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THE PROBLEM OF REACTIVITY IN PATHOLOGY

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THE PROBLEM OF REACTIVITY IN PATHOLOGY

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INTRODUCTION

This collection is dedicated to Academician A. D. Speranskiy on his 65th birthday. The eminent Soviet scientists A. D. Speranskiy student of I. P. Pavlov, is widely known to the scientific and medical world as a very great pathophysiologist -- experimenter and theoretician. His many years of fruitful investigation have, to a great extent, made possible the success of the reconstruction of Soviet medical science on the basis of the principles of Pavlovian nervsim /nervizm/.

A boundless belief in the strength of progressive science, the depth and definitiveness of his theoretical constructions, irreconcilability to set-patterns and routines in scientific investigations, daring and skill in the construction of his experiments, a keen power of observation -- these are the things which primarily draw attention to the creative make-up of A. D. Speranskiy. As a real innovator, he has always sought and continues to seek out unknown pathways in science, opportunities for new generalizations, original approaches to the analysis of the very complex phenomena in the realm of pathology and medicine.

After beginning his independent scientific activity more than a quarter of a century ago, A. D. Speranskiy created his own definitive trend in pathology. The small laboratory originally under his leadership was, after several years, converted into a large department of the All-Union Institute of Experimental Medicine imeni A. M. Gor'kiy. On the base of this department, the Institute of General and Experimental Pathology of the Academy of Medical Sciences USSR (now, the Institute of Pathological and Experimental Therapy) was created in 1944; A. D. Speranskiy continues to be its director to this day.

Hundreds of systematically performed investigations devoted to the study of neural mechanisms of disease, recover and therapy, a series of monographs by A. D. Speranskiy himself, which have exerted an importance influence on the development of Soviet medical science, a series of new effective therapeutic methods, tens of masters' and doctorate theses -- this is a brief summary of the investigative work of the scientific body headed by A. D. Speranskiy. It should be added that the criticism of methodologically erroneous theories in medical science, developed under the guidance of A. D. Speranskiy, is to his credit.

The authors of the articles published in the present collection, the numerous students and co-workers of A. D. Speranskiy along with the entire scientific medical body heartily wish dear Aleksey Dmitriyevich health and new, great successes in his highly useful work for the benefit of Soviet medical science on the occasion of his 65th birthday.

This collection, which includes chiefly the work of co-workers of the Institute of General and Experimental Pathology of the Academy of Medical Sciences USSR, gives a fairly complete conception of the character and volume of the investigations being conducted in the Institute at the present time, and of the changes which have been made here following the Joint Session of the Academy of Sciences USSR and the Academy of Medical Sciences USSR Dedicated to the Problems of the Physiological Teaching of I. P. Pavlov.

As is well known, certain errors committed in the past by A. D. Speranskiy and his students were subjected to criticism at this session. They consisted, specifically, of a certain underestimation of the role of the higher centers of the brain in the development and elimination of pathological processes and in the inadequate analysis of the concrete physiological mechanisms through which the picture of disease is mediated and by which recovery is achieved. It may be asserted that both the group headed by A. D. Speranskiy and he himself accepted this criticism properly and have done much to surmount the shortcomings in his work. The Ninth Session of the Science Council on the Problems of the Physiological Teaching of I. P. Pavlov noted in its resolution the successful course of reconstruction of the work of the Institute of General and Experimental Pathology. The material presented in the present collection confirms, it seems to us, the correctness of this evaluation of the activity of the Institute. In contrast to the other publications of similar type, which combine articles that are entirely different in their topics and are in no way connected with one another, the present collection is topical. All the articles are dedicated to a single topic -- the problem of reactivity in pathology. The scientific session of the Institute of General and Experimental Pathology which was held at the beginning of 1953, and which evoked the lively interest of the scientific was also dedicated to this problem. A large portion of the material presented at that session has been included in the present collection.

It is well known that the term "reactivity" introduced about 50 years ago has obtained an unusually extensive distribution in pathology and medicine.

It is revealing, in connection with this, that while in physiology the development of the teaching about reactivity was indisruptibly connected with the intense study of the reactivity of the nervous system (of neural tracts, centers and the nervous system as a whole), in pathology reactivity was for a long time considered to be something independent, by means of which it was possible to explain readily everything which had previously not been understood. It became clear only comparatively recently that the analysis of the phenomena belonging in the field ofpathology by means of the concept "reactivity" does not transcend the bounds of phenomenology and is purely formal in essence. The question arose as to the mechanisms of reactivity of the complex organism, which is varied and changes during the course of the disease.

At the present time, hardly anyone will dispute the neural nature of these mechanisms. Nevertheless, such concepts, even very recently, were frequently considered to be unfounded in pathology and openly hostile reactions were not uncommonly encountered. His consistent and decisive defense of the idea of the neural origin of reactions determining the reactivity of the complex organism are to the great credit of A. D. Speranskiy. Everything on which the group led by him has worked in the past 25 years has in one way or another been connected with this cardinal question of pathology and medicine.

Speaking of the neural mechanisms of reactivity, it should primarily be kept in mind that the fruitful study of this problem is possible only on the basis of the Pavlovian reflex principle. The occurence of disease, the severity and characteristics of the disease process in the final analysis are determined by the character and fate of the intermediate reactions developed in the body under the influences of a specific stimulus (the cause of the disease) and the accompanying, preceding or subsequent, additional non-specific stimuli. Here, it should be kept in mind that any direct influence on a tissue is never limited to a purely localeffect. It should be added to this that the level of reactivity of the body, which is determined by the interaction of a very large number of reflex reactions, can be markedly altered through a disturbance of the developmental conditions of any one of them.

The difficulties of physiological analysis of the neural mechanisms of reactivity, therefore, are very great. The decisive role here should naturally belong to experimental pathology. By consciously simplifying the relationships, changing the condition of the body in a desired direction, utilizing the rich arsenal of physiological and other special methods, it [experimental pathology] is even today in a position to do much for the understanding of the mechanisms of reactivity and of the means of regulating it. The investigations of I. P. Pavlov are examples of this kind of work.

This collection, which is being brought to the attention of readers, is of interest specifically from this point of view. Despite the differences in the objects under observation and the methods used, the investigations, the results of which have been presented in this collection, supplement one another in a manner of speaking. In some of them the chief attention is given to a study of the role of the afferent portion of the nervous system in the development of the pathological processes and in the creation of immunity. Other work has been devoted to an analysis of the significance of the central nervous structures in disease and resistance. Finally, the changes are analyzed which occur during the course of the disease and recovery in the effector organs. Thus, all the links of the reflex arcs, along which the reactions proceed which create immunity, disease and recovery, and which determine the level and characteristics of reactivity of the body, are brought into the sphere of the investigations. In these investigations, questions of the interaction of the basic pathological (specific) and the supplementary (non-specific) stimuli and of the significance of the time factor in the effect of each of them occupy important places.

As the reader can see, the material which is included in this collection has been obtained as a result of investigations performed with the use of the most varied methods -- physiological, histological, biochemical, serological, and others. However, it is not their variety which is most important here. It is significant that the majority of the works were accomplished on the basis of a complex of different methods, and that the method of physiological analysis is the main one in all the investigations. It should be noted particularly that in comparison with previous investigations of the Institute, significantly greater attention has been given to the study of the part of the higher centers of the nervous system, and particularly of the cerebral cortex, in the occurrence, development and outcome of pathological processes, and in the reaction of the body to the effect of extraordinary stimuli. This question has been decided by different means and by different methodological approaches. The most important of them is the classic method of conditioned reflexes. Methods of extirpation, oscillography, drug effects, histological and biochemical investigations of brain tissue were also used.

As a result, the physiological analysis of the processes under study has undoubtedly become much more profound and precise. An important index of this is the fact that in many works the investigations have been brought to the stage where the results of them even at the present time can be directly useful for the practice of public health.

It goes without saying there are still shortcomings in the activity of the Institute. The work presented in the present collection is not devoid of them either, naturally. The authors of the works here and the entire scientific body of the Institute are very much interested in the development of a principled working criticism of the investigations carried out in the Institute. All comments on this subject will be studied and considered attentively.

In recent years, the Institute has considerably extended and strengthened its connections with scientific-research and teaching medical institutions of the periphery. Therefore, we felt it was possible to publish in this collection a series of works which have been carried out outside of the walls of the Institute but which are similar in their character to those which are being carried on in the laboratories directed by A. D. Speranskiy.

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Doctor of Biological Sciences

BRANCHES OF STUDY OF THE PHYSIOLOGICAL MECHANISMS OF ACTIVE IMMUNIZATION AND THE PERSPECTIVES OF INCREASING ITS EFFECTIVENESS

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The problem of immunity to infectious diseases occupies one of the central places in pathophysiology. The question of the mechanisms by means of which the state of immunity is attained is, in its turn, most important here, because a profound penetration into its inner essence is the most reliable approach to the regulation of immunity.

Undoubtedly, among the reactions which assure the creation of immunity, the chief significance belongs to those which alter the sensitivity of the body itself. These reactions are frequently not connected with any changes in the indices of the so-called anti-infectious immunity (phagocytosis, antibodies) and consist of the rapid functional reconstruction of the body which returns it to the state of equilibrium with the disruptive conditions of the environment. These protective reactions of the body to the antigen are essentially no different from reactions to other stimuli and rather represent a category of processes characterizing the normal activity of the body, because reasons for their occurence and development are constantly encountered. As far as the nature of these reactions is concerned, no doubt exists; there is hardly anyone at this time who would dispute their neural origin.

The study of the neural mechanisms of immunity, begun by Academician A. D. Speranskiy about 25 years ago, is proceeding successfully, especially in recent years, by virtue of the complete victory of Pavlovian teaching in Soviet physiology and medicine. However, the number of works devoted to this subject are still not so great as might be expected. The attempt of investigators to understand the evaluate the part of cortical mechanisms in the development of non-specific immunity (which cannot be connected with the activity of special anti-infectious adaptations) is particularly valuable.

Indisputably, real progress in immunology is possible specifically along this line -- the branch of knowledge concerned with the mechanisms of the adaptative reactions of the complex organism to existence under conditions of prolonged or brief contact with the causal organisms of infectious diseases.

Up to the present time, a larger part of the investigations has been devoted to a more specific problem -- specific prophylaxis of infections by means of so-called active immunization.

As is well known, the study of vaccination was begun at the time of the excellent discoveries of Jenner and Pasteur. Later, work was conducted basically in the direction of the widest application of this method of prophylaxis and of increasing its effectiveness by means of obtaining purified antigens and simplifying the method of immunization. Progress here has been considerable. We need mention only the fact that, thanks to the use of active immunization, the incidence and mortality rate of smallpox, diphtheria, tetanus and a number of other infections have dropped sharply.

At the same time, it should be noted that the development of the theory of active immunization for a long time proceeded almost exclusively according to the plan of the simplified conceptions of Virchow and Ehrlich.

The mechanism of active immunization has been summarized as being simply an accumulation of specific antibodies and an increase of the phagocytic activity of elements of the reticulo-endothelial system. The effect of the antibodies, particularly of the antitoxins, was, in its turn, considered to be most elementary: their role was reduced to the neutralization of the microbial toxins, in the same way that base neutralizes acid in the test-tube.

I. I. Mechnikov was the first who came out decisively against this vicious system of views. He justifiably asserted that immunity which is created as a result of active immunization is a very complex phenomenon, and he pointed to the importance of a change in the sensitivity of the body in this process.

In accordance with the state of the theory, a set pattern and routine prevailed in giving the inoculations. This was noted, for example, in a completely identical approach to the development of methods of immunization against different infectious diseases.

Nevertheless, this empirical work of many years' duration made possible the demonstration of a number of specific rules and regulations and the accumulation of a large number of facts which were of essential significance for the elucidation of the mechanisms of active immunity. Only relatively recently has a departure come about, among the majority of immunologists, from the traditional but extremely erroneous schemata of Virchow and Ehrlich by virtue of the development of criticism of Virchovian ideas in pathology and the gradual assertion of the basic principles of the teaching of I. P. Pavlov. An attempt was evidenced among Soviet investigators, as P. F. Zdrodovskiy said, to see a reflection of the general rules and regulations of nervous system activity established by I. P. Pavlov, N. Ye. Vvedenskiy and A. A. Ukhtomskiy in the special rules and regulations of immunogenesis.

It would be incorrect, however, not to mention that there were investigators among the Soviet Pathophysiologists and immunologists who, in their creative research in the field of immunology, had been guided by the basic postulates of the teach of I. P. Pavlov long before the combined session of the two academies [Academy of Sciences and Academy of Medical Sciences]. Aside from A. D. Speranskiy and his students, G. V. Bygodchikov, A. D. Ado, N. V. Puchkov, A. N. Gordiyenko and others should be named here.

It must be granted that although the great majority of investigators who are devoting themselves to the study of the problem of active immunization now have the proper methodological attitudes, nevertheless, in the evaluation of a number of questions of principle there is still no unanimity of opinions, and a certain theroretical dispersion is occurring. Numerous cases still exist where the authors have limited themselves to a purely declarative acceptance of the teaching of I. P. Pavlov.

The present communication is devoted to certain theoretical generalizations and factual data on the problem of active immunization which have been obtained in the Laboratory of Physiology of Immunity of the Institute of General and Experimental Pathology of the Academy of Medical Sciences USSR.

The experimental material was obtained by D. F. Pletsityy, L. L. Aver'yanova, R. Ya. Zel'manovich, V. D. Kucherenko.

As is well known, the prototype of immunity created under the influence of artificial active immunization is post-infectious immunity, which is different in intensity and duration for various diseases. This circumstance is often forgotten, although it serves as the starting point for very important conclusions. As a matter of fact, the specific mechanisms of development of individual infectious diseases are different, and, accordingly, the immunity occurring in these diseases is also different.

A physiological study of the mechanism of the immunizing effect o individual antigens is unthinkable except on the basis of a thorough knowledge of the mechanisms of the development of the corresponding infectious diseases. The particular rules and regulations of immunogenesis after the injection of various antigens differ from one another to the same degree as do the pathogeneses of the corresponding diseases. It seems indubitable, for example, that inadequate progress in the matter of the specific prophylaxis of typhoid and dysentery are explained primarily by the lag in the study of pathogenesis of these diseases.

True, a small-scale reproduction of the specific disease picture (for example, in anti-smallpox vaccination) occurs in a number of cases in the course of ummunogenesis after immunizations with live vaccines. However, even here, a knowledge of the fine mechanisms of development of the pathological progress would undoubtedly make possible an improvement in the methods of immunization.

The investigation of the pathogenesis of individual diseases, like the study of the mechanisms of immunogenesis, can be effective only under the conditions of physiological analysis of these processes. The theoretical basis for such an analysis should be the principle of nervism, the Pavlovian reflex theory. A study of the time factor and of the role of the site of application of the antigen are, in their turns, of quite considerable interest in the system of investigations of the mechanisms of development of disease and immunogenesis on the basis of the physiological teaching of I. P. Pavlov.

It is well known that one and the same stimulus produces reactions which are different in intensity and in form depending on the place where it is acting in the body. This difference is maintained even when the stimulus acts a long time and even where, in time, it enters the general circulation. Therefore, the process which arises at the very beginning of action of the stimulus is particularly important and exerts a long and strong effect on the subsequent state of the body.

A. M. Bezredka was the first investigator who gave special attention to the role of the site of application of the antigenic stimulus in the development of active immunity. His ingenious experiments in this direction are widely known. However, the error of the theoretical starting points of A. M. Bezredka, which are based on concepts of tissue, cellular reception of antigens, finally brought his investigations into a blind alley.

A. D. Speranskiy propounded a new idea with regard to this question, espousing the belief of the neural reception of antigens in the complex organism. The accuracy and fruitfulness of this approach can hardly be disputed.

Our investigations, which are devoted to the study of the role of the site of application of the antigenic stimulus, are connected with the study of tetanus.

In experiments on rabbits it has been established that a necessary condition for tetanus is the action of threshold doses of tetanus toxin on the neuroreceptor structures of skeletal muscles. In those cases where there are no such conditions, for example, after the injection of a lethal dose of tetanus toxin subcutaneously in the upper part of the ear or into a skin flap, tetanus does not occur.

The neuroreceptor apparatus of skeletal muscles is, thus, a specific system, in its own way, with respect to tetanus toxin.

On the basis of these data it might be expected that the stimulation produced by tetanus toxoid would also be most effective after intramuscular injection.

Experiments were done on rabbits. The animals of the control group were immunized by means of the subcutaneous injection of tetanus toxoid; the toxoid was injected into the experimental rabbits intramuscularly. For the rest, the experimental conditions were similar for both groups of animals.

Immunization was accomplished according to the following outline. First, the rabbits were injected with two cubic centimeters of toxoid each; then, after three weeks, a second injection of the toxoid in a volume of one cubic centimeter was given. After 45 days, the animals were revaccinated by means of the injection of one cubic centimeter of toxoid.

The dynamics of the immunity were evaluated according to the antitoxin level in the blood of the animals. Blood for determining the antitoxin titer was taken at the following times: before the onset of the experiment, 21 days after the first injection of the toxoid (directly before the second inoculation), 14 days after the second toxoid injection, before revaccination and 14 days after it. The antitoxin content in the blood serum was calculated according to the usually accepted method.

The results of the experiment showed significantly higher contents of antitoxin in the blood of the experimental animals (which had been injected intramuscularly with toxoid) than in the controls (which had been immunized subcutaneously). The titer of toxoid in the blood of the experimental animals after the first immunization was five to ten times higher than in the control animals; after revaccination, on the average, twice as high.

Similar experiments were recently performed by us on monkeys. These experiments confirmed the results described above. The question of the intramuscular immunization of people to tetanus comes,up, because the methods of immunization accepted at the present time cannot be considered theoretically well grounded or maximally effective.

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Similar work on the review of immunization methods which are firmly included in anti-epidemic practice (with an account of the characteristics of the pathogenesis of the infections) is also beine conducted by other authors. Thus, the data of N. A. Khmel'chenok deserve attention, namely, that in experiments on rabbits the injection of diphtheritic toxoid into the area of the lymphoid pharyngeal ring of Pirogov [Waldeyer's ring] shows a distinct advantage over the subcutaneous route. These data have found confirmation in the therapy of people (N. A. Khmel'chenok).

Indirect proof of the accuracy of this point of view concerning the correlation of specific rules and regulations of immunogenesis with the mechanisms of development of corresponding diseases has been furnished by our experiments on the study of the characteristics of the reactions of immunity at different ages.

Here also the principle of the investigations is connected with the experimental material obtained in the analysis of the pathogenesis of tetanus. Mice and rabbits which are born with inadequately developed nervous systems do not show the clinical picture of tetanus in the early stages of postnatal ontogenesis. The typical reaction to the effect of tetanus toxin is established in them with the development of the nervous system -- the inclusion of afferent systems, the establishment of reciprocal relationships in the central nervous system and the development of complex forms of reflex activity. In animals born with relatively well developed nervous systems, those capable of independent life beginning with the first few days (guinea pigs), the typical manifestations of tetanus can be found immediately after birth.

It turned out that the immune reactions of the animals undergo exactly the same evolution in the course of ontogenesis. New born rabbits, for example, are completely inert in the immunological sense. No significant accumulation of antitoxin occurs in the blood of these animals following the effect of parenteral injections of tetanus toxoid, and no immunological reconstruction of the body comes about. With age, as the nervous system is developed, parallel with the development of the capacity for reproduction of the specific reaction to the administration of tetanus toxin, the immunological reactivity of these animals is increased and at the end of the first month of life attains adult levels. Guinea pigs, right from the first few days of life, are no different from adults in the immunological sense.

As has been seen, even in ontogenesis the development of the mechanisms of disease and immunity proceed in parallel, which once again emphasizes the capacity of mutual conditioning of these processes.

Investigators working in the field of practical immunology have long noted the tremendous importance of the time factor in the development of active immunity.

