСТЕМУ

Б. БОХУШ, Э. ЭНДРЁЦИ и К. ЛИШШАК

В ходе выработки и стабилизации оборонительного условного рефлекса у крыс авторы путем анализа осуществления рефлекса и спонтанных межсигнальных реакций исследовали действие хлорпромазина, местно инъицированного в область ретикулярной формации среднего мозга.

Введение 5—50 мкг хлорпромазина в ретикулярную формацию среднего мозга в значительной мере снизило число спонтанных межсигнальных реакций при неизменном осуществлении рефлекса.

При введении 0,1 мг/100 г веса тела хлорпромазина во всю систему снижается только число межсигнальных реакций, в то время как введение количества 0,2—1,0 мг/100 г веса тела задерживает также осуществление оборонительного условного рефлекса, вернее двигательную реакцию на безусловное раздражение.

Результаты исследований указывают на роль ретикулярной формации среднего мозга в организации спонтанных, целеустремленных двигательных реакций.

ДЕЙСТВИЕ ПЕРЕСЕЧЕНИЯ ЧРЕВНОГО НЕРВА В ПРЕДОХРАНЕНИИ ОСТРОГО ПОРАЖЕНИЮ ПОЧЕК У СОБАК

А. ФЕКЕТЕ, И. ТАРАБА и М. ВИШИ

У собак с односторонней нефректомией авторы вызывали временным зажимом почечной артерии патологическую картину, подобную острому почечному поражению у людей. После пересечения чревного нерва у животных при действии той же травмы эта патологическая картина не развивалась, и при преходящих симптомах в пределах 14 дней наступала полная реституция. У двух групп животных клинически и на основании выживаемости удалось выявить значительную разницу. Результаты указывают на нейрогенные факторы в патогенезе острого почечного поражения.

МЕДИКАМЕНТОЗНОЕ ПРЕДОТВРАЩЕНИЕ ЭКСПЕРИМЕНТАЛЬНОЙ ОСТРОЙ ПОЧЕЧНОЙ НЕДОСТАТОЧНОСТИ

И. ТАРАБА

Острое азотемическое уремическое состояние, вызванное временным зажимом почечной артерии, удалось предотвратить применением хлорпромазина. Между получившей это средство и неполучившей его контрольной группами выживаемость показывает достоверные отклонения, а гистологическая картина — соответственно выживаемости — менее значительные изменения. На основании действия хлорпромазина, парализирующего вегетативные субкортикальные центры, указанный факт предотвращения обращает внимание на патогенную роль тонуса вегетативной или симпатической нервной систем.

ИЗМЕНЕНИЕ ПОЧЕЧНОЙ ФУНКЦИИ ПОСЛЕ НОВОКАИНОВОЙ ИНФИЛЬТРАЦИИ, ПРИМЕНЕННОЙ ПРИ ОСТРОМ ПОЧЕЧНОМ ПОВРЕЖДЕНИИ

А. ФЕКЕТЕ и И. ТАРАБА

У собак в поверхностном морфин-эфировом наркозе при помощи зажима почечных артерий в течение 2 часов вызывали состояние, напоминающее острое почечное поражение у людей. Это состояние в течение нескольких дней привело к олигурически-азотемическому состоянию и к смерти. Если при этой травме проводилось новокаиновая инфильтрация, то преобладающее большинство животных выживала. Согласно результатам острых опытов, проведенных на оставшихся в живых животных, под влиянием новокаинового лечения анурическое состояние прекращается, количество крови, протекающей через почки повышается и экстракция ПАГК улучшается. Принимая во внимание сосудорасширяющее действие новокаина результаты экспериментов говорят за сосудосуживающую этиологию посттравматического анурически-азотемического состояния.

ДЕЙСТВИЕ ЗАДЕРЖИВАЮЩИХ УГЛЕВОДНЫЙ ОБМЕН, СОЕДИНЕНИЙ, НА ДЕКСТРАНОВОЕ АНАФИЛАКТОИДНОЕ ВОСПАЛЕНИЕ

П. ГЁРЁГ и Л. СПОРНИ

Авторы исследовали действие известных соединений, отравляющих обмен глюкозы, на декстрановое анафилактоидное воспаление. Малоновая кислота, малеиновая кислота, арзенат и фторид в не токсической дозе оказывают интенсивное задерживающее действие на возникновение декстранового отека. Задерживающее действие малоновой кислоты можно прекратить фумаровой кислотой и янтарной кислотой. Вышеуказанные яды, действующие на обмен веществ прекращают действие инсулина, повышающее анафилактоидный отек.

Обсуждается предположительная роль заторможенности обмена глюкозы в задержке воспаления.

ОБ АДРЕНЕРГИЧЕСКИХ БЕТА-РЕЦЕПТОРАХ МИГАТЕЛЬНОЙ ПЕРЕПОНКИ

Л. ДЬЁРДЬ, Й. МОЛЬНАР и М. ДОДА

Авторы исследовали адренергические бета-рецепторы мигательной перепонки кошек и выявили, что сокращение мигательной перепонки, вызванное раздражением шейного симпатического нерва, вливанием адреналина, дачей амфетамина, эрготамина и толазолина, можно расслаблять дачей **l-dl**-изопреналина. Указанные действия можно антагонизировать дачей дихлороизопротеранола.

ДЕЙСТВИЕ РАЗДРАЖЕНИЯ БЛУЖДАЮЩЕГО НЕРВА И АЦЕТИЛХОЛИНА НА МЕРЦАТЕЛЬНУЮ СПОСОБНОСТЬ СЕРДЦА МЛЕКОПИТАЮЩИХ ПРИ РАЗЛИЧНОЙ ТЕМПЕРАТУРЕ ТЕЛА

Л. СЕКЕРЕШ и Дь. ПАПП

Авторы исследовали действие раздражения культи правостороннего периферического блуждающего нерва, а также инъекции или вливания ацетилхолина на мерцательную способность сердца на сердце наркотизированной кошки **in situ**, далее на сердце кошки, изолированном по методу Лангендорффа при различной температуре тела или в случае применения перфузионной жидкости различной температуры. Снижение фибрилляционного порога, вызванное раздражением блуждающего нерва или ацетилхолином при нормальной температуре тела, более выраженное, то есть гипотермия повышает чувствительность сердечной мышцы к влиянию блуждающего нерва. Кроме этого при гипотермии после раздражения блуждающего нерва и инъекции или вливания ацетилхолина чаще наблюдается аритмия и желудочковое мерцание, чем при нормальной температуре тела. Все сказанное, однако, относится только к вызывающим аритмию или мерцание действиям блуждающего нерва, так как отрицательное хронотропное действие раздражения блуждающего нерва и введения ацетилхолина при гипотермии выраженно понижено. Авторы обсуждают возможное причины упомянутого отклонения, а также механизм повышенного фибриллярного действия ацетилхолина и раздражения блуждающего нерва, наблюдаемого при гипотермии.

ПРОИЗВОДНЫЕ N/1ω-АМИНОАЛКИЛ/-ФТАЛИМИДА — НОВАЯ ГРУППА СОЕДИНЕНИЙ АНТИФИБРИЛЛЯРНОГО ДЕЙСТВИЯ

Л. СЕКЕРЕШ, К. ХИДЕГ, О. Х. ХАНКОВСКИ и Дь. ПАПП

Исходя из структуры прокаинамида, авторы получили новую группу соединений с антифибриллярным действием, замещенные производные алкиламина фталимида. В настоящей работе авторы исследуют — при неизменном радикале фталимида — действие изменений, вызванных во вторичной аминовой группе, а также в длине алкиловой цепи, на антифибриллярную активность этих производных. Замещение радикалов диэтиламина, диметиламина или морфолина во вторичную аминовую группу не означало никакого преимущества с точки зрения антифибриллярного действия. Но введение пиперидиновой группы на это же место в значительной мере повысило действие, которое после удлинения алкиловой цепи продолжало повышаться, хотя в последнем случае повысилась также и токсичность и снижающее действие на кровяное давление. Замещение пиперазинового кольца выраженным образом повышает антифибриллярное действие, но одновременно также и токсичность соединения. Содержащее 4 алкиловые группы соединение N-метил-пиперидина обладает противомерцательной активностью на предсердии 1,7 раз, а на желудочке в 2,5 раз большей, чем хинидин, причем токсичность соединения только 1,6 раз больше токсичности хинидина.

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Biochemia

THE EFFECTS OF FEEDING, FASTING AND ADRENALINE ON THE GLUCOSE-6-PHOSPHATASE ACTIVITY OF THE LIVER

By

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(Received September 17, 1963)

The G-6-P-ase activity of the liver has been studied in response to feeding different diets and to fasting. It has been found that G-6-P-ase activity increased not only during fasting, but also on feeding diets lacking glucose or dextrin.

It has been demonstrated that G-6-P-ase activity changes significantly during the period between feedings; it decreases a few hours after feeding, then it increases and remains high until well after the next feed.

It has been shown that adrenaline is capable to increase hepatic G-6-P-ase activity *in vivo*, provided the activity was initially low.

The important role of G-6-P-ase* in the blood sugar producing activity of the liver is well known. As a result of the phosphorylation of glycogen G-1-P, then under the effect of phosphoglucomutase G-6-P is formed. G-6-P is split by G-6-P-ase into free glucose and inorganic phosphate. Thus, the direct blood sugar producing enzyme is G-6-P-ase.

The organism regulates blood sugar production also with G-6-P-ase itself. According to Ashmore *et al.* (1954), in diabetes the G-6-P-ase activity of the liver increases by about 100 per cent, while others have found increases amounting to 40 to 60 per cent (Langdon and Weakley 1955; Patrick and Tulloch 1957; Harper 1959). Insulin treatment *in vivo* normalizes G-6-P-ase activity.

In vitro, however, insulin is ineffective. Langdon and Weakley (1955) have suggested that part of the microsome-bound G-6-P-ase is present in a masked form in the normal liver, and this overmasking ceases in diabetes.

The increase of G-6-P-ase activity may reduce the sugar utilisation by the liver even when the activity of glucokinase (the enzyme directly involved in the phosphorylation of sugar) does not change. The highly active G-6-P-ase

* Abbreviations used in this paper:

G-6-P-ase:	glucose-6-phosphatase
G-1-P:	glucose-1-phosphate
G-6-P:	glucose-6-phosphate
F-6-P:	fructose-6-phosphate
EDTA:	ethylenediamine tetraacetate

may namely reconvert to glucose the G-6-P formed under the effect of glucokinase and thus, through a summation of the actions of the two enzymes, glucose utilisation decreases.

It has been demonstrated in man that diabetes increases and insulin (or carbutamide) normalizes G-6-P-ase activity (PATRICK and TULLOCH 1957). The effects observed in diabetes with and without insulin treatment make it obvious that insulin plays an important role in keeping G-6-P-ase activity at a normal level, or in the diminution of its activity.

These results have been confirmed by evidence obtained in glucose infusion experiments (HAWKINS *et al.* 1959). Glucose infusion diminishes namely G-6-P-ase activity in the normal rat, but not in the diabetic animal. It may be surmised that the insulin produced in response to glucose infusion is responsible for the decrease of G-6-P-ase activity. This is indicated also by the fact that glucose, if administered over a certain period of time, causes no further reduction, and, also, that after insulin pre-treatment the infusion of glucose does not diminish the activity.

All these make it likely that insulin reduces the G-6-P-ase activity of the liver.

G-6-P-ase activity changes in the opposite direction, *i.e.* increases, in response to food deprivation and to the feeding of certain kinds of diet (ASHMORE *et al.* 1954; FITCH *et al.* 1959; FREEDLAND and HARPER 1957, 1958a, 1958b, 1958c; HARPER 1959; LANGDON and WEAKLEY 1955; WEBER and CANTERO 1954, 1957a, 1957b). Numerous authors have claimed that the increase of activity resulting from fasting would be due to a concentration of the liver, although at the same time there is no increase in the activity of other hepatic enzymes. Studying the effects of various diets, FREEDLAND and HARPER (1957, 1958a, 1958b) and HARPER (1959) have shown that protein, fat, galactose and fructose increased, glucose and dextrin decreased G-6-P-ase activity. They called this phenomenon *primary adaptation*, by which the organism provides for blood sugar production from glycogen. They have shown the primary adaptation, *i.e.* the increase of G-6-P-ase activity, to set in as soon as the diet contained less than 30 per cent dextrin.

In the present study an attempt has been made to find a correlation between the increase of G-6-P-ase activity caused by fasting and that caused by feeding a glucose-free diet, and to establish whether adrenaline was capable of increasing the activity of the enzyme, and how this depended upon the diet and the time elapsed since feeding.

Materials and Methods

Male albino rats, weighing 150 to 200 g, were used. The animals were maintained on a mixed diet and were fasted for 24 hours before beginning the experiment. Then they were fed for 1 to 3 days a fat (butter), protein (casein), or carbohydrate (dextrin-maltose)

diet. Food and water were allowed *ad libitum*. On completion of the experiment the animals were stunned, decapitated and exsanguinated. The liver was taken out without delay and frozen with solid carbon dioxide. Then from the frozen liver small specimens were taken, weighed, homogenized with quartz dust and chilled distilled water, to get a 10 per cent homogenate.

G-6-P-ase activity was determined according to CORI and CORI (1952). Activity was estimated from the liberated amount of inorganic P. Incubation lasted 15 minutes, at 30° C and pH 6.8. The results were related to 100 mg fresh liver and 100 g final body weight.

$$\text{G-6-P-ase activity} = \frac{\text{activity of whole liver} \times 100}{\text{body weight}}$$

Inorganic P was estimated by the method of TAUSSKY and SHORR (1953).

Results and Discussion

The effects of fasting and different diets on the G-6-P-ase activity of the liver

In this group it was studied how G-6-P-ase activity was influenced by fasting and by the different kinds of diet. The results are presented in *Table I*.

The results shown in *Table I* are represented graphically in *Fig. 1*.

Table I

Effect of fasting and of different diets on G-6-P-ase activity of rat liver

Number of animals	Diet quality and duration in days				Glucose-6-phosphatase	
					concentration	activity
	1	2	3	4	μg P/15'/100 mg liver	mg P/15'/100 g body weight
5	mixed				261 ± 26.4*	10.00 ± 1.34*
6	mixed	fasting			447 ± 6.5	14.78 ± 1.42
5	mixed	fasting	fat		418 ± 22.1	14.96 ± 0.85
7	mixed	fasting	fat	fat	499 ± 40.6	17.58 ± 1.91
3	mixed	fasting	fat	fasting	503 ± 23.7	17.70 ± 1.15
6	mixed	fasting	protein	protein	515 ± 45.6	19.06 ± 2.61
6	mixed	fasting	dextrin	dextrin	315 ± 7.2	12.00 ± 0.32

* ± standard error of the mean

The data in *Table I* and *Fig. 1* indicate that fasting for one day increased the hepatic G-6-P-ase concentration and also the activity related to 100 g body weight. Activity remained high during the fatty diet. A slight further increase ensued when the fatty diet was continued, or the animal was again fasted. Activity was increased also on feeding protein for two days. It was only the feeding of glucose (dextrin) which caused the activity to decrease.

According to the above results, it is not the fasting, or not the fasting alone that increases the concentration or the activity of G-6-P-ase. G-6-P-ase level is always high when there is no glucose in the diet and decreases, when the animal is fed glucose. The decrease may be ascribed to an increased secretion of insulin; this would be indirectly responsible for the decrease in the quantity of G-6-P-ase. This has been confirmed by those experiments in which G-6-P-ase activity decreased directly in response to the injection of insulin in the liver of normal rats (ASHMORE *et al.* 1956; HAWKINS *et al.* 1959).

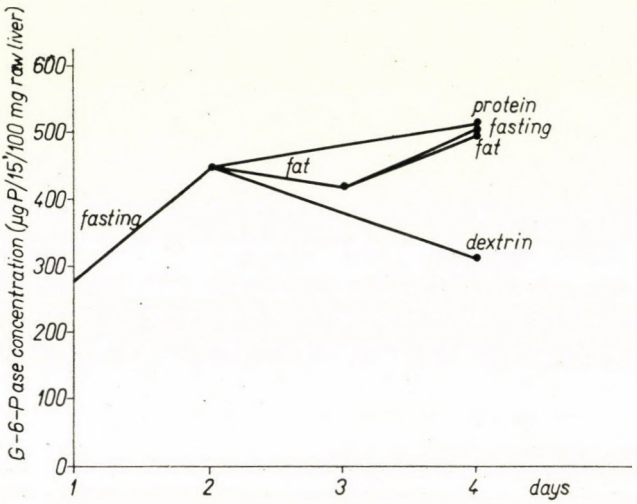

Fig. 1. Effects of fasting and diets on G-6-P-ase concentration of the rat liver. The single points represent the mean values of the results presented in *Table I*

Daily variations of G-6-P-ase activity

The opposite effects of fasting and feeding food containing glucose on G-6-P-ase activity make it questionable whether it is the higher or the lower values that may be considered to represent the normal.

In further experiments we have therefore investigated whether the G-6-P-ase activity showed daily variations in the period between feedings. The animals were fed a standard diet* *a.m.* once a day, between 7 and 9 o'clock. After feeding the animals were allowed exclusively water until fed again the next day. After two days of feeding the standard diet, G-6-P-ase activity was determined immediately after feeding, then 3, 6, 12 and 24 hours later. The results are shown in *Fig. 2.*

* The composition of the standard diet (FREEDLAND and HARPER 1958a) was dextrin-maltose, 65 per cent; casein, 25 per cent; sunflower seed oil, 7.5 per cent; yeast, 1 per cent; Ca lactate, 1 per cent; NaCl, 0.5 per cent.

Fig. 2 shows that immediately following feeding, concentration and activity of G-6-P-ase were high. Three hours later activity was significantly lower. This was followed by a gradual increase until at 24 hours activity was higher than immediately after feeding. This value reflects the condition before the next feeding.

The results make it clear that even animals fed a standard diet show wide variations in G-6-P-ase activity depending on the time elapsed since

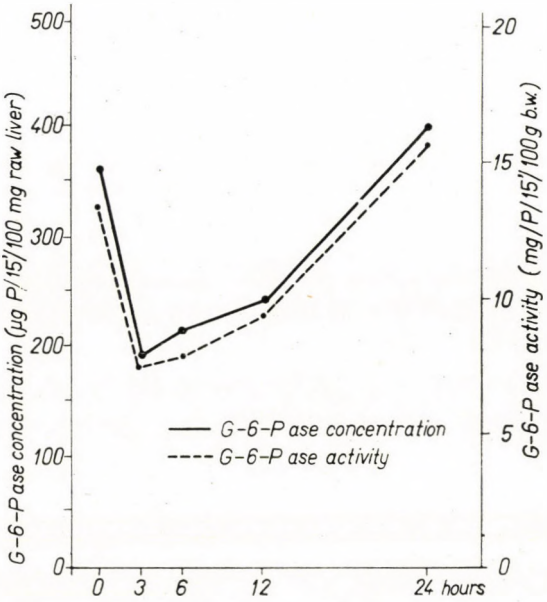

Fig. 2. Daily changes in G-6-P-ase activity in the interval between feedings. The points represent mean values obtained in 4 to 7 experimental animals

feeding. This explains the wide range of variations in the G-6-P-ase values when the time of feeding is uncertain. It is also visible that during feeding the animals show activity values similar to those obtained during a short fast and it is only several hours later that activity decreases significantly.

Thus, G-6-P-ase activity shows daily variations correlated with feeding. This would be observable, of course, less clearly if the animals were fed several times a day.

It may therefore be assumed that the G-6-P-ase activity of the liver is regulated by two factors, insulin and adrenaline. As activity decreases exclusively on feeding a high glucose (or dextrin) diet and does not decrease in response to feeding fat, protein or fructose, it seems justified to claim that in the daily variations displayed by normal animals *(e.g.* in the period following alimentary

hyperglycaemia) it is the insulin which is responsible for the decrease of G-6-P-ase activity. The increase of activity observed later after feeding may be due either to an increased production of adrenaline, or to a decrease in the secretion of insulin.

Effect of adrenaline on G-6-P-ase activity

On the basis of the above, we have examined whether adrenaline would enhance G-6-P-ase activity. The animals were maintained for two days on the dextrin-maltose diet, to reduce the G-6-P-ase activity of the liver. Three hours after the last feeding one group was treated with 0.25 to 0.30 mg adrenaline subcutaneously, the other group served as control. Thirty minutes to 1 hour after the injection of adrenaline the animals were killed and the G-6-P-ase activity of the liver was determined. The results are presented in *Table II*.

Table II

Effect of adrenaline on G-6-P-ase activity of the rat liver

G-6-P-ase concentration μg P/15′/100 mg liver		G-6-P-ase activity mg P/15′/100 g body weight	
Control	Adrenaline	Control	Adrenaline
300	375	12.0	15.7
305	380	12.2	16.6
305	380	11.1	19.0
310	430	11.4	15.9
335	445	11.9	14.7
340	465	13.4	20.0
	470		13.5
	480		14.9
	510		20.2
	535		20.4
	550		23.3
	628		17.0
315 ± 17	470 ± 22	12.0 ± 0.3	17.6 ± 0.8
$0.0001 < p < 0.001$		$0.0001 < p < 0.001$	

± standard error of the mean

The data in *Table II* show that in response to adrenaline G-6-P-ase activity increased significantly. The difference between the two groups was highly significant in the case of G-6-P-ase activity related to both 100 mg fresh liver tissue and 100 g body weight.

However, adrenaline did not increase G-6-P-ase activity, when this was high before the adrenaline had been injected. When the animals were fed fructose* instead of dextrin-maltose, activity was high and adrenaline caused no further increase *(Table III)*.

Table III

Effect of adrenaline on G-6-P-ase activity of rats maintained on fructose diet

G-6-P-ase concentration μg P/15′/100 mg liver		G-6-P-ase activity mg P/15′/100 g body weight	
Control	Adrenaline	Control	Adrenaline
380	380	17.7	16.9
385	410	16.3	18.4
420	420	18.8	15.1
435	470	18.7	19.3
510	530	21.2	20.7
615	550	22.8	24.9
	575		25.9
457 ± 36	477 ± 28	19.3 ± 2.1	20.2 ± 4.1
$0.05 < p < 0.5$		$0.05 < p < 0.5$	

± standard error of the mean

Thus, the enhancing effect of adrenaline on low G-6-P-ase activity was observed exclusively after dextrin-maltose feeding. The question therefore arose, whether adrenaline only antagonized the insulin effect (*i.e.* abolished the inhibition caused by insulin), or did it actually activate G-6-P-ase.

In connection with this it may be asked, to what should G-6-P-ase activity be related? Changes in the condition of the liver (in its glycogen, protein and water contents, cell count, *etc.*) may make namely difficult to determine whether its G-6-P-ase activity has increased or decreased. It is usual to give the values of G-6-P-ase concentration per 1 g liver tissue and those of activity per 100 g body weight. If the two values change parallel in the same direction, as in the above experiments, the results are acceptable. Misleading results would be obtained by relating the values to the protein or solids content of the liver.

* The composition of this diet was, fructose, 65 per cent; casein, 25 per cent; sunflower seed oil, 7.5 per cent; yeast, 1 per cent; Ca lactate, 1 per cent; NaCl, 0.5 per cent.

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FLUORESZIERENDE KOMPONENTEN IN ELASTIN

Von

ILONA BANGA, JOLANDA MAYLÁTH-PALÁGYI und A. JOBBÁGY

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UND KLINIK FÜR HAUT- UND GESCHLECHTSKRANKHEITEN
DER MEDIZINISCHEN UNIVERSITÄT BUDAPEST

(Eingegangen am 5. Februar 1964)

In den Fraktionen des aus Elastinpulver nach PARTRIDGE hergestellten Oxalsäure-Hydrolysates wurden Löslichkeit und Fluoreszenz der fluoreszierenden Substanz untersucht.

Die Substanz, deren Fluoreszenz mit dem Filter 365 gemessen wurde, blieb nach Ausschütteln mit Lipoidlösungsmitteln zu etwa $^4/_5$ in der wäßrigen Phase, während $^1/_5$ in der Fettlösungsmittelphase erschien. Nach der auf die Oxalsäure-Hydrolyse folgenden Salzsäure-Hydrolyse verschwand die Fluoreszenz nicht, sondern wurde eher stärker. Daraus wird der Schluß gezogen, daß die gemessene Fluoreszenz nicht von Polypeptiden stammt.

Die Eigenschaften der untersuchten fluoreszierenden Substanz stimmten nich mit denen des sog. »gelben Pigmentes« überein. Gelbfärbung und Eiweißgehalt der mittels Oxalsäure-Hydrolyse gewonnenen Fraktionen änderten sich nämlich parallel, während die spezifische Fluoreszenz annähernd gleich blieb. Die untersuchte Komponente wies auch andere Löslichkeitsverhältnisse auf als das gelbe Pigment. Aus diesen Feststellungen folgt, daß nicht die Fluoreszenz der farbigen Substanz untersucht wurde.

Es wird in Übereinstimmung mit anderen Autoren die Meinung vertreten, daß das Elastinmolekül verschiedenartige fluoreszierende Stoffe enthält.

In den Mittelpunkt der biochemischen Erforschung der Haut und Bindegewebe trat vor einigen Jahren die Entdeckung, daß ein Bestandteil des Elastinmoleküls über Fluoreszenz verfügt. SZENT-GYÖRGYI (1957) wies als erster auf den engen Zusammenhang zwischen der Fluoreszenz einer Substanz und ihrer Energieübertragungsaktivität im Stoffwechsel hin. So darf man voraussetzen, daß der fluoreszierenden Substanz im Elastin eine Rolle im Abbau und Aufbau der elastischen Fasern zufällt. PARTRIDGE und DAVIS (1955) schrieben über das gelbe Elastinpigment, ohne dessen Fluoreszenzvermögen wahrzunehmen. LABELLA (1957) vertrat als erster die Meinung, dieser Substanz müsse eine metabolische Funktion zugeschrieben werden. Nach seiner Hypothese handelt es sich um ein ungesättigtes Lipoid, das an der Aufrechterhaltung der Elastinmolekülstruktur mitwirkt. Die Fluoreszenz des Elastinpigmentes entdeckte LOOMEIJER (1958). Die von ihm aus Salzsäurehydrolysat isolierte geringe Pigmentmenge war wasserunlöslich, aber äther- und chloroformlöslich. KÄRKELÄ und KULONEN (1959) führten die partielle Elastin-Hydrolyse mit verschiedenen Enzymen, Säuren und Basen durch. Das isolierte, gelb fluoreszierende Pigment vermochten sie auf das 8—10fache anzureichern. Ihrer Ansicht nach bindet sich diese Substanz eng

an die Peptidkette. LOOMEIJER (1961) teilte mit, die Arterienwand der Säuger fluoresziere, wenn sie mit 350—400 mμ UV-Licht bestrahlt wird und isolierte von der Elastin-Peptidkette eine gesättigte Fettsäure mit 12 Kohlenatomen, die seiner Ansicht nach für die Fluoreszenz verantwortlich sei. LABELLA (1961, 1962) und LABELLA und LINDSAY (1963) trennten das gelbe Elastinpigment von einem farblosen Stoff, der bei 405—440 mμ fluoresziert. WALFORD und Mitarbeitern (1961) isolierten aus Elastolysat eine pigmentreiche und eine pigmentarme Fraktion. PARTRIDGE und Mitarbeitern (1963) stellten aus Elastin zwei Substanzen her, die THOMAS und Mitarbeitern (1963) »Desmosine« bzw. »Isodesmosine« nannten. Die Untersuchungsergebnisse von SINEX und FARIS (1962) sprechen gleichfalls dafür, daß für die Fluoreszenz von Elastin zwei verschiedene Komponenten verantwortlich sind.

Wie vorstehende Ausführungen zeigen, darf der fluoreszierende Elastinbestandteil berechtigterweise auf großes Interesse rechnen. Aus den hier angeführten wenigen Angaben über diesen Forschungsbereich geht aber auch hervor, daß die Mitteilungen über seine Eigenschaften widersprechend sind. Aus diesem Grunde befaßten auch wir uns mit der Untersuchung der fluoreszierenden Elastinkomponente.

Experimentelle Materialen und Methoden

Als Ausgangsmaterial diente das von PARTRIDGE und Mitarbeitern (1955) hergestellte Elastinpulver, aus dem wir folgende Stoffe gewannen:

a) Nach der Vorschrift von PARTRIDGE und Mitarbeitern (1955) mit partieller Oxalsäure-Hydrolyse hergestellte Elastinlösung, die sämtliche 5 Fraktionen — auch α- und β-Elastin — enthielt und die wir durch Vermengung der 5 getrennten Fraktionen gewannen. (Diese Lösung bezeichnen wir im weiteren kurz als Stammlösung.)

b) Die Fraktionen (I, II, III, IV, V) der vorigen Stammlösung jeweils gesondert.

c) Die III. Fraktion der Stammlösung, die auf Grund ihrer Koazervatbildungsfähigkeit wahrscheinlich reines α-Elastin enthielt. Sie färbte sich am kräftigsten gelb und ihr Eiweißgehalt war am höchsten.