The essence of the matter here consists in the fact that even a single antigenic stimulation leaves a stable trace in the body, which is sometimes maintained for life. The duration of this residual reaction undisputably points to the fact that the keeper of it is the nervous system. Frequently, a second immunizing stimulus not only completely restores the reaction which had been extinguished with time but also notably increases it, sometimes by many times. Therefore, we are dealing here with a purely physiological phenomenon, which has received the name summation. The result of the interaction of the two immunizing stimulations is dependent primarily on the duration of the interval between them. Careful study of this subject by P. F. Zdrodovskiy has given appreciably practical results. The so-called law of intervals was formulated which to a certain degree has made possible the elimination of empiricism in immunization.

It is impossible to avoid seeing reflections of general laws of dynamics of neural excitation, which were established by N. Ye. Vvedenskiy, in these rules and regulations of immunology: in both places, the reactions to simulations are determined primarily by the properties of the preceding stimulation and by the period which has elapsed since the time of its application. With such an approach, the reactivity of the body ceases to appear as a mechanism which has been set once and for all, but appears rather to be in a shifting category which is continuously changed throughout life.

However, for a long time one circumstance made it possible for opponents of the physiological trend in immunology to object to the ideas of I. P. Pavlov, N. Ye. Vvedenskiy and A. A. Ukhtomskiy in the analysis of the rules and regulations of active immunity. The objection consisted in the fact that the usual physiological reactions progress very rapidly, while immunological reactions require long intervals of time for their accomplishment.

The lack of a sound basis for these objections is obvious. It is well known that many physiological processes, for example, the formation and reinforcement of the conditioned reflexes, are also developed over long periods of time. However, it seems most improbable that the development of immunological reactions could not be recorded in short periods of time (with which physiology usually deals).

We also occupied ourselves with the elucidation of the dynamics of immunity several years ago. At the same time we set before ourselves the task of progressing toward the understanding of those interrelationships which are constructed in the process of elaboration of active immunity between immunity proper and antibody-formation.

Our first investigations were devoted to the analysis of the physiological essence of the so-called negative phase of immunity, the presence of which has long been noted by a number of authors in the early stages of development of active immunity. We performed two series of experiments here.

In the first of them, mice served as the experimental animals. The dynamics of anti-tetanus immunity was investigated in them following a single and a repeated (at interval of 10 days) immunization with tetanus toxoid.

The degree of immunity was evaluated on the basis of the survivability of the animals following the injection of a single lethal dose of tetanus toxin after a single immunization, or of two single lethal doses following a double immunization. In addition, account was taken of the times of onset of the disease, its duration, and also the intensity of the manifestations of tetanus. The observations were conducted for a period of 10 days after the first and second injections of toxoid. Every day, one group of mice was investigated, so that the possibility existed of observing the development of immunity day after day for 10 days after the first and second inoculations.

These experiments showed that the response of the body to the immunizing stimulus is of a complex phasic character. The level of immunity created fluctuates periodically: the phases of definite immunity are replaced by periods immunological depression ("negative phase"). It turned out that the curves depicting the dynamics of immunity following single and double immunizations of the animals were basically unitypical, even though they proceeded on different levels.

In evaluating these data from the point of view of physiological concepts, the phasic character of the development of immunity should apparently be regarded as a manifestation of the dynamics of the specific neural process. In doing this, the negative phase, ceases to appear as an accidently and paradoxical phenomenon but is seen as a regularly repeated stage of development of the state of immunity.

Analogous experiments have been done on rats with the difference, however, that investigations were also conducted of the antitoxic properties of their blood in parallel with direct determinations of the immunity of the animals at various times after single and double injections of the toxoid.

In the first of these experiments, which was performed on 600 rats, account was taken of the dynamics of immunity to a lethal dose of tetanus toxin and of antibody-formation in the animals on the first, tenth, twelfth and fifteenith days after the administration of one cubic centimeter of tetanus toxoid, In the second experiment, in which 600 rats were also utilized the dynamics of immunity to the two lethal doses of tetanus toxin were studied at the same intervals after the second injection (10 days) of toxoid (twice, using 0.5 cubic centimeters each time).

It was shown that the survival rate of animals following injections of one and of two lethal doses of tetanus toxin in singly and doubly immunized animals is affected in the same way as in the experiments on mice. Here also, the periods of increase in immunity were regularly replaced by periods of decrease of it, although after a second injection of the toxoid the general level of resistance is increased.

The second experiment showed a definite lack of correlation between the degree of immunity of the animals and the content of tetanus antitoxin in the blood. The titer of antitoxin in the blood of the animals is, for a certain period of time, directly dependent on the time which has elapsed since the day of immunization. No such correlation could be found with respect to resistance (immunity proper). For example, on the fifth day after the second injection of toxoid, the content of antitoxin in the blood was equal to 1/300 antitoxin units, while the survival rate of the rats after the injection of two lethal doses of toxin was 76 percent. On the seventh day, with the same antitoxin titer only 42 percent of the rats survived. On the 10th day the amount of antitoxin in the serum reached 1/100 antitoxin units, whereas the survival rate dropped to 62 percent compared with the fifth day.

On the basis of these data which establishe the wave-form course of the process of development of active immunity, it might be possible to anticipate the presence of phasic changes of immunity at small intervals of time, measurable in hours, minutes and seconds. The experiment was performed on 600 rats. The animals were immunized twice with tetanus toxoid according to the same plan as was used in the preceding experiment. Later, the animals of each group were injected with two lethal doses of tetanus toxin directly after the second injection of toxoid at one, five, 12, 16 hours and, finally, after one day. Before each injection of toxin the antitoxin content in the blood was determined in addition to keeping a record of the survival rate of the animals.

As the results of this experiment showed, the immunity of the animals increases sharply immediately after the application of the second immunizing stimulus, becoming much greater than in the control rats, which had been immunized once.

After an hour, however, a notable decrease of the intensity of the immunity occurs, and after five hours it becomes almost two times weaker than in the first minute after the second injection of toxoid. At 12 hours, the resistance reaches the initial level, becoming equal to the immunity ofonce-immunized animals. Sixteen hours after the second immunization, a small decrease is again noted in the degree of immunity, which at the end of the day is replaced by an increase in it.

As might have been expected, the fluctuations in the strength of the immunity of the animals in these short periods was not accompanied by changes of the figures of antitoxic activity of the blood. The antitoxin titers of the blood sera of the animals remained unchanged for the entire experiment and were equal to 1/300 antitoxin unit.

The most important conclusion which should be drawn from the results of this experiment is that the immunity of animals can be changed instantaneously through the influence of an immunizing stimulus.

On the basis of the experiments presented above, the following conclusion may justifiably be drawn. The condition of immunity which is developed through the effect of immunizing stimuli (active immunity) is the result of a specific reconstruction of the entire body, the final expression of which is the creation of a new, as a whole higher, level of resistance and of immunological reactivity. The accumulation of specific antibodies is only one of the manifestations of this reconstruction thereby, even in the event we are dealing with the special form of immunity which has received the name antitoxic immunity. Therefore, the non-specific (not associated with phagocytosis and antibodies) component of immunity is, as has long ago been stated by A. D. Speranskiy, predominant in the mechanism of the so-called active artificial immunity.

In connection with the results of these experiments which show that the reactivity and the immunity of the body can be altered in very short time intervals, we set about developing methods of active immunization, new in principle.

If immunity and the reactions of immunity can be altered in very short periods of time through the influence of a specific immunizing stimulus, it means that it can also be changed in the desired direction by artificial means in the same periods of time.

The data of our experiments give evidence to the effect that with the proper intervals between inoculations and a definite number of inoculations the entire immunization cycle can be limited to several hours.

The immunity of rats and rabbits immunized by this accelerated method did not lag behind the resistance of control animals which had been vaccinated according to plans adopted in immunization of groups of people using many-day intervals between antigen injections.

Recently, we made the same observations on monkeys. Just as in the experiments on rats, so in the case of monkeys we studied the immunity to tetanus which was attained by immunization of the animals with tetanus toxoid. Similar results were obtained recently also during the utilization of the method of accelerated immunization for the creation of active immunity against dysentery (experiments on rabbits).

On the basis of the orientation experiments, it may be thought that the use of the plan of accelerated immunization developed by us might be effective also in those cases where they are carried out on a background of an infectious process which has already begun. We have used these methods successfully some time after injecting animals with one or two lethal doses of tetanus toxin.

A single injection of tetanus toxoid does not exert any effect on the development of tetanus intoxication, whereas the fractional administration of this dose according to a definite plan often gives positive effects. This permits us to hope for the possibility of utilizing tetanus toxoid as an agent for the specific therapy of tetanus.

It seems to us that the most important task confronting immunology today is a concentration of efforts for the future development of rapid and reliable methods of immunization which do not require many-day intervals between inoculations. The data presented show promise for investigative research in this direction.

THE ROLE OF NERVE-REFLEX MECHANISMS IN THE FORMATION OF ANTIBODIES

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I. I. Mechnikov emphasized the importance of humoral factors in the formation of acquired immunity. (I. I. Mechnikov, Immunity in Infectious Diseases, Medgiz, 1947, p 309).

Humoral phenomena in acquired immunity are expressed as the appearance of specific antibodies in the blood serum. The antibodies are not the chief nor the only cause of acquired immunity, and the mechanism of their action cannot be reduced to the antigen-antibody reaction. However, the prophylactic and therapeutic significance of immune sera cannot be disputed, and, therefore, the study of the physiological mechanisms of their formation and the search for methods of increasing their effectiveness remain important problems of immunology.

The study of the physiology of acquired immunity in the portion of it which is associated with the formation of antibodies has, in our opinion, three basic aspects; first, the investigation of the intimate biochemical essence of the processes of interaction of the antigen and the fermentative systems of the cellular elements of the macrophage system as a result of which the protein complexes are formed which play the part of the antibodies: secondly, the investigation of the physiological trigger mechanisms of the formation of antibodies and, finally, the physiological mechanism of their preventive and therapeutic effects.

Until recently, the investigation of these problems was made from the standpoint of the cellular pathology of Virchow, while the formation of antibodies was regarded only as the result of the purely direct contact of the antigens with the cellular elements. Ehrlich's ingenious hypothesis of "side-chains" reigned in immunology, and the mechanism of action of prepared antibodies injected into the body was regarded from the point of view of the antigen-antibody reaction.

Nevertheless, the penetration of the infective nidus into the body, or the administration of antigen with the aim of creating artificial immunity leads to the development of a series of complex reflex reactions in the body.

However, until very recently the reflex mechanism of the reactions to the antigenic stimulus was stubbornly ignored, although it is hard to understand how the stimulus can miss the nervous system, which reacts most quickly and precisely to any stimulation and produces the corresponding effect. In 1937, A. D. Speranskiy wrote, "It is dreadful to contemplate what would have become of physiology if nerve influences had been excluded from the processes which it studies so persistently as they have been excluded from pathology until recent years." (A. D. Speranskiy, Elementy postroyeniya teorii meditsiny (Elements of Construction of a Theory of Medicine) published by VIEM [All-Union Institute of Experimental Medicine], 1937, p 328). The role of nerve reflex mechanisms in the pathogenesis of infection and in immunogenesis have been extensively shown by facts obtained in the laboratory of A. D. Speranskiy.

Despite this, the conception of the leading role of the nervous system in immunogenesis has still not found universal acceptance.

In the present work data are being presented of complex investigations which were devoted to three subjects: 1) the influence of cortical regulation on the formation of serum antibodies, 2) the role of the nerve reflex component and the receptor zone of the primary application of the antigen in the formation of antibodies, and 3) the elaboration of antibodies under the influence of chemical factors of nerve excitation.

The works of co-workers of A. D. Spernaskiy's laboratory (I. P. Bobkov and A. L. Fenelonov A. L. Fenelonov, A. V. Ponomarev, S. I. Lebedinskaya, O. Ya. Ostryy, A. Ya. Alymov, D. F. Pletsityy) showed the influence of the primary site of application of the antigenic stimulus on the character of development of the infectious disease and the formation of immunity. Based on the work of the authors named, we investigated this subject through the examples of the creation of immunity following vaccinations against rabies, tetanus, and dysentery.

In the experiments of Ye. I. Kurbatova with anti-rabies vaccination the previous data of Akker were confirmed, to the effect that the intracutaneous administration of antirabies vaccine in the Fermy method produces the strongest immunity, which sets in after shorter periods than with subcutaneous vaccination. Experiments were performed on a large number of rabbits. It turned out, therein, that as early as 10 days after the conclusion of the intracutaneous vaccination the rabbits survived subdural infections with 10 lethal doses of fixed virus. The rabbits which had been given the vaccine subcutaneously withstood no more than two to five minimal lethal doses, even 30 days after the conclusion of the immunization. It is very interesting that inoculations of Philips' vaccine by the intracutaneous method did not give better results than were obtained by subcutaneous injections. The strength of the immunity and the periods of its development were in this case no different from the results of the subcutaneous inoculations given according to the Fermy method. It was not possible to raise the degree of immunity by an additional intracutaneous injection of phenol in the capacity of an additional stimulus. The experiments of A. K. Volchikhina with anti-tetanus vaccination gave analogous results.

In the experiments of A. K. Volchikhina, another law was shown clearly. It appeared that with very large doses of toxoid (up to 13 cubic centimeters) and short intervals between the injections the differences between receptive zones levelled off, and the creation of antitoxin decreased significantly.

These data coincide with the data of the investigations of P. F. Zdrodovskiy's laboratory; P.F. Zdrodovskiy showed the significance of summation of the immunogenic stimuli and the phenomena of the so-called worst suppression of immunogenesis after subcutaneous immunization. It must be supposed that an optimum frequency and strength of the immunogenic stimulus exists for each receptor zone, which is determined by the physiological lability of the given sense organ and its relationship to the effector organs of immunogenesis.

With immunizations by corpuscular vaccines, the optimal receptor zones maintain their significance, but here they are different. Thus, in the experiments of E. V. Konovalov and T. A. Solov'yeva, who used single immunizations of rabbits with heat-killed dysentery vaccine prepared from the Flexner dysentery strain No 942 (W), it was established that the highest titers of agglutinins are seen after the injection of the vaccine intravenously, somewhat smaller ones after injections into the capsule of the knee joint, considerably lower titers after subcutaneous injections, and the lowest, after intraperitoneal injections.

At the same time, in the still unfinished experiments of T. A. Kukhareva the best effect in immunizing against gas gangrene was obtained with vaccination into the joint, and the poorest, with intravenous administration. Naturally, the question arose: to what extent does this difference depend on the properties of the receptor zone specifically and the characteristics of the reflex mechanism in each individual case? With the aim of deciding this question, E. V. Konovalov and T. A. Solov'yeva performed experiments on the study of the dynamics of immunity after vaccinations in a denervated joint and in a joint with an intact innervation.

Denervation of the joint was accomplished by means of transection of the sciatic and femoral nerves and smearing the blood vessels with ammonia.

For the purpose of excluding the effect of the operation itself on immunogenesis, the denervations of the joints were performed in all the experimental and control rabbits. The vaccination was performed in the experimental rabbits in the denervated joint, and in the joint of the opposite extremity in the controls (this joint had also been denervated in the control rabbits).

The agglutinin titers after a single vaccination into the denervated joint were considerably less than after the injection of the antigen into the joint with the innervation intact. Revaccinations of the control rabbits in the denervated joints did not increase the antibody titers. Revaccinations of the rabbits in the joints with innervations intact, those which had been vaccinated in the denervated joint capsules, sharply increased the agglutinin titers. It does not appear possible to explain these results by an alteration of the rate of resorption from the denervated joint, because special experiments in which the resorption rates of Bacilli prodigiosum from denervated and normal joints were determined showed that they were the same.

The same rules and regulations apply in principle to the enteric immunizations of animals with impaired innervations of the gastrointestinal tracts. Thus, in the experiments of Ye. Ye. Kostromina with the enteric administration of dysentery vaccine to rabbits in which the vagus nerves had been transected under the diaphragms, the maximal agglutinin titers barely reached the minimal titers for the control animals, which had not been subjected to vagotomies.

Thus, on the basis of the experiments presented and also the data in the literature (A. Ya. Alymov and D. F. Pletsityy) we should conclude that the intensity of immunogenesis depends on the frequency and strength of the immunogenic stimulus, on the primary site (receptor zone) of application of the antigen and on the physiological condition of the peripheral end of the sense organ.

In any method of immunization, however, not only the condition of the peripheral end of the sensory organ and the primary site (receptor zone) of application of the antigenic stimulus but also the state of excitability of the other sensory organs is of primary significance to the intensity of immunogenesis. Thus, it has been established by the experiments of L. A. Pervushina that when the excitability of the nervous system is increased by strychnine, and dysentery vaccine is administered intraperitoneally, immunogenesis is increased markedly.

M. Ya. Sergeyeva, after increasing the excitability of the nervous system, also obtained an increased formation of agglutinins (by three to four times, compared with controls) as a result of enteric vaccination with dysentery vaccine.

In the state of chloral-hydrate anesthesia, however, the formation of agglutinins was markedly decreased. It should be noted that narcotic inhibition of immunogenesis is most intense when the narcosis is used in the first stages of vaccination and very slight when the narcosis is used only during the period of revaccination, which also definitely speaks for the reflex mechanism of the first phase of immunogenesis. These rules and regulations were established by numerous experiments of our colleagues N. P. Yefimova and L. V. Kalygina during the study of the effect of narcosis on the intensity of formation of antitoxin after immunization of rabbits with diphtheria toxoid. These data completely coincide with analogous data of other authors, particularly with the data of P. F. Zdrodovskiy's laboratory.

The decrease in immunogenesis in the state of anesthesia is also connected with a decrease in the absorptive function of the macrophage (reticulo-endothelial) system of I. I. Mechnikov. In our experiments, performed in conjunction with L. I. Raykher and A. P. Kobyl'skiy, and in the experiments of A. P. Kobyl'skiy it was established that in the state of anesthesia a delay in the removal of Bacilli prodigiosus microbes from the blood stream occurs, and parallel with this, the intensity of formation of agglutinins also decreases. For purposes of excluding any individual peculiarities of the rabbits, the control and the basic experiments were always performed on one and the same animals at intervals of seven to 14 days. It turned out, by so doing, that the injection of an emulsion of a culture of Bacillus prodigiosus during anesthesia was always accompanied by a delay in its disappearance from the blood stream compared with the initial injection, which was given without anesthesia, and it did not produce any increase in the titer of agglutinins. At the same time, the injection of Bacillus prodigiosus without anesthesia always led to a marked increase of the agglutinin titer and to a more rapid removal of these microbes from the blood stream compared with the initial injection during anesthesia. The microbes were removed most vigorously at the time of the second injection made without anesthesia. This is explained by the fact that the original injection of the microbes, when the animals are in a state of anesthesia, does not leave any residual state of increased antigenicity following it.

In the regulation of the formation of antibodies a significant role must be played also by the chemical factors of nerve excitation,

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which represent, according to A.A. Ukhtomskiy, "the chemical tail" of the process of excitation, prolonging the action of the nerve stimulus and preparing the substrate for the reception of subsequent nerve impulses at its new level of physiological lability. A study of the influence of the chemical factors of nerve excitation on immunogenesis is particularly important in view of the fact that the immune reactions occur at lengthy intervals which far exceed the periods of time during which the nerve stimuli begin and end.

Our co-workers have studied the effect of the chemical factors of nervé excitation on the formation of antibodies during immunization.

N. P. Yefimova and L. V. Kalugina showed in experiments on rabbits that against the background effect of physostigmine, acetylcholine injected daily in the intervals between immunizations leads to a very marked increase of the antitoxic titer of diphtheritic antiserum. Using the same method of administration, adrenalin reduces the formation of antitoxins by several times. This effect of the mediators is intimately related to the condition of cortical activity. Although acetylcholine stimulates immunogenesis, even on the background of anesthetic exclusion of cortical regulation, even though to a lesser degree than without anesthesia, adrenalin, on a background of anesthesia, completely suppresses the formation of antitoxins.