Im folgenden beschreiben wir ausführlich die zur Untersuchung der angeführten Stoffe ausgearbeiteten Methoden.

a) Die Stammlösung untersuchten wir von zwei verschiedenen Gesichtspunkten. Wir beobachteten die Löslichkeitsverhältnisse ihrer fluoreszierenden Komponente sowie ihre Fluoreszenz sowohl in der Stammlösung als auch in den mit Lipoidlösungsmitteln hergestellten Fraktionen. Da sich ein Teil der verwendeten Lösungsmittel — Chloroform, Äther, Petroläther — nicht mit Wasser vermengte, separierte sich die wäßrige Phase nach dem Schütteln von der lösungsmittelhaltigen, und zwischen den beiden bildete sich eine geringere oder größere Niederschlagsmenge. Bei Gebrauch der sich mit Wasser vermengenden Lösungsmittel — Azeton, Methylalkohol — gab es nur eine wasser-lösungsmittelhaltige Phase und entweder keinen oder nur sehr wenig Niederschlag.

Bei der Fraktionierung gaben wir die 10fache Lösungsmittelmenge zu 10 ml der untersuchten Lösung. Das Ausschütteln erfolgte im Scheidetrichter, die getrennten Phasen wurden voneinander separiert, ihr Volumen bestimmt, und danach untersuchten wir sie gesondert. Von Azeton nahmen wir — anders als von den anderen Lösungsmitteln — das 20- bzw. 40fache Volumen für die Untersuchungen.

Die Untersuchung wurde mit dem Fluoreszenz-Adapter des *Hilgerschen Spektrophotometers* unter Verwendung des Filters 365 vorgenommen. Als Standard diente mit n/10 Schwefelsäure zubereitete Chininsulfatlösung in der Konzentration von 1 μg/ml oder 0,1 μg/ml, je nach der Stärke der gemessenen Fluoreszenz. Als Blindprobe benutzten wir das betreffende Lösungsmittel. Bemerkt sei, daß wir im Interesse einer Vergleichbarkeit der Ergebnisse sämtliche

Berechnungen so vornahmen, als ob die Standardlösung von 0,1 μg/ml zur Bestimmung benutzt worden wäre. Nachfolgend wollen wir die Untersuchungsmethoden und Berechnungen ausführlich beschreiben. Die hierbei gewonnenen Resultate waren zumeist niedrigere Zahlen als 1, weshalb wir diese Werte mit 100 multiplizierten. Das Ausmaß der Fluoreszenz-Lichtintensität des fluoreszierenden Stoffes geben wir somit in dimensionslosen fiktiven Einheiten (arbitrary unit) an.

Die Untersuchung der Stammlösung erfolgte aus der 100fachen Verdünnung. Die Fluoreszenz-Lichtintensität (im weiteren Lichtintensität) der Standardlösung mit 100% annehmend, errechneten wir, wieviel μg schwefelsaurer Chininsulfat-Fluoreszenz der gemessene Lichtintensitätswert entspricht. Die so gewonnene Zahl mit der Verdünnung (100) multiplizierend erhielten wir die auf 1 ml Stammlösung entfallende Fluoreszenz. In Kenntnis der Tatsache, wieviel Eiweiß 1 ml der untersuchten Lösung enthält, errechneten wir auch die auf 1 mg Eiweiß entfallende sog. spezifische Fluoreszenz.

Fernerhin untersuchten wir die Fluoreszenz der aus der Stammlösung mit verschiedenen Lipoidlösungsmitteln gewonnenen Fraktionen.

Die wäßrige Phase wurde vor der Messung auf 30—60 ml ergänzt, wonach wir sinngemäß ebenso vorgingen wie bei der Untersuchung der Stammlösung.

Die Lipoidlösungsmittel enthaltende Phase wurde vor der Bestimmung auf 30—60 ml eindestilliert. Die Untersuchung stimmte mit der vorstehend beschriebenen überein.

Den Niederschlag untersuchten wir nur dann, wenn er wasserlöslich war. Die aus 10 ml Stammlösung gewonnene Präzipitätsmenge wurde in wenig dest. Wasser aufgelöst und dann das Volumen auf 30—60 ml ergänzt. Im weiteren gingen wir nach dem geschilderten Verfahren vor.

Im Falle von einem mit Wasser vermischbaren Lösungsmittel wurde die wasser- und lösungsmittelhaltige Phase ebenso eindestilliert wie die lipoidlösungsmittelhaltige. Die Untersuchungs- und Berechnungsmethode war dieselbe.

Den Eiweißgehalt bestimmten wir nach der modifizierten *Folinschen Methode* (LOWRY und Mitarbeitern 1951). Die Werte für die Kalibrationskurve stellten wir nach *Kjeldahl* aus der Stammlösung fest.

b) Aus der I.—V. Fraktion der Oxalsäure-Hydrolyse nach PARTRIDGE (1955) führten wir Fluoreszenz- und Eiweißuntersuchungen durch, um zu ermitteln, wie diese beiden Faktoren zusammenhängen. Mit den Fraktionen der Stammlösung erfolgten die Untersuchungen auf die gleiche Weise.

c) Die III. Fraktion der Oxalsäure-Hydrolyse nach PARTRIDGE (1955) wurde aus den bereits angegebenen Gründen gesondert untersucht. Die Löslichkeits- und Fluoreszenzuntersuchungen nahmen wir sowohl am Material selbst als auch an dem daraus zubereiteten Salzsäure-Hydrolysat vor. Letzteres wurde so hergestellt, daß wir den Stoff mit 2 N Salzsäure 24 Stunden lang bei 100° C in einem Bombengefäß hielten. Die Löslichkeits- und Fluoreszenzuntersuchung der Soffe war dieselbe wie bei der Stammlösung. Als Lösungsmittel wurde nur Chloroform benutzt. Bei Untersuchung des Eiweißgehaltes im Salzsäurehydrolysat wurde eine Tyrosinkurve aufgenommen. — Nach unseren Feststellungen hat die infolge der Neutralisierung entstandene Salzkonzentration die Intensität der nach der *Folinschen Methode* gewonnenen Farbe nicht beeinflußt.

Ergebnisse

Die nachfolgend mitgeteilten Untersuchungsergebnisse stellen die Mittelwerte mehrerer Bestimmungen dar.

a) 1 ml Stammlösung enthielt 8,6 mg Eiweiß und 9,2 mg Trockensubstanz. Die Fluoreszenz von 1 ml Lösung machte 37, die spezifische Fluoreszenz 44 aus *(Tabelle III)*.

Nach Extraktion der Stammlösung mit verschiedenen Lösungsmitteln traten folgende Resultate zutage:

Mit Chloroform ausgeschüttelt, war die Fluoreszenz in der wäßrigen Phase am stärksten; hier fanden wir 84% der gesamten festgestellten Fluoreszenz *(Tabelle I)*. Der Eiweißgehalt dieser Phase betrug 7,7 mg/ml, 90% des

Eiweißgehaltes der Stammlösung. Die spezifische Fluoreszenz ergab 39 *(Tabelle II)*. In der chloroformhaltigen Phase stellten wir 16% der Fluoreszenz fest *(Tabelle I)*. Die Fluoreszenz des Niederschlags wurde nicht bestimmt, weil dieser wasserunlöslich war. Mit 20%igem NaOH und konz. H_2SO_4 ließ er sich in Lösung überführen, und nach der Analyse bestand er zu 90% aus organischen und zu 10% aus anorganischen Stoffen. Im Niederschlag bestimmten wir 15% des Trockensubstanzgehaltes und 9% des Eiweißgehaltes der Stammlösung.

Tabelle I

Prozentuale Verteilung sämtlicher Fluoreszenzwerte

Untersuchte Substanz		Fluoreszenz		
		in der Lösungsmittelphase, %	in der wäßrigen bzw. wäßriglösungsmittelhaltigen Phase, %	im Niederschlag,* %
Mit Chloroform	fraktionierte Elastin-Stammlösung	16	84	—
„ Äther		6	88	6
„ Petroläther		0	100	—
„ Azeton		89		11
„ Methylalkohol		100		kein Niederschlag
III. Fraktion der Oxalsäure-Hydrolyse	mit Chloroform ausgeschüttelt	13	87	—
	nach Salzsäure-Hydrolyse mit Chloroform ausgeschüttelt	18	82	kein Niederschlag

* Das Zeichen — bedeutet, daß wegen Unlöslichkeit des Niederschlages keine Untersuchung vorgenommen wurde.

Nach Ätherextraktion wies die wäßrige Phase die stärkste Fluoreszenz, 88% des bestimmten Gesamtwertes auf *(Tabelle I)*. Der Eiweißgehalt (8,4 mg/ml) machte 98% des Eiweißgehaltes der Stammlösung aus. In der ätherhaltigen Phase fanden wir 6% der gemessenen Gesamtfluoreszenz *(Tabelle I)*. Der Niederschlag war wasserlöslich, seine Fluoreszenzuntersuchung ergab dasselbe Resultat wie die der ätherhaltigen Phase *(Tabelle I)*. Deren Eiweißgehalt erwies sich als 4% des Gehaltes der Stammlösung. Die auf 1 mg Eiweiß entfallende Fluoreszenz war in der wäßrigen Phase 28, im Niederschlag 66 *(Tabelle II)*.

Wurde zum Ausschütteln Petroläther verwendet, so fanden wir überhaupt keine Fluoreszenz in der Lösungsmittelphase, vielmehr verteilte sich die Fluoreszenz zwischen dem Niederschlag und der wäßrigen Phase. Da wir

Tabelle II

Spezifische Fluoreszenzwerte in den nach Extraktion der Stammlösung und III. Fraktion gewonnenen Phasen

Untersuchte Substanz		Fluoreszenz	
		in der wäßrigen bzw. wäßrig-lösungsmittelhaltigen Phase	im Niederschlag*
Mit Chloroform	fraktionierte Elastin-Stammlösung	39	—
„ Äther		28	66
„ Petroläther		36	—
„ Azeton		49	48
„ Methylalkohol		78	kein Niederschlag
III. Fraktion der Oxalsäure-Hydrolyse	mit Chloroform ausgeschüttelt	29	—
	nach Salzsäure-Hydrolyse mit Chloroform ausgeschüttelt	192	kein Niederschlag

* Das Zeichen — bedeutet, daß wegen Unlöslichkeit des Niederschlages keine Untersuchung vorgenommen wurde.

den Niederschlag nicht untersuchten, wurde die Gesamtfluoreszenz in der wäßrigen Phase gemessen *(Tabelle I)*. 97% (8,3 mg/ml) des Eiweißgehaltes der Stammlösung erschienen in dieser Phase. Die spezifische Fluoreszenz wurde mit 36 festgestellt.

Tabelle III

Spezifische Fluoreszenzwerte in der Stammlösung und in den Fraktionen der Oxalsäure-Hydrolyse

Untersuchte Substanz		Fluoreszenz
Stammlösung		44
Fraktionen der Oxalsäure-Hydrolyse	I.	42
	II.	37
	III.	48
	IV.	43
	V.	nicht meßbar

Bei Anwendung von Azeton blieben — aus den bereits angeführten Gründen — nur 44% des Eiweißgehaltes der Stammlösung in der wäßrig-

lösungsmittelhaltigen Phase, deren Fluoreszenz 89% der festgestellten Gesamtfluoreszenz ausmachte. Im Niederschlag ermittelten wir 11% der Gesamtfluoreszenz *(Tabelle I)*. Der auf 1 mg Eiweiß entfallende Wert war in der Flüssigkeit 49, im Präzipitat 48 *(Tabelle II)*.

Nach Extraktion mit Methylalkohol blieben nur 39% des Eiweißgehaltes der Stammlösung in der untersuchten alkoholisch-wäßrigen Phase, deren Fluoreszenz — da beim Ausschütteln kein Niederschlag entstand — der unserseits gemessenen Gesamtfluoreszenz (100%) entsprach *(Tabelle I)*. Der auf 1 mg Eiweiß entfallende Wert war 78 *(Tabelle II)*.

b) Die Untersuchung der Fraktionen der Oxalsäure-Hydrolyse nach PARTRIDGE (1955) ergab, daß der Eiweißgehalt der I., II. und IV. Fraktion einander nahesteht, während in der III. Fraktion etwa das Doppelte dieser Werte angetroffen wurde. (Die V. Fraktion enthielt praktisch kein Eiweiß.) Die spezifische Fluoreszenz in der I.—IV. Fraktion betrug 37—48; signifikante Abweichungen traten nicht zutage *(Tabelle IV)*.

Tabelle IV

Eiweiß- und spezifische Fluoreszenzwerte der Oxalsäure-Hydrolyse-Fraktionen

Fraktion	Eiweißgehalt mg/ml	Fluoreszenz
I.	9,6	42
II.	8,0	37
III.	19,9	48
IV.	10,0	43
V.	0,1	nicht meßbar

c) Die im Verlauf der Oxalsäure-Hydrolyse separierte III. Fraktion wurde gesondert untersucht. Ihre spezifische Fluoreszenz erwies sich als 48 *(Tabelle III)*. Nach Ausschütteln der Substanz mit Chloroform stellten wir 87% der Gesamtfluoreszenz in der wäßrigen Phase, 13% in der Lösungsmittelphase fest *(Tabelle I)*, die spezifische Fluoreszenz der wäßrigen Phase war 29 *(Tabelle II)*. Hydrolisierten wir die III. Fraktion mit Salzsäure, und erfolgte das Ausschütteln hiernach mit Chloroform, so fanden wir 82% der Gesamtfluoreszenz in der wäßrigen und 18% in der chloroformhaltigen Phase *(Tabelle I)*. Der spezifische Wert in der wäßrigen Phase war 192 *(Tabelle II)*.

Besprechung

Nach den obigen Untersuchungsergebnissen fanden wir, wenn die Elastin-Stammlösung mit verschiedenen Lipoidlösungsmitteln fraktioniert

wurde, die höchsten Fluoreszenzwerte in der wäßrigen Phase. In der lipoidlösungsmittelhaltigen Phase und im Niederschlag wurden niedrige Werte ermittelt *(Tabelle I)*. Ähnliche Resultate ergab die Untersuchung der III. Fraktion und des daraus hergestellten Salzsäure-Hydrolysates *(Tabelle I)*. Demnach hat weder die Oxalsäure-Hydrolyse noch die nachfolgende Salzsäure-Hydrolyse in der Chromoforgruppe des Elastinmoleküls eine Veränderung zustande gebracht, unter deren Wirkung diese in die Lipoidlösungsmittelphase übergegangen wäre. Es scheint somit, daß die fluoreszierende Elastinkomponente noch eng an die Eiweißstoffe gebunden war und daher als Lipoprotein in der wäßrigen Phase erschien. LOOMEIJER (1958) isolierte aus dem Salzsäure-Hydrolysat von Elastin ein gelbes Pigment, das sich in Wasser nicht, wohl aber in Fettlösungsmitteln auflöste.

Betrachten wir die spezifischen Fluoreszenzwerte *(Tabelle II* und *III)*, und vergleichen wir sie mit denen der Elastin-Stammlösung, so können wir folgendes feststellen: Identisch sind die Ergebnisse in der azetonhaltigenwäßrigen Phase, in deren Niederschlag sowie in der I.—IV. Fraktion der Oxalsäure-Hydrolyse. Niedrigere Werte als bei der Elastin-Stammlösung ermittelten wir meistens in den wäßrigen Phasen. In diesen Fällen war die Fluoreszenz, wie das Ausschütteln mit Äther zeigte, im Niederschlag angereichert. Erhöhte Werte traten auch in der methylalkoholisch-wäßrigen Phase zutage. Letzteres kann auf der Esterifizierung mit der Carboxylgruppe des Pigmentstoffes beruhen. Den auffallendsten Wert (192) ergab das Salzsäure-Hydrolysat der III. Fraktion. Nachdem wir die Polypeptidbindungen (zumindest teilweise) vermutlich gespalten haben und der fluoreszierende Stoff dennoch erhaltengeblieben ist, ja infolge der Hydrolyse sogar in einen isolierten Zustand gelangte, darf der Schluß gezogen werden, daß die unserseits gemessene Fluoreszenz nicht von Polypeptiden stammt. Nach unserer Meinung muß es sich um eine niedermolekuläre Substanz handeln, die unter Wirkung der Hydrolyse keine Veränderung erleidet (»Desmosine?«).

Die Eigenschaften dieser Substanz stimmen nicht mit denen des »gelben Pigmentes« von PARTRIDGE und DAVIS (1955), LABELLA (1957, 1961, 1962), LABELLA und LINDSAY (1963), KÄRKELÄ und KULONEN (1959) sowie LOOMEIJER (1958, 1961) überein. Bei unseren Untersuchungen haben sich nämlich Färbung und Eiweißgehalt der Oxalsäure-Hydrolysefraktionen parallel verändert. Die am stärksten gelbe III. Fraktion enthielt das meiste Eiweiß, dagegen war ihre spezifische Fluoreszenz (48) nicht signifikant höher als die der anderen Fraktionen *(Tabelle IV)*. Wir wiesen auch bereits darauf hin, daß die unserseits untersuchte fluoreszierende Substanz andere Löslichkeitsverhältnisse zeigte als das isolierte gelbe Pigment (LOOMEIJER 1958). Im Sinne dieser Feststellungen haben wir nicht die Fluoreszenz des gelben Pigments untersucht.

Auch hiernach hat es also den Anschein, daß das Elastinmolekül mehr fluoreszierende Stoffe enthalten muß, als LaBella (1961, 1962) und LaBella und Lindsay (1963), Sinex und Faris (1962) sowie Partridge und Mitarbeitern (1963) behaupten.

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Physiologia

THE EFFECT OF PROGESTERONE ON ADRENAL CORTICOSTERONE AND ALDOSTERONE SECRETION IN THE FEMALE RAT

By

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The effect of various doses of progesterone was investigated on adrenal corticosterone and aldosterone secretion *in vivo* and *in vitro*.

Daily administration of 1.5 mg/100 g of progesterone for six consecutive days significantly increased corticosterone secretion. When used at a dose level of 3 mg/100 g, or, of 5 mg/100 g the drug was without effect, while in a dose of 10 mg/100 g it markedly diminished corticosterone production.

Aldosterone secretion was enhanced by the daily administration of 10 mg/kg of progesterone.

The changes in adrenal weight were in accordance with those found in corticosterone secretion.

Under conditions *in vitro*, low concentrations of progesterone as precursor increased the synthesis of both corticosterone and aldosterone. High concentrations, however, caused the aldosterone production substantially to decrease with only a slight concomitant diminution in corticosterone synthesis.

Progesterone treatment has been demonstrated in our previous experiments to increase glucocorticoid secretion in both the dog and the rat (TELEGDY and ENDRŐCZI 1959; TELEGDY *et al.* 1962). Similarly, LANDAU and LUGIBIHL (1961) found enhanced aldosterone secretion after the administration of progesterone. On the other hand this hormone has been shown to be capable of causing adrenal atrophy (VAN REES 1959), an effect shared with 6α-methyl-17α-hydroxyprogesterone (EDGREN *et al.* 1959; GLENN *et al.* 1959; HOLUB *et al.* 1961; LOGETHETOPOULOS *et al.* 1961). As to the progesterone-induced augmentation of aldosterone secretion the results thus far obtained are not quite unequivocal, as several authors have succeeded but partially in demonstrating or corroborating this effect (GORNALL *et al.* 1960; LAYNE *et al.* 1962; STARK 1962a, 1962b; STARK and KOSSMANN 1963).

The differences in experimental results may be accounted for by several factors, such as the dose of progesterone administered, the quality of the gestagen used as well as the differences in both the experimental animals and experimental procedures. To avoid the influence of these factors, we have attempted to study the effect of progesterone in the same animal species and for an identical period of time. Female rats were used in the experiments and the influence of progesterone on adrenal corticosterone and aldosterone secretion was studied both *in vivo* and *in vitro*.

Methods

Inbred adult female albino rats weighing from 120 to 200 g were used. The animals were kept under standard environmental conditions and on a standard diet. For treatment *in vivo*, progesterone (Glanducorpin, Richter Fa.) in oil was administered subcutaneously for six consecutive days.

The animals were grouped as follows:

1) controls, 0.4 ml oil/day
2) 1.5 mg/100 g of progesterone/day
3) 3 mg/100 g of progesterone/day
4) 5 mg/100 g of progesterone/day
5) 10 mg/100 g of progesterone/day

After treatment had been brought to an end, we measured the rate of adrenal corticosteroid secretion. For this purpose the animals were anaesthetized with 250 mg/100 g of hexobarbital sodium and the corticosterone and aldosterone content of adrenal venous blood was determined according to the method described previously (TELEGDY *et al.* 1962). Corticosterone was measured in each animal individually, while aldosterone in the pooled blood from 6 to 8 rats. The corticosterone secretion rate was expressed in terms of μg/h/100 g body weight/adrenal; that of aldosterone, in μg/h/kg body weight/adrenal.

After blood collection, the adrenals were removed, cleaned and weighed on a torsion balance with an accuracy of 0.1 mg.

The investigations *in vitro* were carried out on the adrenal glands of normal, untreated animals. From the rats killed by decapitation the adrenals were removed, cleaned and sliced with a razor blade. Corticosterone synthesis was studied in *Krebs—Ringer* bicarbonate buffer containing 200 mg/100 ml dextrose at pH 7.6. The gas phase consisted of 95 per cent oxygen and 5 per cent carbone dioxide. The tissue was incubated for 30 minutes at 38° C; thereafter the medium was replaced by a progesterone-containing *Krebs—Ringer* solution and incubation was continued for an additional period of two hours (GIROUD *et al.* 1958).

The experimental arrangement in these experiments was as follows:

1) controls
2) 10 μg of progesterone
3) 100 μg of progesterone
4) 200 μg of progesterone
5) 300 μg of progesterone

After incubation the medium was poured off and its corticosteroid content was determined.

Extraction, paper chromatography and identification of corticosteroids were performed as described previously (ENDRŐCZI and YANG 1960). Quantitative estimation of corticosterone was made by means of the tetrazolium blue reaction.

Aldosterone was determined by using a microscale tetrazolium reaction, a procedure developed in this laboratory (ENDRŐCZI and YANG 1960)

Corticosterone and aldosterone productions have been expressed in terms of μg/100 mg tissue/hour.

Results

Fig. 1 shows corticosterone and aldosterone secretion.

Corticosterone secretion in control animals treated only with oil averaged 13.3 ± 3.8 μg/100 g/h. After administration of progesterone in a dose of 1.5 mg/100 g this value rose to 17.2 ± 4.1 ($p < 0.02$). After 3 mg/100 g of progesterone the rate of corticosterone secretion was 13.7 ± 4.2 μg/h/100 g, showing, thereby, no significant difference from the control values, while after 5 mg/100 g it decreased to 10.6 ± 3.4 μg/h/100 g ($p < 0.05$), and, after 10 mg/100 g, to 9.7 ± 2.0 ($p < 0.01$).

Aldosterone secretion in control animals treated with oil was 1.1 μg/h/kg. A value of 0.9 μg/h/kg was found after the administration of 1.5 mg/100 g of

progesterone, 1.03 μg/h/kg after 3 mg of the drug 1.04 μg/h/kg after 5 mg, and 2.5 μg/h/kg after 10 mg of progesterone.

Adrenal weights are shown in *Fig. 2*. Control values (oil-treated animals) averaged 28.0 $\pm$ 6.4 mg/100 g. A value of 39.7 $\pm$ 5.0 mg/100 g was found

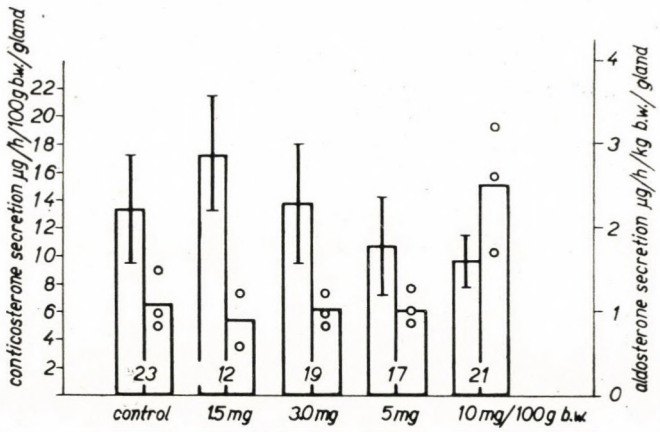

Fig. 1. Secretion rate of corticosterone and aldosterone after various doses of progesterone.
First column: corticosterone (and standard deviation)
Second column: aldosterone (and standard deviation)

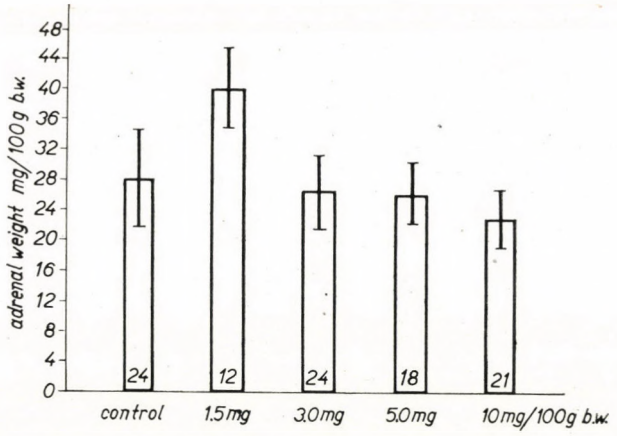

Fig. 2. Changes in adrenal weight after various doses of progesterone

after 1.5 mg/100 g of progesterone ($p < 0.001$), 26.6 $\pm$ 4.8 after 3 mg (no difference from the control), 25.9 $\pm$ 3.8 after 5 mg/100 g of progesterone (again no significant difference from the control) and 23.1 $\pm$ 3.8 mg/100 g after giving 10 mg/100 g of progesterone ($p < 0.001$).

The results of our experiments *in vitro* are demonstrated in *Table I.* Progesterone at increasing concentrations up to 200 μg/100 mg tissue augmented corticosterone and aldosterone production. On the other hand, at a dose level of 300 μg/100 mg, the drug considerably diminished aldosterone synthesis with a minimum decrease in corticosterone production.

Table I

Effect of progesterone on the production of corticosterone and aldosterone in the rat adrenal in vitro

Number of animals	Weight of incubated tissue, mg	Progesterone added μg/100 mg tissue	Production in μg/100 mg/h of	
			Corticosterone	Aldosterone
8	237	none	4.0	0.55
8	244	10	5.1	1.55
8	235	100	5.6	1.64
8	230	200	5.8	2.05
8	242	300	5.5	0.68

Discussion

The results of our investigations indicate that the adrenal response to progesterone treatment depends on the dose of progesterone administered in a certain period of time. There are differences in changes occurring in glucocorticoid and mineralocorticoid secretion and also between the behaviour of hormone production *in vitro* and *in vivo.* According to the findings the changes induced by progesterone in the synthesis and secretion of various types of corticoids are elicited by different mechanisms.

Small doses of progesterone increase corticosterone secretion in the rat and both hydrocortisone and corticosterone secretion in the dog (TELEGDY and ENDRŐCZI 1959; TELEGDY *et al.* 1962). Progesterone is well known for its role played in adrenal corticosteroid synthesis and both *in vitro* and perfusion experiments unequivocally demonstrated this hormone to be a precursor for adrenocortical steroids (AYRES *et al.* 1960; GIROUD *et al.* 1958; HECHTER *et al.* 1951). The synthesis-enhancing effect of small progesterone doses in our experiments can be, thus, explained on the basis that, at least as far as experiments *in vitro* are concerned, an increased amount of the precursor is present in the system. In this connection, however, an indirect action *via* the pituitary has also to be taken into account.

Large doses, such as 10 mg/100 g, of progesterone lead to a substantial diminution of corticosterone secretion. Relatively high concentrations do not increase synthesis *in vitro.* In fact, they elicit a slight diminution in secretion,

as compared with values found at lower progesterone levels. However, this decreased synthesis still exceeds the control one. Comparison of our findings *in vivo* and *in vitro* suggests that the inhibitory action exerted by systematically administered large progesterone doses on the pituitary-adrenocortical system is not due to a direct action on the adrenal cortex but to an influence on higher hypothalamic or pituitary systems. The ability of progesterone to antagonize compensatory adrenocortical hypertrophies (HECHT-LUCARI and LUCISANO 1960) also favours this view.

The changes in adrenal weight run parallel to the alterations in corticosterone secretion.

The progesterone-effect on aldosterone secretion depends primarily on the dose administered. *In vivo*, small doses are ineffective. Under conditions *in vitro*, however, even low progesterone concentration did enhance aldosterone synthesis, a finding in accordance with the data of other authors (AYRES *et al.* 1960; GIROUD *et al.* 1958). Large doses of the drug increased the secretion rate *in vivo* but decreased it *in vitro*. These observations, too, suggest that these effects are to be accounted for by different mechanisms. LANDAU and LUGIBIHL (1958) and LANDAU *et al.* (1955) demonstrated large doses of systematically administered progesterone to counteract the salt-retention caused by corticosteroids and to be, thus, a competitive antagonist of aldosterone. The sodium-loss thereby induced would then lead to a secondary overproduction of aldosterone. These results have, however, been corroborated but in part (GORNALL *et al.* 1960; LAYNE *et al.* 1962; STARK 1962a, 1962b; STARK and KOSSMANN 1963). Our investigations have shown that large doses of progesterone are only capable of increasing aldosterone production. The mechanism of this action seems to be identical with that suggested by LANDAU and LUGIBIHL (1958). The above-cited contradictory data may be probably accounted for by an inadequate dosage of progesterone. Our data obtained *in vivo* after the administration of large progesterone doses are in accordance with the findings of SINGER (personal communication).