M. D. Poptsova showed that even with the enteric route of vacination of rabbits by liquid dysentery vaccine, perenteral injection of acetylcholine chloride increases the formation of agglutinins by two to four times. It is important to note that under the influence of acetylcholine chloride high titers of agglutinins are maintained even two and a half months after the conclusion of the vaccination, whereas in control rabbits a sharp drop in the titer occurs by this time -- almost to the initial level. This stimulating effect of acetylcholine chloride occurs only in the presence of a definite dose of the mediator. An increase of the dose of the acetylcholine chloride proves to be ineffective and produces a faster drop in the agglutinin titers after the conclusion of the vaccination.

In principle, the same results were obtained by D. V. Berezhnykh with the parenteral method of administering dysentery vaccine. Parallel with an increase in the agglutinin titer under the influence of acetylcholine chloride, using parenteral vaccination against dysentery, the intensity of the phagocytic reaction was also increased. Intensification of the phagocytic reaction in rabbits which have received acetylcholine chloride was less than the increase in the agglutinin titers. The phagocytic number of the index of intensity of phagocytosis in the experimental animals only slightly exceeded those in the control animals, which had been injected with physiological saline solution instead of acetylcholine chloride.

In the experiments of D. V. Berezhnykh the vaccinations were performed three times at intervals of seven days, and the determination of the agglutinin titer was made, as in the experiments of other workers, before the second and third injections of the vaccine and at 10, 20, 30 and 70 days after the third vaccination. In the intervals between the vaccine injections the rabbits of the control group were injected every other day with physiological saline solution, while the rabbits of the experimental group were injected with acetylcholine chloride. The experiments were performed on 24 rabbits. Seventy days after the conclusion of the vaccination and 91 days after the beginning of the experiment, at which time the agglutinin titers of six rabbits (two controls and four experimental) had dropped markedly and equalled from 1:20 to 1:80, the control rabbits were injected with physiological saline solution only, while the experimental rabbits were injected with acetylcholine chloride in the usual dosage. After 8 days, the agglutinin titers had not increased in the control animals, while in three out of four experimental rabbits receiving acetylcholine chloride it reached 1:160, 1:240 and 1:320. (Analogous results were obtained in P. F. Zdrodovskiy's laboratory by A. A. Klimentova (1948) and I. Ya. Uchitel' (1949). It should be noted that these authors did not inject the mediators in the process of vaccination proper). In this experiment of D. V. Berezhnykh apparently a conditioned reflex restoration of immunogenesis was seen. It should be emphasized especially that the conditioned reflex restoration of agglutinin formation was produced by acetylcholine chloride but not by physiological saline solution. It is probable that not just any stimulus coinciding in time with the action of the unconditioned-reflex immunogenic stimulation is suitable for the conditioned-reflex restoration of agglutinin formation but rather only an adequate stimulus which constantly accompanies neural regulation of the complex biochemical processes of antibody formation. Apparently, only such a conditioned-reflex stimulus can renew the entire complex reaction chain underlying antibody formation. This is, perhaps, just the key to the resolution of those contrdictions which occur in the investigation of the question of the part of the conditioned-reflex mechanism in the formation of antibodies.

The entire complex of our investigations of the neural regulation of antibody formation and of data existing in the literature make it possible for us to express the supposition that antibody formation occurs in a biphasic form. In the first phase, it is accomplished according to a reflex mechanism with a definite receptor zone determining the original intensity of immunogenesis, and in the second phase it is continued through the direct contact of the antigen with the effectors. Neuro-humoral regulation, which assures the prolongation of action of nerve stimuli, plays an essential part here. The regulatory role of the central nervous system in the second phase of antibody formation completely corresponds to I. P. Pavlov's conception of the function "...of trophic nerves, which determines, in the interest of the body as a whole, the precise measure of the definitive utilization of this material by each organ." (I. P. Pavlov, Polnoye sobraniye sochineniy /A Complete Collection of His Works/, 1951, Vol I, p 582.)

It should be emphasized that the mechanism of immunity can in no way be reduced merely to the formation of antibodies. Antibodies are only one of the links in the complex chain of activity of the body's protective devices. It must be supposed further, that the therapeutic and prophylactic effect of immune sera cannot be reduced simply to the neutralization reaction of the antigen by the antibody. In all probability, antibodies which are injected from without or which are formed in the body are themselves the stimuli of definite sense organs, and they reflexly inhibit pathological reactions which occur under the noxious influence of infectious agents. Thus, for example, tetanus antiserum probably inhibits the generalization of excitation produced in the motor center by the effect of the tetanus toxin. The experiments of M. D. Speranskiy, completely confirmed by the experiments of our co-worker, R. A. Khrustaleva, showed that tetanus antiserum when injected into the body with some delay after the injection of tetanus toxin, prevents the development of tetanus only on a background of increased excitability in the nervous system of the animals from the effect of strychnine. These experiments permit us to make the assertion above. This fact, which would appear to be paradoxical at first glance, can be understood correctly in the light of the teaching of I. P. Pavlov and A. A. Ukhtomskiy on the interrelationship of excitation and inhibition.

Here is what A.A. Ukhtomskiy wrote on this subject in his time. "Our school sees in them (in inhibition and excitation -- G.P.) the result of a conflict of excitations in the neural elements, and it /the school/ is inclined to connect the decline in impressibility by a stimulus, which is noted in the Weber-Flexner Law, with inhibition. Since the phenomena of immunity and resistance are also connected with the latter, the bridge between the concepts of nerual and humoral inhibition is outlined." A. A. Ukhtomskiy writes further: "For our part, we have become convinced that physiological science is, indeed, progressing in its entirety as a common front. The research of the humoralists and that of the neurologists are intertwined to a much greater extent that appears at first glance, and they will mutually contribute ideas to another until a really general theory is constructed which regards the various forms of stimulation and excitation as particular instances." (A. A. Ukhtomskiy, A Collection of His Works, published by the Leningrad State University, 1950, Vol I, p 324).

ON THE ROLE OF THE NERVOUS SYSTEM AND ITS HIGHER CENTERS IN THE MECHANISMS OF REACTIVITY CHANGES IN TOXIC-INFECTIOUS PROCESSES AND IN IMMUNITY

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A. D. Speranskiy and his co-workers have accumulated facts for the past 25 years from the various fields of pathology, including also that of infectious diseases. These facts have shown that the reactivity of the body may be altered to a significant degree by various types of operations on the nervous system. A series of facts was established, demonstrating the role of nerve-reflex mechanisms in the processes of reactivity changes of animals to various toxic-infectious stimuli.

The study of the action of extraordinary stimuli on the peripheral ends of the sensory organs in the mechanisms of compensation and protaction occupied an important place in our work.

I. P. Pavlov pointed out that "once the normal, or specific, stimuli are established for the appropriate organ, the physiological problem comes up of analyzing the mechanisms of action of these stimuli or of determining on what specifically they act and where the point of their application is located." (I. P. Pavlov, Complete Collection of His Works, 1946, Vol II, p 465).

As is well known, it has also been shown in our combined investigations with A. Ya. Alymov that a certain dose of streptoccocci when injected intravenously into the ears of rabbits led to their deaths from septicemia in 80 percent of the cases. The same dose of microbes injected into the femoral vein brought on the deaths of only an insignificant number of animals (34 percent).

Thus, not only the character of the pathological process but also simultaneously, the character and degree of the compensatory capacities of the body were determined by the site of injection of the microbes. If the reactivity of the neural apparatus in the area of the femoral vein is altered (by means of novocaine injection), then, disturbances of the compensatory functions of the body, and deaths of the animals occur to the same degree as after injection through the auricular vein.

G. N. Kryzhanovskiy and M. S. Delitsina established the fact that

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one and the same dose of tetanus toxin, which is incapable of causing disease when injected intracutaneously, subcutaneously, into a tendon, spinal cord, brain or even into the subarachnoid space, causes disease even with a fatal outcome when it is injected intramuscularly.

A particularly striking effect is obtained, as N. S. Delitsina has shown, when this dose of toxin is injected into the masticatory muscles of rabbits. These muscles, as is known, in rodents are in a state of increased excitability of a dominant type. Under these experimental conditions, all the animals died in the next few days with signs of marked trismus and opisthotonus.

Analogous facts were obtained in the experiments of Z. I. Sobiyeva in the study of the disease mechanisms of anaerobic infections.

A dose of microbial cutlture which did not produce the disease when injected intracutaneously proved to be entirely adequate to cause a fatal disease when injected intramuscularly.

The same kind of rules and regulations were noted also in our experiments with M. D. Speranskiy in the study of the pathogenesis of diphtheria.

In order to demonstrate the importance of pathological stimulation of appropriate nerve apparatus in disease mechanisms, special experiments were performed.

G. N. Kryzhanovskiy established the fact that if the nerve branches leading to the isolated muscle of a skin-muscle preparation are adequately transected the injection of tetanus toxin, under these conditions, did not cause the disease.

The significance of pathological forms of stimulation of nerve apparatus in muscles in the disease mechanisms of anaerobic infections was revealed by the investigations of Z. I. Slbiyeva. It was shown that under the conditions of transection of the posterior roots of the spinal cord, that is, with a disruption of the afferent portion of the reflex arc, the development of anaerobic infection is markedly inhibited if the microbe is injected into a muscle of the deafferentated extremity. An especially marked decrease in the mortality rates of animals is shown when they are infected 30 days after the operation indicated, whereas almost all the control animals infected by the same dose die in the first few days after the infection.

The same effect was seen with the simultaneous disruption of afferent and efferent links of the "reflex arc" by means of transections of the three basic mixed nerves of the posterior extremity (sciatic, femoral, obdurator).

Thus, by sparing the body to some degree from excessive pathogenic sti,ulation, we can make possible its equilibrium with the environment, even when there are virulent disease organisms or active toxins in its internal milieu.

Basing ourselves on the law established, we can approach the search for planned methods of acting on the body for purpose of prophylaxis and therapy. In other words, based on the mechanism of neural activity determining the reactivity of the body to environmental factors, it is possible to help the body surmount extraordinary stimulations even under conditions which under other circumstances would inevitably cause disease or even the death of the animal.

In the experiments of G. N. Kryzhanovskiy and Z. I. Sobiyeva data were obtained which showed the possibility of preventing the development of tetanus and gas gangrene by injecting the animals with tetanus toxin or anaerobic microbes in novocaine solution (one to 10 percent).

The experiments of G. N. Kryzhanovskiy with the injection of tetanus toxin mixed with novocaine led to results which extended the earlier data obtained by S. I. Lebedinskaya in this connection. At the same time, G. N. Kryzhanovskiy showed that for inhibiting the development of tetanus the injection of novocaine does not have to be made exactly at the same place where the toxin is injected. Under conditions where tetanus develops from the injection of toxin into one of the posterior extremities, an elimination of the muscular rigidity can be attained temporarily by injecting novocaine into the opposite extremity or into a muscle of one of the anterior extremities. In doing this, as has been shown by electrophysiological analysis, the increased electrical activity in the nerves of the extremity involved by tetanus disappears.

The same law was revealed in our experiments performed in conjunction with M. D. Speranskaya. The injections into guinea pigs of absolutely lethal doses of diphtheria toxin following the preliminary injections of two percent novocaine solution into the same sites do not produce the disease in many animals.

Thus, the facts presented again confirmed the very important role of reflex mechanisms in the processes of disease and resistance of animals to toxic-infectious stimuli. The important role here belongs to functional alteration of the threshold of reactivity of the nerve apparatus.

It has been shown in our previous experiments that one and the same dose of tetanus antiserum can exert different prophylactic and therapeutic effects depending on the site of injection of the serum and of the toxin.

It has been established in the experiments of G. N. Kryzhanovskiy that the best effect from the use of tetanus antiserum for prophylactic purposes has been obtained from the intramuscular injection of it.

Precisely the same law was established in the work of Z. I. Sobiyeva: gas gangrene antiserum exerted the greatest prophylactic and therapeutic effects when injected intramuscularly.

Thus, the data obtained in the study of the role of non-specific stimuli in the mechanisms of reactivity changes with respect to toxic-infectious agents have been developed in a new way in experiments using specific stimuli.

If so-called "passive" immunity is connected with the site of injection of the serum, as the development of the disease is connected with the site of injection of the toxin, then it is important to elucidate the significance of the same correlation in the development of active immunity.

It was established in the experiments of L. N. Fontalin that one and the same dose of tetanus toxoid when injected intravenously, intramuscularly or into the spleen stimulated the elaboration of antibodies in different ways. Whereas no signs of antibodies were seen on the 30th day of immunization when the toxoid was injected in certain doses intravenously and intrasplenically, a distinct increase of the antibody titer was observed when the same dosage of toxoid was injected intramuscularly.

In order to elucidate the role of the nerve apparatus of muscles in the processes of active immunization, L. N. Fontalin performed the following experiment.

A group of animals (21 rabbits) was immunized with precipitated tetanus toxoid into muscles which had their innervations disrupted.

Simultaneously, 22 control rabbits were immunized by the same method but using normal muscles. As a result, on the 30th day of immunization the antibody titers were three times lower in animals of the experimental group than in the control animals in which the toxoid had been injected into normally innervated muscles.

Even more striking data were obtained by L. N. Fontalin by immunizing rabbits under the same conditions but with unchanged tetanus toxoid. Here, the differences in antibody titers on the 30th day after immunization were even greater: the antibody titers of animals of the experimental group were 18 times lower than the antibody titers of the control animals.

Thus, it follows from the material presented that the mechanisms of disease and of protection cannot be dissociated from one another, and that the reflex functioning of the nervous system underlies these mechanisms.

The material presented, as is seen, showed that the most reactive innervation zones with respect to a certain pathogenic agent are the zones through which it is easiest to cause the protective reaction of the body with respect to this pathogenic stimulus.

Our work, therefore, completely confirmed the statement by I. P. Pavlov that "extraordinary stimuli acting in the capacity of pathogenic agents represen the specific stimuli of those protective devices of the body which are intended to fight against the corresponding pathogenic agents." (I. P. Pavlov, Complete Collection of His Works, 1946, Vol II, p 350).

We ascribe tremendous importance to the study of the effect of the functional condition of the higher central nervous system centers -- the cerebral cortex -- in increasing the resistance of the body to the toxic-infectious stimuli and in the stimulation of the immunological processes of defense.

We established in investigations of previous years that non-specific stimulation of the nerve-receptor apparatus of the lung reflexly increases the resistance of the body to tuberculosis. Further study of the processes which belong in this category, conducted by N. A. Kryshova and her co-workers in the Institute of Physiology of the Academy of Sciences USSR, showed that protective inhibition occurring in the cerebral cortex is of great importance in the therapeutic effect of the method proposed by our Institute of treating extrapulmonary forms of tuberculosis and certain other diseases of non-tuberculous etiologies by intravenous injections of bismuth carbonate suspensions.

Z. I. Sobiyeva studied the effect of inhibition of the higher centers of the brain on the compensatory processes of the body. By injecting animals repeatedly with certain doses of bromides and later infecting them with anaerobic microbes, she noted that under these conditions the duration of life of the infected animals was increased by more than three times compared with the controls. The same picture was seen when animals were infected on a background of anesthesia produced by amytal.

If an intensification of the processes of cortical inhibition (from the effect of bromide or amytal) leads to an increase in the resistance to microbes, then it is important to clarify how the reactivity of the body is altered when the animals are infected on a background of increased cortical excitability brought about by the action of phenamin. The experiments of Z. I. Sobiyeva showed that after repeated injections of phenamin into rats their resistance is decreased, and such rats die in larger numbers from gas gangrene than do the controls.

The role of the central nervous system in the compensatory mechanisms in tetanus was noted in the experiments of N. G. Kryzhankovskiy. The removal of one hemisphere of the brain and the injection of minimally pathogenic doses of tetanus toxin into the muscles of the corresponding anterior extremity produce and considerably aggravate the the disease compared with unoperated animals. This is expressed in a shortening of the incubation period and in a lengthening of the duration of the disease.

The data of L. N. Fontalin are in complete accord with these experiments; they showed that after the removal of one hemisphere and the injection of tetanus toxoid into the muscles of a posterior extremity (on the side corresponding to the removed hemisphere) a decrease in immunogenesis occurs. On the 21st day after immunization, the antitoxin titer in the blood of such animals was, on the average, 2.5 times less than in the control animals. Thus, the result of removal of one hemisphere was, on the one hand, an aggravation of tetanus, and, on the other, a disturbance of the process of creating immunity to tetanus. This means that the disease processes and the processes of immunological defense are most inteimately connected with the functionalstate of the higher centers of the central nervous system.

At present, we are utilizing the method of functional change of the reactivity of the body by means of cooling the head for purposes of intensification of the processes of inhibition. Our experiments performed in conjunction with M. D. Speranskaya showed that in white rats in whcih the head-cooling was performed for one and half hours there was an increase noted in the resistance to anaerobic infection.

When the cooling is performed before the infection of the animals, the morbidity rate and the mortality rate of the latter are significantly decreased. If the cooling is performed after the infection, it does not exert any prophylactic or therapeutic effect, and the animals die in the same numbers as the controls.

The analysis of the physiological mechanisms underlying this fact is being continued at this time.

We also established (in the preliminary experiments) the fact that following repeated head-coolings a condition is created wherein the application of a bag with sand at room temperature to the head of the animal instead of ice gives the same effect as was obtained with the preliminary cooling, namely, the resistance of the body to anaerobic infection is increased.

Out of 18 such animals infected with lethal doses of anaerobic microbes none of them died from gas gangrene, whereas in the control group of 18 rats 14 died from this disease.

The question of altering the reactivity of the body to anaerobic infections by the conditioned-reflex method is a subject for our future investigations.

Therefore, it has been possible to observe rules and regulations in the processes of infectious-disease pathology and immunity, which were established by the study of the physiology of nervous activity, particularly of the physiology of the cerebrum.

Under conditions of altered reactivity of the higher centers of the central nervous system an inactive stimulus may become an active one, and, contrariwise, extraordinary stimuli may lose this property.

I. P. Pavlov mentioned that the pathologic process "... can also be well analyzed, that is, reduced to known physiological processes and their various fluctuations..." (I. P. Pavlov, Twenty Years of Experience in the Objective Study of Higher Nervous Activity (Behavior) of Animals, 1938, pp 738-739).

In his time, I. P. Pavlov proposed a different viewpoint from concepts that immunity is manifested only in leucocytosis and reactions of the "antigen-cell-antibody" type without consideration of the very important role which the nervous system plays in the processes of immunity.

He welcomed new facts which disclosed the power of the nervous system and its capacity to regulate the defense immunological reactions leading to an equilibrium of the body with the microbial medium.

I. P. Pavlov pointed out that at present conditioned-reflex changes of immunity are known which pertain to the first signal system.

Of late, more and more facts are being accumulated which show the correctness of the conclusion drawn by I. P. Pavlov in his time. Here should be mentioned not only the work accomplished under the leadership of A. D. Speranskiy but also the great number of investi-

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gations performed in the laboratories of K. M. Bykov, A. G. Ivanov-Smolenskiy, V. N. Chernigovskiy, A. O. Dolin, P. F. Zdrodovskiy, A. D. Ado, V. S. Galkin, A. N. Gordiyenko, G. V. Vygodchikov, N. V. Puchkov, G. V. Peshkovskiy and many other Soviet scientists.

On the basis of everything which has been presented, the conclusion may be drawn that the reactivity of the body, that is, its relationship to the environment, normally as well as in pathology, is determined by the analyzer systems /consisting mostly of sensory organ units/, which play a most important role in the formation of the body's response reactions produced by the effect of various environmental stimuli on it. Reactivity and its changes are expressions of the degree of quantitative and qualitative reactions which underly the equilibrium and maintenance of the unity of the body with the conditions of its existence.

The reactivity of the peripheral nerve apparatus and that of their higher representatives to stimuli of the environment increases precipitously on ascending the evolutionary ladder, which increases the defensive functions of the body. I. P. Pavlov wrote that in the higher animals "The neural apparatus....was made reactive to the highest degree, that is, it was made accessible to the most varied phenomena of the external world." (I. P. Pavlov, Twenty years of Experience in the Objective Study of Higher Nervous Activity, 1938, p 67).