In our experiments high concentrations of progesterone inhibited aldosterone synthesis *in vitro*. This may be explained on the basis of the drug's general inhibitory action on cell metabolism (BROWNIE *et al.* 1954).

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CENTRAL NERVOUS LOCALIZATION OF CORONARY REFLEXES

By

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The nervous centres of the vasomotor reflexes elicitable from the coronaries have been localized by sectioning the brain stem at different levels in dogs anaesthetized with morphine-chloralose. The following reflexes were studied:

a) the coronary sinus reflex evoked by inflating a balloon inserted into the sinus, ensuring the unaffected blood outflow;

b) the coronary depressor reflex evoked by increasing pressure in the entire coronary vascular bed by blocking the outflow from the sinus; and

c) the coronary chemoreflex, evoked by the injection of protoveratrine.

None of the above reflexes could be elicited when the bulbar vasomotor centre was lesioned. When the section ran between the pontobulbar junction and the upper one-third of the mesencephalon the coronary depressor reflex, similarly to the carotid sinus reflex, could be elicited, while the coronary sinus reflex could not. The coronary sinus reflex was elicitable exclusively when the entire mesencephalon was intact. The coronary chemoreflex behaved like the coronary sinus reflex.

It has been concluded that the circulatory stabilization brought about by the coronary sinus reflex is realized through more complex, and differentiated nervous structures, than blood pressure drop induced by the coronary depressor reflex, which can be sharply distinguished from the former effect.

In recent years it has been demonstrated that the hypotensive reflex elicited by the stimulation of the baroreceptors of the coronary sinus differs sharply in many important features from the well-known buffer reflexes (SZENTIVÁNYI and JUHÁSZ-NAGY 1959, 1961, 1962). The coronary sinus reflex is characteristically long-lasting (of stabilizer nature), extremely sensitive to hypnotics, and we explained its reactions extending diffusely to the somatic spheres by the peculiar, complex central nervous connections of the reflex. In the present series of experiments we divided the brain stem at different levels, in order to determine the segments functioning as the "centres" of the reflex and to check the validity of our hypothesis. It seemed reasonable to extend our studies to the coronary chemoreflexes elicited by veratrine, in the genesis of which the receptors of the coronary sinus play a decisive role (JUHÁSZ-NAGY and SZENTIVÁNYI 1961), as well as to the coronary depressor reflex elicitable from the area of the left coronary bed but not from the sinus proper. This last reflex is different from the coronary sinus reflex and similar to the buffer reflexes in its properties (SZENTIVÁNYI and JUHÁSZ-NAGY 1962; GONZALES-SENATOS and ERLIJ 1959). It has been assumed that comparative studies of these three reflexes would confirm once again the identity of the coronary

sinus reflex and the coronary chemoreflex, the substantial differences existing between these two reflexes and the coronary depressor reflex.

Some of our results have been presented at the Congress of the *Hungarian Physiological Society* (1962), (JUHÁSZ-NAGY and SZENTIVÁNYI 1963).

Methods

The experiments were conducted on 45 dogs of either sex. The conclusions were drawn on the basis of the results obtained in 33, technically suitable preparations. After pretreatment with 1 to 2 mg/kg morphine, the animals were lightly anaesthetized with chloralose. The chest was opened in the right 4th intercostal space, under artificial respiration. Through the right auricle a double-bore cannula with a balloon attached to it was introduced into the coronary sinus. The outflow tube of the cannula was connected with the femoral vein; thus the outflow of sinus blood remained unaltered even after the balloon had been inflated, *i.e.* the coronary sinus reflex had been elicited. The coronary depressor reflex was evoked by clamping the polyethylene tube ensuring outflow, *i.e.* by increasing the pressure in the *entire* left coronary system. In the latter case, too, the balloon was kept inflated, in order to block sinus outflow completely. In the cases when the coronary sinus reflex elicited by inflating the balloon caused by itself a significant fall in blood pressure, the coronary depressor reflex, in accordance with our earlier investigations (SZENTIVÁNYI and JUHÁSZ-NAGY 1962), often failed to increase the effect, while in other experiments it enhanced the hypotensive reaction. The coronary chemoreflex was elicited by injecting 1 to 2 μg/kg protoveratrine (Puroverin, Sandoz) intravenously, or by injecting 0.5 to 1.0 μg/kg into the left ventricle. To prevent blood clotting, at intervals small doses of heparin were injected into the cannula. Systemic anticoagulant treatment was not applied, because this would have greatly enhanced the hazard of cerebral haemorrhage during the intracranial interventions. Blood pressure was measured in the femoral artery, by means of a mercury manometer.

From the calvary of the animal the soft parts and periosteum were pushed off. The cranial bone was cut linearly by means of a circular saw in the area of the intended section, determined by measuring the distance from the external occipital protuberance and bregma. After having elicited the reflexes several times, the section was made by means of a blunt-edged metal plate, then the elicitation of the reflexes was attempted again. The precise site of the section was controlled after death in every case. In one experiment only one section was done, to reduce traumatic side effects to a minimum. The projections of the main levels of the sections are shown in *Fig. 1.*

Results

On grounds of the results obtained, the experiments may be divided into three groups.

1. Sections touching the vasomotor centre in the medulla oblongata (area between planes I and II of *Fig. 1*).

2. Sections led through the pons, as well as through the lower two-thirds of the mesencephalon (between planes II and III in *Fig. 1*).

3. Sections led at, or superior to, the upper boundary of the mesencephalon (superior to plane III in *Fig. 1*).

(i) After making the sections belonging to Group 1, as well as after haemorrhages in the medulla oblongata demonstrated as side-findings at autopsy, neither the coronary reflexes, nor the carotid sinus reflex, was elicitable. One of the five such experiments is shown in *Fig. 2.*

(ii) In the 11 experiments of this group the reflexes always behaved in the same way, although the sections were made at different levels in the different animals. After division of the brain stem the coronary sinus reflex invariably disappeared, while both the coronary depressor and the carotid sinus depressor reflexes remained elicitable.

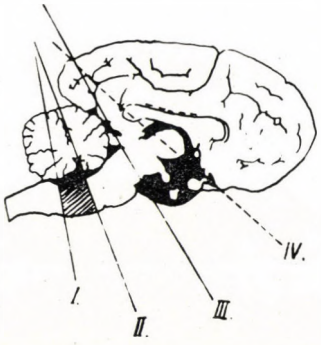

Fig. 1. Diagrammatic representation of the main planes of brain stem section. Explanation see in text

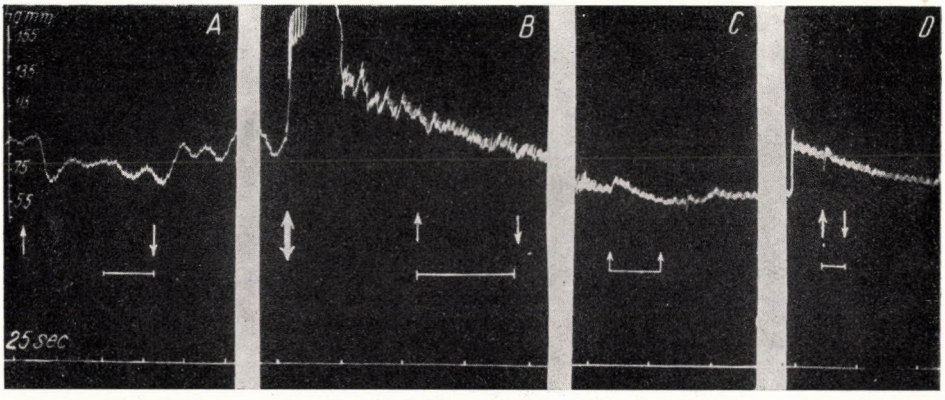

Fig. 2. The section in the midline of the medulla oblongata abolishes every reflex studied. Explanation of signs: ↑ inflation of the balloon to evoke the coronary sinus reflex. ↓ deflation of the balloon. ⊢ indicates blocking of outflow from the sinus toward the femoral vein. ↕ bulbar section. ↑_↑ pulling the carotids to evoke the carotid sinus reflex. Between *C* and *D* the animal received 150 ml blood. Time signal: 25 sec

a) The section ran at the pontobulbar junction, or near it in 5 animals. Subsequently the coronary sinus reflex could never be elicited, while the coronary depressor reflex remained elicitable, though at decreased intensity, in 4 animals.

b) The sections cutting the pons at different levels, or affecting the lower two-thirds of the mesencephalon (6 animals) abolished the coronary

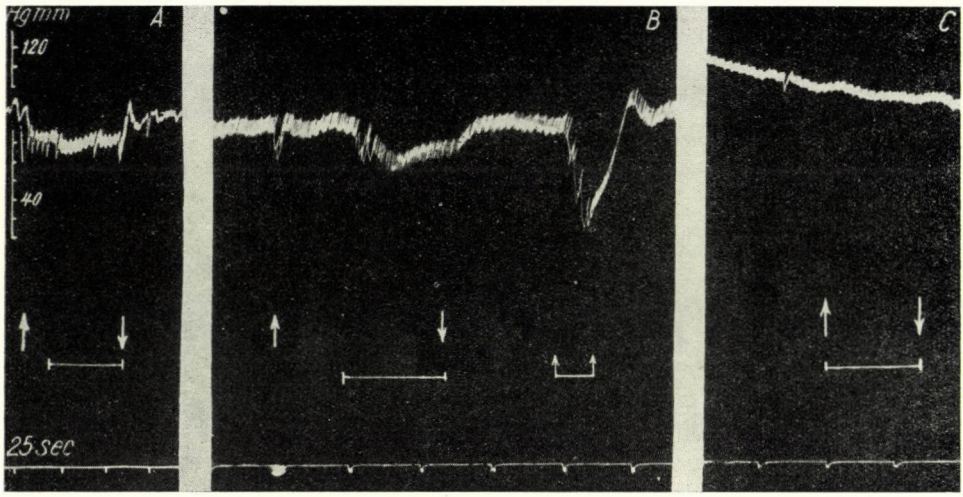

Fig. 3. Section at the upper margin of the pons. Subsequently the coronary sinus reflex could not be evoked, while the coronary depressor reflex persisted. *A:* before section. *B:* after section. *C:* after bilateral vagotomy. Signs as in *Fig. 2*

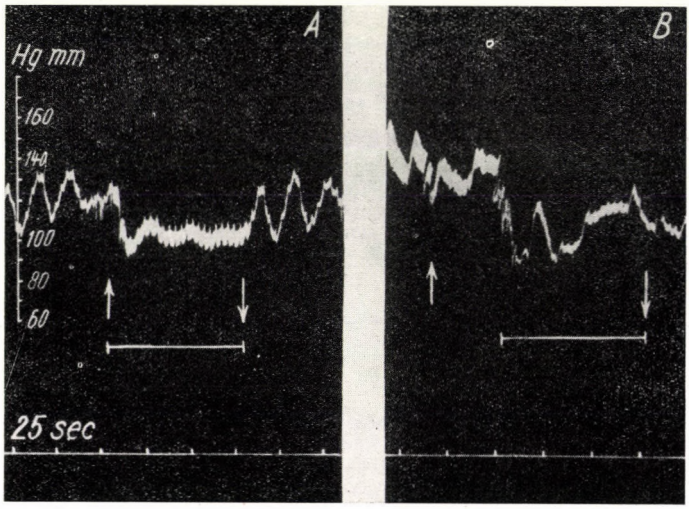

Fig. 4. Section in the middle of the mesencephalon. *A:* before section. *B:* after section. Signs as in *Fig. 2*

sinus reflex. Only a moderate slowing down of the heart rate lasting for a few strokes *(Fig. 3)*, or depressions not exceeding 10 mm Hg were observed exceptionally in one or two cases *(Fig. 4)*. As opposed to this, the coronary depressor reflex remained elicitable in every one of the 6 experiments *(Fig. 3* and *Fig. 4)*.

The elicitability of the coronary chemoreflex was like that of the coronary sinus reflex. As *Fig. 5* indicates, a significant hypotension of the stabilizer type resulted from the injection of 30 μg of protoveratrine, which began to fade only after a delay of 8 minutes.

If the brain stem had been cut between the lower and middle thirds of the pons, protoveratrine caused transient and less marked hypotension, *i.e.* the effect induced by the drug has changed both quantitatively and qualitatively. The residual effect was presumably due to an excitation of the buffer

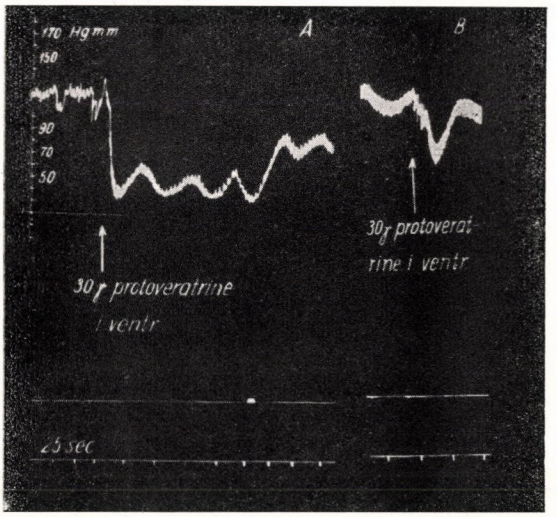

Fig. 5. Effect of pontine section on the coronary chemoreflex. *A:* before section. *B:* after section. On the upper base line the signal indicates that the kymograph has been stopped for 8 minutes

reflexes, the latter may also contribute to the development of reflex hypotension after higher doses of veratrine (DAWES and COMROE 1954; FERNANDEZ and CERLETTI 1955). Protoveratrine in low doses produced no effect.

(iii) In the 17 animals of this group the section was made at, or above, the rostral margin of the mesencephalon. After this section all the three reflexes persisted. Such an experiment is demonstrated in *Fig. 6*. Likewise, carotid sinus and coronary depressor reflexes remained elicitable, when the section running from the splenium corporis callosi to the optic chiasma had separated the entire pallium, affecting partly the brain stem nuclei but not injuring the connections between hypothalamus and mesencephalon (Plane IV in *Fig. 1*). This experiment is demonstrated by *Fig. 7*.

After sections led at the upper margin of the mesencephalon no major change resulted usually in systemic blood pressure *(Fig. 6)*. In these experiments (10 animals) the coronary sinus reflex was elicitable immediately after

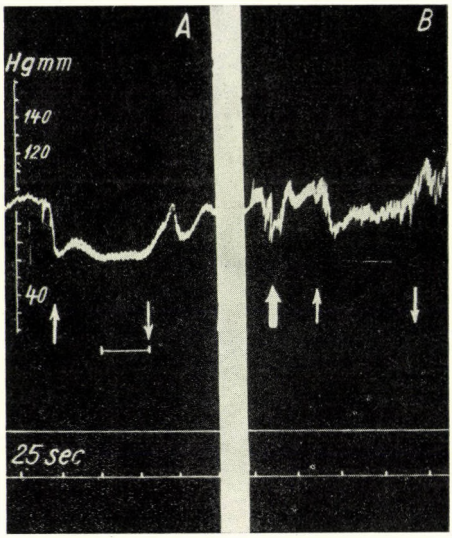

Fig. 6. After leading the section at the upper margin of the mesencephalon the coronary sinus reflex remains elicitable. *A:* before section. *B:* after section. ↑ re-insertion of the cannula into the coronary sinus. Other signs as in *Fig. 2*

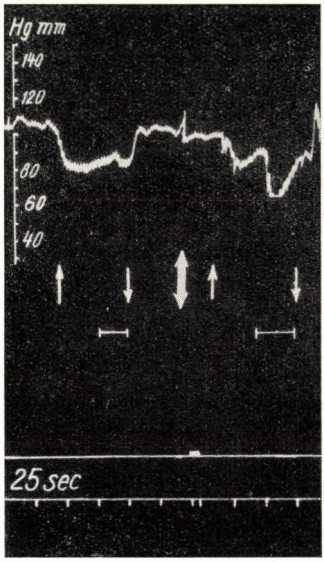

Fig. 7. Effect of oblique midbrain section (plane IV, *Fig. 1)*. Explanation see in text. Signs as in *Fig. 2*

sectioning. In a further group (7 animals), however, blood pressure increased gradually to 200—250 mm Hg in a few minutes after performing the section, and persisted at that level, often till the end of the experiment. This phenomenon seems to be due to a traumatic excitation of some upper brain stem

system, because at necropsy no evidence suggesting some other mechanism (*e.g.* increased cerebral pressure caused by intracranial haemorrhage) was revealed. It was remarkable, however, that while the phenomenon lasted, the coronary reflexes could be evoked in none of the cases; in 5 of 7 such animals stimulation of the receptor was ineffective, and in 2 a paradoxical pressor response was obtained. The reflexes could be only elicited if section was not followed by a pressor reaction, or if this had ceased after a lapse of time.

The results obtained in Group 3 suggest that the elicitability of the coronary sinus reflex requires the unaffected function of the entire mesen-

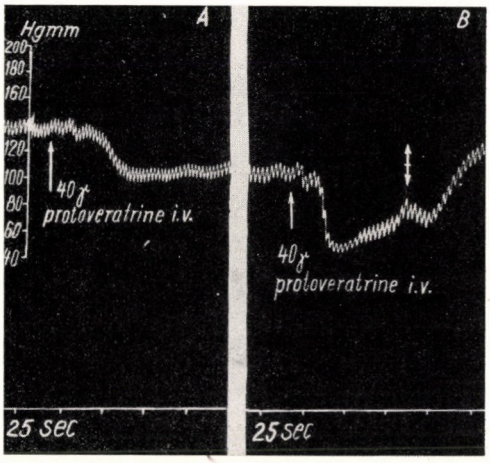

Fig. 8. After sectioning in the uppermost portion of the mesencephalon the coronary chemoreflex is elicitable. *A:* before section. *B:* after section. ↕ bilateral vagotomy

cephalon. The same applies, according to our results, to the coronary chemoreflexes. As shown in *Fig. 8*, the durable hypotension, *i.e.* the reflex stabilization of blood pressure in response to protoveratrine, set in even after the brain stem had been severed at the upper margin of the mesencephalon, in sharp contrast to the results obtained in Group 2 *(Fig. 5)*. The complete analogy in the behaviour of the coronary sinus and coronary chemoreflexes supplies thereby a strong indirect proof of the validity of our earlier finding that these two reflexes are fundamentally identical.

Discussion

Our experiments have revealed that the two reflexes elicitable from the left coronary vascular bed and showing many opposite properties, *viz.* the coronary sinus reflex and the coronary depressor reflex, possess sharply distinct localizations in the central nervous system. The coronary depressor

reflex, like the buffer reflexes, is elicitable namely even when the medullary vasomotor centre is separated from the higher nervous segments. In contrast, the coronary sinus reflex can only be elicited when the mesencephalic structures remained in connection with the lower vasomotor centres.

The fact that the coronary sinus reflex is a complex phenomenon, distinct in its properties (hypotension of stabilizer nature, "all or nothing" reflex response, trigger-like activity, *etc.*) from the buffer-type depressor reflexes, fits well into the observation that the former reflex is realized in more complicated central nervous structures. According to our earlier investigations (SZENTIVÁNYI and JUHÁSZ-NAGY 1962), the coronary depressor reflex, whose receptors presumably lie in the wall of the coronary arteries, possesses properties identical with those of the buffer reflexes. Correspondingly, the latter reflex is more resistant to the effect of hypnotic drugs than the extremely sensitive coronary sinus reflex, and until now this circumstance has been used in the first place for the differentiation of the two reflexes (SZENTIVÁNYI and JUHÁSZ-NAGY 1962). The present findings have made it possible to distinguish them more reliably.

Our veratrine experiments may be considered to represent the first effort in order to locate the central nervous connections of the coronary chemoreflex. The results have supported the earlier finding that the coronary sinus and the coronary chemoreflexes had the same receptor zones (JUHÁSZ-NAGY and SZENTIVÁNYI 1961). For evoking the coronary chemoreflex, the bulbar vasomotor centre is just as insufficient as for evoking the coronary sinus reflex. After the pons has been transected, veratrine does not induce the characteristically persisting hypotension, only a temporary, slight depressor effect could be observed at the most. Using small doses stimulating only the coronary sinus receptors protoveratrine produced no effect. When, however, the brain stem has been divided at the superior margin of the mesencephalon, veratrine exerts its stabilizing effect without fail.

The results would mean more if we knew as much about the function of the systems controlling circulation in the upper brain stem as we know about the bulbopontine centres. This, however is by far not the case (UVNÄS 1960; OBERHOLZER 1960). From the investigations of ALEXANDER (1946) BACH (1952), LINDGREN and UVNÄS (1956), AMOROSO *et al.* (1954) OBERHOLZER 1955) we know well the location of the pressor fields in the medulla oblongata and of the "depressor points" corresponding to the sites of synapse of the aortic arc and carotid sinus afferents. Recently, PÓRSZÁSZ *et al.* (1962) have proved that the effects of these buffer reflexes are based on a direct inhibition of the vasomotor centre, at the level of the medulla oblongata. It may be surmised that the coronary depressor reflex, the behaviour of which after transection of the brain stem was always analogous to that of the buffer reflexes, attacks the bulbar vasoconstrictor system in a similar fashion.

We know much less about the role played by the mesencephalic nervous elements in the control of blood pressure than about the function of the bulbar structures serving the same purpose.

It remains certain that significant pressor and depressor responses could be evoked from the mesencephalon mainly by stimulating the tegmentum and the central grey matter (DANILEWSKY 1875; PRUS 1899; HESS 1938; THOMPSON and BACH 1950), although it is unclear whether these effects arise as a result of a stimulation of independent "centres" or of pathways merely passing through this area. DANILEWSKY and PRUS, the pioneers in this field of research in the past century, already emphasized the complexity of the circulatory effects of mesencephalic stimulation; *i.e.* the blood pressure reactions to the stimulation of these structures were accompanied by vegetative and somatic effects extending to several spheres, which is indicative of a higher, integrative activity of the mentioned system. The specificity of the mesencephalic vasomotor reaction, postulated by the above-mentioned authors, has recently been confirmed (HUNSPERGER 1956). It is also beyond doubt that at least some of the mesencephalic vasomotor elements form a system absolutely independent of the function of the bulbar vasomotor centre (LINDGREN 1955; LINDGREN and UVNÄS 1956).

The fact that the elicitability of the coronary sinus reflex is bound to the intactness of the mesencephalon fits well into those outlined above. We reported earlier that the coronary sinus reflex could not be considered such a specific circulatory reflex as the classical buffer reflexes are. For example, the somatic reflex inhibition elicitable from the former is much more intense than the similar effect of the buffer reflexes (SZENTIVÁNYI and JUHÁSZ-NAGY 1961). The protracted stabilizing effect of the reflex, as well as its often observable trigger-like activity are by themselves indicative intimate connections with such complex nervous centres as actually exist in the mesencephalon. At the same time we do not want to claim that the stabilization of blood pressure induced by the coronary sinus reflex is based merely upon the alterations of mesencephalic function, when the nervous system is absolutely intact. All we wish to emphasize in this regard is that the mesencephalon may be considered to be the lowest cerebral segment the intactness of which is required for the stabilization to take place at all.

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MECHANISM OF THE HYPOTHALAMIC CONTROL OF TSH SECRETION: EXPERIMENTS IN VITRO

By

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The effect of hypothalamic extract, posterior pituitary extract and synthetic oxytocin on TSH release, ^{32}P uptake and oxygen consumption of anterior pituitary slices of the dog and rat has been examined *in vitro*.

It has been shown that the hypothalamic and posterior pituitary extracts induced a considerable increase in TSH release of the anterior pituitary slices. A similar effect of oxytocin has been observed earlier.

In addition, the hypothalamic and posterior pituitary extracts and synthetic oxytocin produced a considerable rise in ^{32}P uptake and oxygen consumption of the anterior pituitary slices.

While there are several data indicating the neurohumoral nature of the hypothalamic control of TSH secretion by the anterior pituitary (HARRIS 1955; BROWN-GRANT *et al.* 1957; NIKITOVICH-WINER and EVERETT 1958), the results of the experiments aiming at isolation and determination of the hypothalamic substance responsible for this control are divergent. Several authors have observed that the hypothalamic extract increased TSH secretion considerably (BARTHOLOMEI and MARCHETTO 1955; OTTAVIANI and AZZALI 1955; BAKKE and LAWRENCE 1958; SCHREIBER 1956) while others have failed to note such an effect (REICHLIN *et al.* 1963; FLORSHEIM *et al.* 1957).

There is a similar lack of uniformity of the results concerning the nature of the substance responsible for the control. Many authors have pointed to the importance of the hormones of the posterior pituitary. Some of the data indicate that the hormones of the posterior pituitary increase TSH secretion and thus may play a role in the control (FRAJA and MARTINI 1953; DUBREUIL and MARTINI 1956; BOTTARI 1957; ADAMS and PURVES 1955; MAEDA 1962; ROSNER *et al.* 1962; GARCIA *et al.* 1962; GILBERT-DREYFUS *et al.* 1960), while according to others they exert no such effect (ARIMURA *et al.* 1956; ISLER 1959; CROSSON *et al.* 1960).

SHIBUSAWA *et al.* as well as SCHREIBER have demonstrated a specific hypothalamic substance (TRF) which acts on TSH secretion (SHIBUSAWA *et al.* 1956a, 1959; SHIBUSAWA 1960; SCHREIBER 1961).

In earlier experiments we have called attention to the possible regulative role of the posterior pituitary hormones first of all of oxytocin (KOVÁCS and VÉRTES 1962). The present experiments were concerned partly with the effect

of the posterior pituitary hormones on the uptake of ^{32}P by the anterior pituitary and on its oxygen consumption, and partly with the effect of the hypothalamic and posterior pituitary extracts on the TSH release, ^{32}P uptake and oxygen consumption of the anterior pituitary.

Methods

The experiments were carried out on surviving slices of the anterior pituitary of dogs and rats. Likewise, the extracts used in these experiments were prepared from the hypothalamus and posterior pituitary of dogs and rats. To obtain the necessary tissues the dogs and rats were killed by an intracardial injection of air and by stunning, respectively, the skull was opened immediately and the brain and pituitary were removed.

The posterior lobes were carefully separated, the anterior lobes were halved and their weight was determined with a torsion balance with 0.2 mg precision. In the case of rats the half-anterior lobes were placed in incubation tubes holding 0.4 ml buffer solution. Each tube contained the halves of the anterior pituitaries of two rats; a pair of tubes containing the pituitaries of the same two animals were treated together, one of the pair serving as control. In the case of dogs the sliced and measured halves of the anterior pituitary lobes were paired, as in the case of the rats, and placed in incubation tubes containing 1.5 ml buffer solution. As incubation medium, *Krebs-Ringer* bicarbonate buffer, pH 7.4, containing 300 mg per 100 ml of glucose, and 2 μC of $Na_2H^{32}PO_4$ per tube was used.

The hypothalamic extract was prepared from dog and, in some cases, rat hypothalamus. The excised pieces of hypothalamus contained that part of the base of the brain which was continuous with the hypophysis and also the brain tissue forming and surrounding the wall of the third ventricle. As a control, cortical tissue of identical weight from the parietal region, and in the case of rats from the temporal region was used. Their weights having been determined, the excised pieces of hypothalamus and cortex were placed into acetone at 0° C for 48 hours. The acetone was changed twice. Then the tissue was pulverized, extracted with 2 N acetic acid, centrifuged, washed twice with 2 N acetic acid, and the supernatant was evaporated *in vacuo* and the residue dissolved in the buffer. When preparing the extract from the posterior hypophyses of the rats and dogs, the posterior hypophyses were homogenized with 0.25 per cent acetic acid, centrifuged and washed twice with 0.25 per cent acetic acid. After centrifugation the supernatants were evaporated *in vacuo* and the residue was dissolved in buffer solution.

Immediately before use, the pH of the extracts was adjusted to 7.4 with 10 per cent $NaHCO_3$, then volumes of 0.1 ml were measured in test tubes, which thus contained amounts corresponding to about $^1/_5$ of a dog hypothalamus (200 mg wet tissue), and to half a posterior pituitary, respectively. An identical volume of cortical extract or buffer solution was measured into the control tubes.

When examining the effect of oxytocin, 1 I. U. of synthetic oxytocin (Richter, Budapest) adjusted to pH 7.4 was added to the corresponding tubes, and the same amount of buffer solution to the control tubes. Subsequently, the tubes were incubated in an oxygen atmosphere at 37° C for 60 minutes.

After incubation the TSH content of the media was determined *in vitro* as described previously (KOVÁCS and VÉRTES 1962). After incubation the tissue pieces were washed three times in large amounts of distilled water in order to remove the residual isotope, then they were incinerated at 400 $\pm$ 20° C, and their radioactivity was determined with an end-window *Geiger-Müller* tube.

The oxygen consumption of the slices of the dog anterior pituitary was determined with the usual *Warburg* method in 5 ml *Warburg* flasks containing 0.7 ml of buffer solution. Into each flask one half of an anterior pituitary was placed, the flasks being arranged in pairs so that one pair of flasks held the two halves of the anterior pituitary of the same animal. One of the flasks in each pair served as a control. The extracts and oxytocin were used in the same amounts as described above.

Results

After incubation of the anterior pitui tary slices with the dog hypothalamic extract the *in vitro* uptake of ^{131}I by the thyroid slices in response to the incubating medium was considerably higher than after treatment with the medium of anterior pituitary slices incubated with cortical extract *(Fig. 1)*.

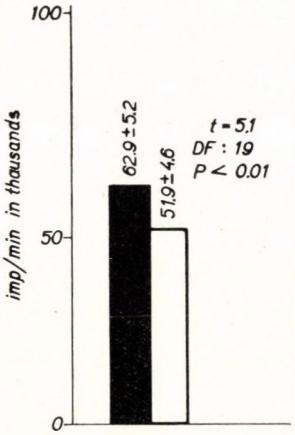

Fig. 1. Effect of the medium of slices of dog anterior pituitary incubated with dog hypothalamic extract (black column) and dog cortical extract (white column) on ^{131}I uptake by dog thyroid slices

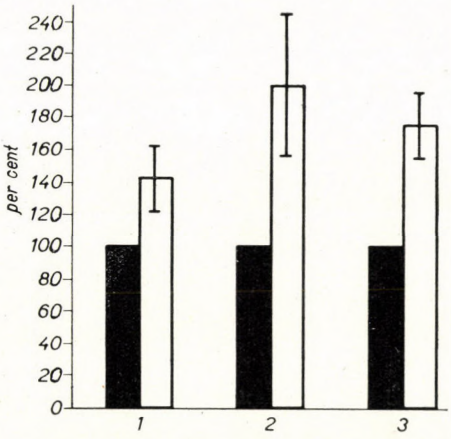

Fig. 2. Effect of dog hypothalamic extract (1), rat posterior pituitary extract (2), and synthetic oxytocin (3), on oxygen consumption of dog anterior pituitary slices. The values shown are percentages of the control values (black columns), which were taken for 100 per cent, on the basis of oxygen consumption computed in μl of O_2/mg of wet anterior pituitary tissue/hr. In each of the three cases the pairs of columns represent mean values of oxygen consumption by the anterior pituitary of five dogs

As compared with the controls the uptake of ^{131}I by the thyroid slices also increased after incubation with the medium of anterior pituitaries incubated with the posterior pituitary extract *(Table I)*.

The hypothalamic and posterior pituitary extracts used in these experiments exerted no direct influence on the uptake of ^{131}I by the surviving thyroid slices.

The effect of the extracts and synthetic oxytocin on the uptake of ^{32}P by the slices of the anterior pituitaries is shown in *Table II*. It can be seen that, as compared with the controls, the hypothalamic extract, the posterior pituitary extract and synthetic oxytocin considerably increased ^{32}P uptake by the slices of the anterior pituitary during incubation. The extracts and synthetic oxytocin caused a similar increase in the oxygen consumption of the slices of the anterior pituitaries *(Fig. 2)*.

Table I

Effect of the medium of rat anterior pituitary slices incubated with rat posterior pituitary extract on ^{131}I uptake by rat thyroid slices

Imp./min/100 mg of thyroid tissue

Incubated with posterior pituitary extract	Control
32,200	26,600
56,000	47,000
37,500	31,200
12,300	12,000
26,600	24,000
26,000	21,900
15,600	10,900

Table II

Effect of dog hypothalamic extract, rat posterior pituitary extract, and synthetic oxytocin, on ^{32}P uptake by dog and rat anterior pituitary slices

Imp./min/10 mg anterior pituitary tissue

Anterior pituitary of dogs	Hypothalamic extract	Cortical extract	Posterior pituitary extract	*Krebs-Ringer* bicarbonate buffer	Oxytocin	*Krebs-Ringer* bicarbonate buffer
	2384	1928	8000	6000	2130	1800
	2739	1913	8670	7660	1890	1510
	3727	3363	3016	2413		
	1600	840	6150	3260		
	3000	1940				
	2550	1000				
	2870	2290				
	3100	1800				
Anterior pituitary of rats						
	14,280	9142	25,000	24,000	16,625	15,500
	18,888	13,610	22,300	11,000	16,545	8090
	14,971	9668	22,850	19,450	12,800	7600
	13,085	7567	28,100	12,100	14,250	9650
			19,000	15,800	17,650	12,910
					18,120	16,710
					16,700	15,320

Discussion

The hypothalamic and posterior pituitary extracts have been observed to exert a considerable TSH-releasing effect. The same effect of oxytocin has been observed in our earlier experiments (KOVÁCS and VÉRTES 1962). In addition to the increased TSH release a similar increase in ^{32}P uptake by the anterior pituitary slices and in their oxygen consumption was observed in response to the extracts as well as oxytocin.

It has been shown (BARTHOLOMEI and MARCHETTO 1955; OTTAVIANI and AZZALI 1955; BAKKE and LAWRENCE 1958; SCHREIBER 1956) that hypothalamic extracts increase TSH secretion. In several experiments SHIBUSAWA *et al.* (1956a, 1956b; SHIBUSAWA 1960) have demonstrated the presence of the factor acting on TSH secretion, first of all in the anterior hypothalamus and in the posterior hypophysis, and, in a smaller quantity, in the cerebral cortex, and also in the body fluids; they also succeeded in isolating this factor in a relatively high degree of purity and termed it TRF. REICHLIN *et al.* (1963) on the other hand, found TRF ineffective in the rat.

SCHREIBER (1961) also isolated a hypothalamic substance which he found to induce a considerable increase in TSH secretion.

The increase in TSH release observed on the effect of hypothalamic and posterior pituitary extract in the present experiments seems to confirm the existence of a hypothalamic factor controlling TSH secretion.

As to the nature of the hypothalamic factor in question, according to SAITO and TANI (1960), SHIBUSAWA's TRF would represent a specific substance which acts on TSH secretion but has no ACTH-releasing effect or oxytocic and vasopressor activity. According to ABÉ (1961) the substance is neither a protein nor a lipid, but in all probability a peptide.

SCHREIBER *et al.* (1963) also found a substance of peptide nature to be effective in influencing TSH secretion.

The controlling role of the posterior pituitary hormones has often been questioned. However, more and more data now indicate that they enhance the activity of the anterior pituitary-thyroid system. In earlier experiments we also observed a strong TSH releasing effect of oxytocin. Oxytocin also proved to be effective in preventing the decrease in anterior pituitary-thyroid function in rats with anterior hypothalamic lesion, while in normal rats oxytocin treatment caused no significant change in the activity of the anterior pituitary-thyroid system (KOVÁCS *et al.*, in press). It is therefore undecided whether the posterior hormones exert a specific controlling effect on TSH secretion, or their action is nonspecific.

The present results do not permit conclusions as to the precise nature of the hypothalamic factor. Since the extracts used in these experiments had a strong oxytocic activity, the possible role of oxytocin cannot be excluded.

The experiments of SCHREIBER and KMENTOVÁ (1959) and SOBEL (1961) revealed a relationship between TSH secretion and the acid phosphatase activity of the anterior pituitary. Although the importance of the change in acid phosphatase activity is not clear, it doubtelessly points to a change in phosphorus metabolism. The fact that in the present experiments the hypothalamic and posterior pituitary extracts and synthetic oxytocin, each of which was found to increase TSH release, were all causing a considerable increase in ^{32}P uptake by the anterior pituitary slices, might be brought into connection with the increase in the acid phosphatase content of the anterior pituitary in the case of increased TSH secretion. On the basis of the latter observation, the increased ^{32}P uptake shows a relationship with the effect of the extracts and oxytocin on TSH release. The increase in oxygen consumption of the anterior pituitary slices in response to the extracts and oxytocin may also be connected with their action on TSH release. This is indicated also by LEVEY's data (LEVEY and ROBERTS 1957) according to which in conditions associated with increased TSH secretion there occurs a considerable rise in the oxygen consumption of the anterior pituitary.

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THE EFFECT OF NERVE DEGENERATION AND REGENERATION ON THE CALCIUM-RELEASE IN THE POST-JUNCTIONAL CYTOPLASM OF THE MOTOR END-PLATE

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Supramaximal stimulation as well as the *i.p.* injection of carbaminoylcholine or anticholinesterases, result in a characteristic cytological alteration (granularization) of the post-synaptic sarcoplasm ("sole plate") of the myoneural junction. The granules appear to contain liberated calcium. Degeneration of the motor nerve soon leads to the cessation of this kind of well-localized calcium liberation, whereas a linear reaction appears at the surfaces of the muscle fibres. The linear reaction persists until the regeneration of the motor nerve fibres, when, however, the original and well-localized calcium liberation returns again. It is assumed that calcium liberation is due to synaptically released acetylcholine and that the linear reaction in the denervated muscle is due to the expansion of the acetylcholine-sensitive areas.

It has been reported previously (CSILLIK and SÁVAY 1963) that electrical stimulation of the motor nerve results in the appearance of histochemically detectable free calcium in the sole-plasm (post-junctional* cytoplasm) of the motor end-plate. It has been found, furthermore, that a similar calcium release could be achieved by injecting animals intraperitoneally with neostigmine or carbaminoylcholine (SÁVAY and CSILLIK 1964) which suggests that the post-junctional calcium liberation is a consequence of the depolarization of the post-junctional membrane. In the present paper, the calcium-release in denervated muscle will be described, as well as the effect of nerve regeneration on the calcium-releasing post-junctional structures. From these experiments it appears that post-junctional calcium-release is regulated by neural influences in the same way as the acetylcholine-sensitivity of the muscle.

Methods

Albino rats of 200—250 g body weight were used. The left hemidiaphragm of 16 animals was denervated intramuscularly by means of the following procedure.

Under pentobarbital anaesthesia, laparotomy was performed, the intramuscular course of the left phrenic nerve in the diaphragm was identified and the ventral branch of the nerve was transected by an intramuscular incision, directed parallel to the course of the muscle fibres. Care was taken to perform the incision as small as possible (1—2 mm long) and to close the abdominal wound quickly. These precautions are necessary to avoid a sudden death of the animals from pneumothorax.

* The term "post-junctional" seems to be more adequate than the widely used expression "post-synaptic".

As a result of this operation, nerve fibres in the ventral part of the hemidiaphragm undergo degeneration, while those in the dorsal part remain intact.

To evoke calcium-liberation in the post-junctional cytoplasm, either neostigmine (1 mg/kg) or carbaminoylcholine (1 mg/kg) was injected intraperitoneally. 6—10 minutes later (when not dead spontaneously) the animals were killed by decapitation. The left hemidiaphragm was excised immediately, and samples from both the ventral and the dorsal part were frozen in solid carbon dioxide for 30 minutes.

Histochemical staining of free calcium was achieved by a heavy metal exchange reaction. 20 μ thick sections were cut in a cryostat and transferred immediately in the frozen state to acetone (2 minutes), floated in 2 per cent sodium barbital solution (30 seconds), treated with 5 per cent cobalt nitrate or lead nitrate solution (5 minutes), washed thoroughly in distilled water (3 times 2 minutes) and developed subsequently in 1 per cent yellow ammonium sulphide solution. Finally, the sections were washed again in distilled water and mounted either in glycerol or, after dehydration, in Permount.

Results

In muscles with an intact innervation, the intraperitoneal injection of neostigmine or carbaminoylcholine resulted in the appearance of histochemically detectable calcium in the post-junctional cytoplasm of the motor end-plate, confined to granules 1—2 μ in size. In sections treated with the heavy metal exchange reaction the localization of free calcium was indicated by a brown or black colour. In control sections, stained with a 1 per cent aqueous solution of *Alizarin red*, in the same localization a granular red precipitate could be observed. Unfortunately, the red colour of the calcium-alizarin complex is unstable and the preparations stained by this method fade completely in the course of a few days. Sections stained by the exchange reaction did not show any signs of alteration in several months.

The pattern of calcium-release resembles superficially that seen in cholinesterase-stained sections *(Fig. 1)*. Under high power, however, it is obvious that, while cholinesterase activity is located in the post-junctional membrane ("subneural apparatus"), the heavily stained granules in calcium-stained sections are confined to the cytoplasms of the fundamental cells of the sole plasm *("Doyère-hillock") (Fig. 3)*. In these preparations, also the outlines of the fundamental nuclei were usually stained.

The reaction obtained in calcium-stained sections is distinctly different from the pattern seen in supravitally lead-treated preparations. The supravital lead reaction (SÁVAY and CSILLIK 1959) as well as the optical birefringence after lead treatment (CSILLIK 1963) shows the same localization as the cholinesterase reaction. If employed supravitally, lead combines with lipoproteins in the post-synaptic membrane. On the other hand, lead "stains" the released calcium as a result of an exchange-reaction, provided the muscle tissue or section has been briefly pre-fixed in acetone.

On the first and second postoperative days, injection of neostigmine or of carbaminoylcholine resulted in practically identical patterns of calcium liberation as in normal muscles. On the third day after transection of the

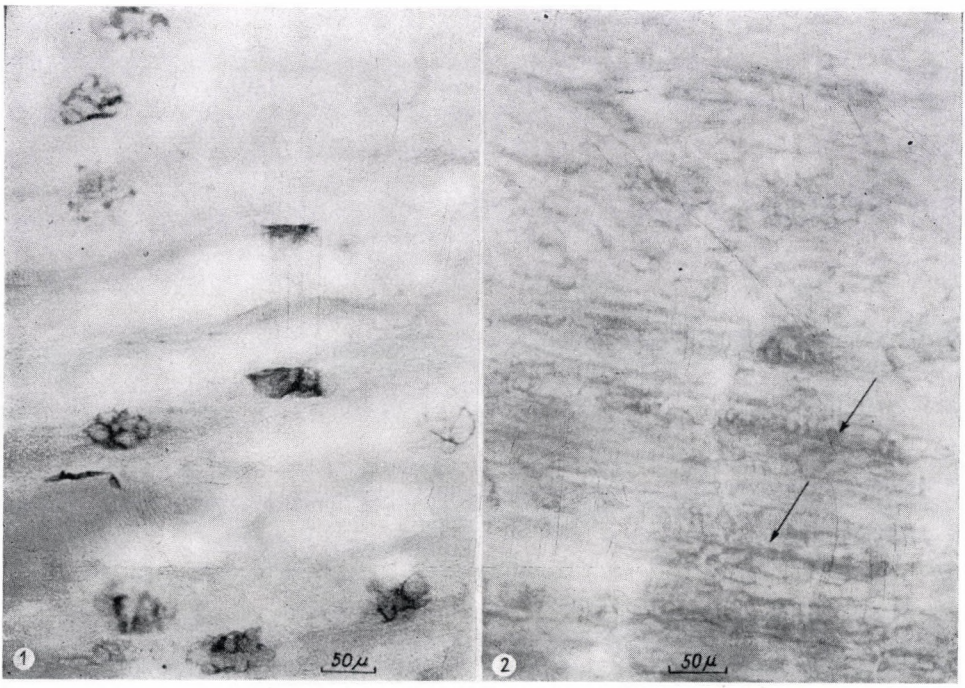

Fig. 1. Calcium release in the post-junctional cytoplasm of motor end-plates in the rat diaphragm, 6 minutes after intraperitoneal injection of 1 mg/kg of carbaminoylcholine. × 200

Fig. 2. Calcium release in the denervated diaphragm of the rat, 4 days after intramuscular transection of the phrenic nerve, 6 minutes after intraperitoneal injection of 1 mg/kg of carbaminoylcholine. No calcium release in the late motor endings; some release at the surface of muscle fibres (arrows). × 200

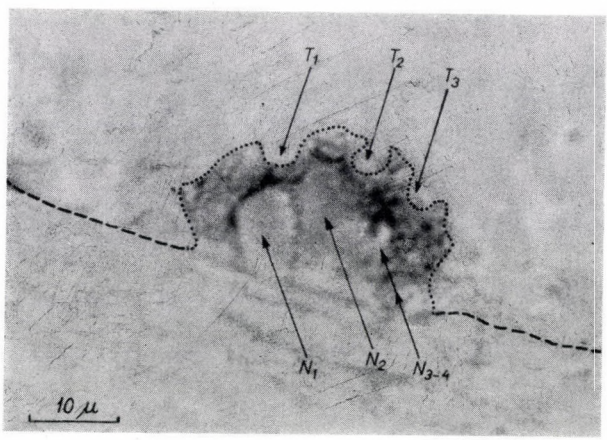

Fig. 3. Cross section of a motor ending in the rat's diaphragm, 8 minutes after intraperitoneal injection of 1 mg/kg of carbaminoylcholine. Calcium release clearly confined to the cytoplasms of the fundamental cells, making up the *Doyère-eminence*. $N_{1-2-3-4}$: fundamental nuclei. T_{1-2-3}: synaptic gutters of the telodendrial nerve branches. Dashed line: surface of muscle fibre. Dotted line: surface of *Doyère-eminence*. × 1500

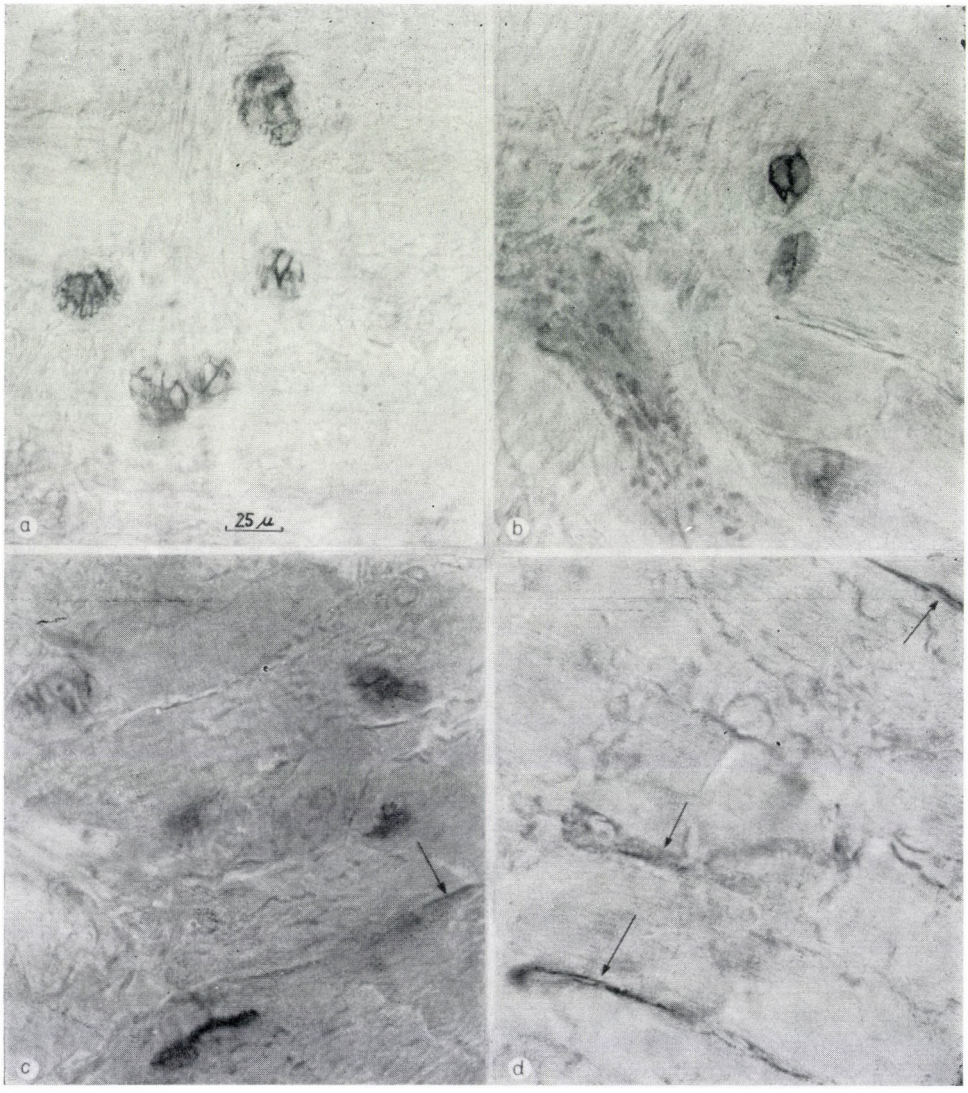

Fig. 4. Alterations in the morphology of the calcium-releasing structure as a result of nerve degeneration. 8 minutes after 1 mg/kg of carbaminoylcholine intraperitoneally. ×400

a) Normal muscle: calcium-release confined to motor end-plates:

b) 40 hours after denervation: in some end-plates strong calcium-release, in others the reaction is much weaker (B and C). D: degenerated nerve bundle;

c) 72 hours after denervation. Weak calcium-release still present in the end-plates. At the arrow the first signs of a "surface reaction" are to be seen:

d) 180 hours after denervation. No calcium release in the late end-plates; linear reaction appears at the surface of muscle fibres (arrows)

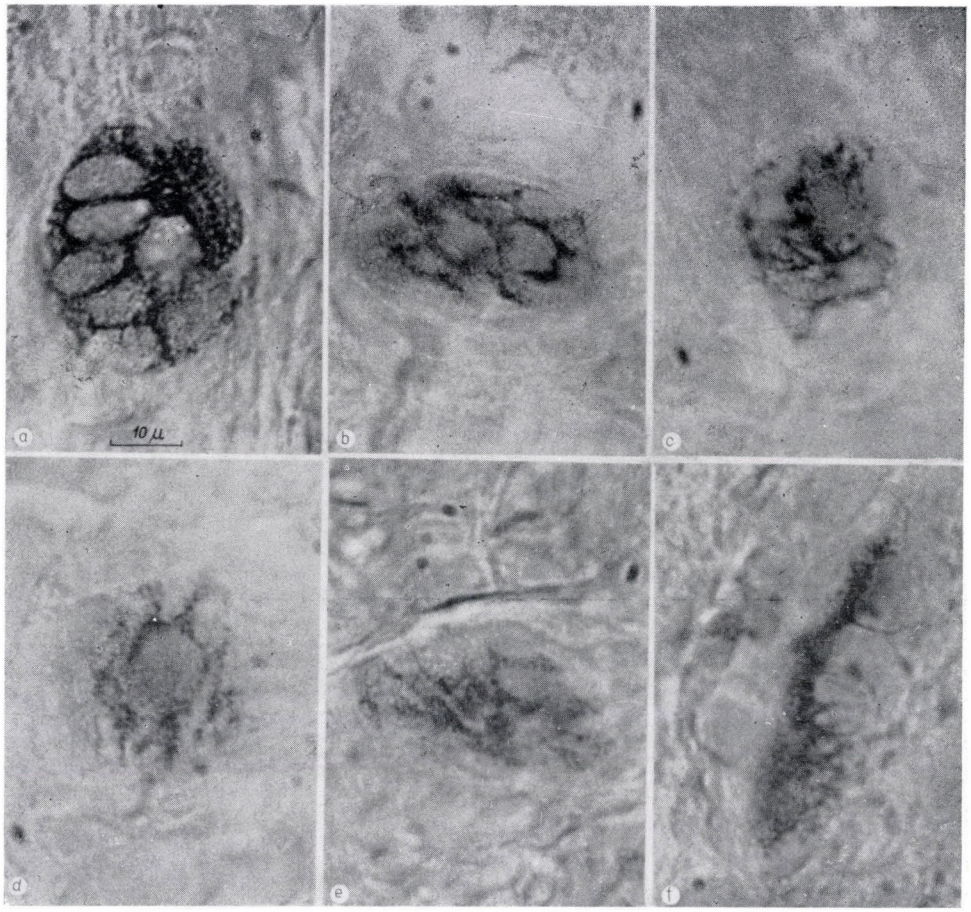

Fig. 5. Alterations in the morphological appearance of the calcium-releasing structures of individual myoneural junctions. Carbaminoylcholine, 1 mg/kg intraperitoneally, 8 minutes. ×1500

a) normal
b) normal
c) 24 hours after denervation
d) 40 hours after denervation
e) 48 hours after denervation
f) 72 hours after denervation

Note that in all cases the heavily stained granules indicating calcium release are located in the cytoplasms of fundamental cells; nuclei are devoid of calcium-reaction, although nuclear membranes show up in the patterns

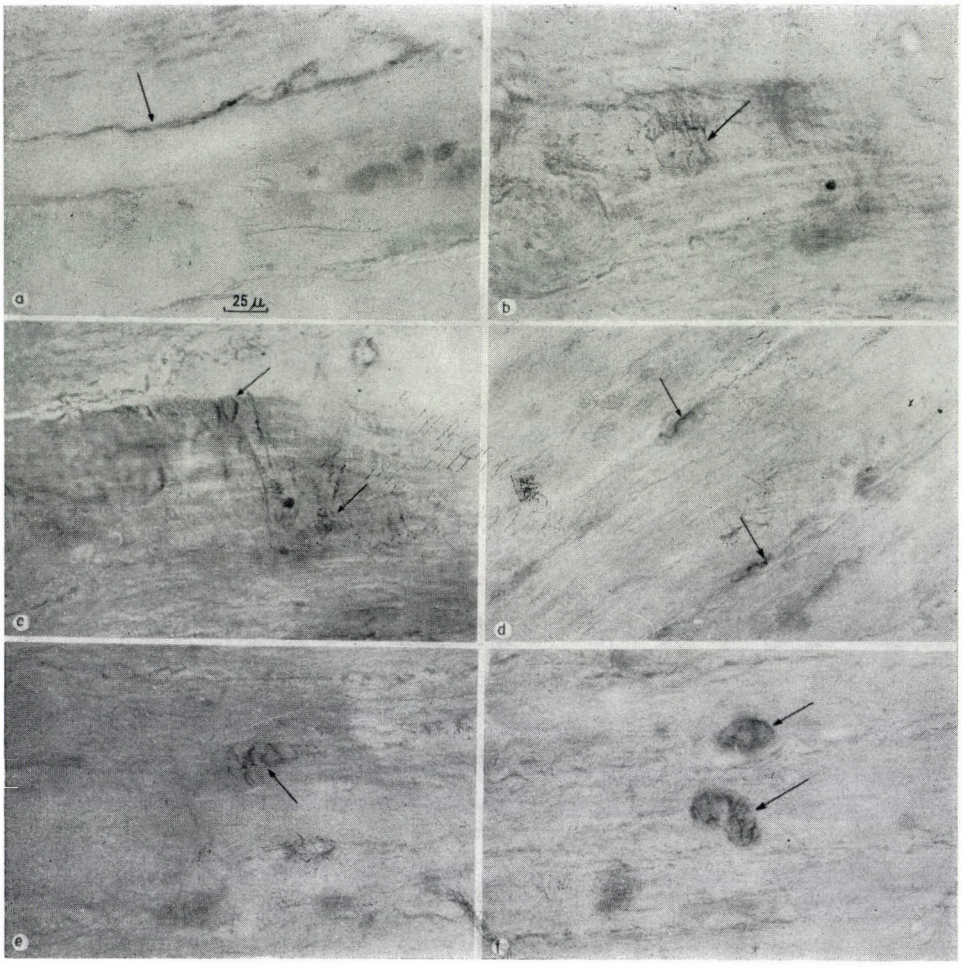

Fig. 6. Influence of nerve regeneration on the morphological pattern of the calcium releasing structure. Carbaminoylcholine, 1 mg/kg intraperitoneally, 8 minutes. ×400

a) Linear reaction on the surface of a muscle fibre, 28 days after transection of the nerve;

b) First signs of localized calcium-release around fundamental nuclei, at the regenerating nerve endings (arrow). 30 days after transection of the nerve;

c) The same as *Fig. 6/b*;

d) Calcium-releasing structures in a cross-section, 36 days after transection of the nerve; nearly complete regeneration;

e) Regenerated calcium-releasing structure, 36 days after transection of the nerve. Although morphologically nearly normal, this structure still shows a weak calcium-release;

f) Two calcium-releasing structures at regenerated nerve endings, 36 days after transection of the nerve (arrows)

nerve, the reaction became slightly weaker and faded or disappeared on the fourth postoperative day *(Fig. 2)*. From the fifth postoperative day on, neither neostigmine nor carbaminoylcholine was capable of evoking calcium liberation. The fine structural alterations of the calcium-releasing cytoplasm are shown in *Fig. 5*.

The end-plate area remained irreactive during several weeks after transection of the phrenic nerve. On the seventh postoperative day, however, peculiar linear structures appeared on calcium-stained sections of neostigmine- or carbaminoylcholine-treated muscles. These structures were located usually in the neighbourhood of the late end-plates. Their length increased gradually in the next few days and reached 150—200 μ two weeks after denervation. As seen in *Fig. 4/d*, these linear structures actually correspond to rows of fine granules, located immediately under the muscle surface membrane, amongst the elongated nuclei of the muscle.

The pattern of these "linear structures" persisted until the 28th day, when the first regenerating sprouts of nerve fibres could be detected in the muscle *(Fig. 6/a)*. It appears that as soon as the regenerating nerve fibres approached the abandoned end-plate region, the "linear reaction" ceased and gave place to a well-localized calcium liberation in the cytoplasm of the fundamental nuclei of the end-plate *(Fig. 6/b—e)*. The reaction, weak at the first approach of regenerating nerve fibres, became more and more intense during the next days. 36 days after the nerve had been transected, the calcium-liberation evoked by neostigmine or by carbaminoylcholine was nearly the same as in normally innervated muscles, with the exception of scattered, still only weakly reacting end-plate areas *(Fig. 6/f)*.

Discussion

It is a well-known fact that muscle activity is accompanied by a release of bound calcium which takes place throughout the entire length of the muscle (Bianchi and Shanes 1959). It appears, therefore, that the well-localized calcium staining in the end-plate territory, observed in our experiments on normal muscles, represents only that specific compartment of muscle calcium the release of which is closely associated with end-plate activity. Accordingly, in the following the term "calcium release" will be used to designate the liberation of this end-plate-bound compartment only; all other sources of muscle calcium, unrelated to junctional activity, will be neglected.