MATERIAL ON THE QUESTION OF THE ROLE OF THE CEREBRAL CORTEX IN RESISTANCE TO TETANUS

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In recent years, much factual material has been accumulated which sheds light on the importance of the condition of the nervous system as a whole and of certain individual links of it in the process of the disease, tetanus.

It has been shown that the injection of tetanus toxin into an area with various peripheral nerve receptor apparatus and also a disturbance of the central nerve connections can produce a radical inversion of the process in connection with clinical manifestations of the disease and the complete development of the disease. Claude Bernard showed that the development of tetanus can be eliminated by acting on the effector structures (intoxication of the myoneural endplate with curare).

Therefore, inteferences with the pathological process in tetanus at the afferent, central and effector parts of the reflex arc can be equally effective in affecting the outcome of /the disease.

The elucidation of the role of the cerebral cortex in the development of the tetanus disease process and in the resistance to tetanus is of particular interest.

The role of the cerebral cortex in the development of tetanus, as in the majority of pathological processes generally, apparently consists chiefly of the compensation of disorders produced by the disturbance of normal activity of lower neural structures.

The method of putting animals into a drug-induced sleep may be used to advantage for studying the functions of the cerebral cortex in the development of tetanus and of the resistance to tetanus, along with other methods of investigation.

This method has been extensively utilized in recent years not only in experimental pathophysiological investigations but also clinically in the treatment of a number of diseases.

Sleep therapy is based on the work of I. P. Pavlov, which was devoted to the experimental studyvof the pathogenesis and therapy of certain disorders of higher nervous activity, chiefly of neuroses. Based on concepts of the protective role of inhibition in the cerebral cortex, I. P. Pavlov proposed utilizing prolonged sleep in the capacity of a therapeutic measure in certain mental illnesses. This kind of therapy has at the present time obtained vary wide-spread distribution and recognition in a number of mental illnesses.

Later, the use of prolonged sleep was proposed also for the therapy of internal diseases (F. A. Andreyev). Considerable clinical experience which has been accumulated in this direction shows that the given method is very effective in certain internal diseases (hypertension and peptic ulcer).

In hypertension, peptic ulcer, and other diseases, where the primary and leading pathogenetic link is apparently the presence of cortical disorders, the effectiveness of this method can be evaluated within the plan of I. P. Pavlov's conceptions of protective cortical inhibition.

The understanding of the mechanism of the therapeutic effect of prolonged sleep in other internal diseases, in the development of which the chief roles do not belong to cortical disorders, is much more complex.

In tetanus, the combined administration of narcotic agents and of tetanus antiserum has been used with relative success for a long time now. However, experimentally, the effect of narcosis and prolonged sleep on the course of tetanus intoxication has been entirely inadequately investigated. There is very little reference to this subject in the literature.

Recently, appropriate investigations were conducted in the laboratory of V. S. Galkin (work of V. A. Koslov). It was shown on experiments on mice and rats that under the influence of prolonged (lasting for tens of hours) cyclonal anesthesia an inhibition occurs of the development of tetanus during the entire duration of the anesthesia in the animals. The same effect has also been noted in cases where animals already sick were placed under anesthesia. It should be noted that the experiments mentioned were conducted under conditions of injecting the animals with very large doses of the tetanus toxin (100 to 1000 MLD).

Brief periods of anesthesia according to V. A. Kozlov's data, did not affect the length of life of animals inoculated with tetanus. Bilyard in experiments on guinea pigs and N. V. Golikov in experiments on rabbits note that in doing this the course of tetanus is, on the other hand, made more severe. Thus, N. V. Golikov, after anesthetizing rabbits which were in states of local tetanus, observed that immediately after coming out of the anesthesia, signs of general tetanus developed in the animals for a short time. Anesthesia, therefore, made possible the demonstration of the process.

The present investigations do not help much in the elucidation of the role of the cerebral cortex in the development of thepathologic process and of resistance in tetanus. As a matter of fact, in all the quoted works anesthesia was achieved by means of compounds which act not only on the cortex but simultaneously also on subcortical centers. However arbitrary the division of anesthetics into "cortical" and "basal" might be, nevertheless, within certain limits selectivity of their actions on various parts of the central nervous system actually does occur.

For solving the problem posed it was necessary, naturally, to make use of an anesthetic agent which would act primarily on the cerebral cortex. As is well known, chloral hydrate is such an agent.

Our observations were made on rabbits.

The preliminary experiments showed that it is most convenient to give the chloral hydrate to the animals by mouth. After being diluted in distilled water which had been heated to 25-30° C, the anesthetic was introduced directly into the stomachs of the rabbits by means of a thin rubber stomach tube, to the end of which was attached a small funnel.

It was established that 15 cubic centimeters of 20 percent aqueous solution of chloral hydrate introduced into the stomach represents the highest dose that a rabbit can tolerate. Under the influence of this quantity of the substance the animals fell into a deep sleep, which lasted 15-27 hours.

A dose equal to 10 cubic centimeters of 10 percent aqueous solution of chloral hydrate produced only a light sleep in the animals which lasted four to eight hours.

The experiment was performed on 30 rabbits weighing two to two and a half kilograms -- 15 experimental rabbits and 15 controls. Rabbits of each group were given one MLD of tetanus toxin (30 mouse MLD per kilogram of weight) under the skin of the outer surface of the left thigh. Ten to twenty minutes after the injection of the toxin the experimental animals were given chloral hydrate anesthesia.

The rabbits were injected with 15 cubic centimeters of 20 percent

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aqueous solution of chloral hydrate. After several minutes, they became sluggish and did not move much, and after 10-15 minutes they began to fall asleep. After 20-30 minutes they were usually in a deep sleep. The rabbits for the most part lay on their sides. The depth of the anesthesia gradually increased, and the maximum was reached seven to ten hours after the production of anesthesia. The respiration of the animals became superficial, often hardly noticeable, the body temperature decreased by several degrees, the corneal reflex disappeared. A weak reflex muscular reaction could be noted only through the effect of strong pain stimuli (prick, pinch).

At the end of 12-14 hours, occasionally earlier, the first period of awakening occurs in the animals. The rabbits get up, take food: for a certain time (30-50 minutes) they are in a state of partial wakefulness, and then they fall asleep again. This sleep comes upon them unexpectedly: in a number of cases it was possible to find sleeping rabbits with their heads burried in their troughs.

The second phase of sleep lasts six to eight hours. Sleep at this time is considerably more superficial: the animals respond even to weak stimulations and can easily be aroused.

Then, the second period of awakening and wakefulness follows, during which the rabbits get up, eat, move about (unsteady gait).

The last stage is the phase of interrupted sleep. In this, the rabbits wake up periodically -- every 30 to 60 minutes. Gradually, the waking period becomes more prolonged, and the periods of sleep become shorter and shorter. Sleep becomes superficial; in the last few hours, the rabbits sleep sitting up. The existing state of inhibition of the central nervous system, specifically of the cerebral cortex, can be judged only by the immobility of the animals and by the closed eyes.

From the short characterization presented of the hypnotic effect of the dose of chloral hydrate used, it is seen that the inhibition of the central nervous system developing under the influence of the anesthetic agent is not the same during different periods of sleep.

On the basis of the observations presented, the following concept may be constructed of the dynamics of this inhibition.

In the very first phase, chloral hydrate undoubtedly acts chiefly on the cerebral cortex. However, afterwards, its action diffuses to lower structures of the brain and spinal cord. At the peak of the first period of sleep a deep inhibition of the entire central nervous system is seen which is so considerable that it is more accurate to speak here not of sleep but rather of anesthesia. Recovery of the active states of various central nervous system centers occurs in reverse order, as the animals emerge from the condition of narcosis: first, the spinal centers are released from inhibition, later, the brain stem and subcortical ganglia, and, finally, the cortex. In the last period of action of chloral hydrate, sleep is achieved, therefore, apparently exclusively on account of the inhibition of the cortex.

Later, the rabbits were injected daily with 10 cubic centimeters of 10 percent aqueous solution of chloral hydrate. Under the influence of this dose an unusual sleep state developed in the animals, which was not accompanied by a diffusion of inhibition to the subcortical area of the brain or to the spinal cord.

The animals became adynamic, and 20-30 minutes after the injection of chloral hydrate, a light sleep came upon them. The rabbits remained in the sitting positions, lowered their heads and closed their eyes. On the first day, they remained in this condition for 10-12 hours, and on the second to third day, for six to eight hours. Under the influence of strong stimuli (prick, jolt), the animals awoke for a short time. Sound stimuli also brought the animals out of the state of sleep: the rabbits opened their eyes, moved their heads, sometimes moved about, but fell asleep again right away. The effect of the chloral hydrate continued for several hours after the conclusion of the period of the distinctly evidenced somnolent state, which expressed itself in general inhibition and lack of mobility of the animals.

The picture described above was notably changed with the development of tetanus intoxication in the animals. On the second to third dayafter injecting the rabbits with tetanus toxin, the hypnotic dose of chloral hydrate mentioned produced a progressively diminishing effect. In the period of the expressed general tetanus it was almost inactive and did not exert any notable hypnotic effect.

The development of tetanus in the experimental animals was characterized by the following peculiarities.

The first injection of the chloral hydrate, which produced profound anesthesia, inhibited the appearance of the signs of tetanus. While in the majority of control animals, distinct phenomena of local tetanus could be noted 36 hours after the injection of toxin, there were no pathological disorders in the experimental animals at that time. However, later (48-52 hours after the injection of the toxin), the picture of tetanus in both groups of animals became the same. The temporarily inhibited pathological process thus developed in an accelerated fashion when the animals came out of the anesthesia.

Later, with the generalization of the process, a more rapid development of tetanus was found in the experimental animals. Their deaths occurred in the majority of cases sooner than in the control rabbits. A protocol of one of the experiments is presented below.

Rabbit No 2904. Weight 2,700 grams	Rabbit No 1701. Weight 2,200 grams
Experimental	Control

9 January 11:00 o'clock

One MLD of tetanus toxin (30 mouse MLD per kilogram of weight) was injected subcutaneously into the external surfaces of the left thighs of both rabbits. Fifteen cubic centimeters of 20 percent chloral hydrate solution were introduced into the stomach of the experimental rabbit (No 2904) 15 minutes after the injection of the toxin.

9 January 12:00 o'clock

Experimental	Control
Rabbit sleeping with even deep sleep, lying on side. Withdraws extremity when paw is pricked with a needle or pinched. Corneal reflex maintained. Does not react to sound stimuli.	Rabbit completely healthy; no abnormalities in behavior.

9 January 5:00 p.m.

Experimental	Control
Superficial, hardly detectable respiration. Temperature 35.4° C. No corneal reflex. Does not react to painful stimuli.	Same as above.

9 January 7:00 p.m.

Experimental	Control
Same	Same

10 January 8:30 a.m.

Experimental	Control
Rabbit sleeping. Lies on abdomen with snout buried in feeding trough. Part of the food is eaten. Respiration is deep and startorous. In response to a strong sound stimulus, the rabbit opens eyes, raises head, but then	An incipient predominance of tonus of the extensor muscles of the left posterior extremity is noted. When thrown on the floor from a height of 20 to 50 centimeters, the rabbit, on alighting, extends the hind paw.

Rabbit No 2904, Weight 2,700 grams (cont'd)	Rabbit No 1701, Weight 2,200 grams (cont'd)
falls asleep again. Reacts to painful stimuli. Temperature 36.2° C.	
10 January 10:20 a.m.	
The rabbit awoke, went for his feeding trough. Gait unsteady. Eats. Tonus of the left hind paw is normal.	During locomotion of the animal, an extensor rigidity of the left hind paw is noted.
10 January 1:00 p.m.	
Rabbit sits motionless with closed eyes -- light sleep. Wakes up in response to slight sound or tactile stimuli.	Same as above.
10 January 4:00 p.m.	
The rabbit does not sleep, is alert, has normal gait. No signs of tetanus. Ten cubic centimeters of 10 percent chloral hydrate introduced through gastric tube.	Distinct local tetanus
10 January 7:00 p.m.	
The rabbit sits motionless with closed eyes, with the head drawn in, On stimulation, opens eyes, moves head, but then immediately falls asleep again. No signs of tetanus.	Same as above
11 January 9:30 a.m.	
The rabbit does not sleep. Marked local tetanus. At 10:15 a.m., 10 cubic centimeters of 10 percent chloral hydrate introduced through gastric tube.	Evident local tetanus
11 January 11:00 a.m.	
Rabbit sleeps lying on side.	Same as above

Muscles of the left hind paw are weak.

11 January 11:45 a.m.

Rabbit woke up. As soon as it stood up, local tetanus appeared again in full measure.

Same as above

11 January 1:00 p.m.

Rabbit sits with closed eyes. On stimulation, it opens eyes and moves about, and then again begins to doze. The signs of tetanus do not disappear during sleep.

Marked local tetanus

11 January 5:00 p.m.

Rabbit is not sleeping. Marked local tetanus. When placed on the left side with a rapid movement, the rabbit goes into a condition of general tetanus.

Marked local tetanus. When placed on the left side with a rapid movement, the rabbit easily returns to its original position.

12 January 9:30 a.m.

Rabbit is not sleeping. Marked local tetanus. Tonic contraction of the muscles of the trunk and anterior extremities is seen. On strong painful stimulation, brief convulsive attacks develop in the animal. Given 10 cubic centimeters of 10 percent chloral hydrate.

Marked local tetanus. A certain tonic contraction of the muscles of the trunk and anterior extremities.

12 January 1:00 p.m.

Rabbit sits motionless, with closed eyes. The left hind paw is extended backward, and the muscles of the trunk are contracted. Is aroused by sound stimulation, but then falls back into a somnolent state.

Marked local tetanus. When placed on the left side with a rapid motion the rabbit goes into a state of generalized tetanus.

12 January 1:40 p.m.

Under the influence of sound stimulation (door was slammed) the rabbit awoke, made several steps and went into an attack of generalized tetanus. The attack lasted for three minutes.	Same as above

12 January 4:30 p.m.

The rabbit shows marked contractions of the muscles of the trunk and all the extremities. When stimulated, it goes into a state of generalized tetanus.	Same as above

12 January 9:00 a.m.

Rabbit is in state of generalized tetanus. Immobile. Muscles of trunk and extremities are markedly contracted. The head is thrown slightly backward. Ten cubic centimeters of ten percent chloral hydrate solution given.	When stimulated, rabbit develops brief attacks of generalized tetanus. Capacity for locomotion is preserved.

13 January 10:15 a.m.

Rabbit is not sleeping. Condition of the animal is serious. Extremities extended, head thrown back, tail raised. Trismus.	Same as above

13 January 4:30 p.m.

Rabbit in very severe condition. Muscles contracted to their limits. Opisthotonus, trismus. Respiration is superficial. It was not possible to administer chloral hydrate.	Rabbit lies immobile. Marked contraction of the trunk and extremity muscles, twitching of front paws.

14 January 8:30 a.m.

Rabbit in agonal state. Impossible to administer chloral hydrate.	Rabbit in state of generalized tetanus.

14 January 11:10 a.m.

Rabbit died. Same as above.

14 January 4:30 p.m.

Same as above.

15 January 12:00 noon

Same as above.

16 January 9:00 a.m.

Rabbit in very severe condition. Does not respond to stimulation. Respiration hardly detectable.

16 January 3:30 p.m.

Rabbit died.

As seen from the protocal presented, the course of tetanus in the experimental rabbit was more severe than in the control. This found its reflection in the earlier generalization of the process and the significantly smaller total duration of life in the experimental animal (which died 50 hours sooner than the control).

The experimental rabbits infected with tetanus died sooner than did the control animals (see Table).

The Effect of Chloral Hydrate Anesthesia on the Length of Life of Rabbits Infected with Tetanus Toxin

Designation of group	No of rabbits	Average length of life of rabbits after injection of toxin in days.	Mortality rate of rabbits according to days after the injection of the toxin											
			5th	6th	7th	8th	9th	10	11	12	13	14	15th	16th
Experimental rabbits	13	6.38	1	2	7	1	2	-	-	-	-	-	-	-
Control rabbits	13	9.07	-	1	3	2	4	1	-	-	1	-	-	1

Note. Two experimental rabbits which died following the first administration of chloral hydrate are excluded from the record; two control rabbits which became sick but which did not die are also excluded.

The total duration of anesthetic sleep in the experimental animals, which was established orientatively, was 25-45 hours.

Thus, the inhibition of the higher centers of the brain which develops under the influence of chloral hydrate is not only incapable of eliminating the pathological process in tetanus, but even notably decreases the protective, compensatory capacities of the sick body.

This conclusion is not unexpected. The effect of chloral hydrate on animals infected with tetanus can be explained in the following way: under normal conditions of body activity the cerebral cortex exerts an inhibitory influence on the functional activity of the lower centers of the central nervous system. Under pathological conditions, as are being observed here, particularly in tetanus, such inhibitory influences on the part of the cortex acquire particularly great importance, because the strength of the bodily resistance to the developing disease process is to a considerable degree determined by them. Naturally, the substitution of an active condition of the cortex by an inhibitory one leads to the cessation of action of this protective mechanism and, therefore, to an increase of the pathological process developing in the subcortical structures of the central nervous system.

Evidently, the generally accepted evaluation which has recently been made of drug-induced sleep as a kind of universal therapeutic agent and the consideration of its mechanism of action on a plan with the idea of I. P. Pavlov of protective cortical inhibition are disputable. It might even be said that these views not uncommonly contradict the Pavlovian conception of the leading role of the cerebral cortex in the activities of higher animals forms.

As a matter of fact, does it not appear strange that, at a time when a question of the body's life or death is at stake, the cutting out or weakening of the functions of the cerebral cortex, the organ which accomplishes the supreme control of the normal course of the vital processes, should be a useful measure? Is it right to speak of the protective role of cortical inhibition in these cases?

It must be supposed that an active, alert condition of the cerebral cortex, not an inhibited one, is a most important condition for the successful resistance of the body to the development of disease. Not only the shorter length of life of experimental animals but also the increase of functional activity of the cerebral cortex in rabbits in the process of development of tetanus speak specifically for such a point of view.

If the dose of chloral hydrate which produces sleep in health animals is inactive at the peak intensity of the manifestations of tetanus, then this fact should, evidently, be understood as meaning that the body itself actively counteracts the development of an inhibitory state in the cerebral cortex. What considerations can be adduced to the effect that this countereffect must be blocked rather than supported?

In connection with what has been stated, it is fitting here to present the very interesting observations of V. N. Popov, which, unfortunately, have not been published.

Several cats were inoculated with lethal doses of tetanus toxin. During the period of generalized tetanus they were divided into two groups -- experimental and control. The experimental animals daily began to be subjected to adequate biological stimulation of many hours' duration (direct proximity of mice). As a result of such a prolonged excitation, three cats, which were in states of generalized tetanus, recovered.

However, abundant clinical and experimental material nevertheless mentions definitely the effectiveness of the method of drug-induced sleep therapy in many diseases, particularly in certain infectious diseases. It apparently should be considered that an irradiation of inhibition occurs thereby to a significant depth, leading to a general diminution of reactivity of the central nervous system. Facts showing that under conditions of drug-induced sleep inhibition of a number of defense reactions also occurs speak for such a conception of the therapeutic effect mechanism of drug-induced sleep.

THE ROLE OF THE PERIPHERAL NERVE APPARATUS IN THE MECHANISM OF ACTION OF TETANUS ANTISERUM

T. V. Mitina

The department of pathological physiology (Head -- Professor I. I. Fedorov) of the L'vov Medical Institute and The Laboratory of the Physiology of Immunity (Head -- Professor A. Ya. Alymov, Corresponding Member of the Academy of Medical Sciences USSR) of the Institute of General and Experimental Pathology

It has been shown through the work of Soviet scientists, mainly that of A. D. Speranskiy and his co-workers, that the so-called passive immunity cannot be reduced to a simple neutralization of toxins which are circulating in the blood.

Immune serum is a stimulus of sensory apparatus which play an important role in the development of "passive" immunity.

The response reaction to stimulation depends on the time of action of the new stimulus and the functional state of the receptors.

The administration of specific serum to the body produces different effects depending on which nerve apparatus the serum acts on first. Thus, the so-called passive immunity is, as a matter of fact, active; it is dependent on the general physiological rules and regulations to the same degree as all the other processes which occur in the complex organism.

The fact that immune sera act on the nerve endings has been established not only in physiological experiments but also confirmed by the histological investigations of Ye. A. Kirillov (1949), who showed that as early as 15 minutes after the subcutaneous and intramuscular administrations of sera there could be seen, in addition to the general tissue reaction, a reaction on the part of the peripheral nervous system.

By acting on the sensory nerve apparatus in a definite way, conditions may be prepared for the more effective action of tetanus antiserum.

This thought also underlay our experimental investigation.

We used the stimulation of sensory apparatus by glucose solution for intensifying the effect of the serum. The choice of glucose as

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a stimulant was dictated by the fact that a stimulating effect of glucose on sensory nerve endings in blood-vessel walls was demonstrated in the laboratory of I. I. Fedorov.

The experiments were done on 164 guinea pigs. The experimental animals, which had been infected with tetanus toxin, were injected with five cubic centimeters of 20 percent glucose solution and the corresponding antitoxic serum in such a small dose that it in itself would not prevent the development of intoxication.

Control animals were injected with the serum and the toxin only. The injections were always given intramuscularly in the area of the upper third of the right thigh.

The mixture of tetanus toxin, antitoxin and glucose was prepared <u>ex tempore</u>.

First we used a dose of serum equal to one antitoxin unit. The experiment was performed in the following way. All the animals were divided into three groups. The animals of the first group (four guinea pigs) were given only tetanus toxin in an amount equal to one MLD. All these guinea pigs became ill with tetanus and died on the fourth day after the injection of toxin. The second group (six guinea pigs) were injected with tetanus toxin in an amount equal to one MLD in a mixture with one antitoxin unit of tetanus antiserum. Marked local tetanus developed in all the animals of this group. Finally, guinea pigs of the third group (also of six animals) were injected with toxin in a mixture with tetanus antiserum and five cubic centimeters of 20 percent glucose solution. In all the animals of this group the manifestations of tetanus developed later than in the guinea pigs of the control groups, proceeded in an appreciably milder fashion, and recovery occurred quicker.

As seen from the results of this experiment, glucose solution made possible an intensification of the therapeutic effect of the serum.

In another experiment we used a smaller dose of antitoxin, namely, 0.1 antitoxin unit. The animals were also divided into three groups. The guinea pigs of the first group were injected with toxin alone in an amount of three MLD, as a result of which all four guinea pigs quickly died with signs of generalized tetanus. The animals of the second group (six guinea pigs) were injected with a mixture of three MLD of toxin and 0.1 antitoxin unit of antitoxin. In this case, the serum proved to be entirely ineffective, and all the guinea pigs died with signs of generalized tetanus. The animals of the third group (10 guinea pigs) were injected with three MLD of tetanus toxin, 0.1 antitoxin unit of tetanus antitoxin and five cubic centimeters of 20 percent glucose solution. None of these 10 guinea pigs became sick with tetanus.

The intensifying action of glucose was particularly well demonstrated in this experiment: the dose of serum which had been completely ineffective by itself proved to be effective when injected in conjunction with glucose.

Later, we injected a still smaller quantity of antitoxin -- 0.01 antitoxin unit; toxin was also injected in the same dose as previously -- three MLD. The animals of the first group, which had been injected with three MLD of tetanus toxin, became sick with tetanus, and they all died with signs of generalized tetanus. The animals of the second group (12 guinea pigs) received mixtures of three MLD of toxin and 0.01 antitoxin unit of antitoxin; here, the two guinea pigs died with signs of generalized tetanus, while the remaining 10 marked local tetanus occurred. The animals of the third group (16 guinea pigs) received three MLD of tetanus toxin, 0.01 antitoxin unit of antitoxin and five cubic centimeters of 20 percent glucose solution. No tetanus infection was produced under these conditions. On the third day after the injection of the mixture mentioned, slight disturcances in motion were observed in seven guinea pigs of this group. These disappeared after two days.

As seen from the results, the intensifying effect of glucose manifested itself very distinctly in this experiment also.

In the next experiment we used a still smaller dose of tetanus antitoxin, namely, 0.004 antitoxin units. Two groups of animals were subjected to the experiment. The animals of the first group (16 guinea pigs) were injected with the dose of antitoxin mentioned and one MLD of tetanus toxin. Well expressed local signs of tetanus developed in all the guinea pigs. The animals of the second group (also 16 guinea pigs were injected with mixtures of one MLD of toxin, 0.004 antitoxin units of antitoxin and five cubic centimeters of 20 percent glucose solution; of this group, four guinea pigs did not develop tetanus; the incubation period was increased in five guinea pigs; the disease in one guinea pig was limited to asymmetry of the extremity. In the majority of the guinea pigs, rapidly progressive local manifestations of tetanus developed.

It follows from the data presented that in this case also glucose intensified the effect of the antitoxin.

In the next experiments, we used the same dose of antitoxin (0.004 antitoxin units) but with a significantly smaller quantity of toxin (three MLD).

In one of these experiments, the control group of guinea pigs (eight animals) received a mixture of antitoxin and tetanus toxin; one guinea pig of this group died from generalized tetanus; in five guinea pigs, local manifestations of tetanus developed; in two, the process was limited to an asymmetry of the posterior extremities. Eight experimental animals were injected with a mixture of antitoxin, tetanus toxin and five cubic centimeters of 20 percent glucose solution; in five animals, no tetanus developed; in the other three, signs of asymmetry of the extremities were seen.

The intensifying effect of glucose was demonstrated quite distinctly in the given experiment also.

Afterwards, we used entirely small doses of the antitoxin (0.0004 antitoxin units) and quite considerable quantities of the toxin (three MLD).

Four control guinea pigs which were injected with antitoxin and tetanus toxin in the doses mentioned died on the third day with signs of generalized tetanus. Out of five experimental guinea pigs which received, in addition to antitoxin and tetanus toxin, five cubic centimeters of 20 percent glucose solution, two died with signs of generalized tetanus, one did not become sick, local tetanus develope in one and signs of asymmetry of the extremities were seen.

The result of this experiment also demonstrates the capacity of glucose to intensify the effect of tetanus antiserum.

In the last experiment, we utilized a negligible dose of serum (0.00004 antitoxin units) and three MLD of tetanus toxin. This dose of serum when injected in a mixture with glucose did not prevent the development of tetanus, but death of the experimental animals occurred two days later than in the control animals which had received the indicated mixture of antitoxin and toxin without glucose.

With the use of an injection of 0.000004 antitoxin units and three MLD of tetanus toxin, the control and experimental animals died at the same time.

From the data presented, it follows that the administration of glucose in 20 percent solution simultaneously with tetanus antiserum increases the effect of the latter.

Without excluding the role of glucose as a nutritional agent intensifying the metabolism at the site of injection and also exerting a detoxifying effect (V. V. Vasil'yeva, 1941), a special distinction should be given to the property of glucose of stimulating the sensory neural apparatus.

The results of our preceding investigation establishing the capacity of glucose to alter the course of experimental tetanus intoxication after subcutaneous and intracardiac administration of it are also in complete accord with the data presented in the present report (T. V. Mitina, The Effect of Stimulation of the Sensory Nerve Apparatus of the Heart on the Course of Experimental Tetanus Intoxication. In the book: On the Mechanisms of Disease and Recovery, L'vov, 1951).

The mechanism of action of glucose on the nerve-receptor apparatus consists, apparently, of increasing their excitability, which then induces the rapid development of defense reactions even with small quantities of the specific serum.

The effect of glucose on the sensory apparatus was also confirmed in our next experiment.

Guinea pigs were injected with a mixture of antitoxin, tetanus toxin, and glucose simultaneously with a one percent novocaine solution. It turned out that the injection of this mixture did not prevent the development of tetanus or the deaths of the animals. Thus, after the injection of a mixture of four cubic centimeters of one percent novocaine solution, five cubic centimeters of 20 percent glucose solution, 0.1 antitoxin units of antitoxin and three MLD of tetanus toxin, all the seven experimental animals became ill with tetanus and died from it. At the same time, in 10 guinea pigs which were given the same mixture without novocaine, no signs of tetanus developed. It may be supposed, thus, that glucose, by acting on the sensory nerve endings, changes their initial states, increases their excitabilities and, by the same token, prepares an appropriate background for the action of the serum.

The results of our experiments coincide completely with the data of M. D. Speranskaya, who observed a significant increase of the effect of tetanus antiserum experimentally on rabbits after it was injected on a background of strychnine intoxication of the animals. Strychnine, in the opinion of M. D. Speranskaya, increases the excitability of the peripheral nerve sensory endings.

THE EFFECT OF A DISRUPTION OF HIGHER NERVOUS ACTIVITY ON THE DEVELOPMENT OF DIPHTHERITIC INTOXICATION AND OF ARTIFICIAL IMMUNITY TO DIPHTHERIA

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Recently, many investigations have been made, devoted to the study of the role of the functional state of the higher central nervous system centers in the development of infectious diseases and the development of immunity. In this group are primarily the numerous and quite contradictory experiments on the study of the effect of anesthesia on the processes of infection and immunity.

Investigations on the effect of disruption of higher nervous activity on the course of bacterial intoxications are of great interest (L. I. Kotlyarevskiy, L. S. Gorsheleva and L. Ye. Khozak).

However, the datz obtained in these experiments are still far too inadequate to permit one to consider that the question of the effect of the functional state of the higher centers of the central nervous system on immunogenesis and the development of disease is conclusively solved.

This also stimulated us to perform experiments on animals for the study of the development of diphtheritic intoxication and immunity to this disease in various functional conditions of the cerebral cortex.

The experiments were performed on guinea pigs, in which the change of the functional state of the cerebral cortex, under the given experimental conditions, produced the elaboration of a conditioned-defense reaction, leading to a disruption of higher nervous activity.

An electric current served as the unconditioned stimulus. A bell was used in the capacity of a conditioning stimulus for one group of animals; for the other group, the light of an electric light was used.

The elaboration of a conditioned-defense reflex was accomplished in a special chamber of the Ye. A. Vladimirova type which had been modified somewhat by us.

The chamber consists of two different-size boxes with glass side-walls connected by a corridor. The floor of the larger box can be pulled out; it contains transversely-situated holes. In the areas between the holes there are exposed electric wires which connect up with a source of electric current (the city lighting circuit).

On the roof of the larger box there is a recess for an electric light bulb with an external switch; the button of the bell is also located here.

The guinea pigs were placed one by one into the large box of the chamber. After a certain time, the bell was rung (conditioning stimulus) with a subsequent (after a second) switching on of the electric current (unconditioned stimulus).

In doing this, a voltage was selected, with the aid of a transformer, to which the animals responded with motor defense reactions. Usually, it was 90 volts.

After two to three combinations of the bell and current, a conditioned defense reflex was elaborated by the guinea pigs. To the combination of the light and the current the reflex was elaborated after five or six experimental sessions.

Afterwards, a single ring of the bell or flash of the electric light was adequate to put the animals into a state of marked excitation and to make them run squeaking into the opened door of the corridor, through which they entered the chamber of the other box. The sound conditioning stimulus thereby produced a more marked excitation of the guinea pigs than did the light.

The experiments were conducted daily at one and the same time over the course of a month. In the experimental animals there was frequently noted a disorder of the intestinal function (diarrhea), ulcerative keratitis developed, and a loss of weight was observed (the weight dropped by 40-100 grams). Two guinea pigs died with the signs described above. One guinea pig with the same signs recovered three days after the experiments were concluded. A scar and a circumscribed corneal opacity remained on its affected eye. It is very interesting that following injection of it with 30 MLD of diphtheria toxin, it again developed an ulcer at the same spot on the cornea ("second blow", according to A. D. Speranskiy).

The experiments were performed on 40 guinea pigs.

In the first series of experiments, the conditioned-defense reaction was elaborated in experimental guinea pigs weighing 400 grams by means of daily experiments for a month. After this, they were all injected with 1.5 MLD of diphtheria toxin. At the same time, the same dose of diphtheria toxin was injected also into control guinea pigs of the same weight which had not been subjected to the effects of any additional stimuli.

In the group of animals where the bell served as the conditioning stimulus, all the experimental guinea pigs (five in number) died eight to seventeen hours after they had been injected with diphtheria toxin. Of the five control guinea pigs, only two died; here, the deaths of the animals did not occur immediately after the injection of the toxin, as was the case in the group of experimental guinea pigs, but rather on the third day.

In another group of experiments, where the electric light was used in the capacity of a conditioning stimulus, all the experimental animals died 18-24 hours after they had been injected with diphtheria toxin; of five control guinea pigs one survived; the others died on the third day.

In the second series of experiments the experimental guinea pigs, weighing 350 grams, were immunized with a single injection of diphtheria toxoid, after which the conditioned defense reaction was elaborated in them.

Control animals of the same weight were immunized at the same time as the experimental animals and were not subjected to any additional procedures.

Thirty days after the immunization, all the guinea pigs (experimental as well as controls) were injected with 30 MLD of diphtheria toxin each.

All five experimental animals of the first group (the conditioning stimulus was the bell) died 16-33 hours after the injection of the toxin. Of the five control guinea pigs of this group two died on the third day after the injection of the toxin; the remaining three survived.

In the second group (the flashing of an electric light was used as the conditioning stimulus), all five experimental guinea pigs died 18-24 hours after the injection of toxin. Of the five control animals, one guinea pig survived, while four died on the third day.

The investigations which were performed show that for the elaboration of the conditioned defense reaction the strongest conditioning stimulus is the sound stimulus (bell). N. A. Podkopayev also mentions this.

The conditioned defense reflex was elaborated more quickly in the animals to the combination of the sound stimulus with the unconditioned stimulus (current) than to the combination of light with the same unconditioned stimulus. The conditioned defense reflex which was elaborated in the guinea pigs to the use of the sound stimulus was accompanied by quite an appreciable excitation and marked somatic disorders. The latter also serve as indicators of the fact that a disruption of higher nervous activity occurs in the animals in the elaboration of the conditioned defense reflex to an electric current. N. A. Podkopayev refers this stimulus to the group of disruptive stimuli.

The experiments performed by us show, therefore, that a disruption of higher nervous activity aggravates the course of diphtheritic intoxication and inhibits the development of antitoxic immunity from the vaccination of animals with diphtheria toxoid.

ON THE QUESTION OF THE MECHANISM OF ACTION OF DIPHTHERIA ANTISERUM

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In our preceding investigations the significance of the pathological forms of stimulations of individual sensory zones of the tongue was shown with respect to the disease mecha isms of diphtheria.

The tasks confronted us of elucidating the role of these zones in the mechanism of the body's increase in resistance to the given disease.

With this aim in view, an experiment was performed on 123 guinea pigs weighing 240-250 grams, which were divided into four groups. The first control group was injected intravenously with one MLD of diphtheria toxin.

The second group of animals was injected intravenously with one MLD of diphtheria toxin and, immediately after this, with 0.001 antitoxin units of diphtheria antiserum also intravenously.

The third group of animals was injected intravenously with one MLD of diphtheria toxin and, immediately after this, with 0.001 antitoxin units of diphtheria antiserum into the root of the tongue.

The results of this experiment are presented in the Table.

DEATH OF GUINEA PIGS FROM DIPHTHERIA INTOXICATION FOLLOWING THE INJECTIONS OF DIPHTHERIA ANTISERA INTO DIFFERENT SENSORY AREAS

No of froup	Site of injection of diphtheria toxin	Dose of toxin	Site of injection of diphtheria antiserum	Dose of serum	Number of animals	Number of them which survived	Number of them which died	Percentage which died
1	Vein	1 MLD	--	--	33	4	29	87.8
2	Vein	Same	Vein	0.001 AU	29			
3	"	"	Tip of tongue	" "				
4	"	"	Root of tongue	" "				

As seen from the Table presented, 87.8 percent of the animals in the control group died. The intravenous administration of 0.001 antitoxin units of diphtheria antiserum, just like the injection of it into the tip of the tongue, decreases the mortality rate of the animals (to 58.6-57.1 percent) but to an appreciably smaller degree than the injection of the same dose of serum into the root of the tongue (33.3 percent).

Thus, one and the same dose of serum exerted different effects depending on the site of injection of it. This difference cannot be explained, under the conditions of our experiment, by an unequal resorption of the serum into the blood. In injecting the serum into the blood its effect was the same as when it was injected into the tip of the tongue, although in the latter case the absorption time of the serum was lengthened. Moreover, the same dose of serum, when injected into the root of the tongue, where the resorptive capacities do not differ essentially from those which are in effect after the injection of the serum into the tip of the tongue, showed a significantly greater effect, increasing the survival rate of the animals by 25 percent compared with the two preceding groups.

The facts obtained give us cause to consider diphtheria antiserum not only as an agent which neutralizes the toxin but also as a specific stimulus of the sensory nerve apparatus. This stimulation is the primary link of the reflex process which alters the reactivity of the body to a pathological stimulus.

Such a conception finds its confirmation in those experiments where novocaine was injected into the root of the tongue. Under these conditions also, it was possible to prevent the deaths of the animals from diphtheria in a significant percentage of the cases, even though a letha, dose of diphtheria toxin had been injected into the novocainized zone of the tongue. (O. Ya. Ostryy and M. D. Speranskaya, On the Role of Pathological Stimulations of the Sensory Nerve Apparatus in the Disease Mechanisms of Diphtheria in Experiment (published in this collection)).

These data speak for the fact that it is possible to increase the resistance of the body to diphtheria by nonspecific and specific stimuli (novocaine, diphtheria antiserum) if they are injected into a definite sensory zone. By the same token, the question comes up again as to the evaluation of the mechanisms of action of serum prophylaxis and therapy in a number of toxic-infectious processes.

From the experimental data presented it follows also that the site which proved to be most reactive with respect to diphtheria toxin simultaneously was also the most effective for the action of specific serum.

The rules and regulations noted make it possible to outline new perspectives in the research for effective forms of experimental therapy of this disease.

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ANATOMICAL INVESTIGATIONS OF THE NERVOUS SYSTEMS OF ANIMALS INFECTED WITH ANAEROBIC INFECTIONS UNDER CONDITIONS OF ALTERED BODILY REACTIVITY

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The problem of our investigation was the anatomical study of the nervous system reactions in the pathological process which develops in animals after they are infected with anaerobic infections.

According to the data in the literature, many infectious diseases are accompanied by extensive anatomical changes in the nervous system. They have been noted in tuberculosis, typhus, septicemia, leprosy, pneumonias, etc. (B. I. Lavrent'yev, M.L., Borovskiy, S.S., Vayl', L.I. Falin, V.F. Lashkov and others).

In the literature devoted to the study of gas gangrene, data have been presented chiefly concerning the anatomical changes of the peripheral tissues and of the nervous system directly at the site of involvement. There are only individual indications of changes of neural elements above the focus of involvement (A. N. Chistovich, A. A. Polyantsev).

Different data have been obtained concerning the changes in the central nervous system in gas gangrene. A. A. Arapov did not find any anatomical changes in the central nervous system specific for gas gangrene. A. V. Smol'yannikov found characteristic nervous system changes and came to the conclusion that they are of decisive significance in the cause of death of experimental animals from gas gangrene.

In selecting the methods of investigation we were guided by the data on the development of neural dystrophic processes obtained in A. D. Speranskiy's laboratories.

By using the method of an additional influence on the body, we attempted to change the anatomical reactions of the nervous system and of the organs to the infectious agent, and by means of anatomical and electrophysiological study of the various central and peripheral nervous system centers to reveal the nature of these changes and come to an understanding of their significance for the course and outcome of the disease.

Guinea pigs weighing 300 grams were infected with gas gangrene (The infection was accomplished by R. Ya. Zel'manovich), by the injection of a bacterial suspension of B. perfringens in a mixture with 0.1 cubic centimeter of 40 percent calcium chloride into the right gastrocnemius muscle. The right tibial nerve on the side of the involvement was transected before or after infecting the animals. In different experiments, different variants of this operation were used, namely: a) transection of the nerve, b) transection of the nerve and injection of 0.95 cubic centimeters of 70 percent alcohol into its peripheral segment, c) transection of the nerve, injection of one to two drops of vitamin B_1 into its peripheral sigment and subsequent suturing of the nerve ends.