The fact that acetylcholine (accumulated as a result of a long-lasting stimulation or preserved by a cholinesterase inhibitor) or an acetylcholine-like substance (carbaminoylcholine) leads to such an easily detectable histological phenomenon like the appearance of calcium, makes it possible to

decide from a histological section whether or not the post-junctional membrane is depolarized. Yet one cannot decide from the morphological pattern whether the strictly localized calcium-release in the end-plate territory is due

(i) to the fact that only this restricted area is sensitive to acetylcholine;

(ii) or that the acetylcholine-sensitive area is more widespread but only a restricted area is capable of releasing calcium;

(iii) or that both the calcium-releasing and the acetylcholine-sensitive areas are, in fact, more widespread, and the restricted localization of liberated calcium is due to the fact that only the cellular elements of the sole-plate take up calcium, wherever it has been liberated.

Investigations carried out by means of a micropipette application of acetylcholine (GINETZINSKY and SHAMARINA 1942; AXELSSON and THESLEFF 1959; THESLEFF 1960; DIAMOND and MILEDI 1962; MILEDI 1960a, 1960b) suggest that the acetylcholine-sensitive area is about 20 times larger than the actual size of the end-plate and, consequently, that of the calcium-liberating structure of the sole plate. Thus possibility (i) appears to be ruled out by these experiments.

On the other hand, the fact that not only the granules in the fundamental cell cytoplasm (which are most probably identical with the large siderophilic mitochondria called "telosomes" by NOEL 1957) but also the nuclear membranes of these cells and, occasionally, also adjacent parts of the sarcoplasmic reticulum stain for calcium, suggest that liberated calcium might be attached to various structures in the vicinity of its liberation. It seems highly improbable, namely, that nuclear membranes should be the main stores of bound calcium. However, considering that the sarcoplasmic reticulum extends to the whole length of the muscle fibre, and, in spite of this, only those parts of the reticulum which are close to the end-plate territory show signs of calcium staining (after prolonged effect of the releasing procedures), it stands to reason to assume that calcium is liberated in the cytoplasm of the fundamental cells in the sole plate, and it is attached to several cytological elements in its very neighbourhood. Therefore, it appears that possibility (ii) is the correct answer to the question where acetylcholine acts and where calcium is liberated.

The lack of neostigmine-induced calcium liberation after degeneration of the motor nerve terminals could be a simple result of the acetylcholine depletion of the nerve endings after degeneration. Consequently, the cholinesterase inhibitor cannot preserve any transmitter by impeding with the hydrolysis of the ester (the junctional acetylcholinesterase remains virtually intact for several months after transection of the nerve (SÁVAY and CSILLIK 1956). The fact, however, that not only neostigmine, but also carbaminoylcholine was ineffective in inducing calcium-liberation from denervated sole plates suggests that the post-junctional membrane, and/or the post-junctional cytoplasm, have undergone serious alterations during this period. It has been

shown by one of us (CSILLIK 1963) that the molecular structure of the lipoprotein material in the post-junctional membrane shows a re-arrangement shortly after denervation. It seems, therefore, that these molecular alterations change the physiological properties of the membrane in such a way that it cannot any more react with the transmitter (or transmitter-like substances) in the normal way.

The appearance of a slighter, but spatially more extended type of calcium liberation on the surface of denervated muscle fibres several days after transection of the motor nerve is a sign of the spreading of calcium-liberating stores. This is, in all probability, a consequence of the spreading of acetylcholine-sensitive areas, demonstrated also by the authors using the micropipette application technique (AXELSSON and THESLEFF 1959; GINETZINSKY and SHAMARINA 1942; MILEDI 1960a, 1960b; THESLEFF 1960). It is remarkable that here again the size of the acetylcholine-sensitive area appears to be about 20 times larger than the size of the calcium-liberating zone, since the values given by the afore-mentioned authors are in the range of 2—4 mm, while the length of the calcium-stained "linear structures" is 100—200 μ. It appears, therefore, that the calcium-liberating area actually represents a small, central zone of the acetylcholine-sensitive area, supporting again the validity of the assumption (ii) mentioned on p. 340.

As a result of nerve regeneration, the calcium-releasing area becomes restricted again to the region of the sole-plate. In these experiments, "regeneration" is in fact a simple re-innervation of the late end-plate territories, since previous studies have shown that during such a relatively brief period the structure of the post-junctional elements remains more or less intact and the regenerating nerve fibres simply grow into the abandoned end-plate areas (CSILLIK and SÁVAY 1958). The spatial restriction of the calcium-liberation runs parallel with the restriction of acetylcholine sensitivity, as shown by MILEDI (1960a, 1960b). Exactly the same kind of spatial restriction of acetylcholine sensitivity (DIAMOND and MILEDI 1962) and of calcium liberation (SÁVAY and CSILLIK 1964) was found during the development of muscle innervation in foetal and new-born muscles. It appears, therefore, that extrajunctional calcium-releasing areas are suppressed by the appearance of the innervation apparatus just in the same way as extrajunctional acetylcholine-sensitive areas are, both in the course of nerve regeneration and during ontogenetical development.

Acknowledgement

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A SIMPLE DEVICE FOR RECORDING THE MOVEMENTS OF UNRESTRAINED ANIMALS

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A device for the recording of phasic movements of freely moving experimental animals is described. The essence of the device consists in a small magnet fixed on a fine spring over an induction coil. The current induced by the movement of the magnet occurring in the course of phasic movements of the animal is amplified by any common biological amplifier and can be recorded simultaneously with the electrical manifestations of the brain. Oscillations of the system are damped by an electrically non-conductive fluid *(e.g.* mineral oil). The sensitivity of the device, adjustable within wide limits according to the actual requirements, allows the recording of the finest, even non-visible movements, *e.g.* vibrations caused by a cat's purr, heart beat, *etc.* The recording device can be fixed to the head of the animal, its small weight (5.5 g) does not interfere with the execution of any actions.

In the investigation of the neural organization of behaviour there is a frequent requirement for the simultaneous analysis of the movements of the experimental animals and of the changes of cerebral electrical activity. The usefulness of the electromyographic method for registration of movements of freely moving animals in experiments lasting for several months is doubtful because of the accompanying complications (displacement of electrodes, infections, *etc.*). Therefore efforts have been made to use simpler procedures instead of the complicated electromyographic method (DELGADO and STOLWIJK 1962; HECHT and BIELECKE 1960); one of the most common consists in cinematographic recording (DELGADO 1956; HUNTER and JASPER 1949; SCHWAB *et al.* 1954; STEWART *et al.* 1956).

In an earlier paper we described a simple device for recording startle reactions (SZABÓ *et al.* 1962). This method proved suited to register the slightest movements of the animal. At present we shall discuss a more developed variety of the device and its application in conditional reflex experiments.

The instrument which is fixed to the head of the experimental animal consists of a cylindrical metal casing containing a magnet attached to a spring and a mild iron coil. The device is 12 by 12 by 14 mm in size and 5.5 g in weight *(Fig. 1)*. The coil is made of copper wire 0.03 mm in diameter, its electrical characteristics are R = 800 Ohm, n = 1200.

The working principle of the device is as follows *(Fig. 2)*. A small magnet is attached to a helical spring. The lines of force starting from the magnet cross the turns of the coil. In the case of a relative displacement of

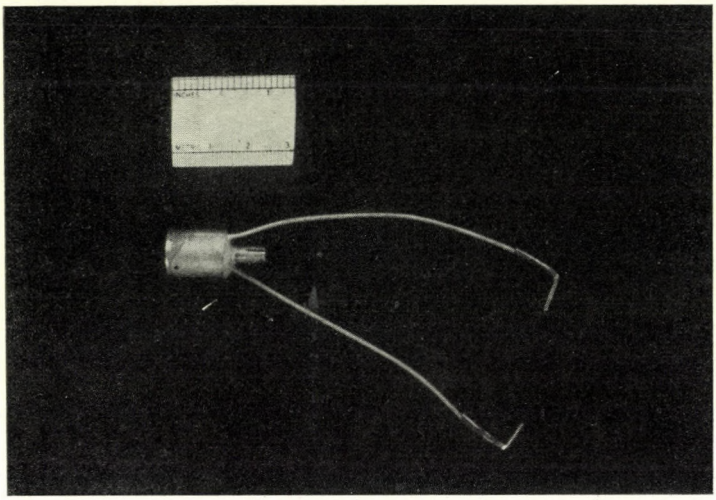

Fig. 1. Device used for recording movements

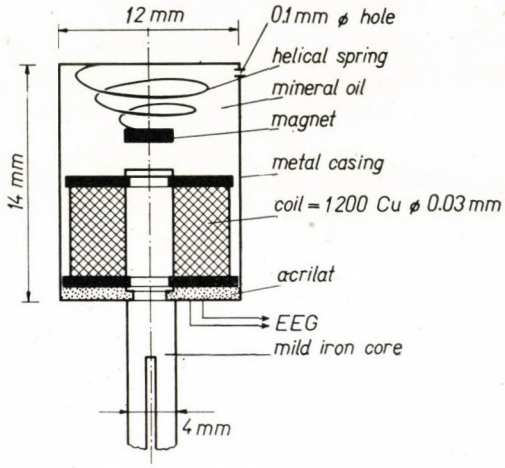

Fig. 2. Cross-section scheme of device (further details see in text)

the magnet, the number of lines of force crossing the coil undergoes a change and a potential difference is produced. This potential is amplified and recorded (ECG, EMG, EEG). In the course of head movements the relative displacement of the magnet is due to inertia.

During a moderate displacement of the head the amplitude of the potential given by the instrument is about 0.3—3 mV independently of the direction of movement. This independence is a consequence on the one hand of the relatively large air hole and of a specially developed magnetic field,

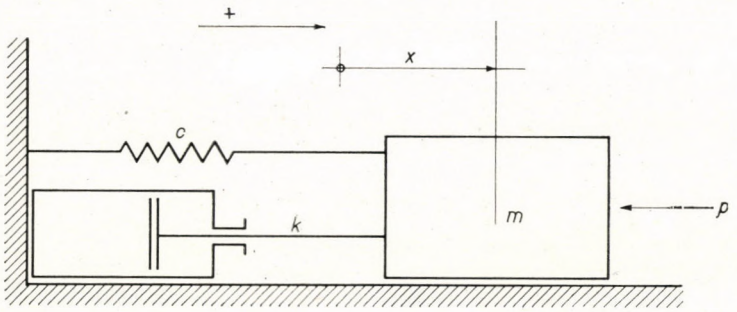

Fig. 3. Mechanical model of the device (further details see in text)

on the other hand of the fact that the constant of the helical spring as a mechanical swinging system is not changed whatever the direction of movements.

In the first version of our device (SZABÓ *et al.* 1962) the swinging system was damped by air, which resulted in a long lasting subsidence of oscillations. In order to adapt the device for the requirements of behavioural purposes — selective recording of different components of complex locomotor acts — the damping time of the system had to be shortened. To meet this requirement, the use of a non-conducting fluid as damping medium seemed promising. By using paraffin oil, damping of about 6 decibels could be reached.

The degree of damping was calculated on the basis of the following considerations. In the mechanical model of the device *(Fig. 3)*, the movement of its swinging system could be characterized by the differential equations

$$x'' + \frac{k}{m}x' + \frac{1}{mc}x' = 0$$

where c = the spring constant; m = magnet of m mass; x = the direction of displacement of magnet caused by the inertia of $P = ma$ force, and k= its damping in proportion to velocity.

The solution of the equation is

$$x = c_1 e^{\lambda_1 t} + c_2 e^{\lambda_2 t}$$

where both λ_1 and λ_2 are roots of the characteristic equation. After substitution, $\lambda^2 = x''$, $\lambda = x'$, $1 = x$, the characteristic equation is

$$\lambda^2 + \frac{k}{m}\lambda + \frac{1}{mc} = 0$$

and its roots are

$$\lambda_{1,2} = -\frac{k}{2m} \pm \sqrt{\left(\frac{k}{2m}\right)^2 - \frac{1}{mc}}.$$

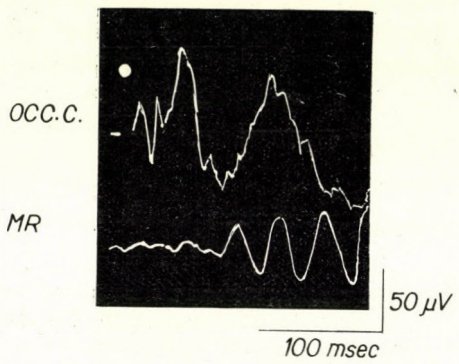

Fig. 4. Simultaneous oscilloscopic records of an occipital evoked potential elicited by flash (upper beam), and a startle reaction (lower beam)

The feature of the movement of the swinging system depends on the sign of radical quantity. If it is negative then the masspoint performs only a single sweep, if it is positive then oscillations of a damped character ensue where the logarithmic ratio of the subsequent deviations is

$$\vartheta = ln \frac{x_1}{x_3} = \frac{kT}{2m}$$

or in decibels

$$\vartheta_{db} = 20\, lg \frac{x_1}{x_3}.$$

The degree of damping given by this value of numeric dimension depends on the quality of the damping medium (in the present case the density and viscosity of the fluid applied).

A hole 0.5 mm in diameter was drilled in the side wall of its metal case to fill the *motimeter* with the damping fluid. Re-filling of the system if necessary can be accomplished through this hole by means of an injection syringe. The

closing of this aperture is inadvisable since the casing may crack due to the fluid's thermal expansion. The capillary forces keep the fluid from flowing out from the casing.

This method was successfully used for a comparative analysis of startle reactions and cortical evoked potentials elicited by light stimuli *(Fig. 4)*, (KLINGBERG and GRASTYÁN 1963).

A record obtained during alimentary conditional reflex performance is shown in *Fig. 5*. The conditional stimulus was a click of 1.5 cps frequency. The first click elicited a startle reaction (a), subsequently the cat turned its

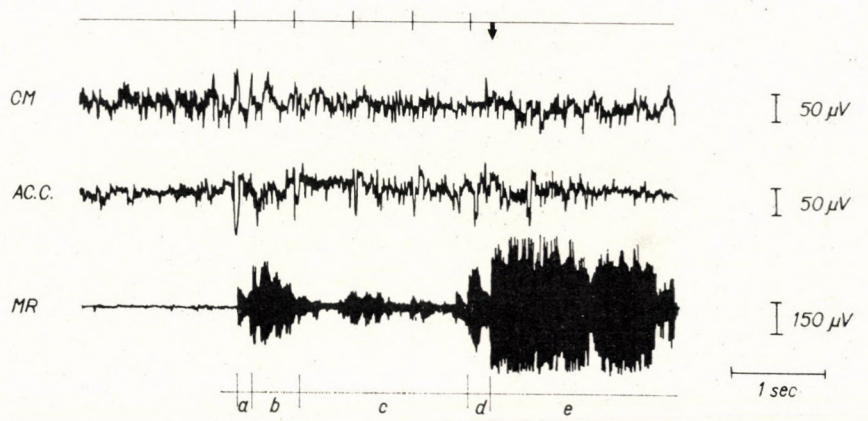

Fig. 5. Simultaneous recording on electroencephalograph of movements occurring during a conditional reflex trial and the electrical activity of two brain-regions
CM: right centre median
AC. C.: left acoustic cortex
MR: Registration of movement
Conditional stimuli (clicks) and the beginning of conditional motor act are shown by the short vertical bars and arrow on the first line, respectively

head toward the loudspeaker (b), hereupon it looked upwards with slow orienting movements at the feeding device (c), then put one of its forepaws on the feeding device (d), finally jumped up and started to eat (e).

The registration of a cat's purr is demonstrated in *Fig. 6*. This often disturbs recording movements, therefore its recognition is important. In a moment marked with an arrow the floor of the box was inundated with water. Purring immediately ceased indicating that the recorded material was not identical with simple breathing.

As seen in *Fig. 7*, by applying the damping fluid the self-oscillation of the system was considerably decreased and even such slight movements as displacement of the head due to cardiac contraction may be recorded.

When evaluating the recordings obtained by the described instrument, the following should be taken into account:

1. Any changes of the kinetic conditions of the head of the animal are reflected by the instrument. The voltage of the electrical potential produced is proportional with the positive or negative acceleration appearing during displacement of the head. There are no electrical signs in the intervals of movements of uniform velocity apart from the duration of damping.

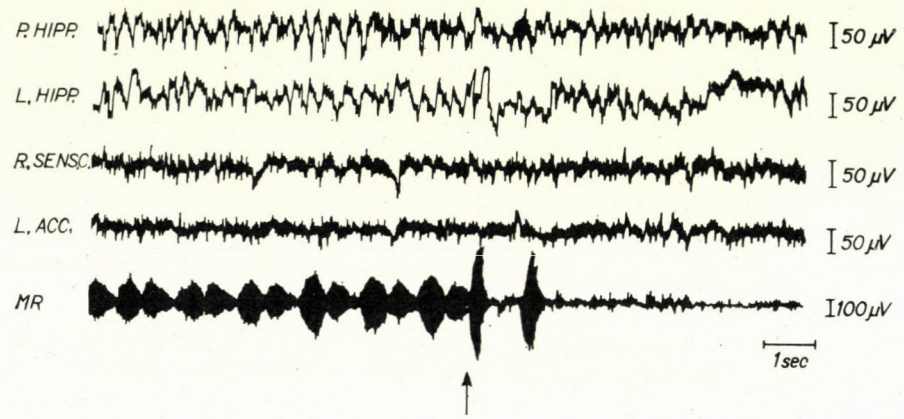

Fig. 6. Recording cat's purr simultaneously with the electrical activity of different brain regions on EEG

R. and L. HIPP.: Right and left hippocampus
R. SENS. C.: Right somato-sensory cortex
L. AC.C.: Left acoustic cortex
MR.: Recording of movement
Further descriptions see in text

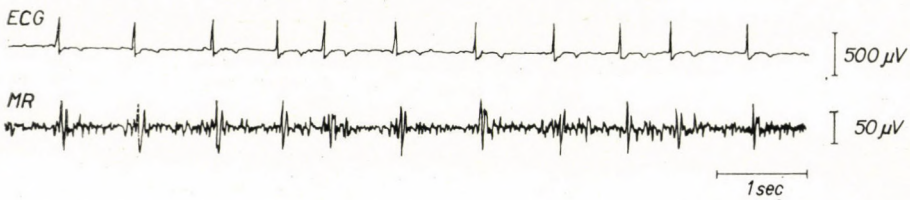

Fig. 7. Recording of head movements due to cardiac contractions simultaneously with electrocardiogram

2. No references are gained in regard of the direction of movement. The instrument is so designed as to be able to give electrical signs of any smaller movements occurring in any direction. Its direction characteristics form a distorted sphericity. This distortion is very slight and does not cause any drawback.

3. The sensitivity of the system can be adjusted within wide limits with the help of the amplifier the actual requirements of the experiment. Any of the biological amplifiers (EEG, EMG, ECG) can be used successfully.

4. The delay of the recording system is well below the biological requirements.

5. One of the main drawbacks of the recording system consists in the fact that the contribution of different body regions in the production of the records is not revealed.

The method presented seems to be suitable for physiological, pharmacological and psychological experimentation, especially if the analysis of a closer relationship of movements and electrical manifestation is required.

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Pathophysiologia

ВЛИЯНИЕ ПОПЕРЕЧНЫХ УСКОРЕНИЙ НА ТЕЧЕНИЕ НЕКОТОРЫХ ПАТОЛОГИЧЕСКИХ ПРОЦЕССОВ

НАТАЛЬЯ К. СИМЕОНОВА

КАФЕДРА ПАТОЛОГИЧЕСКОЙ ФИЗИОЛОГИИ, КИЕВСКОГО МЕДИЦИНСКОГО ИНСТИТУТА
КИЕВ, УССР

1. Белые мыши, перенесшие действие поперечных перегрузок в 30 ед. в течение 3 минут, живут в условиях острой гипоксии и перегревания более продолжительное время, чем контрольные. Эти животные обнаруживают большую выживаемость при электротравме и введении стрихнина.

2. У крыс, перенесших действие 30-кратных перегрузок в течение 2 минут, ослаблены компенсаторные реакции со стороны внешнего дыхания на разрежение атмосферы, однако, выживаемость при этом повышена.

3. Гипотермия в той степени, которая возникает у крыс при 2 минутном вращении при 30 ед., не может быть причиной гипореактивного состояния и понижения чувствительности организма к недостатку кислорода.

4. Можно предположить, что вращение в центрифуге при больших перегрузках способствует развитию в центральной нервной системе запредельного торможения, которое снижает чувствительность организма к последующему действию различных патогенных факторов, в том числе и гипоксии.

В условиях космического полета организм человека или животного испытывает на себе действие факторов космического пространства — невесомости и радиации, а при старте и приземлении — действие значительных перегрузок. Выполнение фигур высшего пилотажа также связано с действием больших перегрузок на организм летчика.

Изучению функциональных сдвигов и компенсаторных механизмов при действии ускорений посвящено большое количество работ. Большинство авторов считает, что при этом наиболее выраженные сдвиги возникают в системе кровообращения и внешнего дыхания.

Что касается функционального состояния центральной нервной системы, то в настоящее время получены данные, свидетельствующие о развивающемся торможении. Это показано в опытах по изучению рефлекторной деятельности на животных и человека, биоэлектрической активности головного мозга, психологических проб (В. И. Бабушкин и соавт. 1956; В. В. Усачев 1956; Б. М. Савин и З. К. Сулимо-Самуйлло 1958; Г. В. Изосимов и А. Н. Разумеев 1962; А. С. Барер 1962).

В теоретическом и практическом отношении важно изучить состояние организма в ближайшее и отдаленное время после перенесения перегрузок. Например, интересно исследовать, как развиваются патологические процессы, защитные реакции и процессы восстановления, если организм, перенесший такое воздействие, попадает в неблагоприятные условия (недоста-

ток кислорода, высокая или низкая температура, травматизация, интоксикация, утомление). Важно также изучить реактивность этих животных к лекарственным веществам. Этому вопросу посвящены экспериментальные исследования Н. Н. Зайко и Н. К. Симеоновой (1962), В. Е. Белай, П. В. Васильева и С. П. Колчина (1963).

В настоящей работе будут представлены данные о реактивности животных, перенесших поперечные перегрузки, к дополнительному воздействию различных патогенных факторов.

Методика

В опытах были грызуны — 116 белых мышей и 115 белых крыс. Животные фиксировались в контейнерах и подвергались вращению в центрифуге радиусом 90 см со скоростью 174 оборота в 1 минуту. При этом они испытывали действие радиального ускорения в 30 ед., направленного вентро-дорзально, т. е. поперечно.

Результаты

В зимней серии опытов из 37 мышей после 3-минутного вращения погибли 7. Весной наблюдалось повышение устойчивости организма к действию этого фактора, и после 3-минутного вращения выживали все мыши. Температура тела сразу после вращения была снижена на 2—3°, потом в течение 30 минут продолжала падать еще на 1—2°. Восстановление температуры затягивалось на 2—2,5 часа. Обнаружилось, что параллельно с развивающейся постгравитационной гипотермией изменяется реактивность организма.

В первой серии опытов на 30 мышах исследовалась устойчивость животных, подвергшихся влиянию перегрузок, к острому кислородному голоданию. Через 5—7 минут после вращения опытную и контрольную мышь помещали в барокамеру. Давление понижали до 200 мм ртутного столба. Наблюдения показали, что, находясь в совершенно одинаковых условиях, контрольное и опытное животное по-разному относятся к действию этого патогенного фактора — всегда первым погибало контрольное животное.

Во второй серии опытов переменный электрический ток параллельно подводился контрольному и опытному животному. Электроды вводились подкожно. Контрольные мыши погибали от удара током напряжением 80 вольт. Опытные оставались живыми. Если для гибели контрольной в редких случаях требовалось повторное включение тока, то опытная мышь все же оставалась живой. Она могла дополнительно перенести еще несколько ударов.

В третьей серии изучали течение судорог и выживаемость после введения азотнокислого стрихнина. Раствор в разведении 1:1000 вводили внут-

рибрюшинно из расчета 0,15 мг на 100 г веса. Большинство контрольных мышей (17 из 26) погибли через 4—7 минут от первого же судорожного приступа. У мышей, перенесших вращение, тоже развивались судороги, продолжавшиеся иногда около часа. Однако, большинство животных остались в живых (20 из 26). Если животное погибало, то смерть наступала через 10—17 минут и позже после введения стрихнина.

В четвертой серии опытов в условиях перегревания при температуре 45—50° 8 контрольных мышей погибли через 3—5 минут. У вращавшихся животных (8 мышей) явления перегревания были выражены меньше. Судороги и смерть наступали позже, либо животное оставалось в живых, если перегревание прекращалось.

Возникает вопрос, каковы причины наблюдаемых изменений реактивности.

Можно предположить, что вращение в центрифуге при больших перегрузках способствует развитию в центральной нервной системе запредельного торможения, которое снижает чувствительность организма к последующему действию различных патогенных факторов. Второе предположение касается роли гипотермии, развивающейся во время вращения. Действительно, у белых мышей после вращения температура была снижена на 2—3°, и последующие воздействия производились на этом фоне. Необходимо было выяснить роль этого фактора в изменении реактивности. Наконец, важно было оценить влияние больших перегрузок на компенсаторные возможности организма.

В связи с этим мы предприняли исследования на крысах, у которых изучали течение острой гипоксии и некоторые механизмы защиты на фоне предварительного действия на организм радиального ускорения.

Эти опыты были проведены на 115 белых крысах самцах весом 140—200 г. Острое кислородное голодание создавалось в барокамере емкостью 7,3 л при равномерном снижении барометрического давления за 3 минуты 10 секунд до 200 мм ртутного столба, что соответствует 10 000 м над уровнем моря. В этих условиях большинство животных выдерживалось до гибели. Животные фиксировались, велась непрерывная запись дыхания, на каждом километре «подъема» регистрировалась ЭКГ, в ходе опыта измерялась температура тела при помощи электротермометра, вмонтированного в барокамеру. Кроме того, учитывалась продолжительность жизни животного с момента достижения «высоты» 10 000 м и до гибели.

Предварительно на группе здоровых животных (16 крыс) были установлены некоторые показатели течении гипоксии в данных условиях.

Исходная температура тела у контрольных крыс была 37—38°. В результате опыта температура падала и в момент смерти была около 35°. Как видно на *рис. 1*, исходная частота пульса (500 ударов в 1 минуту) сохраняется до «высоты» 5000—6000 м. На этой высоте частота пульса начинает

быстро падать на уровне 8000—9000 м нарушается ритм сердечных сокращений. Ни в одном случае не было обнаружено компенсаторного учащения пульса.

Особый интерес при подъеме на высоту представляет дыхание. Соответствующие данные представлены на *рис. 1* и *2*, причем, на *рис. 1* представлены усредненные данные об изменениях частоты дыхания, а на *рис. 2* приведены две типичные пневмограммы, на которых можно видеть не только

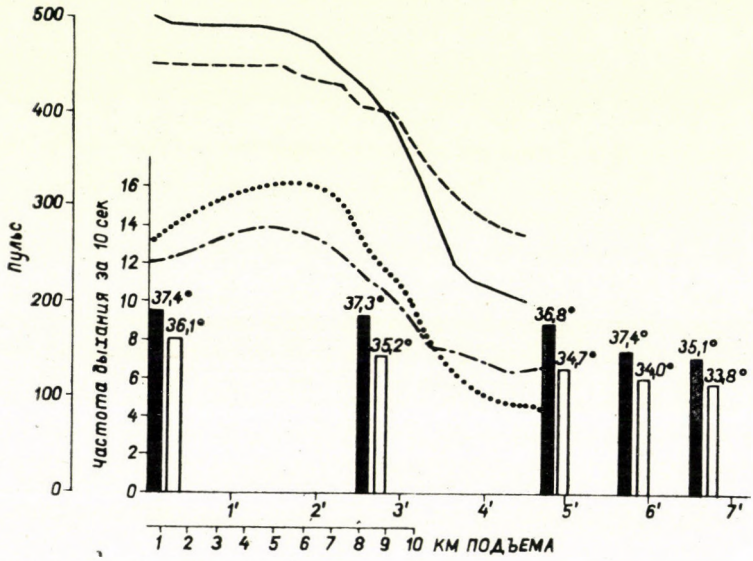
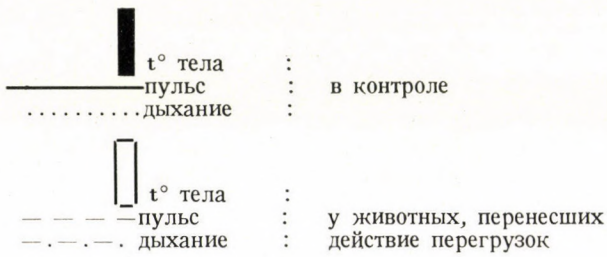


Рис. 1. Изменение температуры тела, частоты дыхания и пульса при разрежении атмосферы



изменение частоты, но и другие особенности дыхания. На пневмограмме видно, что исходное дыхание у контрольных животных очень лабильно и составляет в среднем 80 в 1 мин. (13 за 10 сек.). Тотчас же после начала разрежения воздуха наступает компенсаторное учащение дыхания, которое далее нарастает и удерживается на максимальных цифрах на высоте 4500—6500 м. На 6000 м дыхание становится аритмичным, судорожным, частота

критически падает, скоро наступает смерть животного. Средняя продолжительность жизни в барокамере (с момента достижения высоты 10 000 м) для контрольных животных в данной серии опытов равна 3 мин. 5 сек.

Во второй группе было 25 животных, которых сначала подвергали вращению в центрифуге при 30 ед. в течение 2 минут, а потом «подъему» в барокамере. Сразу после вращения у крыс наблюдаются вестибулярные реакции, через 3—4 минуты животные становятся почти неподвижными, для их поведения характерна общая двигательная заторможенность. Тем-

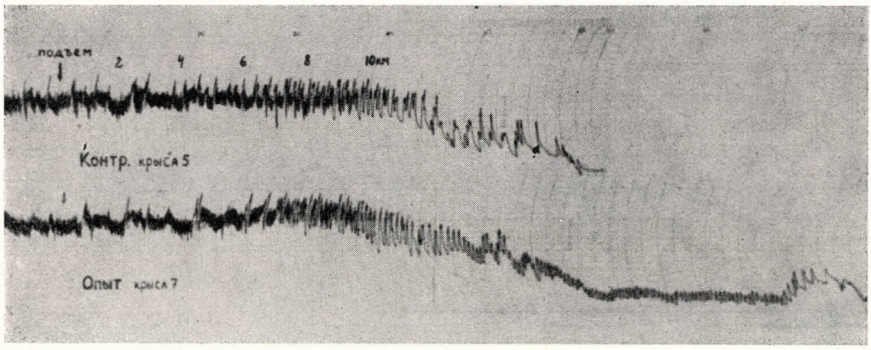


Рис. 2. Изменение дыхания при разрежении атмосферы. (Пневмограммы контрольного и опытного животного)

пература тела к концу вращения была снижена на 0,8—1,2°. Интересно отметить, что у крыс после вращения в течение некоторого времени (10—20 мин) температура продолжает падать еще на 1° и только после этого начинает повышаться. Большинство опытных животных мы помещали в барокамеру через 7—10 минут после вращения, при этом температура тела была ниже исходной на 1—1,5°.

Сопоставляя реакцию на разрежение атмосферы контрольных и опытных, мы обнаружили, что в этом отношении имеется существенное отличие. У опытных животных само вращение приводило к некоторому снижению частоты дыхания, и до начала «подъема» в барокамере дыхание было в среднем 72 против 80 в 1 минуту. После того, как начинается разрежение атмосферы, компенсаторное учащение дыхания наступает не сразу, причем, само по себе учащение выражено меньше, чем у интактных животных *(рис. 1)*. В общем, у животных, перенесших перегрузки, компенсаторная реакция дыхания на разрежение атмосферы более вялая и менее продолжительная. Уменьшение частоты и расстройство ритма дыхания наступает у опытных животных раньше, чем в контроле, однако, не так резко. Дальнейшее течение гипоксии может быть различным — либо наступает смерть животного, либо происходит своеобразная перестройка — дыхание становится более

частым и ритмичным, иногда имеет выраженную периодичность *(рис. 2* и *3)*. При этом температура тела падает до 32—34° С. Появление этого периода сказывается на увеличении продолжительности жизни. Следует отметить, что для опытных животных такое течение гипоксии более типично. На нашем материале такая перестройка у опытных животных наблюдалась в 66%, у контрольных — только в 30%. Именно этим объясняется перекрест кривых дыхания, который можно видеть на *рис. 1* — сначала частота дыхания в контроле выше, чем в опыте, а на 10 км это соотношение изменяется в пользу опытных животных.

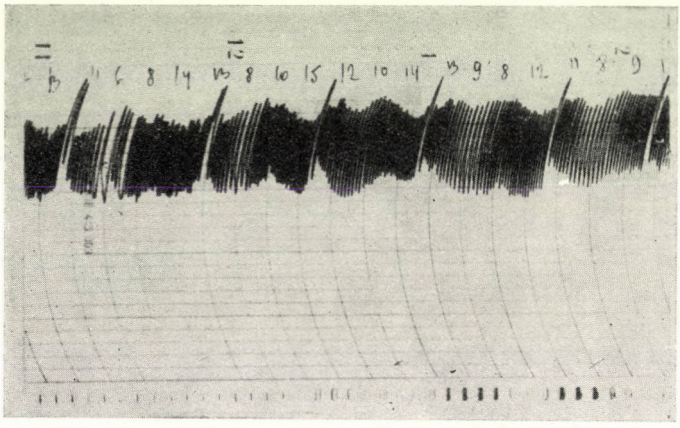


Рис. 3. Периодическое дыхание у опытного животного на высоте 10 км

Что касается изменений пульса, то нетрудно заметить, что и здесь наблюдаются подобные же соотношения. На *рис. 1* видно, что у животных, подвергшихся вращению, частота пульса перед подъемом в барокамере несколько меньше, чем у контрольных крыс. Во время подъема компенсаторное учащение пульса отсутствует как у контрольных, так и у опытных. По мере развития гипоксии происходит уменьшение частоты сердечных сокращений, однако, у вращающихся животных это расстройство происходит более постепенно, и на 9000 м наблюдается перекрест кривых, свидетельствующих о том, что у опытных брадикардия прогрессирует медленнее.

Температура тела вращающихся животных также падает во время опыта в барокамере. В результате эти крысы погибают при более низкой температуре, чем интактные животные (33—34°).

Нам казалось интересным сопоставить реакцию со стороны внешнего дыхания с продолжительностью жизни животного в барокамере. При этом оказалось, что, несмотря на более вялую компенсацию, у опытных животных продолжительность их жизни не снижается, а повышается. В данной серии в среднем она равна 5 мин. 20 сек. против 3 мин. 5 сек. в контроле,

Таблица I

Продолжительность жизни контрольных животных и животных, подвергшихся действию перегрузок, в условиях пониженного барометрического давления

Время года	Фиксация животного	Степень разрежения атмосферы в мм рт столба	Скорость разрежения атмосферы мм рт. ст./мин.	К-во животных: Общее / К—Оп	Средняя продолжительность жизни К — Оп	Степень достоверности
Май	Не фиксировано	180	166	23 / 13—10	4'15''—6'51''	$p < 0{,}01$
Май	Не фиксировано	200	168	9 / 6—3	8'45''—11'15''	$p > 0{,}3$
Январь	Фиксировано	200	280	6 / 3—3	1'40''—3'26''	$p < 0{,}01$
Февраль	Фиксировано	200	270	14 / 5—9	4'10''—7'18''	$p > 0{,}05$

причем, если только 15% контрольных животных жили более 3 минут, то среди опытных — 85% животных жили более этого срока. В *таблице I* представлены данные о продолжительности жизни контрольных и опытных животных в условиях пониженного барометрического давления. Опыты ставились в различное время года, при этом вариировали степень и скорость снижения атмосферного давления. Из этих данных следует, что средняя продолжительность жизни вращавшихся животных выше таковой в контроле.

Учитывая, что во время вращения в центрифуге температура опытного животного падает на 1,0—1,5°, мы сочли необходимым поставить дополнительный контроль с охлаждением крыс другим способом, не подвергая их действию радиального ускорения, и посмотреть их реакцию на разрежение атмосферы. В результате 5—7 минутного охлаждения в рефрижераторе температура крыс падала на 1—1,5°. В отличие от крыс, перенесших вращение, эти животные восстанавливают свою температуру сразу после прекращения охлаждения, что сопровождается интенсивной мышечной дрожью. Кроме того, если у вращающихся крыс частота дыхания уменьшена по сравнению с нормой, а также уменьшена реакция внешнего дыхания на разрежение атмосферы, то у крыс после 5-минутного охлаждения наблюдается увеличение частоты дыхания и увеличение компенсаторной реакции внешнего дыхания на остро развивающуюся гипоксию *(рис. 4)*. Создается впечатление, что 5-минутное охлаждение оказало стимулирующее влияние на защитные реакции организма. Вероятно, поэтому охлажденные животные жили в барокамере дольше интактных — 7 мин. 26 сек. против 3 мин. 30 сек.

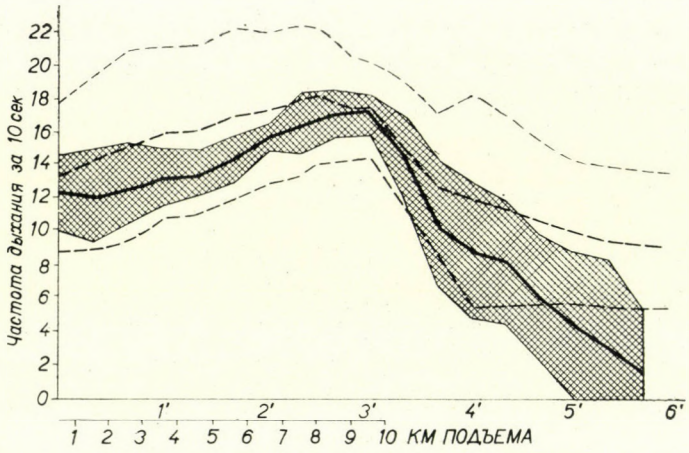


Рис. 4. Изменение частоты дыхания при разрежении атмосферы в контроле (сплошная линия) и у животных, подвергшихся охлаждению (прерывистая линия). Черное и белое поле — разброс в контроле и в опыте

Обсуждение

В опытах на белых мышах было установлено, что эти животные после вращения в центрифуге при 30 ед. в течение 3 минут живут в условиях острой гипоксии и перегревания более продолжительное время, чем контрольные. Выживаемость после электротравмы и введения стрихнина была выше в группе опытных животных, чем в контроле. Дополнительные опыты были поставлены для того, чтобы выяснить влияние ускорений на компенсаторные возможности организма.

Известно, что у крыс в механизмах защиты при понижении содержания кислорода во вдыхаемом воздухе большую роль играет учащение дыхания. Наши данные свидетельствуют о том, что под влиянием радиального ускорения у животного ослаблены защитные реакции со стороны внешнего дыхания при последующем действии острой гипоксии.

Возникает вопрос, каким образом вращение в центрифуге привело организм в гипореактивное состояние.

Обращает на себя внимание то обстоятельство, что у животных, перенесших вращение, температура снижена на 1—1,5° и в течение всего опыта в барокамере была ниже, чем в соответствующие моменты у контрольных животных *(рис. 1)*. В отдельной серии опытов мы создавали гипотермию такой же степени другим способом, не подвергая крыс вращению. При этом наблюдались изменения, противоположные тем, которые наблюдаются после вращения. По литературным данным (П. М. Старков 1957, Б. А. Сааков 1957) начальное снижение температуры тела на 1—2° само по себе вызывает состояние возбуждения и гиперреактивности. Изучая сосудистые реф-

лексы на введение вазопрессивных веществ, Б. А. Сааков наблюдал их повышение в начальном периоде охлаждения.

Нам кажется, что гипотермия в той степени, которая возникает у крыс при 2-минутном вращении при 30 ед., не может быть причиной наблюдаемого гипореактивного состояня и понижения чувствительности организма к кислородному голоданию.

В литературе есть указания на то, что перегрузки являются сильным раздражителем для центральной нервной системы, в результате чего в ней развивается запредельное торможение. Эти данные получены на основании изучения условных и безусловных рефлексов и биоэлектрической активности коры головного мозга. Нам кажется, что в наших опытах тоже содержатся данные, свидетельствующие о развивающемся торможении — уменьшение частоты дыхания и сердечных сокращений, уменьшение рефлекса дыхательного центра на разрежение атмосферы, общая двигательная заторможенность. По-видимому, снижение температуры во время вращения и прогрессирующее снижение ее после вращения тоже может в некоторой степени зависеть от центрального расстройства терморегуляции. А из литературы известно, что запредельное торможение снижает чувствительность организма к недостатку кислорода.

Торможением нервной системы можно легко объяснить, почему белые мыши после вращения оказались менее чувствительными к повреждающему действию электрического тока. Ведь исход электротравмы зависит от исходного функционального состояния нервной системы.

Причину того, что вращавшиеся животные не погибают от смертельной дозы стрихнина и лучше переносят перегревание еще предстоит подробно изучить. Во всяком случае, состояние нервной системы может влиять на исход и этих патологических процессов.

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ÜBER DIE WIRKUNG DER RADIALEN BESCHLEUNIGUNGEN AUF DEN ABLAUF EINIGER PATHOLOGISCHEN PROZESSE

Von

N. K. Simeonowa

Vorliegende Arbeit enthält Angaben über die Reaktivität der Tiere, welche außer der Beschleunigungswirkung, der Einwirkung verschiedener pathogener Faktoren ausgesetzt waren.

Die Versuche wurden an Nagetieren — 115 Mäusen und 116 Ratten — durchgeführt. Die radiale Beschleunigung wurde mittels einer Zentrifuge mit einem Halbmesser von 90 cm erreicht.

Die Versuche zeigten, daß bei der Beendigung der Drehbewegung die Körpertemperatur der Tiere um 1—2° C niedriger war als zu Beginn des Versuches und diese Senkung hielt dann noch 20—30 Minuten an.

Weiße Mäuse, welche auf die Dauer von drei Minuten einer radialen Beschleunigung von 30 Einheiten ausgesetzt waren, lebten unter Bedingungen der akuten Hypoxie und Hyperthermie länger als die Kontrolltiere. Diese Tiere zeigten eine größere Überlebungsquote bei Elektrotraumen und bei der Einführung von Strychnin.

Bei Ratten, welche auf die Dauer von zwei Minuten einer dreißigfachen Überlastung ausgesetzt wurden, waren die kompensatorischen Reaktionen seitens der äußeren Atmung verringert, doch die Überlebungsquote war größer.

Eine solche Hypothermie, die durch zweiminutige Umdrehungsdauer von 30 Einheiten bei Ratten auftritt, kann nicht der Grund eines hyporeaktiven Zustandes und einer Verminderung der Empfindlichkeit des Organismus einer Hypoxie gegenüber sein.

Man kann annehmen, daß die Wirkung der Drehung in der Zentrifuge bei großen Überlastungen der Entwicklung einer supramarginalen Hemmung im zentralen Nervensystem Vorschub leistet, welche ihrerseits die Empfindlichkeit des Organismus gegenüber der späteren Wirkung verschiedener pathogener Faktoren, u. a. Hypoxie, vermindert.

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Pharmacologia

EFFECT ON BLOOD PRESSURE OF DRUG COMBINATIONS CONTAINING PHENOTHIAZINE DERIVATIVES AND HYDERGIN

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The effect on blood pressure of Hydergin, promethazine and levomepromazine has been studied in anaesthetized cats.

In harmony with the data in the literature it has been found that promethazine does not affect blood pressure appreciably, while levomepromazine is a hypotensive agent.

It has also been shown that, as regards their action on blood pressure, distinction can be made between two groups of phenothiazine derivatives, one enhancing, the other antagonizing, the effect of Hydergin. The most representative drugs of the two groups are promethazine and levopromazine, respectively.

It has also been demonstrated that, if injected following administration of Hydergin, the phenothiazine derivatives reduce blood pressure, while when these derivatives are given first, the effect of Hydergin is reversed, and it will increase blood pressure.

Hydergin is a well-known sympathicolytic drug, which paralyses thermoregulation and has sedative and anaesthesia-potentiating actions, but is devoid of the side effects of chlorpromazine (HAUSCHILD 1956; ISSEKUTZ 1959; ROTHLIN 1934, 1950). For this reason it has been recommended as a substitute of chlorpromazine in *Laborit* and *Huguenard*'s classical "cocktail lytique" (BENZER *et al.* 1955a, 1955b; HUGUENARD 1954; VÉGHELYI 1960) and now Hydergin is often applied in combination with the various phenothiazine derivatives.

As it has already been shown (BÁLINT 1964), with respect to their potentiating activity the phenothiazine derivatives fall into two groups on the basis of how they act when combined with Hydergin.

The drugs of group 1, especially levomepromazine, markedly antagonized Hydergin, while the drugs of group 2, the most representative of which was promethazine, showed a strong synergism with Hydergin.

In the present study we have undertaken to determine whether this dual effect of the phenothiazine derivatives, hitherto not reported by other authors, would be manifest also on the action on blood pressure.

Materials and Methods

A total of 30 cats of either sex, weighing 1800 to 3500 g was used. As an anaesthetic, a mixture of chloralose and urethane, 1 : 7, prepared by the method of THURÁNSZKY and SZEGHY (KOVÁCH 1954) in a 50 per cent aqueous solution, was injected intravenously in doses of 0.4 ml/kg body weight.

Blood pressure was measured by means of an electromanometer attached to a polyethylene cannula inserted into the femoral artery, and was recorded by a *Hellige* multiscriptor (THURÁNSZKY 1962).

The drugs used and their doses were, Hydergin (Sandoz, Basel), 40 μg/kg; levomepromazine (Nozinan, Specia, Paris), 2 mg/kg; promethazine (Phénergan, Specia, Paris), 2 mg/kg. The drugs were injected into a femoral vein.

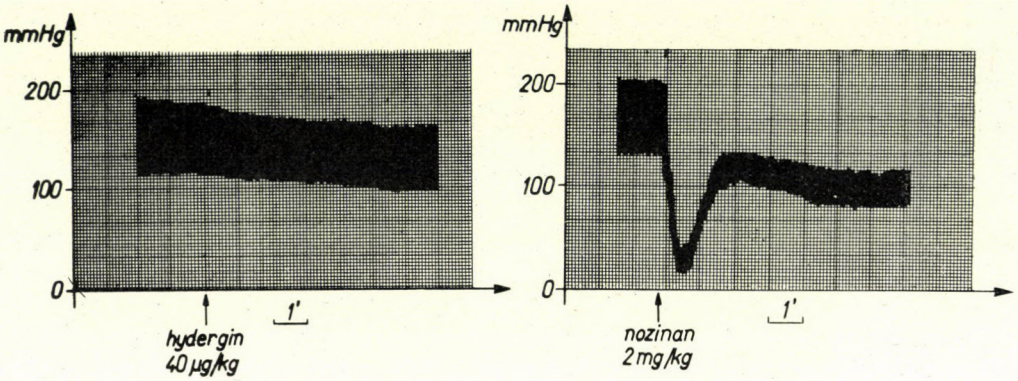

Fig. 1. Hydergin, 40 μg/kg, reduced blood pressure by 15 to 20 mm Hg, for about 10 to 15 minutes

Fig. 2. Levomepromazine, 2 mg/kg, reduced blood pressure by about 80 to 100 mm Hg for $^1/_2$ to 1 minute, then blood pressure remained stable at a level 40 to 50 mm Hg lower than the initial, for 25 to 30 minutes

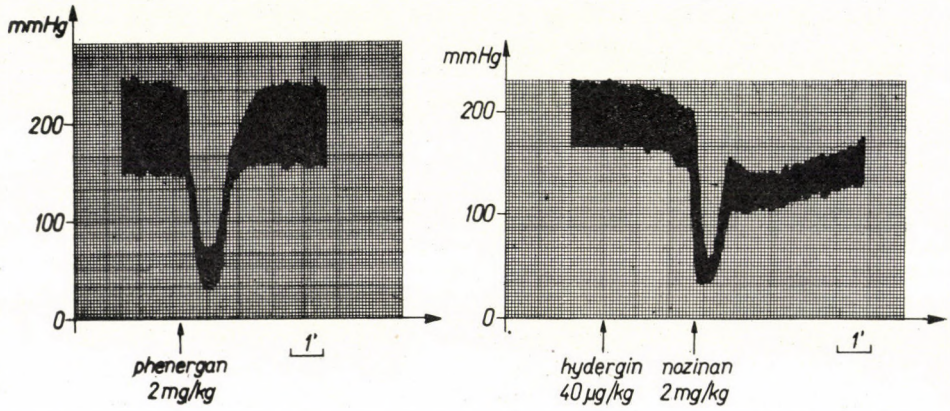

Fig. 3. Promethazine, 2 mg/kg, caused an about 80 to 100 mm Hg drop in blood pressure for about $^1/_2$ min, then it persisted at the initial, or a 5 to 100 mm Hg lower level

Fig. 4. The response to Hydergin (40 μg/kg) followed by 2 mg/kg levomepromazine, was essentially the same as the response to levomepromazine alone, except that normalization resulted sooner, in 5 to 10 minutes

In the first step, we examined the blood pressure response to 40 μg/kg Hydergin, 2mg/kg levomepromazine and 2 mg/kg promethazine, in 2 animals each.

These results are shown in *Figs 1, 2,* and *3.*

Subsequently, we set up groups of four animals each, and determined the blood pressure response to the following combinations.

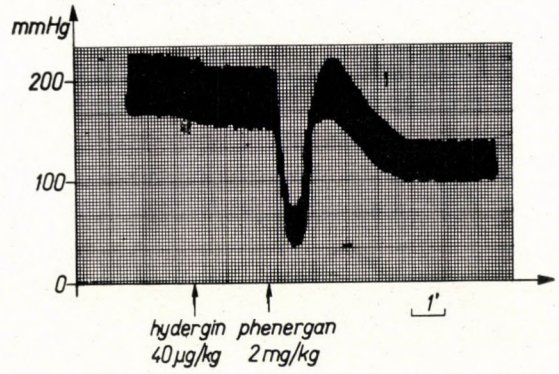

Fig. 5. When the 40 µg/kg dose of Hydergin was followed by the injection of 2 mg/kg promethazine, after the initial 80 to 100 mm Hg fall lasting about half a minute, blood pressure returned to the initial level for a while, then decreased, to stay for about 25 to 30 minutes at an approximately 40 to 50 mm Hg lower level, then it slowly rose

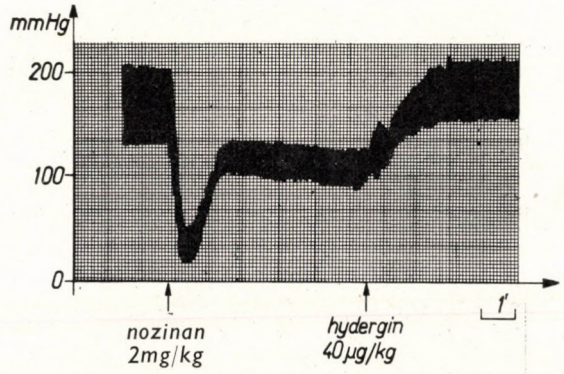

Fig. 6. The injection of 2 mg/kg of levomepromazine produced the lasting hypotensive response described above. When 40 µg/kg of Hydergin was then administered, the effect of the latter was reversed and blood pressure rose to the initial, or to an about 5 to 10 mm Hg higher level

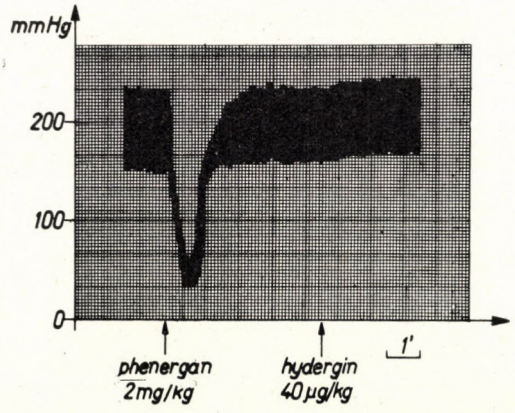

Fig. 7. About 3 minutes after the effect of 2 mg/kg of promethazine was over, 40 µg/kg of Hydergin did not influence blood pressure which was the same as that measured before the administration of promethazine, or increased it by 5 to 10 mm Hg

Table I

No. Drug	Number of animals	Direction of change of blood pressure	Interval between administration of drugs, minutes	Duration of maximum fall of blood pressure, minutes	Size of maximum fall in blood pressure, mm Hg	Size of maximum rise of blood pressure, mm Hg	Effect
1. Hydergin		↓		10	15	—	
2. Hydergin				15	25	—	
Hydergin, total	2			10—15	15—25	—	reduced
3. Levomepromazine		↓		25	40 (100)	—	
4. Levomepromazine				30	50 (80)	—	
Levomepromazine, total	2			25—30	40—50	—	reduced
5. Promethazine		↓ ↑		$^1/_2$	(80)	—	
6. Promethazine				1	(100)	5—10	
Promethazine, total	2			$^1/_2$—1	(80—100)	0—10	—
7. Hydergin + levomepromazine			3	5	40 (60)	—	
8. Hydergin + levomepromazine		↓ ↑ ?	3	8	45 (80)	—	
9. Hydergin + levomepromazine			3	10	55 (100)	—	
10. Hydergin + levomepromazine			3	11	50 (80)	—	
Hydergin + levomepromazine, total	4		3	5—10	40—50	—	antagonistic
11. Hydergin + promethazine			3	25	40 (60)	—	
12. Hydergin + promethazine		↓ ↑ ↓	3	27	45 (70)	—	
13. Hydergin + promethazine			3	30	50 (100)	—	
14. Hydergin + promethazine			3	30	50 (80)	—	
Hydergin + promethazine, total	4		3	25—30	40—50	—	synergistic

15. Levomepromazine + Hydergin			3	3	40 (80)	—	
16. Levomepromazine + Hydergin		↓ ↑	3	3	50 (100)	5	
17. Levomepromazine + Hydergin			3	3	50 (80)	10	
18. Levomepromazine + Hydergin			3	3	50 (100)	5	
Levomepromazine + Hydergin, total	4		3	3	40—50	0—5—10	reversed
19. Promethazine + Hydergin			3	1	(80)	—	
20. Promethazine + Hydergin		↓ ↑	3	1/2	(100)	10	
21. Promethazine + Hydergin			3	1/2	(80)	5	
22. Promethazine + Hydergin			3	1	(100)	5	
Promethazine + Hydergin, total	4		3	1/2—1	(80—100)	0—5—10	reversed
23. Hydergin + levomepromazine				1	(80)	—	
24. Hydergin + levomepromazine		↓ ↑		4	(60)	—	
25. Hydergin + levomepromazine				7	(80)	—	
26. Hydergin + levomepromazine				5	(100)	—	
Hydergin + levomepromazine, total	4			1—5	(80—100)	—	antagonistic
27. Hydergin + promethazine				30	45 (80)	—	
28. Hydergin + promethazine		↓ ↑ ↓		27	50 (100)	—	
29. Hydergin + promethazine				25	55 (80)	—	
30. Hydergin + promethazine				28	50 (75)	—	
Hydergin + promethazine, total	4			25—30	45—50	—	synergistic

a) Hydergin, 40 μg/kg, followed 3 minutes later by levomepromazine, 2 mg/kg, or promethazine, 2 mg/kg.

b) Levomepromazine, 2 mg/kg, or promethazine, 2 mg/kg was injected 3 minutes before the administration of Hydergin (40 μg/kg).

c) Hydergin (40 μg/kg) was injected together with 2 mg/kg levomepromazine, or 2 mg/kg promethazine.

The results obtained in these experiments are shown in *Figs 4, 5, 6, 7, 8* and *9*.

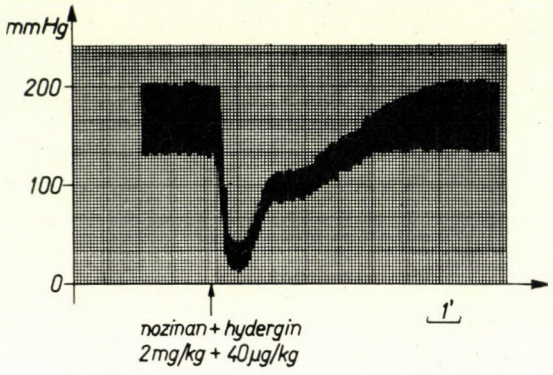

Fig. 8. The response to the combined administration of 40 μg/kg Hydergin and 2 mg/kg levomepromazine was similar to, but more protracted than, the response to promethazine in that there was no lasting hypotensive response

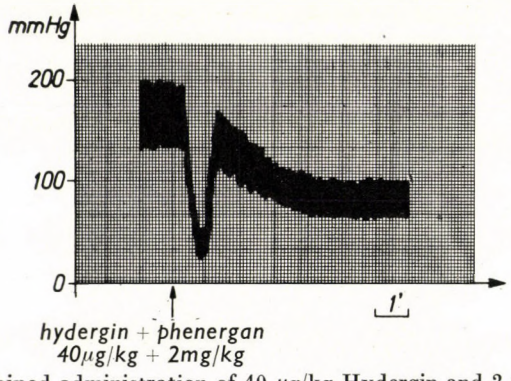

Fig. 9. On the combined administration of 40 μg/kg Hydergin and 2 mg/kg of promethazine blood pressure was reduced by about 50 mm Hg for about 25 to 30 minutes

Results

The results are presented in detail in *Table I.*

It must be emphasized that the changes of blood pressure in the different groups were always identical within the limits of normal physiological variations, *i.e.* the tendency of the changes was always the same in the individual animals of the same group.

Discussion

According to data in the literature, promethazine has hardly any action on the autonomic centres and vegetative functions, and thus does not significantly influence blood pressure (*Specia* 1960b, VÉGHELYI 1960). This has been confirmed by our results, since promethazine by itself did not reduce blood pressure significantly and lastingly in any of the cases.

Our results confirm also the observation (*Specia* 1960a, VÉGHELYI 1960) that, both levomepromazine and Hydergin are hypotensive agents.

Promethazine, which otherwise influences blood pressure for a short time only, if injected together with, or immediately after, Hydergin, produced a substantial and lasting fall in blood pressure.

It was remarkable that, if given before the injection of Hydergin, promethazine did not lower blood pressure. What resulted was a slight increase of blood pressure, a reversal of the Hydergin effect. The same was observed in the case of levomepromazine.