In the experiments where the operation was performed on the nerve after the infection, the times of the operation were varied: immediately after the infection, one hour after the infection, three hours after the infection, and six hours after the infection.

Guinea pigs served as controls. They were divided into three groups, namely, those in which: 1) only nerve trauma was produced, 2) only calcium chloride was injected into the muscle, and 3) trauma of the nerve was produced and calcium chloride was injected into the muscle. In addition, a series of experiments was performed for determining the rheobase and chronaxie of the tibial nerves in the infected guinea pigs and guinea which had simply been injected with calcium chloride intramuscularly.

The following were subjected to anatomical investigation: gastrocnemius muscles, skin of the thighs and feet and their neural structures, sciatic nerves, intervertebral ganglia on the side of the infection and on the opposite side, the lumbosacral and sometimes also the cervicothoracic segments of the spinal cord and in a part of the animals, the anterior two fifths of the cerebral cortex.

The preparations were stained by the Nissl, Bil'shovskiy or Campos, Bil'shovskiy-Gross in the Lavrent'yev modification or Mark modification methods, and for fat.

In the first stage of the work the problem was reduced to establishing the nature of the nervous system changes in the fatal and nonfatal cases. Ten guinea pigs were investigated; of this number, seven died at different periods (from one to forty days) after the infection and three survived and were killed after six and a half months. Through a comparison of the histopathological changes in the spinal cord, intervertebral ganglia and peripheral segments of the nervous system and also of those in the muscles and skin of the animals which died and of those which survived, the following was established. In all of the animals which died after being infected with anaerobic infections changes were found in all the peripheral nervous system sections studied by us and in the spinal cord both on the side of the infection and on the opposite side (Fig. 1).

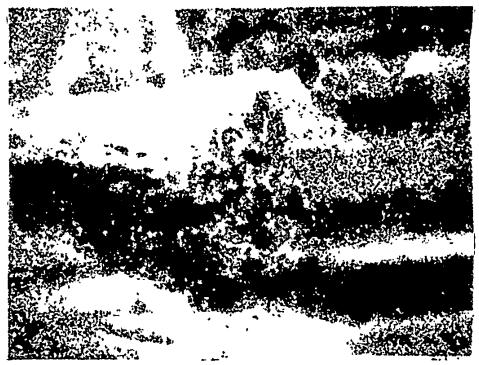

Fig. 1. Decomposition of nerve fibers in fasciculi of the left gastrocnemius muscle of guinea pig which died a day after being infected without nerve trauma (staining by the Bil'shovskiy-Gross-Lavrent'yev method).

In the animals which died, severe necrotic and inflammatory processes predominated. It should be noted here that severe, often irreversible nerve cell changes were found in the spinal cords of the guinea pigs which died. Rarefaction and dissolution of the Nissl substance, coarse vacuolization, shrivelling of cells, deformation of their nucleoli, deposition of glia around the ganglion cells, neuronophagia, and also ghost cells were seen.

In the guinea pigs which died two days after being infected without additional nerve trauma, the changes in the cervical and thoracic segments of the spinal cord were expressed to a greater extent than in the lumbosacral area. In all the animals which died after that, more severe changes were seen in the sensory cells of the posterior horns and in the internuncial neurons than in the motor cells of the anterior horns.

The involvements of the motor cells of the spinal cords in animals which died at short intervals after being infected have the character of so-called reversible changes and are expressed to a greater extent on the side of the involvement than on the opposite side; in guinea pigs which are sick for a long period of time, the changes are severe, irreversible and are expressed approximately to the same degree on both sides. Inflammatory foci were seen in the area of the posterior horns in certain guinea pigs.

In animals which died following the infection, where the latter had been preceded by trauma of the tibial nerve, in contrast to the cases where infection was caused without accompanying trauma, Wallerian degeneration (Fig. 2) rather than necrosis was found in the right tibial nerve below the site of the transection. Various anatomical

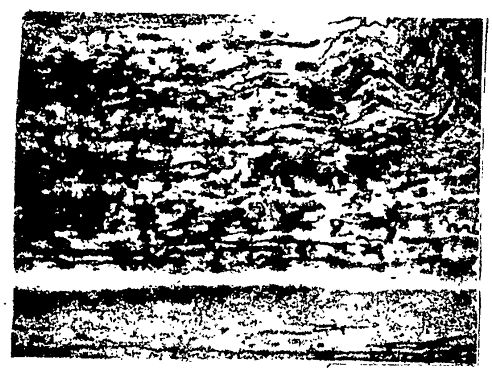

Fig. 2. Wallerian degeneration of nerve fibers of the right tibial nerve in a guinea pig which died three days after being infected; the infection was preceding by transection of this nerve and alcoholization if its peripheral segment. The alcohol-treated nerve fibers are preserved (staining by the Bil'shovskiy-Gross-Lavrent'yev method).

changes, including also signs of irritation, occurred in the contralateral neural structures of the spinal cords of these animals. The anatomical changes in the contralateral intervertebral ganglia and sciatic nerve were somtimes expressed to a greater degree than on the side of the involvement.

Microscopic study of the nervous systems of the surviving experimental animals, which were then killed six and a half months after being infected, revealed milder anatomical changes in the peripheral system -- intervertebral ganglia, sciatic nerves, terminal neural apparatus of the gastrocnemius muscles -- than in the guinea pigs in the same series of experiments which had died. There were no changes in the spinal cords of these animals.

The presence of a neuroma at the site of transection of the right tibial nerve and also neurotization of the peripheral segment of this nerve and of the right gastrocnemius muscle in the surviving animals permit us to draw the conclusion that regeneration of the peripheral nerve is possible under the conditions of gas gangrene.

It should be noted that the severity and character of the anatomical changes at the periphery are associated with the severity of the involvement of the nerve cells of the spinal cord and probably depend on the state of the latter and of the higher centers of the central nervous system;

A guinea pig which died after 40 days and also the surviving guinea pigs may serve as examples of such an association. In the former, severe degenerative changes and marked signs of irritation of the neural structures of the contralateral muscle (Fig. 3) were seen, which coincided with the severe inflammatory process in the intervertebral ganglia and with severe disease of the ganglion cells in these ganglia and in both halves of the spinal cord; the motor nuclei were also involved here.

In the surviving guinea pigs the changes in the peripheral nervous system were expressed to a much weaker degree than in those which died; the signs of irritation of the neural structures of the contralateral muscle could be characterized as quiescent; the microscopic picture of the intervertebral ganglia also attests to the former existence in them, apparently, of a noe quiescent pathological process. In these guinea pigs, there were no changes in the nerve cells or in the stroma of the spinal cord.

Study of the skin of these 10 infected guinea pigs showed that its neural structures are better impregnated with silver than the normal. This attests to the presence of some kind of physiological condition of the cutaneous neural structures different from the normal, and apparently reflecting the latered reactivity of the body as a whole.

We have also observed appreciable pathological changes of these nerve structures in the skin of degenerative and reactive character, on the side of the infection as well as on the opposite side. In the nerve fibers there were seen an inequality of the calibers, varicosity, separation of the fibers, edema, vacuolization, cork-screw shaped convolutions and fragmentation of the axis cylinders. Along with these changes of the axis cylinders there were noted periaxonal changes which were expressed unequally in different animals; they consisted in an increase of the dimensions of the nuclei of the neurolemma, near which reticulated structures were found. The latter sometimes were of oval or cylindrical forms. Within them or alongside them, the axis cylinder could be found; it maintained its integrity. It may be supposed that these reticulated structures arose from the myelin sheath and the neurolemma.

In the neural apparatus of the encapsulated and non-encapsulated terminal corpuscles changes of the axone calibers and signs of varicosity were found; no degenerative changes were observed. It could be noted thereby that the character and intensity of the changes of the neural structures of the skin depend on the duration and severity of the pathological process developing in the body and on the outcome of this process. With a brief course of the disease and a fatal outcome, degenerative changes of the cutaneous neural structures predominate, and these are expressed to a greater extent on the side of the infection. With a prolonged course of the disease and a fatal outcome, the signs of irritation of the axones predominate; these are expressed both on the side of the infection and on the opposite side. In the surviving animals the changes of the neural structures of the skin were expressed to an appreciably milder degree, are encountered less often than in the animals which died, and have the character of signs of irritation on the side of the infection as well as on the opposite side.

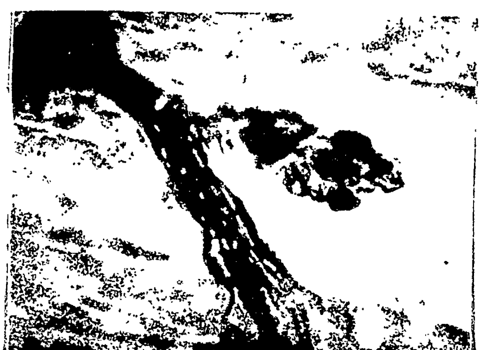

Fig. 3. Neuroma of motor nerve ending of left gastrocnemius muscle of guinea pig which died 40 days after being infected, which was preceded by a transection of the right tibial nerve and alcohol-treatment of its peripheral segment (staining by the Bil'shovskiy-Gross-Lavrent'yev method).

The next stage of our work was conditioned by the need for differentiating the nervous system changed produced by the microbial factor from the changes produced by supplementary procedures -- nerve trauma, injection of calcium chloride into the muscle and the combination of nerve trauma with the injection of calcium chloride into the muscle. In all, 18 guinea pigs were investigated, of which 12 guinea pigs were infected; some were subjected to additional nerve and some were not. Six guinea pigs were subjected only to the supplementary procedures without being infected. The guinea pigs were killed seven and 21 days after these procedures.

In the presence of nerve trauma alone, only degenerative and regenerative processes occur in the traumatized nerve; no changes were found in the central nervous system. Changes of the neural structures of the muscle on the opposite side were also absent. The solution of calcium chloride, in the same concentration which we used for injection into the muscle, produces not only necrosis of the muscle tissue but also degeneration of the neural structures of this muscle, degeneration of the nerve innervating it, so-called reversible and irreversible changes in the intervertebral ganglia and signs of primary irritati6n of the motor nerve cells of the spinal cord (Fig. 4). The

Fig. 4. Degeneration of the right tibial nerve after the injection of calcium chloride into the right gastrocnemius muscle (staining by the Bil'shovskiy-Gross-Lavrent'yev method).

combination of the two procedures mentioned led to an intensification of the signs of primary irritation of the nerve cells of the spinal cord.

Pathological changes of the nervous system of the infected guinea pigs differed from those which had not been infected, but which had been subjected to the effect of calcium chloride. Thus, in the guinea pigs which had been infected without additional nerve trauma, an inflammatory process was found in the pia mater and in the cerebral cortex, expressed to a greater extent on the left than on the right (Fig. 5).

Fig. 5. Inflammatory changes of the pia mater of the cerebrum of a guinea pig which had been infected without nerve trauma (Nissl stain).

Signs of primary irritation in the intervertebral ganglia occur sooner in the infected guinea pigs than in the guinea pigs in which nerve trauma had been produced by the injection of calcium chloride into the muscle. The inflammatory process in membranes of the intervertebral ganglia and also the so-called reversible and irreversible changes in the nerve cells of these ganglia were sometimes expressed to a greater extent on the so-called healthy side than on the side of the intervention in the cases of the guinea pigs which had been infected without supplementary influences as well as in the guinea pigs which had been simply injected intramuscularly with calcium chloride. The regeneration of the traumatized nerve was delayed in the infected animals in comparison with those which had not been infected.

Signs of irritation in the opposite gastrocnemius muscles of infected guinea pigs with severe involvements of the infected paws were expressed to a much greater extent than in the remaining animals.

Certain observations point to the role of the time factor in the development of the pathological process in the experimental guinea pigs.

Thus, in guinea pigs which had been operated six hours after the infection, neurotization of the nerve and muscle were expressed better than in the other infected animals and approximately the same as in guinea pigs which had been subjected simply to an operation on the nerve. Hence, the conclusion may be drawn that under certain conditions of infection, regeneration of the traumatized nerve is possible. With an operation on the nerve after the infection (especially after six hours) a lessening of the anatomical changes is seen in the intervertebral ganglia and improvement of the process of regeneration of the traumatized nerve compared with what occurs in other animals investigated.

For studying the functional state of the nerve innervating the infected muscle and that of the corresponding nerve of the opposite side, the rheobase and chronaxie of this nerve were determined in guinea pigs which had simply been injected intramuscularly with calcium chloride (control).

Determinations of the rheobase and of chronaxie were made after three and six hours, then at one, three and five days after the procedure. These determinations showed a decrease and then a loss of excitability of the nerve innervating the involved muscle, in the experiment as well as in the control; the excitability was not restored for the entire period of the observation.

In the opposite nerve, a decrease of the excitability was noted during the first few days in the experiment as well as in the control. However, in contrast to the experimental animals, in the controls the magnitudes of the rheobase and chronaxie were afterwards restored almost to normal.

These facts indicate the fact that infection of the body produces more constant reflected changes than does the injection of calcium chloride intramuscularly.

As seen from the data obtained by us, calcium chloride, when injected in certain concentrations into the muscle produces pathology not only in the muscle tissue but also of the nervous system, which apparently alters the reactivity of the body. Such an alteration of the reactivity of the body facilitates the development of disease under conditions of introduction of anaerobic infections.

Certain agents used by us, however, exerted favorable effects on the outcome of the disease.

By additional procedures it was, therefore, possible to alter the reactivity of the body, under some conditions, in the direction of intensifying its "physiological measures" against disease, and under other conditions, contrariwise, in the direction of weakening these "measures".

As has been indicated above, in guinea pigs sick with anaerobic infections, the cutaneous nerual structures were demonstrable with much greater prominence than in the healthy animals. Making use of this, we managed to obtain a better conception of the structure and distribution of the neural apparatus of the skin of the thigh and foot in gui ea pigs and to construct an outline of their innervation.

While we do not have the opportunity to present the data obtained in detail, we shall discuss only certain, we believe, new facts in this connection.

It was shown that the innervation of each section of skin is characterized not only by a certain number and structure of the neural formations but also by a definite linkage of them. We observed the grouping of certain neural apparatus and also the linkage of the encapsulated corpuscles with the free terminal branches. In all the investigated areas and in all the layers of the skin small non-capsulated balls were found, which were formed along the courses of bundles of nerve fibers by concentric rings of the nerve fibers which made up these bundles. We have not found any descriptions of neural structures like these small balls in the literature available to us.

The following hypothesis may be expressed concerning the function of these balls. They serve, possibly, just like other neural structures, for the transformation of neural excitation or else for its intensification. If this hypothesis is correct, then, in addition to the peripheral nerve structures which transform an external stimulation into neural excitation, it is possible to accept the existence also of structures which transform the neural excitation which is already progressing along the neural pathways of the skin.

BIOCHEMICAL CHANGES IN THE CENTRAL NERVOUS SYSTEM FROM VARIOUS TOXIC-INFECTIOUS PROCESSES

M. Sh. Promyslov

Physiological analysis of the mechanisms of disease and recovery can be made substantially more profound by including in the analysis those biochemical changes which occur, thereby, in the central nervous system.

Unfortunately, at the present time the number of investigations which might disclose the biochemical essence of the change in reactivity of the body in the toxic-infectious processes is still inadequate.

At the same time, it is hard to imagine that the profound changes which occur in these processes are without pertinence to the trophic function of the nervous system, or that they do not produce appreciable biochemical changes in the body.

I. P. Pavlov ascribed great importance to physico-chemical investigation for obtaining knowledge of the essence of the processes which occur in nerve tissue. "It can hardly be disputed," he wrote, "that an accurate theory of all neural phenomena will be obtained by us only through a study of the physico-chemical process which occurs in nerve tissue; knowledge of its phases will give us a complete explanation of all the external manifestations of nerve activity, their sequence and associations." (I. P. Pavlov, Collection of His Works, 1949; Vol III, p 346).

We studied the changes of the nitrogenous substances of the brain and spinal cord after the actions of various toxins on the animal body. The experiments were performed on rabbits.

Changes of the nitrogen metabolism of the brain usually are identified with changes of protein metabolism. However, such an identification is not justified, especially in the investigation of nerve tissue. Nerve tissue, specifically brain tissue, contains 60 percent lipids; the spinal cord, 70 percent. The nitrogen of all the lipids comprises 14 to 15 percent of the total nitrogen of the brain and 23 to 24 percent of the total nitrogen of the spinal cord. Therefore, we studied three separate fractions of the brain tissue: a trichloracetic extract, protein nitrogen and lipid nitrogen. It was established thereby that the changes produced by the toxins in the brain and spinal cord are distinguished by specific qualitative and quantitative characteristics in the presence of a developing pathological process.

In contrast to the action of diphtheria toxin, which produces a diminution of the proteins in the brain as well as in the spinal cord, the action of tetanus and gas gangrene toxins lead to diminution of the nitrogenous lipids only in the brain. With generalized tetanus and gas gangrene, no disturbances of the metabolism of nitrogenous substances are seen in the spinal cord. It should be noted also that in local tetanus we did not find any changes in the nitrogen balance either in the brain or in the spinal cord. In addition, a lethal dose of tetanus toxin produces unitypical qualitative changes in the brain, regardless of the site of injection and of the clinical picture of tetanus. At the same time, the quantitative relationships, which are expressed in observable changes, are varied.

Because the basic nitrogenous lipids of nerve tissue are phospholipids and cerebrosides, we decided to clarify the question as to which of these compounds is diminished in general tetanus and which, in gas gangrene.

With this aim in view, we determined the phosphorus and nitrogen of all the lipids of the brain and for purposes of characterizing them we took the relationship of phosphorus to nitrogen in normal rabbits and in rabbits which had been injected with lethal doses of tetanus toxin and B. perfringens toxin. It was found, in doing this, that the quantity of phosphorus of the cerebral phospholipids does not change in the experimental animals comparied with the normals; the relationship of phosphorus to nitrogen, however, increases significantly. This made it possible for us to draw the preliminary conclusion that in generalized tetanus and gas gangrene a decrease occurs in the cerebrosides of the brain.

In order to convince ourselves of the accuracy of this conclusion we worked out a method of determining the brain cerebrosides. Separation of the brain tissue lipids was accomplished by successive six-hour extractions with ether, acetone, and a 1:1 mixture of chloroform with methyl alcohol, respectively. Then, the solvent was distilled off, and the lipids were subjected to hycrolysis. After this, the galactose was determined quantitatively by the colorimetric method. The determination was carried out by means of anthrone, with which galactose gives a blue coloration in concentrated sulfuric acid. The auantity of galactose found multiplied by 4.6 (the conversion factor) gave the quantity of cerebrosides of the brain.

These determinations confirmed our preliminary conclusions. In effect, in generalized tetanus and gas gangrene a decrease occurs in the cerebrosides of the brains of rabbits.

It is interesting that the latter investigations showed the presence of a larger quantity of cerebrosides in the spinal cords of rabbits than in their brain; nevertheless, the quantity of cerebrosides in the spinal cord did not undergo any changes in either tetanus or gas gangrene.

The established fact is of interest also with respect to the fact that the biological role of the cerebrosides has still not bee clarified in the processes of metabolism in nerve tissue.

Until recently, investigations were limited simply to noting the fact that cerebrosides are contained in nerve tissue in large amounts.

Thus, two lines of metabolic disturbance of nitrogenous compounds were established by us: 1) in diphtheria -- where a decrease in the cerebral protein nitrogen is seen, and 2) in tetanus and gas gangrene -- where decrease is seen of the nitrogen of the cerebral cerebrosides.

It seems to us that consideration of these facts aids in establishing the fact that diphtheria, tetanus and gas gangrene are diseases in which the developments of the pathological processes are in each case specific and clearly definable involvements of the brain.