The lasting and marked hypotensive response to levomepromazine was abolished by Hydergin in every case, and blood pressure returned to the initial or to a slightly higher level. This reversion of the Hydergin effect was much more marked in the case of levomepromazine that in that of promethazine.

When Hydergin and levomepromazine were injected together, the response was similar to, but more protracted than, the response given to promethazine alone, *i.e.* the lasting hypotensive effect of levomepromazine was absent, and blood pressure soon returned to the initial level.

When levomepromazine was injected after the administration of Hydergin the response was similar to that given to levomepromazine alone; the injected but certainly not yet broken down Hydergin did not modify the levomepromazine effect, only its duration was greatly reduced.

From the results the following conclusions may be drawn.

(i) Our earlier observation that the phenothiazine derivatives either enhance or antagonize the effect of Hydergin has been confirmed as to the effect on blood pressure, if the derivatives were injected together with, or after, Hydergin.

(ii) When the phenothiazine derivatives are injected first, the effect of Hydergin is reversed; it then increases blood pressure, but the increase does not exceed, or exceeds only slightly, the level measured in intact animals.

These facts seem to corroborate our working hypothesis that the phenothiazine derivatives, although very closely similar in chemical structure, may be divided in at least two groups on the basis of their mode of action.

Further studies are in progress to clarify this problem.

Acknowledgement

The author is indebted to M. Németh for technical assistance.

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SOME PHARMACOLOGICAL EFFECTS OF N-ALKYL- AND N-ARALKYL-NOR-TROPINE ESTERS

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The peripheral anti-acetylcholine activity of various N-alkyl-nor-tropine derivatives, such as N_a-n-propyl, isopropyl, n-butyl, 4-bromobenzyl and 4-phenyl-benzyl, has been investigated and compared with the effect of lower homologues of the series.

The *anticholinergic activity* of compounds having substituents in the N_a steric position decreased in the following order: methyl > isopropyl > ethyl > n-propyl.

From the point of view of the *ganglion blocking action* the compounds investigated can be divided into two groups. Of those having a highly space-filling group in N_b position, the N_a-ethyl homologues exhibit the greatest activity, while the corresponding N_a-isopropyl derivatives are considerably weaker in effect. If the N_b group is a methyl radical, it is the N_a-isopropyl derivatives which are more active ganglion blockers than the N_a-ethyl members of the series.

Paralyzing effect on motor end-plates showed no unequivocal changes with alterations in chemical structure.

Several hundreds of new tropine derivatives have been synthetized and investigated in the past decades. These compounds contained the most varying ether or ester groups and different substituents on the tropane skeleton. In the case of quaternary derivatives, the substituent on the nitrogen atom mostly occupies the so-called N_b-steric position, *i.e.* it is oriented toward the pyrolidine ring of the molecule. Only few compounds are known in which the quaternary group has the N_a steric position, in other words in which the original methyl group of the tropane skeleton is substituted by a more space-filling alkyl or aralkyl group oriented towards the ester group of the molecule.

Even though several compounds of this type had been described (BRAUN 1920) they were scarcely subjected to pharmacological analysis. The first N-alkyl-nor-tropine derivatives were synthetized by KEAGLE and HARTUNG (1946) but these compounds have been identified chemically without any detailed investigation into their steric structure. The first large series of N-alkyl-nor-tropine derivatives which were studied also stereochemically have been prepared in this laboratory (NÁDOR *et al.* 1962).

More recently, FRIEDMAN and SMITH (1959) have described the pharmacological actions of N-allyl-benzyl-nor-tropine-HCl. When compared with its methyl analogue, this compound proved to be weaker in its anticholinergic actions but exhibited a higher local anaesthetic activity and its toxicity was also higher. Subsequently, several teams have attempted the synthesis of

N-allyl-nor-atropine. We were the first to describe the preparation of this compound by means of the N-carbobenzyloxy method (NÁDOR, GYÖRGY, DÓDA 1961a); it proved to be some 20 times weaker than atropine in its cholinolytic action. Thereafter, SOYKA and UNNA (1961), as well as DAL RI and SCHMIDT (1961) also synthetized N-allyl-nor-atropine; their preparation, however, was an oil-like non-crystalline substance probably not completely pure. Most recently, DECSI and NÁDOR (1963) have drawn attention to the interesting central nervous (hallucinogenic?) effects of the compound.

Continuing our earlier investigations into the structure-activity relationship of tropane derivatives, the present paper deals with the question of changes brought about in peripheral cholinolytic activity on substituting the original N-methyl group of the tropane skeleton by various N-alkyl radicals. In earlier investigations we have studied a series of tertiary benzylic acid esters and found that the cholinolytic activity was markedly reduced after replacing the methyl group by an N-ethyl radical, but slightly increased in the case of substitution with an N-isopropyl group. In quantitative terms, the anti-acetylcholine activity of the methyl compound amounted to 306 per cent, that of the ethyl analogue to 5 per cent, and that of the isopropyl derivative to 18 per cent of the activity of atropine (GYÖRGY, DÓDA, NÁDOR 1960).

Atropine p-biphenylylmethyl bromide was shown by GYERMEK and NÁDOR (1957) to have a ganglion blocking action 26 times stronger than that of TEA. The N-stereoisomer of this compound, N_a-phenylbenzyl-N_b-methyl atropinium bromide (N-731) possessed only 50 per cent of the activity of the original compound (NÁDOR, GYÖRGY, DÓDA 1961b). Comparison of N-731 with its N_a-methyl analogue, atropine methyl bromide revealed another important feature in structure—activity relationship, as compound N-731 was found to be twice as active as atropine methobromide in blocking ganglionic transmission. This observation indicates that the ganglion blocking activity of a tropine derivative can be increased by augmenting the space-filling of the alkyl group not only in the N_b steric position but also in the case of an N_a-substituent. Substituting the N_a-methyl radical by an N_a-ethyl group in a large number of compounds resulted in a substantial increase in ganglion blocking potency, and two derivatives were found to be about a hundred times more potent than TEA (DÓDA, GYÖRGY, NÁDOR 1963). It was thought that these changes were due to the electron-distributing effect exerted on the nitrogen atom by methyl and ethyl groups, respectively.

Until now a limited number of long carbon-chain N_a-alkyl analogues, mainly tertiary derivatives, has only been investigated, so that the data available do not permit any final conclusion concerning the relationship between chemical structure and pharmacological activity. The present paper deals with the comparison of the peripheral anticholinergic actions of higher

N_a-alkyl homologues (such as propyl, butyl, benzyl, *etc.)* of various tropine esters.

Methods

The anti-acetylcholine action of the compounds was measured on blood pressure, ganglion blocking activity on the superior cervical ganglion, by registering the nictitating membrane contractions on electrical stimulation of cervical sympathetic, and their curare-like effect on the anterior tibial muscle. All experiments were performed on cats anaesthetized with chloralose-urethane, as described earlier (GYÖRGY, DÓDA, NÁDOR 1961). Activities have been expressed in per cent of the atropine, TEA and d-tubocurarine effect, respectively. A tenfold decrease in the sensitivity to acetylcholine was produced in our experiments by 0.005 mg/kg of atropine. Medium effective doses of TEA and d-tubocurarine were 1.5 and 0.18 mg/kg, respectively. The compounds to be investigated were injected into the femoral vein.

Results and Discussion

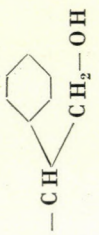
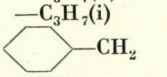
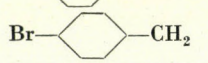
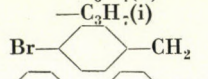
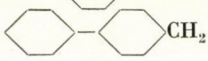
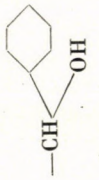
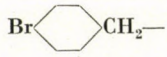
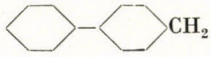
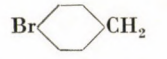
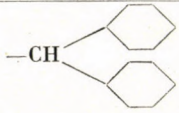
The relationship between chemical structure and pharmacological activity of 18 newly synthetized nor-tropine derivatives has been examined. On the nitrogen atom, the compounds have in N_a steric position various alkyl radicals of a space-filling higher than that of an ethyl group. For the sake of comparison, *Table I* shows not only the activity of these compounds but also contains some earlier data for ethyl and methyl derivatives.

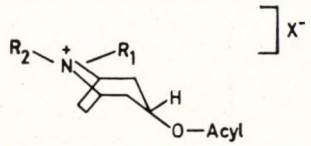
The first question to be dealt with is the anti-acetylcholine effect of the compounds. If the derivatives within the same ester group are divided in sub-groups according to the character of the N_b-radicals the following conclusions can be drawn. With the exception of two compounds it is, as a rule, the N_a methyl group which yields the most active derivatives. This is usually followed by the isopropyl and then by the ethyl analogues. The n-propyl derivatives exhibit, as a rule, weaker activity. It is an interesting feature that the N-isopropyl derivatives are always more effective than the N-n-propyl analogues; thus, the cholinolytic activity of the former compounds in individual ester groups is 13, 31, 73 and 92 times, respectively, higher than that of the N-n-propyl derivatives. For instance, compound N-1044 is seven times more potent than atropine. The N_a benzyl, 4-bromobenzyl and butyl derivatives are, as a rule, weak cholinolytic agents irrespective of their tertiary or quaternary character.

As to the influence of N_a substituents on the ganglion blocking action, the first finding to be mentioned was that the effect on ganglionic transmission does not go hand in hand with that exerted on parasympathetic end-organs, *i.e.* that the antimuscarine and antinicotine effects do not change in parallel. In some cases, such as with N_b methyl quaternary benzylic acid esters, the character of the N_a substituent influences but scarcely the ganglion blocking effect, the activity of methyl, ethyl, n-propyl, and isopropyl analogues being practically identical. As described earlier (DÓDA, GYÖRGY, NÁDOR 1963), introduction of an ethyl group in N_a steric position into a compound having

Table I

Structure and activity of the compounds

Name or Code Nr.	R_1 (N_a position)	R_2 (N_b position.)	Acyl
Atropine	$-CH_3$	H	
N-678	$-C_2H_5$	H	
N-840	$-C_3H_7$(n)	H	
N-839	$-C_3H_7$(i)	H	
N-746		H	
N-761		H	
Atropine methyl bromide	$-CH_3$	$-CH_3$	
N-960	$-C_2H_5$	$-CH_3$	
N-1059	$-C_3H_7$(n)	$-CH_3$	
N-838	$-C_3H_7$(i)	$-CH_3$	
N-732		$-CH_3$	
N-731		$-CH_3$	
Homatropine	$-CH_3$	H	
N-608	$-C_2H_5$	H	
N-630	$-C_3H_7$(i)	H	
Novatropine	$-CH_3$	$-CH_3$	
N-763	$-C_2H_5$	$-CH_3$	
N-1043	$-C_3H_7$(i)	$-CH_3$	
N-239	$-CH_2-$		
N-781	$-C_2H_5$		
N-1041	$-C_3H_7$(i)		
N-310	$-CH_3$		
N-760	$-C_2H_5$		
N-1035	$-C_3H_7$(i)		
N-719	$-C_4H_9$(n)		
Na-154	$-CH_3$	H	
N-679	$-C_2H_5$	H	
N-966	$-C_3H_7$(n)	H	
N-661	$-C_3H_7$(i)	H	
N-963	$-C_4H_9$(n)	H	
Na-158	$-CH_3$	$-CH_3$	
N-990	$-C_2H_5$	$-CH_3$	
N-1062	$-C_3H_7$(n)	$-CH_3$	
N-1044	$-C_3H_7$(i)	$-CH_3$	
N-611	$-CH_3$		
N-790	$-C_2H_5$		
N-717	$-C_5H_{11}$(i)		
Na-148	$-CH_3$	H	
N-676	$-C_2H_5$	H	
N-1045	$-C_3H_7$(i)	H	



X−	Antiacetylcholine effect, Atropine = 100	Ganglion blocking effect, TEA = 100	Curare-like effect, dTc = 100	References
SO_4	100	40		
Br	3.8			4
Cl	1.75	0	0	
Br	54	< 40	0	
Br	0.8	0	0	
Br	0.6	0	0	
Br	930	660	7.3	4
Br	107	46	2.7	4
Br	2.9	208	2.1	
Br	210	160	4.3	
Br	0.17	1 930	7.3	
Br	0.6	1 300 +	2.0	+13
Br	2.0			
Br	0.15	0	0	4
Cl	0.58	30	0	9
Br	3.8	450	7 +	+ 7
Br	12.5	120	3.4	4
Br	2.36	386	≦ 1.8	
Br	1.5	1 900	12	7
Br	0.27	2 380	6	4
Br	0.06	640	appr 3.7	
Br	1.5	4 000		7
Br	0	10 400	11.6	4
Br	0.14	600	appr 2.5	
Br	0.03	150	20	
Br	306	0	0	10
Br	4.5	0	0	4
Br	1.4	< 50	0	
Br	18	—	0	9
Br	0.12	< 50	< 1.8	
Br	2880	160	4.4	10
Br	100	100	appr 2.4	4
Br	7.9	102	2.2	
Br	724	112	2.8	
Br	0.57	150	1	10
Br	1.25	446	15.5	4
Br	0	50	15—20	
Br	4.5	0	0	
Cl	0.06	0	0	9
Cl	0.62	< 35	0	

a small N_b substituent further decreases the originally weak ganglion blocking activity. On the other hand, the same substitution in compounds possessing a highly space-requiring N_b substituent increases the originally high potency. Thus, among the mandelyl esters, the N-ethyl homologues are the most active compounds even in the case of two different quaternary groups (N-781 and N-760). Correspondingly, the N_b methyl derivatives of N_a-ethyl-nor-tropeines are, as a rule, weak ganglion blocking agents, for instance the activity of N-960 only amounts to 46 per cent of that of TEA. Two of the isopropyl homologues, compounds N-838 and N-1043, are more effective than the ethyl derivatives. No marked difference has been found between the effects of isopropyl and n-propyl derivatives.

In these series, it was particularly interesting to study the part played by the N_a radicals in ensuring a maximum ganglion blocking potency. The potency of compound N-760 could not be increased further; in fact, substitution of the N_a-ethyl group by isopropyl or propyl radicals led to a diminution in activity. (Some further N_a-aralkyl derivatives of this series are now being synthetized in this laboratory.) If, however, the N_b quaternary group is a methyl radical instead of the highly space-filling 4-phenylbenzyl group present in compound N-760, interesting alterations in ganglion blocking potency can be produced by further increasing the space-filling of the N_a group. Of the tropyl esters, it is compound N-732, the 4-bromobenzyl homologue, which exhibits the maximum potency. The N-isomer of this derivative N_a-methyl-N_b-bromobenzyl atropinium bromide (N-243), is a compound somewhat weaker in activity than compound N-732 (1550 and 1930 per cent of TEA activity, respectively; GYÖRGY, DÓDA, NÁDOR, to be published). This is an interesting feature as in the case of atropine biphenylyl-methyl-bromide the inversion of N_a and N_b radicals resulted in a 50 per cent decrease of the ganglion blocking activity (NÁDOR, GYÖRGY, DÓDA 1961b) while, in the present case, the same inversion of the spatial arrangement of N-substituents led to a slight increase of potency *(Fig. 1)*.

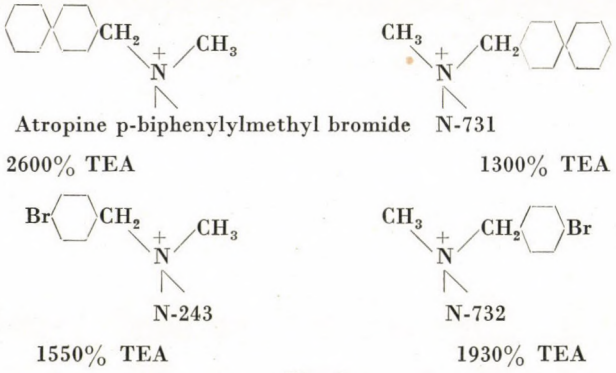


Fig. 1

In the above series, maximum activity was exhibited by the 4-bromobenzyl derivative (N-732). Interestingly enough, the highly active methyl analogue is followed by the weakly effective ethyl, propyl and isopropyl derivatives, and then by the highly active N-732. The question of the generalization revealed by this peculiar behaviour must be decided by further investigations. The final conclusion to be drawn from the present observations is that, in each series, maximum ganglion blocking effectiveness is achieved by different substituents and that, in this group of compounds, it is much more difficult to explore any definite rule in structure-activity relationship than in the series containing N_b substituents (GYERMEK and NÁDOR 1953; DÓDA, GYÖRGY and NÁDOR 1963).

The paralyzing effect of methyl-quaternary tropyl esters on motor nerve endings, goes to a certain degree with their ganglion blocking activity. Atropine methyl bromide and compound N-732 are, also in this respect, the most active members of the series. On the other hand, among the 4-phenylbenzyl quaternary derivatives of the homatropine series the weakest ganglion blocker, compound N-719, shows the highest curare-like activity. With this compound, just like with compound N-717, the ganglion blocking and curare-like activities show no parallelism. Similar differences in individual action occur with the n-propyl and isopropyl analogues, where the latter are more active. These differences are, however, by far not as great as those in anti-acetylcholine activity. It is to be noted that weak propyl and stronger isopropyl derivatives occur as well. This change in molecular structure often leads to a remarkable increase in ganglion blocking activity, at least in compounds with a skeleton other than tropane (WINBURY 1952; CUSIC and ROBINSON 1951).

On the basis of electron-theoretical considerations it has been pointed out by us more than ten years ago that in the case of the homologous series of TMA and TEA type it is the electron-repelling (such as methyl and isopropyl) or the electron-attracting (such as ethyl) character of the N-alkyl group which determines, whether a ganglion-excitatory or a ganglion-inhibitory action is exerted by the molecule. In our opinion, it is this type of investigations and this type of considerations which should be kept in view if one really wishes to explore unequivocally the relationship between chemical structure and pharmacological activity.

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STUDIES ON THE ACUTE CARDIAC ACTION OF STROPHANTHIN IN THE DOG BY MEANS OF CARDIAC DENERVATION

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The acute effects of strophanthin on the innervated and denervated heart have been studied in the dog. After halving the sternum, the heart was totally denervated by cutting the plexuses running to the heart, leaving intact the vagal trunk and the sympathetic chain. In response to strophanthin, blood pressure and cardiac output did not change significantly. In the denervated heart strophanthin induced no bradycardia, disturbance of conduction or arrhythmia; inversion of T and depression of ST, characteristic of the digitalis effect, were only slight, and not constant. On the basis of the results obtained and in the light of the data in the literature, the mode of action of strophanthin on the heart has been discussed.

The cardiac glycosides exert their influence on heart function in two ways, *viz.* (i) by acting on the electrolytes and metabolism of the myocardium; and (ii) by action mediated through the nervous system. In spite of the extensive investigations carried out in this field, it is still unclear which of the significant and characteristic cardiac effects are based on a direct action on the heart, and which are based on action upon the nervous system. The cause of the divergence of the results is to be sought in the first place in differences in the methods of examination. One of them involves the use of surviving heart and heart-lung preparations. This method has the disadvantage that it yields results applicable only with restrictions to the living organism (preparation may profoundly alter the metabolism, electrolyte and water household and the reactivity of the heart). Another method is to section or paralyze pharmacologically the branches of the vagus and sympathicus nerves supplying the heart. The most serious shortcoming of this method is that in this way nervous impulses can be eliminated only partially. In our previous experiments we used a new method, the injection of strophanthin into the isolated cranial circulation, for studying the acute effect of strophanthin exerted through the nervous system. In the present experiments we studied the direct cardiac effects of strophanthin, by total denervation of the heart.

Methods

The heart was totally deprived of its nerves by the method of COOPER *et al.* (1961). The essence of this procedure is that after opening the pericardium the periadventitial nervous plexuses are removed in the proximal parts of the aorta, pulmonary artery, vena cava, pulmonary veins. Likewise, the periadventitia of the blood vessels running in the pulmonary

hilum is removed. The phrenic nerves, as well as the nervous branches running in the anterior and posterior mediastinum and pericardium are divided. The method has the particular advantage of leaving intact the vagal and sympathetic trunks, and by stimulating the cervical vagus or sympathetic it can be ascertained whether cardiac denervation has been successful.

Dogs weighing 10 to 15 kg were used. Anaesthesia was maintained with chloralose (25 mg/kg, intravenously) and ether-oxygen under pressure. On the basis of results obtained in preliminary experiments, the chest was opened by halving the sternum, the pericardium was incised and a 2 per cent solution of procaine was introduced into the pericardial sac, to diminish the excitability of the heart. Then the heart was denervated, penicillin and streptomycin were applied locally, and the chest was closed by layers. To control the effectiveness of cardiac denervation, the cervical vagus was stimulated electrically, recording simultaneously the EEG. (Prior to cardiac denervation, eletrical stimulation of the vagus causes bradycardia and often an atrioventricular block. After denervation vagal stimulation does not affect the heart.) To study the acute cardiac effect of strophanthin, a large dose (0.75 mg) was injected slowly, intravenously. It is namely known that in response to high doses of the drug the characteristic cardiac effect develops within $^1/_2$ hour, and that dogs are less sensitive to strophanthin than humans.

We studied the acute effects of strophanthin in 11 dogs with denervated heart and in 10 control dogs. The ECG was recorded by means of a direct-writing *Cardiomat* apparatus and needle electrodes. Blood pressure was measured in the femoral artery. In some cases the cardiac output was determined by the dye-dilution technique, using *Evans* blue for this purpose.

The ECG was recorded before and 5, 10, 15 and 30 minutes after the injection of strophanthin, then at 30-minute intervals. (In a few control experiments a dose of 0.5 mg was used; the ECG changes corresponded completely with those induced by the 0.75 mg dose, except that the changes developed at a slower rate in both the innervated and denervated hearts.)

Results

The results of our experiments have been tabulated. In the control animals with innervated heart *(Table I)* the characteristic ECG signs were in practically every case:

1. Prolongation of atrio-ventricular conduction (P—Q distance) and of intraventricular conduction (QRS complex).

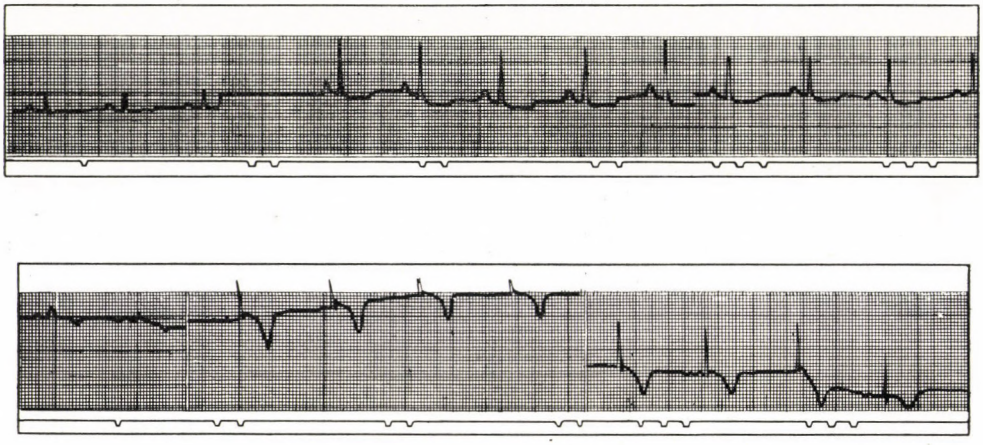

Fig. 1. Innervated heart

Above: Before strophanthin
Below: 90 minutes following the administration of strophanthin. Heart rate is reduced, marked inversion of T, depression of ST

2. Shortening of the QT distance (electrical systole).

3. Inversion of T and depression of ST (usually in the lead with the tallest R).

4. Extrasystolia.

5. Reduction of heart rate.

Following cardiac denervation *(Table II)* the response was significantly different. The most conspicuous change was the absence of bradycardia. Extrasystolia was not observed, either. The changes of T and ST were much less marked than in the controls and appeared in a small percentage of the cases. The reduction of QT distance was also less marked and less frequent. The same was the case with conduction. No significant change was noted in blood pressure and cardiac output, either.

A few characteristic ECG changes are shown in *Figs 1* to *3*.

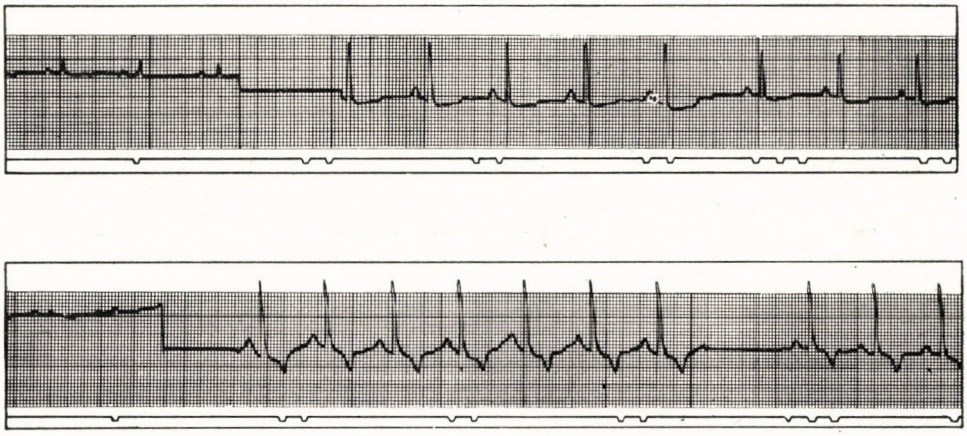

Fig. 2. Denervated heart

Above: Before strophanthin
Below: 90 minutes following the administration of strophanthin. No change in heart rate. Inversion of T. Slight depression of ST in leads II and III

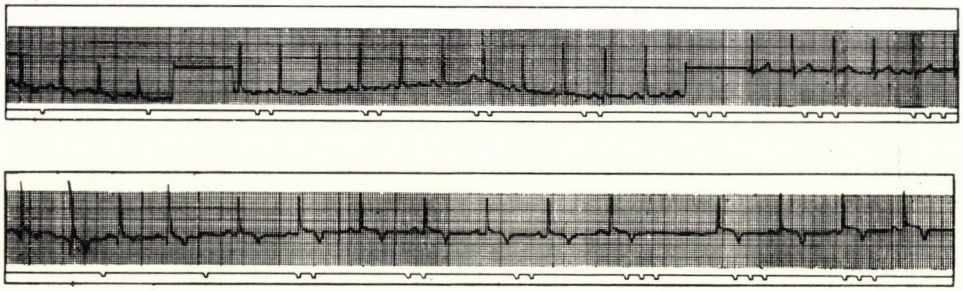

Fig. 3. Innervated heart

Above: Before strophanthin
Below: 90 minutes following the administration of strophantin. Heart rate is reduced; sinus block in lead III; marked inversion of T

Table I

Acute action of strophanthin on the innervated heart

No.	Period	Heart rate	Blood pressure	PQ sec	QRS sec	QT (electrical systole) sec	Change of T wave	Change of ST segment	Arrhythmia	Disturbance of conduction
1.	A	128	110	0.12	0.05	0.28	—	—	—	—
	B	110	110	0.11	0.05	0.27	Inversion of T_2T_3	Depression of ST_2ST_3	Ventricular ES	—
	C	100	105	0.10	0.05	0.26	Inversion of T_2T_3	—	—	—
2.	A	118	95	0.09	0.04	0.30	—	—	—	—
	B	110	100	0.10	0.05	0.27	Inversion of T_2T_3	Depression of ST_2ST_3	Atrial ES	—
	C	100	95	0.11	0.05	0.26	Inversion of T_2T_3	Depression of ST_2ST_3	Atrial ES	—
3.	A	110	100	0.09	0.05	0.28	—	—	—	—
	B	96	95	0.10	0.05	0.26	Inversion of T_1T_2	Depression of ST_1	—	—
	C	90	100	0.11	0.05	0.26	Inversion of T_1T_2	Depression of ST_1	—	—
4.	A	144	105	0.11	0.04	0.24	—	—	—	—
	B	100	110	0.14	0.05	0.24	Inversion of T_2T_3	Depression of ST_2ST_3	Ventricular ES	Temporary AVblock
	C	96	—	0.14	0.05	0.22	Inversion of T_2T_3	Depression of ST_2ST_3	Ventricular ES	Temporary AV block
5.	A	132	90	0.11	0.05	0.26	—	—	—	—
	B	120	90	0.13	0.05	0.24	Inversion of T_2	Depression of ST_2	—	—
	C	110	85	0.13	0.06	0.24	Inversion of T_2	Depression of ST_2ST_3	—	—

6.	A	118	100	0.09	0.04	0.28	—	—	—	—
	B	100	100	0.11	0.06	0.25	Inversion of T_2T_3	Depression of ST_2ST_3	Ventricular ES	—
	C	98	—	0.12	0.06	0.25	Inversion of T_2T_3	Depression of ST_2ST_3	Ventricular ES	—
7.	A	128	105	0.10	0.05	0.27	—	—	—	—
	B	100	—	0.14	0.07	0.26	Inversion of T_1T_3	Depression of ST_1ST_2	Atrial ES	Temporary AV block
	C	90	105	0.16	0.07	0.24	Inversion of T_1T_3	Depression of ST_1ST_2	Atrial ES	Temporary AV block
8.	A	136	105	0.10	0.04	0.25	—	—	—	—
	B	120	105	0.12	0.05	0.24	Inversion of T_2T_3	Depression of ST_2ST_3	—	—
	C	116	105	0.12	0.06	0.24	Inversion of T_2T_3	Depression of ST_2ST_3	Ventricular ES	—
9.	A	130	105	0.09	0.05	0.26	—	—	—	—
	B	114	100	0.11	0.05	0.24	Inversion of T_1T_2	Depression of ST_1ST_2	Ventricular ES	—
	C	110	95	0.11	0.06	0.24	Inversion of T_1T_2	Depression of ST_1ST_2	Ventricular ES	—
10.	A	140	100	0.10	0.05	0.25	—	—	—	—
	B	110	105	0.12	0.06	0.26	Inversion of T_2T_3	Depression of ST_2ST_3	Ventricular ES	—