However, these involvements in tetanus and gas gangrene are qualitatively unitypical in their biochemical expressions.

Evidently, the generality observed here characterizes only the type of functional and organic change in the central nervous system which reflects the unity of the reactive changes connecting the various nosologic forms according to the characteristics of their dystrophic processes; these processes have been shown here to be independent of the characteristics of the causal agent itself. In other words, we have before us a group reaction, not an individually specific reaction.

Only the finding of a basic link in all the varied biochemical processes occurring in the central nervous system can bring us closer to an understanding of all the mechanisms which underlie disease and recovery. The finding of this link will aid us in solving the problem indicated here.

ON THE ROLE OF THE HIGHER CENTERS OF THE CENTRAL NERVOUS SYSTEM IN COMPENSATING FOR DISTURBED FUNCTIONS OF THE BODY

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"The more perfect the nervous system of an animal and the more centralized it is, the more its highest center is a director and distributor of the entire activity of the body, despite the fact that this does not come out clearly and openly." (I. P. Pavlov, Complete Collection of His Works, 1951, Vol III, p 409).

This statement by I. P. Pavlov contains a broad program of comparative-evolutionary investigations. It should, however, be recognized that the role of the higher centers of the nervous system in regulating the functions of the body has been investigated very systematically only with respect to the motor function, where the significance of these centers is manifested in particularly distinct form.

The removal of the cerebrum in fish, amphibians (frongs), and reptiles (turtles) does not produce any constant changes in the motor function: decerebrate fish continue to swim, frongs continue to swim and jump, and turtles continue to crawl.

Beginning with the birds, compensation of the motor function disorders produced by the removal of the cerebrum becomes quite difficult. In pigeons and roosters, for example, the removal of the fore-brain produces distinct disturbances of motor function: decerebrate birds move about very slowly, lose their abilities to take wing by themselves, alight awkwardly when thrown up into the air.

Removal of the cerebral cortex in mammals is compensated with still greater difficulty. In dogs, decortication produces marked disorders of motor function. Coordination is notably affected thereby; the animals walk by throwing their paws forward at a high level, lose their equilibria when pushed, cannot run, permanently lose their capacities or differentiated movements of the front paws -- the ability to dig holes, hold bones, etc.

Disorders of motor function produced by the removal of the cerebral cortex in monkeys is even more poorly compensated.

Thus, in the process of evolution, by virtue of the ever greater centralization of the nervous system, the possibility of replacing functions of some of its parts by those of others becomes more and more limited.

The capacity of the higher centers of the nervous system to regulate the entire activity of the body, and not just the functions of movement and those of the higher sense organs, is suggested by a study of the role of these centers in the process of evolution and in the regulation of the so-called vegetative functions: cardiac activity, respiration, digestion, urinary excretion, etc. These questions have been subjects of investigation in the group directed by us for the period of the past few years.

The investigations mentioned here have been carried on in two directions. On the one hand, we studied the compensation of the functional disorders produced by the operation of decerebration itself; and on the other, the capacity of decerebrate animals to compensate for functional disturbances produced by the effect of extraordinary stimuli on the body.

For the investigation of the mechanisms of compensation we utilized the method of conditioned reflexes, along with the method of decerebration.

In approaching the comparative investigation of the role of the higher centers of the nervous system in the processes of compensation it is important to take into account the characteristics of the brain structure at various stages of evolution.

In fish, amphibia, reptiles and birds, the fore-brain is represented chiefly by the subcortical nuclei, which gradually become more and more prominent, attaining very large dimensions in birds. The layer which covers these nuclei is represented in fish by epithelial cells; in amphibians, by a thin layer of nerve cells. In the reptiles, this layer is increased in size, but in birds it again undergoes a certain reduction with a simultaneous increase in size of the subcortical nuclei.

Only in the mammals, especially in the highly developed animals, is this layer represetned by a very well developed cortex, which predominates over the other parts of the brain.

Therefore, by removing the fore-brain in fish, amphibia, reptiles and birds we are removing a homologous structure represented chiefly by the subcortical nuclei. In mammals, complete removal of the fore-brain (that is, of the cortex and subcortical nuclei) is impossible, because this operation leads to the rapid deaths of the animals. In the process of decortication the subcortical nuclei are to some degree injured and afterwards undergo partial degeneration,

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but this, to be sure, is not equivalent to their complete withdrawal as in removal of the fore-brain in animals of the lower classes.

Taking this circumstance into account, it is impossible to identify the consequences of decortication in mammals with the consequences of removal of the fore-brain in representatives of other classes.

In the comparative investigations of the role of the higher centers of the nervous system in the regulation of cardiac activity we made use of the method of electrocardiography, which made it possible to judge objectively the activity of the heart, although this is not a completely exhaustive functional characterization of it.

If the fore-brain of a fish is removed (the experiments were done on crucians), no changes of the rhythm nor any qualitative characteristics can be noted in the electrocardiogram.

Investigations of the electrocardiograms of frogs before and after removal of the fore-brain showed that after brief (over the course of several minutes) periods of slowing, and sometimes of increased frequency, of the cardiac activity, sinus arrhythmia occurred.

In addition, an increase in the size of the electrocardiographic waves, and, in a number of experiments, splitting of the P wave, is noted, which give evidence of the lack of simultaneous involvement of both atria by the excitation process. The changes mentioned are brief and disappear on the second day.

More marked disturbances of cardiac activity, up to complete cessation of the heart, occur only when the brain is transected at the level of the optic lobes, which was shown by I. M. Sechenov.

Electrocardiographic examinations before and after decerebration in reptiles -- swamp turtles and lizards (agamas and monitors) -- showed that a very brief retardation or acceleration of cardiac activity and an increase in the size of the waves occur in them. However, these disturbances are quite quickly compensated in these cases also.

Very distinct and constant cardiac activity changes occur beginning with the class Aves.

Electrocardiographic investigations showed that the removal of the cerebral hemispheres in pigeons and roosters produces a marked retardation of cardiac activity, in individual experiments going down to 80-100 beats per minute (instead of the normal 300-350). Ten to fifteen days after the operation, the bradycardia becomes somewhat less definite, but is maintained throughout the entire life of the decerebrate animal.

An analogous regular increase in the part played by the higher centers of the nervous system is also noted with respect to the regulation of respiration.

After the removal of the hemispheres in frogs, their respiratory movements slow up for several minutes; sometimes, they accelerate. In some experiments, periodic respiration can be seen on the second day after the operation, but then the respiration returns to normal. The phylogenetically older respiratory movements of the submaxillary muscle of the frog are altered to a lesser degree, after this operation, than pulmonary respiration.

Removal of the hemispheres of reptiles (especially, turtles), as our experiments showed, produces very brief, rapidly compensated changes of the respiratory movements.

Here again, just as in the case of the cardiac functions, permanent respiratory disturbances following removal of the hemispheres develop only in birds. Observations existing in the literature (O. P. Molchanova, B. I. Bayandurov and Pegel' and others), which have also been confirmed by our experiments, show that removal of the hemispheres of birds produces permanent retardation of the respiratory movements and a decrease in metabolic rate, which continues until the animals die.

In general, the same rules and regulations have been established by us also with respect to the function of digestion. Roentgenoscopic examinations of the evacuatory function of the gastro-intestinal tract and a study of the rate of digestion showed that disturbances of these functions are also brief following decerebration of the frog, but are expressed to a greater extent than are disorders of respiration and of cardiac activity. However, after one to two days, these disturbances are also eliminated.

In birds, following the removal of the cerebral hemispheres the functions of the gastrointestinal tract are inhibited markedly and for a long time, as are also the functions of the heart and respiration. Roentgenographic investigations showed that the inhibition of the motor function extends not only to the crop (as has been noted previously in the literature) but also to the entire gastro-intestinal tract and continues until the animal dies.

Finally, we have obtained analogous data in the study of diuresis. After the decerebration of frogs, the output of urine is only slightly altered; in birds after the same operation, the urine output is decreased by two to three times.

Thus, the investigation of the condition of certain vegetative functions in representatives of various classes of vertebrates showed that the disturbances of activity of the internal organs produced by the removal of the hemispheres in fish, amphibians and reptiles are very brief and are easily compensated. Distinct and permanent changes are seen only starting with the class of birds.

Birds, in contrast to all the preceding classes, are warm-blooded. In order to understand the tremendous significance of this fact in the process of evolution, it is sufficient to remember that 95 percent of the energy obtained from food, in the case of the warm-blooded animal in a resting state, goes into the maintenance of a constant body temperature, and only five percent of the energy is spent for the maintenance of the work of the heart, gastrointestinal tract and other systems of the body. This explains the ability of the lower classes, which do not have constant body temperatures (fish, amphibia and reptiles), to survive without food for months, and sometimes even longer.

A constant body temperature is a tremendous step forward in the adaptation of the body to changing environmental conditions.

For purposes of heat output, in addition to certain adaptations of the integument (feathers, wool, sweat glands, etc.), systems which were built up at earlier times (respiration, circulation) continue to be utilized. Thus, for the occurence of homoeothermism the functions of the body had to be made, to a greater degree, subservent to the central nervous system.

This, it seems to us, has also made possible the fact that, beginning with the class of birds, the importance of the higher nervous system centers as directors and distributors of the entire body activity is manifested in particularly distinct fashion.

The more the nervous system regulates the activity of the organs at the periphery, the more it itself must be under the influence of impulses from the periphery, because only with the fulfillment of this condition is central regulation of the activity of the body possible.

I. M. Sechenov directed attention to the "dark sensation" which is conditioned by impulses from the muscles. The possibility of the passage of impulses from the internal organs to the highest centers of the central nervous system has been shown by the experiments of I. P. Pavlov, K. M. Bykov and others. Our experiments permit us to assert that in the process of evolution this stream of impulses should show itself most distinctly beginning with the class of birds. From this point of view, we cannot help but express wonder at the fact that to date only the role of exteroceptive stimulations is being taken into account in the evolution of the brain. Thus, the development of the fore-brain is made dependent on the olfactory organ; of the mid-brain, on the visual organ; and of the hind-brain, on the auditory and vestibular organs. Our data permit us to assert that, in any case, beginning with the class of birds the interoceptors assume great importance in the evolution of the brain, and particularly of its anterior segments.

Experiments on the investigation of the state of the vegetative functions after the removal of the fore-brain have been conducted by us mainly with the aim of further study of the reactions of decerebrate animals to excessive stimulations.

However, as has been shown, these experiments are independently interesting, because they demonstrate graphically the ever increasing importance of the higher centers of the nervous system in the regulation of the vegetative functions. This also is at the basis of the different degrees of recovery of those functional disturbances which are caused by the operation of decerebration itself.

The reactivity to excessive pathological stimulations is altered in accordance with the role assumed by the fore-brain, as our experiments have shown.

The choice of the form of the excessive stimulation requires special discussion. the utilization of biological and chemical stimulations in comparative-evolutionary investigations is made difficult because of the varied spcies sensitivities to these stimuli. This is why we used localized tissue inuury, that is, the method which was widely used in his investigations by the founder of comparative pathology, I. I. Mechnikov. This form of stimulation was also used by I. M. Sechenov, I. P. Pavlov and N. Ye. Vvedenskiy for the investigation of the mechanisms of pathological reactions.

In our investigations, we used, along with localized thermal injury of the tissues, diphtheria and tetanus toxins, intoxications of the animals with mercuric chloride, injections of turpentine into the tissues and disruptions of the coronary circulation of the heart.

The most marked difference in the reactions of the normal and decerebrate animals to excessive stimulation is seen in the class of birds. It is manifested chiefly in the fact that the functional reactions of decerebrate birds to excessive stimulation are, as a rule, more weakly expressed and rapidly cease.

Thus, the acceleration of cardiac activity after various myocardial injuries, which in the normal pigeon continues for several days, is expressed more weakly in the decerebrate pigeon and lasts a total of several hours.

Lung injuries, which in normal birds produce increases in the respiratory rates and of metabolic rates, can in decerebrate birds, on the other hand, produce a retardation of respiration and a decrease in metabolic rate.

The injection of turpentine into the tissues, which in normal birds produces a distinct increase in the temperature, increase in frequency of cardiac activity and respiration, evokes weakened and rapidly abating reactions in decerebrate birds. Functional disorders after mercuric chloride intoxication are expressed to a milder degree in decerebrate birds than in normal birds.

The fact attracts special attention that weakening of the functional reactions is accompanied by a general diminution of resistance to excessive stimulations.

Using the same strengths of excessive stimulations, normal birds, which respond with marked functional reactions, remain alive, while decerebrate birds, in which these reactions are weakened, not uncommonly perish.

From these experiments it follows clearly that functional reactions can have protective natures, and that their occurrences depend on the higher centers of the nervous system.

In connection with the data obtained by us, the work of P. A. Baratynskiy is interesting; this work was carried out at the end of past century according to the suggestion and under the direction of I. P. Pavlov. The author studied the effect of narcotic agents on decerebrate frogs and pigeons. He showed that these agents equally affect frogs from which the cerebral hemispheres have been removed and normal frogs. In decerebrate birds, however, the functional reactions were weakened (specifically, no stage of excitation occured after the narcotic agents), and the resistance to narcotic agents decreased markedly. Therefore, our data coincide with those of the investigation presented.

We have already mentioned that in mammals only the removal of the cortex is possible under conditions of the chronic experiment, because complete removal of the fore-brain, that is, of the subcortical

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nuclei also, rapidly causes the deaths of the animals. This fact in itsle is clear-cut proof of the high degree of centralization which is present in the nervous system in this stage of evolution and of the importance which it assumes for the regulation of the entire activity of the body.

Preservation of the subcorticalnuclei in the process of decortication of mammals permits them to adapt themselves better to the defect than can birds in which the fore-brain is removedas a whole. This is why in mammals in which the cortices have been removed, disturbances of the vegetative functions are expressed more mildly than in decerebrate birds.

As I. P. Pavlov has shown in hissalivary gland experiment, in animals in which the cortex has been removed the conditioned reflex regulation of vegetative functions is impaired. Unconditioned regulation is preserved: food stimulation of the oral cavity continues to produce salivary excretion reflexly. It was shown by the experiments of Zeleniy that six to seven months after decortication the excretion of gastric juice in response to a mock feeding is restored. In time, the evacuatory function of the stomach is restored, as is also the periodicity of hungar. Thus, in mammals in which the cerebral cortex has been removed there is no permanent inhibitionof the evacuatory function which is seen in birds after complete removal of the cerebral hemispheres. After the removal of the cortex in dogs, the normal rates of cardiac activity and respiration are recovered in the first few days.

Investigations which have been conducted in our laboratory have shown that in rats, rabbits and cats removal of the cortex does not produce any distinct electrocardiographic changes. In dogs, a certain lability of the waves is noted. However, this, apparently, depends on the changes of position of the diaphragm following the removal of the cortex. More distinct changes of the electrocardiograms appear after the removal not only of the superficial but also of the basal parts of the cortex; here, the function of the hypothalamic area is distrubed to some degree.

Our investigations showed that in dogs in which the cerebral cortex had been removed the metabolic rates not only did not decrease but they even increased somewhat, which may be associated with their increased motor activity. The amount of urine excreted, its composition and specific gravity showed no deviations from the normal in dogs in which the cortices had been removed.

Therefore, with respect to the functions of the heart, respiration and diuresis of dogs in which the cortices had been removed, the permanent inhibition characteristic of birds in which the hemispheres have been removed was not found.

With the aim of a comparative study of the reflex reactions of normal dogs and dogs in which the cortices had been removed, we undertook the investigation of their olfactory reactions to painful stimulations of the skin by electric currents.

The latter produces a stormy motor reaction, shortness of breath, defecation and urination in operated dogs. These unconditioned reflex reactions are expressed to a somewhat greater extent in them than in normal dogs. After turning off the current, the dog jerked its head and trunk for some time; shortness of breath lasted somewhat longer in it than in the normal, and there was greater inhibition of hunger periodicity.

The difference in the reaction of the decorticate dog to electrocutaneous painful stimulation depends also on the absence of its higher analyzers. The normal dog tries to rip off the electrodes, gnaw through the electric wire, tear itself away from its straps. A dog which has been deprived of its cortex is not able to find the place where the painful stimulation is being applied; it turns its muzzle in the opposite direction, gets hold of one of its front paws (the electrodes are attached to the hind extremities) and begins to bite it, sometimes drawing blood.

In the normal dog, as is well known, even after the second or third application of the painful stimulation, a conditioned reflex reaction is elaborated to the situation, to the experimenter's touching the induction coil, etc. In strength, the conditioned olfactory reaction, motor as well as vegetative (shortness of breath, anuria, etc.), does not lag behind the unconditioned reaction.

Quite naturally, in the decorticate dog no conditioned olfactory reaction occurs.

A clear picture should be obtained of the correlation of the conditioned and unconditioned reflex regulations of mammals for analyzing their reactions to excessive stimulation and for analyzing the role of the cerebral cortex in the compensation for distrubance of functions.

Removal of the cortex in dogs disturbs the conditioned reflex reaction, particularly, coordination. Against this background, additional injuries of the nervous system or of the motor apparatus can altogether deprive the animal of the capacity of standing or walking (E. A. Asratyan). Unconditioned reflex reactions (for example, olfactory reactions to electrocutaneous stimulation) in decorticate dogs, on the other hand, are not only preserved but can even be intensified by virtue of the elimination of cortical inhibition. The same thing is seen with respect to the reactions of decorticate animals to other excessive stimulations also.

In response to localized injury of the stomach mucosa in dogs deprived of the cerebral cortex, vomiting and incessant contractions of the hunger type appear, which are seen also in normal dogs when the same procedure is performed. As our preliminary data show, these disorders apparently may be protracted in dogs in which the cortex has been removed.

Circumscribed myocardial injury in decorticate dogs produces basically the same functional cardiac changes as in normal dogs. However, during the period when these disorders are completely compensated in the normal dog, they can reappear in decorticate animals. Thus, a convulsive attack produced by an electric current in a decorticate dog provokes disorders of cardiac activity but does not cause them in the normal animals which, in the past, suffered from the same myocardial injury.

Our investigations showed that fever produced by turpentine in a decorticate dog is expressed to a greater degree than in normal animals. These data correspond completely with the results obtained by P. N. Veselkin.

In decorticate mammals, the reactions to excessive stimulation, as a rule, are not only maintained, but can even be expressed more vigorously, which is evidenced by a series of investigations which have been carried out in various laboratories.

In summarizing everything which has been said, the conclusion may be drawn that the cerebral cortex plays a great part in equilibrating the reactions of the body with excessive stimuli acting on it. Removal of the cortex, as a rule, produces protracted-course reactions, whereby a disturbance of the compensatory capacity of the operated animals is most often seen.

An attempt to establish the significance and show the specific characteristics of various centers of the central nervous system, particularly the cerebral cortex and subcortical structures, in the defense reactions of the body presents considerable difficulties, in view of the profound interconnections which exist between the individual divisions of the nervous system. These attempts, naturally, come up against both contradictions and exceptions. However, the need for establishing some kind of general orientative rules and regulations is increasing every day as the investigation develops.

Comparative investigations of the reactions to unitypical stimulations in animals with varying degrees of development of various centers of the central nervous system can prove to be very fruitful for the establishment of such rules and regulations. To be sure, it is essential to take into account ecological and other characteristics of various species of animals -- problems which require particular elucidation.

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ON THE ROLES OF DIFFERENT BRAIN CENTERS IN THE REGULATION OF THE GASEOUS EXCHANGE

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In birds, as is well known, the subcortical nuclei reach maximal sizes. In connection with this capacious development of them, the rudimentary cortex in birds is expressed to an even smaller degree than in animals of the preceding class -- the reptiles, and is almost completely absent anatomically. In birds, under conditions of the chronic experiment, the hemispheres may be completely removed, whereas in mammals only the removal of the cortex is possible. Removal of the cortex together with the subcortical nuclei leads to the rapid death of the animals, and attempts at isolated removal of the subcortical nuclei inevitagly are associated with the simultaneous involvement of the cortex.