Periods: A = before strophanthin
B = 30 minutes after strophanthin
C = 90 minutes after strophanthin

Table II

Acute cardiac effect of strophanthin on the denervated heart

No.	Period	Heart rate	Blood pressure	PQ sec	QRS sec	QT (electrical systole) sec	Change of T wave	Change of ST segment	Arrhythmia	Disturbance of conduction
1.	A	110	100	0.11	0.05	0.26	—	—	—	—
	B	116	100	0.10	0.05	0.26	—	—	—	—
	C	108	95	0.11	0.05	0.25	Inversion of T_1	—	—	—
2.	A	132	90	0.09	0.06	0.24	—	—	—	—
	B	128	95	0.09	0.06	0.24	Inversion of T_2 T_3 increased	—	—	—
	C	130	100	0.09	0.06	0.26	Inversion of T_2 T_3 increased	—	—	—
3.	A	124	90	0.10	0.04	0.26	—	—	—	—
	B	120	—	0.11	0.044	0.25	—	Depression of ST_2 ST_3	—	—
	C	124	90	0.11	0.04	0.25	—	Depression of ST_2 ST_3	—	—
4.	A	108	95	0.09	0.05	0.28	—	—	—	—
	B	110	90	0.10	0.06	0.26	Inversion of T_2 T_3 increased	—	—	—
	C	106	—	0.09	0.06	0.26	—	—	—	—
5.	A	136	100	0.10	0.05	0.26	—	—	—	—
	B	130	—	0.10	0.04	0.26	—	—	—	—
	C	140	—	0.10	0.05	0.26	—	—	—	—
6.	A	118	110	0.09	0.03	0.29	—	—	—	—
	B	120	100	0.09	0.03	0.29	—	Slight depression of ST_2 ST_3	—	—
	C	120	100	0.09	0.03	0.29	—	Slight depression of ST_2 ST_3	—	—

7.	A	108	105	0.10	0.05	0.28	—	—	—	—
	B	110	95	0.10	0.05	0.28	—	—	—	—
	C	111	100	0.10	0.05	0.28	—	—	—	—
8.	A	120	100	0.12	0.05	0.26	—	—	—	—
	B	124	100	0.12	0.05	0.26	—	—	—	—
	C	120	100	0.12	0.05	0.26	—	—	—	—
9.	A	124	—	0.10	0.04	0.29	—	—	—	—
	B	128	—	0.10	0.04	0.27	—	—	—	—
	C	124	—	0.10	0.04	0.27	—	—	—	—
10.	A	116	95	0.09	0.06	0.29	—	—	—	—
	B	120	90	0.09	0.06	0.28	—	—	—	—
	C	124	95	0.09	0.06	0.28	—	—	—	—
11.	A	140	110	0.11	0.04	0.28	—	—	—	—
	B	148	105	0.11	0.04	0.26	—	—	—	—
	C	136	100	0.11	0.04	0.26	—	—	—	—

Periods: A = after heart denervation
B = 30 minutes after strophanthin
C = 90 minutes after strophanthin

Discussion

On the basis of our experiments with isolated cerebral perfusion it was assumed that strophanthin acted on heart rate (chronotropic effect) and myocardial excitability (bathmotropic effect) through the nervous system, in the first place. The present results seem to confirm that contention. Following cardiac denervation strophanthin had no negative chronotropic and positive bathmotropic early effect. HEYMANS *et al.* (1932) could prevent in dogs with denervated carotid sinus and aortic arc the bradycardia caused by Oubain. Similar observations have been made in the rabbit by ZIPF and EHRLICHER (1951). All these tend to indicate that the early bradycardia inducing effect of cardiac glycosides is of nervous origin (mediated mainly by the cardiac branches of the vagus). The early bathmotropic effect, too, seems to be preponderantly of nervous origin. If injected into the isolated cerebral circulation, strophanthin promptly induces extrasystolia, while following denervation of the heart even high doses of the drug cause no arrhythmia.

WEINBERG *et al.* (1955) found that strophanthin injected into a cerebral ventricle often produced extrasystolia, which could be prevented by the administration of hexamethonium.

It appears that the inhibitory effect of strophanthin on conduction (negative dromotropic effect) is also based on an indirect cardiac action. In response to the injection of strophanthin into the isolated cerebral circulation, prolongation of PQ, and even atrioventricular block and a broadening of the QRS were observed. These were prevented to a great extent by bilateral vagotomy. Following cardiac denervation the negative dromotropic effect was slight and infrequent. BURSTEIN *et al.* (1948) found that digitalis increased the PQ interval only slightly after the nerves running to the heart had been cut.

The shortening of the electrical systole and the changes of repolarization brought about by strophanthin are based mainly on the drug's direct action on the heart. This is indicated by the fact that these changes develop in the denervated heart, too. In response to digitalis (strophanthin) cardiac metabolism is known to increase and the rate of entry of potassium into the heart muscle slowed down (the intracellular potassium level decreases). The changes of repolarization and the shortening of the QT interval might probably be one to this effect still, in the denervated heart strophanthin causes only slight changes of the T and ST segments suggesting a role of nervous influences. The increase of the work of the hypodynamic heart in response to digitalis is based, of course, decisively upon the drug's direct action on the heart.

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Recensiones

Biophysik

Volume 1, Number 1, 1963. Springer Verlag, Berlin

Editor: B. RAJEWSKY

The experts of biophysics have done excellent work, particularly since the middle of the past century, but allowed, for reasons quite unclear even now, their branch of science to be pushed in the background by other disciplines. Nevertheless, there have been workers who were untiring in their efforts to emphasize the role of primary importance biophysics has to play in the creation of exact biological science, and among them Professor RAJEWSKY, Director of the Max Planck Institut für Biophysik (Frankfurt a. M.) is undoubtedly an outstanding personality.

Recently, the significance of biophysics has been re-discovered and as a result of this, the International Organization of Pure and Applied Biophysics has been founded. RAJEWSKY is on the directorial board and is the Chief Editor of the new periodical entitled "Biophysik". This periodical had to be published, because papers on biophysical problems have been increasing in number, and in the lack of special journals they had to appear dispersed in different periodicals, making a comprehensive view most difficult. According to the announcement, the main fields of interest of the new periodical are radiological biophysics, molecular biophysics, biophysics of the electromagnetic fields, climatological biophysics and medical physics, as well as the technical aspects of biophysics. Papers written in English or French are also accepted, but every original paper is published in German; once in a while comprehensive surveys are also published.

We accept gladly this new biophysical periodical and extend our congratulations to Professor RAJEWSKY, who, after 50 years of scientific work and with 70 years on his shoulders, did not hesitate to accept the responsibilities of Chief Editor, ensuring thereby a high scientific standard for the periodical.

J. ERNST

Acta Histochemica (Supplementband III)

Gustav Fischer, Jena 1963

281 pages, 114 illustrations

Price: DM. 56.50

The volume contains the material of the 7th Symposium of the German "Arbeitsgemeinschaft für Histochemie", held at Münster, October 19—21, 1961, on the histochemistry of mineral substances.

In the first part papers concerning the main subject, in the second part papers on other topics are presented.

The introductory paper (E. SCHÜTTE, Berlin-Dahlem) surveys the role played in metabolism by mineral (mainly inorganic) substances, as the building-stones of the organism. Then follow a number of lectures on the deposition of mineral salts into the cartilage and bones under normal and pathological conditions (E. EGER, Göttingen, autoradiographic studies; K. H. KNESE, Kiel, electronmicroscopic studies; F. HENCK, Kiel, R. M. FRANK, Strasbourg). Comparative histochemical studies have been made to demonstrate the common features of atherosclerosis and bone formation *in vivo* and *in vitro* (J. LINDNER *et al.*, Ham-

burg). On the basis of detailed histochemical and electronmicroscopical studies the demonstration of heavy metals in tissues is described (E. LINDNER, Düsseldorf; R. BÄSSLER, Mainz; F. TIMM, Göttingen; G. LACK and R. NETH, Hamburg; P. GEDIGK, Marburg). The occurrence of zinc in *Paneth*'s cells in the intestines (M. EDER, Munich) and in the eosinophilic granules of the blood and bone marrow cells (D. RASKOVIC *et al.*, Belgrade) has been investigated by histochemical methods. There are detailed reports on element analysis by microincineration (I. KRUSZYNSKI, Liverpool) and by X-rays (A. ENGSTRÖM, Stockholm). Short reports deal with the electronmicroscopic examination of the spodogram (M. ARNOLD *et al.*, Göttingen), the use of the formamido procedure (H. EINBRODT, Göttingen) and the results of microautoradiographic microincineration of skin sections (G. K. STEIGLEDER, Frankfurt a. M.).

The free papers deal with the following problems. Quantitative autoradiographic studies in sections of whole animals (H. KUTZIM, Cologne); the *Schiff*-type reagents and their uses (F. H. KASTEN, College Station, Texas and Giessen); the significance of the star-shaped silver nitrate precipitate in the presence of ATP (A. M. DALEG, Brussels); increase in the activity of lysosomal enzymes following phagocytosis of macromolecular substances (A. E. MEIJER and R. G. J. WILLIGHAGEN, Leiden); quantitative histochemical studies by the "two wave lengths" method (M. J. HARDONK *et al.*, Leiden); the role of metals in formazon chelate formation (H. H. PFEIFFER, Bremen); demonstration of free amino acid groups (H. G. GOSLAR, Tübingen); examination of material transport in explants by fluorochrome (G. H. M. GOTTSCHEWSKI, Mariensee); the chemistry of mucoid glands (H. HARMS, Leverkusen); liver phosphatase following different treatments (H. PETZOLD, Leipzig).

The papers discussing the main subject are clearly outlining the possibilities of the histochemical examination of inorganic tissue building-stones and supply some information as to their role. The evidence presented is most valuable in this field of histochemistry, which is probably the oldest, but in many respects still rather obscure.

Printing and illustrations are good. It is a pity that the work has been published two years after the symposium, because the valuable subject matter has lost much of its actuality.

Z. PÓSALAKY

ANICHKOV, S. V., BELENKII, M. L.:

Pharmacology of the Carotid Body Chemoreceptors

Pergamon Press, 1963.

225 pages, 33 illustrations, many tables

The volume is the English translation (by R. CRAWFORD) of the Russian original published in 1962. The monograph deals with the pharmacology and biochemistry of the carotid body, as well as the physiological significance of the reflexes in which the chemoreceptors play the role of mediators. The data of the literature are discussed in great detail; but the principal feature of the book is that it presents a complete survey of the evidence accumulated during the past 30 years in the Soviet Union, collected in part by the authors and their closest associates.

The monograph consists of three main parts.

In the *introduction* and the *first part* the methods and the data relative to the anatomy and physiology of the carotid body are surveyed. Most of the investigations were carried out in cats, by the isolated perfusion of the sinus region (MOYSEYEV's method), *in situ*. In other studies the carotid body was perfused *in vitro* and the changes in the action potentials of the *Hering* nerve in response to chemicals were recorded (KRILOV's method). A further, most interesting method consists in ligating the blood vessels of the sinus region, suturing the common carotid artery into a *Leersum* loop; this allowed the authors to make studies after wound healing, in unanaesthetized animals.

In general, the anatomical and physiological data presented are wellknown, except for the Soviet works on the changes in the sensitivity of the chemoreceptors of the carotid body in the course of phylo- and ontogenesis. These data indicate that the chemoreceptors of the area studied have an increasing physiological importance with the advance of phylogenetic and ontogenetic evolution, as opposed to the claim made by SCHMIDT and his school that the carotid body is a rudimentary organ and under normal conditions the reflexes connected with it have no significant role to play.

In the *second* and most voluminous *part*, the effect of various pharmacological agents on the chemoreceptors is discussed. The drugs tested are divided into two main groups. From among the agents causing tissue hypoxia the effects of cyanide, sulphides and acides on the chemoreceptors are discussed in great detail, together with the reflexes evoked by them. As far as the latter are concerned, the authors deal not only with the generally known circulatory and respiratory reflexes, but also with the reflex erythrocytosis, with the reflex changes in the functions of the adrenal medulla, pituitary-adrenocortical system, neurohypophysis and gastrointestinal tract. In the group of substances influencing cholinergic and adrenergic processes, the authors describe the effects on the chemoreflexes of acetylcholine, the ethers and esters related to it, the quaternary ammonium bases (tetramethyl-, tetraethylammonium, hexamethonium), the alcaloids of the nicotine group (nicotine, anabasine, coniine, lobeline, cytisine, sparteine, *etc.)*, the synthetic cholinolytic agents, anticholinesterases, as well as of the catecholamines. Finally, in the last chapter of this part, information relative to the substances not included in the above groups (veratrine group, papaverine, xanthine derivatives, inorganic ions, narcotics, histamine, serotonine) is presented.

In the *third part* the authors analyse the mechanisms, significance and physiological role of chemoreception. After a critical discussion of the theories published in the literature, they describe their experiments involving the use of various reducing agents, compounds influencing carbohydrate metabolism, and high-energy phosphate esters, and on the basis of the results obtained they suggest a new solution of the problem raised at the beginning of the chapter. According to the authors, the most important role of chemoreceptors is to relay information to the central nervous system about shifts in the energy balance of peripheral tissues in the unfavourable, catabolic direction. The tonic nature of carotid body function suggests an unstable energy balance of the chemoreceptor cells, and it is due to this lability that the chemoreceptors "inform" the nervous system not when serious metabolic disturbances have already developed, but they are capable of signalling by their impulses even the tendencies to unfavourable changes in metabolic equilibrium. All the reflex reactions connected with chemoreception are aimed at a normalization of tissue metabolism. Finally, conditioned reflex data indicating a cortical control of chemoreceptor function are presented.

The monograph is fascinating and the reader must feel that the authors were right when stating in the foreword that the volume will be of interest not only for those concerned with theoretical sciences or biology, but also for the clinicians.

A. ERDÉLYI

CRABBÉ, J.:

The Sodium-Retaining Action of Aldosterone

Arscia S. A., Brussels, 1963. 119 pages; preface by G. W. THORN

The finer analysis of the mode of action of hormones is one of the fundamental problems of up-to-date medical science. This problem arises also in the case of aldosterone, a hormone playing a role in the maintenance of electrolyte balance, an essential part of the basic vital activities.

J. CRABBÉ has selected for finer analysis the isolated urinary bladder and skin of the frogs *Bufo marinus* and *Rana ridibunda*. He has made many experiments with the bladder of the former species, in the first place. Even the choice of the model is ingenious; in the simple structure not thicker than 200 μ the author could study many such phenomena as could hardly have been studied in higher, more complicated organisms. And while reading the book, we never forget that we are dealing with an experimental model, for which the credit goes to the sound judgement of the author in presenting his subject matter.

In the introduction the author deals concisely with the history of the discovery and the biological significance of aldosterone.

Then experiments are described carried out not only by the author, but mainly by other workers, as regards the effects of aldosterone on renal and extrarenal sodium metabolism. The pertinent hypotheses and theories are outlined, some of which are still subject to controversies. We then learn that the aim is to study one of the many effects of aldosterone, the one by which it influences active sodium transport.

In most experiments the migration of sodium is recorded together with the simultaneous changes in electrical potentials. The close correlation between bioelectrical phenomena and Na-flux from the works of USSING is now confirmed. In the part dealing with the methods, both the basic experimental object and the applied modern methods are described in detail.

In *Chapter 1*, describing the results of the experiments, the author makes it clear that the species of frog used, as compared with higher animals, show a significant aldosterone production and blood aldosterone levels. The biological role of aldosterone is illustrated by the fact that the frogs maintained in distilled water produce more of this hormone than the frogs kept in sodium chloride solution. The response to the administration of aldosterone, too, depends on the environment; the animals kept in distilled water and having more endogenous aldosterone respond less markedly. The author studied the effect of aldosterone not only *in vivo*, but also *in vitro*, by adding the hormone to surviving preparations *(Chapter 2)*. In this case, too, the active Na-flux increased. The experiments *in vitro* made it possible to observe many interesting laws, for example the latency observable also *in vivo* the Na-flux changes only 1 hour after the aldosterone has been added to the system. The duration of the latency does not depend on the concentration of aldosterone, thus it is not caused by a gradual accumulation of aldosterone. The antagonistic effects of spironolactones and other steroids, too, could be observed.

Chapter 3 is one of the most interesting parts of the book. In this we learn about the fate of the aldosterone added to the surviving preparation. Aldosterone is not metabolized to any appreciable extent, nor does it combine irreversibly with the preparation. It can be recovered at the end of the experiment, yet it exerts its effect. Both this part and the experiments relative to the interaction with vasopressin *(Chapter 4)* are discussed in detail.

The book ends with a general discussion and with the conclusions drawn from the obtained results.

The described experiments may be most helpful in the solution of this very important problem and may widen the view of those concerned with such problems.

214 references, many figures and tables increase the value and usefulness of this book.

P. VECSEI

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РЕЗЮМЕ

ВЛИЯНИЕ КОРМЛЕНИЯ, ГОЛОДАНИЯ И АДРЕНАЛИНА НА АКТИВНОСТЬ ГЛЮКОЗА-6-ФОСФАТАЗЫ ПЕЧЕНИ

Дь. БОТ, К. АНДРАШШИ, И. ПОРЧАЛМИ и Дь. ВЕРЕБ

Авторы исследовали изменение активности глюкоз-6-фосфатазы под влиянием различных режимов питания и голодания. Они установили, что активность глюкоза-6-фосфатазы повышается не только после голодания, но и под влиянием таких диет, которые не содержат глюкозу или декстрин.

Авторы выявили, что активность глюкоза-6-фосфатазы в период между кормлениями значительно изменяется, спустя несколько часов после кормления активность снижается, а затем повышается, и глюкоза-6-фосфатаза сохраняет свою высокую активность также во время следующего кормления.

Установлено также, что адреналин может повысить активность глюкоза-6-фосфатазы печени в том случае, если эта активность была низкой.

ФЛЮОРЕСЦИРУЮЩИЕ КОМПОНЕНТЫ В ЭЛАСТИНЕ

И. БАНГА, Й. ПАЛАДЬИ-МАЙЛАТ и А. ЙОББАДЬ

Авторы исследовали растворимость флюоресцирующего вещества и гидролизате, полученном при помощи шавелевой кислоты по методу Партриджа, а также его флюоресценцию, измеряемую на фильтре 365. После взбалтывания с растворителями липоидов прибл. 4/5 часть флюоресценции находилась в водяной фазе, а 1/5 часть в фазе липоидных растворителей. После гидролиза в соляной кислоте флюоресценция не прекратилась, а даже усиливалась, значит, она не происходила от полипептида. — Так как свойства исследовавшегося флюоресцирующего вещества и так называемого «желтого пигмента» не были одинаковыми, авторы пришли к заключению, что они измеряли флюоресценцию не цветного вещества. По их мнению молекула эластина, содержит несколько флюоресцирующих компонентов.

ДЕЙСТВИЕ ПРОГЕСТЕРОНА НА СЕКРЕЦИЮ КОРТИКОСТЕРОНА И АЛЬДОСТЕРОНА НАДПОЧЕЧНИКАМИ КРЫС-САМОК

Дь. ТЕЛЕГДИ и К. ЛИШШАК

Авторы исследовали действие различных доз прогестерона на секрецию кортикостерона и альдостерона надпочечниками in vitro и in vivo.

Установлено, что при дачи 1,5 мг/100 г прогестерона в течение 6 дней достоверно повышается секреция кортикостерона надпочечниками. После дачи более высоких доз (3 и 5 мг/100 г) не удалось выявить значительного отклонения по сравнению с контролями, в то время как после дачи 10 мг/100 г прогестерона выделение кортикостерона значительно снижается.

В отношении секреции альдостерона после дачи 10 мг/100 г прогестерона отмечалось повышение.

Изменения веса надпочечников соответствует секреции кортикостерона.

В условиях in vitro при повышении количества прогестерона временно повышается также синтез кортикостерона и альдостерона. При дальнейшем повышении количества прогестерона синтез альдостерона снижается в значительной степени, в то время как секреция кортикостерона снижается в меньшей мере.

ЛОКАЛИЗАЦИЯ РЕФЛЕКСОВ ВЫЗВАННЫХ СО СТОРОНЫ КОРОНАРНЫХ АРТЕРИЙ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ

Ш. ЮХАС-НАДЬ, М. СЕНТИВАНЬИ, И. ХОРКАИ и Б. ВАМОШИ

У собак, наркотизированных морфином и хлоралозой, авторы локализировали нервные центры вазомоторных рефлексов, вызываемых со стороны коронарных сосудов, методом пересечения ствола мозга на различном уровне. Они исследовали

а) рефлекс со стороны венечного синуса сердца (S. C. Rl.), вызываемый вдуванием баллона, введенного в синус, при обеспечении ненарушенного оттока крови;

б) депрессорный рефлекс со стороны коронарных сосудов (C. D. R.), вызванный повышением общего давления в коронарных сосудах, созданным при помощи прекращения оттока крови из синуса, и

в) хеморефлекс со стороны коронарных сосудов, вызываемый протовертатрином.

В случае повреждения вазомоторного центра продолговатого мозга исследованные рефлексы не проявлялись. Если пересечение проводилось между понтобульбарной границей и верхней третью среднего мозга, C. D. R. удалось вызвать — подобно рефлексу со стороны пазухи сонной артерии — также после пересечения, в то время как S. C. R. никогда не появился, его удалось вызвать только в случае неповрежденности всего среднего мозга. Хеморефлекс венечной артерии удалось вызвать, или он отсутствовал при пересечении на таком же уровне, как и в случае S. C. R. Из проведенных экспериментов авторы делают тот вывод, что стабилизация кровообращения, вызванная S. C. R. осуществляется в более дифференцированных нервных структурах, чем эффект C. D. R, который можно хорошо обособлять от S. C. R.

ДАННЫЕ К МЕХАНИЗМУ ГИПОТАЛАМИЧЕСКОЙ РЕГУЛЯЦИИ СЕКРЕЦИИ ТИРЕОТРОПНОГО ГОРМОНА; ЭКСПЕРИМЕНТЫ IN VITRO

М. ВЕРТЕШ и Ш. КОВАЧ

Авторы исследовали действие вытяжки гипоталамуса, вытяжки задней доли гипофиза и синтетического окситоцина на выделение тиреотропина, а также на поглощение P^{32} и потребление кислорода срезами передней доли гипофиза собак и крыс.

Было установлено, что при действии вытяжки гипоталамуса, вытяжки задней доли гипофиза секреция тиреотропина срезами передней доли гипофиза в значительной мере повышается. О подобном действии синтетического окситоцина авторы сообщили уже в прежней статье.

Вытяжка гипоталамуса, вытяжка задней доли гипофиза и синтетический окситоцин, наряду с повышением секреции тиреотропного гормона в значительной мере повышают также и поглощение P^{32} и потребление кислорода срезов передней доли гипофиза.

ДЕЙСТВИЕ ДЕГЕНЕРАЦИИ И РЕГЕНЕРАЦИИ НЕРВОВ НА ОСВОБОЖДЕНИЕ КАЛЬЦИЯ В ПОСТЮНКЦИОНАЛЬНОЙ ЦИТОПЛАЗМЕ ДВИГАТЕЛЬНОЙ ПЛАСТИНКИ

Б. ЧИЛЛИК и Д. ШАВАИ

Надчелюстное раздражение, а также инъекция карбаминоилхолина или антихолинэстераза вызывают в постсинаптической протоплазме мионеврального соединения, типичное цитологическое изменение (грануляцию). Согласно гистохимическому иссле-

дованию эта грануляция является признаком освобождения кальция. При дегенерации двигательного нервного волокна освобождение кальция в постсинаптической протоплазме прекращается, и происходит более слабая, локализующая на поверхности мышечных волокон, затяжная линейная реакция. Эта реакция продолжается до тех пор, пока двигательное нервное волокно не регенерируется, после чего начинается реакция освобождения кальция нормального размера и нормальной интенсивности, ограничивающаяся на двигательную пластинку. Авторы полагают, что грануляция (освобождение кальция) является индикатором зоны, чувствительной к ацетилхолину, а появляющаяся при денервации линейная реакция отражает повышенную чувствительность мышцы к ацетилхолину (расширение зоны, чувствительной к ацетилхолину).

ПРОСТОЙ АППАРАТ ДЛЯ РЕГИСТРАЦИИ ДВИЖЕНИЙ СВОБОДНО ДВИГАЮЩИХСЯ ЖИВОТНЫХ

И. САБО, Л. КЕЛЛЕНЬИ и Д. КАРМОШ

Дается описание прибора, пригодного для регистрации фазовых движений свободно двигающихся подопытных животных. Аппарат состоит из небольшого магнита, фиксированного на тонкой пружине над индукционной катушкой. В ходе фазовых движений животного магнит перемещается и индуцирует ток в катушке, который можно усиливать любым биологическим усилителем и одновременно отводить с электрическими импульсами мозга. Наблюдаемые осцилляции собственной частоты системы затухаются жидкостью, не обладающей электропроводимостью (парафиновое масло). Чувствительность прибора можно установить в широких пределах, соответственно актуальным требованиям, и это предоставляет возможность для регистрации самых тонких, невооруженным глазом даже незаметных движений животного, например вибраций от мурлыканья или сердцебиения. Аппарат для отведения импульсов закрепляется на голове животного. Вес аппарата небольшой (5 г) и не мешает движению и поведению животных.

О ДЕЙСТВИИ ГИДЕРГИНА И КОМБИНАЦИЙ ЛЕКАРСТВ С СОДЕРЖАНИЕМ ПРОИЗВОДНЫХ ФЕНОТИАЗИНА НА КРОВЯНОЕ ДАВЛЕНИЕ

Г. БАЛИНТ

Автор исследовал действие Гидергина, прометазина (Фенергана) и левопромазина (Нозинана) на кровяное давление наркотизированных кошек.

Он установил, что, в полном согласии с литературными данными, прометазин не оказывает существенного влияния на кровяное давление, в то время как левопромазин имеет гипотензивное действие.

Установлено и то, что в отношении их действия на кровяное давление производные фенотиазина можно разделить на две группы: одна группа оказывает синергическое, а другая группа антагонистическое действие на Гидергин. Наиболее характерными представителями этих групп являются прометазин и левопромазин.

В ходе исследований автор установил также, что после введения Гидергина производные фенотиазина оказывают гипотензивное действие, в то время как после предварительной дачи этих производных действие Гидергина изменяется и становится гипертензивным.

О НЕКОТОРЫХ ФАРМАКОЛОГИЧЕСКИХ ДЕЙСТВИЯХ И-АЛКИЛ- N-АРАЛКИЛ-НОР-ТРОПИНОВЫХ ЭФИРОВ

Л. ДЬЁРДЬ, М. ДОДА и К. НАДОР

Авторы исследовали периферическую антихолинергическую активность нескольких производных N-алкил-нор-тропина (N_a-пропил, изопропил, n-бутил, 4-бромбензил, 4-фенилбензил), сравнивая ее с активностью более низких гомологов.

Порядок силы антиацетилхолинового действия на основании заместителя в положении N_a как правило, следующий: метил > изопропил > этил > n-пропил

В отношении силы *парализирующего ганглии* действия эти соединения можно разделить на две группы: наиболее активными членами серий, содержащих в N_b *положении группу с большим пространственным заполнением,* являются производные N_a-этила. Действие аналогов N_a-изопропила значительно слабее. Если N_b-*группа является метилом,* наблюдается обратное положение: производные N_a-изопропила более активны, чем тем, которые содержат метиловую группу.

Вышеприведенные заместители влияют на действие, парализирующее концевой аппарат поперечнополосатой мышцы, не однозначно, а в различных направлениях.

ИССЛЕДОВАНИЕ ОСТРОГО КАРДИАЛЬНОГО ДЕЙСТВИЯ СТРОФАНТИНА ПОСЛЕ ДЕНЕРВАЦИИ СЕРДЦА У СОБАК

Ф. ШОЛЬТИ, М. ИШКУМ и Ю. НАДЬ

В опытах на собаках исследовалось острое кардиальное действие строфантина в случае иннервированного и денервированного сердца. После вскрытия грудной клетки сердце совершенно денервировали путем парасечения нервных сплетений, идущих к сердцу, при сохранении ствола блуждающего нерва, а также ствола симпатического нерва. При остром действии строфантина кровяное давление не показало существенных изменений. В исследовавшихся случаях минутный объем также не изменился. На денервированном сердце после дачи строфантина брадикардии, расстройства проведения и аритмии не развивалось. После денервации сердца характерные для действия наперстянки инверсия зубца Т и депрессия отрезка ST также развивались лишь в незначительной мере и не оказались прочными. На основании проводившихся экспериментов и литературных данных авторы обсуждают механизм действия строфантина на деятельность сердца.

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