In connection with this, the study of the after-effects of removal of the hemispheres in birds and decortication of mammals are, undoubtedly, of interest in the study of the interrelationship of the cortical and subcortical structures in the regulation of the activity of the body. It goes without saying, here, that it should be taken into account that this comparative method has serious shortcomings, because the subcortical structures of birds cannot be regarded as being completely indentical with the subcortical structures of mammals. However, by virtue of the fact that the uniformity of functions of the corresponding centers of the central nervous system has been clearly shown in various species of animals by comparative-physiological investigations, the use of the comparative-physiological method in solving the problem of the interrelationship of the cortical and subcortical structures can be considered justified.

The present investigation is devoted to the study of the changes of the gaseous exchange in birds in which the hemispheres have been removed and in decorticate dogs.

Data in the literature on this subject are limited. In investigations conducted by B. I. Bayandurov and his co-workers (F. G. Popov, Kudryavtsev) a marked drop in the quantity of excreted carbon dioxide was found in pigeons after the removal of both hemispheres (gaseous exchange was determined by the Pashutin method). A certain time after the operation, an increased excretion of carbon dioxide occurred, but at the same time the weight of the animal also increased. In calculating the excreted carbon dioxide per unit of weight a long-lasting decrease in its quantity was noted; the respiratory quotient was unchanged thereby.

The investigations of O. P. Molchanova in this direction, which, however, were conducted under other methodological conditions (gaseous exchange was determined by the Regnaud method), showed the same rules and regulations: the gaseous exchange in pigeons which had been deprived of both brain hemispheres was often decreased; the respiratory quotient was unchanged.

Along with the decrease in the gaseous exchange in birds after decerebration the rate of the respiratory movements is also slowed. In dogs following decortication, as was shown in the old observations of Danilevskiy, the rate of the respiratory movements is returned to normal.

The methods of removal of the hemispheres in birds and of decortication in dogs used by us did not differ from the generally-accepted methods. The investigation of the gaseous exchange in dogs was accomplished by means of a special mask supplied with respiratory valves. Preliminarily, for one to two months the dogs were trained to breathe in the mask. The expired air, after passing through the expiratory valve, entered gas meters which made it possible to determine the pulmonary ventilation. After certain time intervals (20-30 minutes), tests of the exhaled air were taken, which were analyzed in a Holden apparatus. On the bases of the ventilation data and the qualitative composition of the exhaled air, the oxygen consumption and the carbon dioxide excretion were determined per unit of time, and the respiratory quotient was calculated.

The investigations were conducted in the morning hours, fasting, over a period of from one to two hours.

The decorticate dogs were investigated under the same experimental conditions. Application of the respiratory mask caused, at first, a motor excitation in them, which quieted down after a certain time (different times for different dogs -- from five to 45 minutes). The gaseous exchange was determined when the animal appeared to be in a completely relaxed state. Investigations of gaseous exchange in decorticate dogs were made at various periods after the operation -- a month or more.

In investigating the gaseous exchange in birds, we utilized gas meters, in contrast to preceding authors, which made possible the simultaneous determination of the pulmonary ventilation as well as a more detailed analysis of the gaseous exchange. Because the use of the respiratory mask in birds is technically almost impossible to accomplish, we had to resort to tracheotomy, which was conducted under local anesthesia and under aseptic conditions. The upper outlet of the tracheotomy tube was connected with a respiratory valve, which substituted for the mask. Tracheotomy itself, as is well known, produces a decrease in pul onary ventilation, but because both decerebrate and normal birds were studied under the same conditions in our experiments this factor was equalized out.

We set about the investigation of the gaseous exchange in decerebrate roosters 12-14 days after the operation, at which time the after-effects connected with the operative procedure itself had been eliminated.

The experiments were performed on five adult roosters ($1\frac{1}{2}$ to two years of age) of approximately the same weight ($1\frac{1}{2}$ to two kilograms). The data obtained are presented in Table 1 and Table 2.

Table 1

GASEOUS EXCHANGE IN BIRDS BEFORE REMOVAL OF CEREBRAL HEMISPHERES

No of rooster	Ventilation in cc.	In expired air. % CO_2.	% O_2	% utilization of O_2	O_2 consumption per minute	CO_2 excretion per minute	Respiratory quotient
1	330	5.7	14.65	6.45	21.29	18.81	0.88
	270	5.4	13.85	7.15	19.31	14.58	0.76
2	300	5.53	14.75	6.75	20.25	16.59	0.82
	290	5.48	14.13	7.17	20.79	15.89	0.76
3	330	5.63	13.2	7.3	24.09	18.58	0.77
	400	5.1	13.68	7.44	29.76	20.4	0.69
	330	5.48	13.55	7.5	24.75	18.08	0.73
	390	4.58	14.38	6.69	26.09	17.86	0.68
4	500	5.35	14.0	6.97	34.85	26.75	0.77
	500	5.4	13.85	7.15	35.72	27.0	0.76
	450	5.65	13.6	7.4	33.3	25.43	0.76
5	350	3.83	15.93	5.33	18.7	13.41	0.72
	380	3.6	15.85	5.09	19.3	13.68	0.7

Table 2

GASEOUS EXCHANGE IN BIRDS AFTER REMOVAL OF CEREBRAL HEMISPHERES

No of Rooster	Ventilation in cc.	In expired air		% utilization of O_2	O_2 consumption per minute	CO_2 excretion per minute	Respiratory Quotient
		% CO_2	% O_2				
1	200	5.1	14.1	6.91	13.82	10.2	0.74
	200	5.25	13.73	7.34	14.68	10.5	0.72
2	220	5.05	14.05	6.98	15.36	11.11	0.72
	230	4.98	14.45	6.5	14.95	10.45	0.69
	210	4.35	15.0	5.96	12.51	9.14	0.73
3	290	3.55	15.93	5.01	14.53	10,29	0.77
	190	3.95	14.9	6.2	11.78	7.51	0.64
	130	4.23	14.83	6.21	8.07	5.5	0.68
	140	4.0	17.78	6.34	8.88	5.6	0.63
4	160	4.4	14.48	6.61	10.58	7.04	0.67
	190	4.9	14.15	6.9	13.11	9.31	0.71
	200	4.63	14.93	5.98	11.96	9.26	0.77
	190	4.63	14.48	6.55	12.45	8.79	0.71
5	250	4.5	14.15	7.0	17.5	11.25	0.64
	200	4.95	--	--	--	-9.9	--
	250	5.25	14.05	6.93	13.86	13.13	0.95

From the Tables it is seen that the removal of the hemispheres in roosters does not produce any particular changes in the composition of the expired air. The percentage of oxygen utilization, that is, the quantity of oxygen absorbed from 100 cubic centimeters of ventilated air is no different in decerebrate roosters from that in normal roosters, but the total consumption of oxygen per unit time is decreased considerably. While the normal rooster consumes an average of about 20-25 cubic centimeters of oxygen per minute (minimal quantity in the experiments was 18.7 cubic centimeters of oxygen), the decerebrate roosters, under the same conditions, use a total of only 10-15 cubic centimeters. Such a decrease in the oxygen consumption per unit time was caused by a decrease in pulmonary ventilation of the operated birds (from 300 cubic centimeters to 200 cubic centimeters and less). The carbon dioxide excretion is significantly decreased in exactly the same way with no change in the respiratory quotient. Such a decrease of oxygen consumption and carbon dioxide excretion attests to a decrease in the itensity of the metabolic processes in the bodies of the birds following the removal of both hemispheres of the brain.

Different results were obtained in the study of the gaseous exchange in dogs after the removal of the cerebral cortex (the investigations were performed on three dogs two months to a year after the decortication operation).

In dogs with intact nervous systems the oxygen consumption per kilogram of weight ranges, on the average, from five to seven centimeters per minute. The oxygen consumption in decorticate dogs ranges approximately within the same limits. In two decorticate dogs the oxygen consumption per kilogram of weight was equal to 4.95-7.2 cubic centimeters of oxygen; in the third, somewhat higher -- up to 8.5 cubic centimeters (possibly this was connected with the frequently observed motor excitation resulting from the development of the post-operative scar). All the other indices of gaseous metabolism (oxygen content and CO_2 content in the expired air, the percentage of oxygen utilization, ventilation of the lungs) were practically unchanged after the removal of the cerebral cortex.

These preliminary data indicate that the intensity of the oxidative processes in decorticate dogs is not decreased. As our further investigations showed, the significance of the cerebral cortex in the regulation of the gaseous exchange is manifested particularly distinctly when there are additional influences acting on the decorticate dogs, particularly pathological changes of the lungs. Evidently, the cerebral cortex plays a part chiefly in the adaptation of respiration to the constantly changing conditions of the environment, whereas automatic regulation of respiration (maintenance of it at the level which is average and usual for the given animal), as our experiments on birds also showed, can be accomplished by the subcortical structures.

THE INFLUENCE OF THE FORMATION OF DEFENSIVE CONDITIONED REFLEXES AND OF DISRUPTIONS OF HIGHER NERVOUS ACTIVITY ON THE STATE OF REACTIVITY IN RABBITS

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Studies devoted to disruptions of higher nervous activity which have been accomplished in I. P. Pavlov's laboratory, especially the work of M. K. Petrova, show the tremendous influence of disturbances of the activity of the cerebral cortex in altering the physiological functions of the intact body.

Studies carried out previously have shown the effect of various experimental forms of the pathology of pregnancy in animals on the development of the fetus and on the physiological condition of the newborn (I. A. Arshavskiy, 1950, 1951; I. A. Arshavskiy and L. S. Galeyeva, 1951; L. S. Galeyeva, 1950, 1951).

The purpose of the present work consisted of studying the effect of the formation of conditioned reflexes and disruptions of nervous activity on natural physiological immunity in rabbits.

The method of work on rabbits using conditioned reflexes has not been adequately worked out; in the literature, we were able to find only a few studies on this subject (N. A. Golubev, 1926; A. N. Bregadze, 1929-1935; V. I. Klimova, 1948; O. V. Malinovskiy, 1952; A. A.Volokhov, 1952). A part of the studies mentioned were devoted to the study of the motor food conditioned reflexes; another part, to the study of the defensive conditioned reflexes. V. I. Klimova used pin pricks of the rabbit's paw as the unconditioned stimulus. A. N. Bregadze used an electric current, placing the electrodes on the hind paw; however, the details of his method are not known.

We found no data in the literature concerning disruptions of higher nervous activity in rabbits. Therefore, we shall discuss in greater detail the method of operation with the motor defensive conditioned reflexes and the method of creating disruptions [of the nervous activity] in rabbits.

The conditioned reflexes in rabbits were elaborated in a specially constructed chamber measuring 70 x 45 x 60 centimeters (for this purpose we used the method accepted in the laboratory of A. A. Volokhov). On the front sides of the chamber there is a glass, double-wing door, through which the experimental rabbits are observed. The chamber walls are double and are made of plywood; several layers of asbestos are placed between them for the purpose of better soundproofing. A bell, rattle, siren and two light bulbs are built into the roof of the chamber. One light bulb of 25 candlepower served for constant illumination; the second, of 60 candlepower, was used for elaborating conditioned reflexes. A metronome was placed by the side of the chamber.

First, the rabbit was permitted to become accustomed to its new surroundings, for which it was simply placed in the conditioned-reflex chamber for 20-30 minutes on the first day; on the second day, it was again placed in the chamber for 20-30 minutes, and the electrode was put on its ear; only on the third day was the elaboration of conditioned reflexes begun. The conditioned reflexes were elaborated to the effects of sound and visual stimuli. The experiment consisted of six to 10 associations (per day); there were one to three-minute interruptions between the associations. The various intervals between the associations were haphazard in order to avoid a conditioned reflex for time, which might interfere with subsequent work on conditioned reflexes. Differentiations and inhibitory conditioned reflexes were also created in the rabbits. The unconditioned stimulus was an induction current of a strength adequate to obtain a threshold reaction. The reinforcement lasted a second following five to eight seconds of the isolated action of the conditioning stimulus. In order to avoid the sound of the induction coil, we used the alternating current from the city circuit, the potential difference of which was decreased to five volts by a transformer. The alternating current does not need a breaker, and therefore, the induction coil works noiselessly.

The base of the rabbit's ear was stimulated by the current, for which purpose special electrodes were assembled in the form of a clamp. The latter hung free on the ear and did not fall off when the rabbit moved or shook its ears.

The reaction was considered positive when the rabbit shook its ear, tapped with its hind paws, moved or adducted its ears. Usually, several components of the reaction could be seen which were accomplished separately or together in various combinations.

Disruption of the higher nervous activity was produced by the "confusion" of the negative and the positive stimuli (the positive stimulus was given immediately after the negative conditioning stimulus) or by a "double exchange" of the negative conditioning stimulus for the positive one and of the positive conditioning stimulus for the negative one, and also by an overstrain of the nervous system by high-powered stimuli -- an automobile siren for a strong induction current. In the latter case, the electrodes were placed on the hind paw of the rabbit and sometimes on the ear. The stimulus acted from three to fifteen minutes. If no disruption was obtained the first time, and there were no changes of conditioned-reflex activity on another day, then the experiments were repeated the next day. Some times, the stimulus was used several days straight; however, despite this, in some rabbits no disruption could be obtained.

The character of the disturbance of the cortical activity from the creation of nervous system disruptions was evaluated by the change in the positive conditioned reflexes and differentiations.

For evaluating the change in the reactivity state we selected the determinations of one of the indices of natural physiological immunity, namely, the complement titer. The complement titer was determined in the following way. Three to five cubic centimeters of blood were taken from the marginal vein of the rabbit's ear and placed in a thermostat at 37° C for 30 minutes. Then, dilutions of the serum to be examined were prepared (0.25, 0.12, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01 cubic centimeters) in 0.5 cubic centimeters of physiological solution. One cubic centimeter of the hemolytic system was added to each test-tube, and then, one cubic centimeter of the physiological solution (the physiological solution was prepared using thoroughly boiled distilled water). Thus, the total volume of liquid in which the reaction occurred was 2.5 cubic centimeters. The mixture was shaken up carefully, after which the test-tubes were placed in the thermostat for an hour. Complete hemolysis was designated by three plus-signs; incomplete by two, and hardly noticeable -- by one plus. The smallest quantity of serum which gave visible hemolysis was taken as the complement titer. The experiments were performed on 63 rabbits; 30 of them were gravid.

The motor defensive conditioned reflexes were elaborated in the rabbits to different degrees; in some of them, they were practically not formed at all.

We noted that conditioned reflexes are formed much more rapidly to a bell and a rattle than to the beat of the metronome; as a rule, there was no orientative reflex to the metronome. We connect this with the fact that a tap is a customary sound for rabbits, because they often tap with their hind legs when they are troubled. However, despite the absence of an orientative reflex conditioned reflexes are, nevertheless, formed to the metronome.

Conditioned reflexes to the bell and the rattle are sometimes elaborated after the first one or two combinations. The conditioned reflexes thereby begin to manifest themselves, as a rule, on the first day, and on the third to fourth day they become permanent. Conditioned reflexes to the tap of the metronome appear only on the second to third day, and become permanent still later.

Differentiation /discrimination/ in rabbits is elaborated well and is very stable. It is elaborated best and quickest to different frequencies of the metronome. It should be noted that there was no response reaction to the conditioning stimulus in the first differentiating combinations. It appears the differentiation has occurred suddenly, but after several combinations the same response reaction begins to appear to the differentiating stimulus as to the positive conditioning stimulus, and only after several combinations does the reaction to the differentiating stimulus again disappear, this time for the last time. These data correspond with the data of A. N. Bredadze (1929), who first noted this fact. The elaboration of differentiation in different rabbits occurs at different times. In some rabbits it is impossible to obtain complete differentiation.

We are not in agreement with the opinion of A. N. Bregadze (1935) that in motor defensive conditioned reflexes to an electric current the differentiations are poorly formed and readily destroyed. Our experiments on 48 rabbits (A. N. Bregadze performed them on two rabbits) showed that differentiations are rapidly obtained and, as a rule, are permanent.

Rabbits, like dogs, can be divided according to nervous system types. In our experiments, three groups of rabbits could be distinctly outlined. The first group consisted of those in which both positive and negative conditioned reflexes (differentiations) were well elaborated. The majority of rabbits belong to this group; they correspond to Pavlov's strong well-balanced type.

The second group of rabbits corresponds to the strong unbalanced type of nervous system. Positive conditioned reflexes are elaborated well in them, but differentiations are obtained with great difficulty and are often lost. Rabbits of this group are more sensitive to the current; therefore, for reinforcement they should be given weaker current. After disruption of higher nervous activity, their differentiations are most often lost.

The third group is made up of rabbits in which conditioned reflexes are formed with great difficulty or are not formed altogether. Sometimes, signs of "exhaustion" of the nervous system are seen in them, as in higher nervous activity disruptions, where the first one or two combinations give good conditioned reflexes, and then the conditioned reflex activity is completely absent for a long period of time. Differentiations are obtained well and rapidly in this group of rabbits; they are permanent and are elaborated beginning with the first combination without being changed afterwards. Usually, these rabbits reactpoorly to reinforcement by current; therefore, the current has to be intensified. Disruptions of the higher nervous activity in them most often occur with inhibition -- the positive conditioned reflexes disappear.

We first produced higher nervous activity disruptions by a strong induced current, but the disruptions were obtained rarely by this method, and cases were even observed where the conditioned reflex activity became better expressed. We believe that the intensification of the conditioned reflex activity and the difficulty of obtaining disruptions from a strong induced current in our experiments depend on the fact that we also used the induced current in the capacity of an unconditioned reflex, that is, reinforcement. It might be supposed that in the case of food conditioned reflexes the strong induced current would produce disruptions of higher nervous activity were better, but not always obtained to the automobile siren. Usually, immediately after the stimulation with the siren positive conditioned reflexes disappear; sometimes, paradoxical phases are seen. However, on a different day, the conditioned-reflex activity may be restored, and in certain rabbits is even better expressed than before the disruption. If the disruption is not obtained the first time, then it is sometimes obtained after repeated daily applications of the siren.

Sometimes, the disorders of the conditioned-reflex activity occurred when we proceeded with the elaboration of differentiation and used, right away, a large number of differentiating conditioning stimuli (more than the number of positive conditioning stimuli).

Disruptions of higher nervous activity are obtained also with "confusion" of the negative and positive conditioning stimuli or with a "double exchange." We created the "confusion" in the following way: a negative conditioning stimulus was used, after which immediately, without interruption, a positive conditioning stimulus was administered and a reinforcement with the current. Three such combinations were made, one after the other. "Double exchange" -- is the reinforcement of a negative conditioning stimulus and no reinforcement of the positive one: this was done three times in a row, whereby positive and negative conditioning stimuli were alternated.

The disruption of higher nervous activity were obtained best with combinations of "confusion", "double exchange" and the action of a high-powered stimulus (in these cases, the automobile siren was used). Usually we began with "confusions"; if there were no signs of conditioned-reflex activity disturbances on the second day, "double exchange" was used. On the third day, in case of necessity, the siren was used. Under these conditions, as a rule, a single siren stimulation for three minutes was adequate.

Forty three rabbits with disruptions of the higher nervous activity were observed by us. In the presence of disturbances of cerebral cortical activity we found paradoxical phases, inhibition of the conditioned reflexes, and, much less often, loss of differentiations. The behavior of the rabbits is markedly altered during the disruptions. Some animals becomes more excitable: they are in constant movement, shake their ears, tap with their feet, gnaw the cage, throw the calmp with the electrodes off their ears (although they never did this before the disruption), gnaw through the electric wires from the electrodes, and often show shortness of breath. Other rabbits, on the other hand, become quiet, hide in some corner (always in the same one), usually far from the place where the conditioning stimuli are located, and they assume strained positions. Inhibition of the positive conditioned reflexès is usually noted in such rabbits. The unconditioned stimulus also acts with less effect; the current has to be intensified. From time to time, marked changes in behavior occur in these animals. They begin to move, to shake their ears almost continuously, to tap with the feet, etc., like the rabbits of the preceding group. Such a state sometimes occurs after reinforcement with a strong current and sometimes even without obvious causes. The conditioned reflexes may be restored thereby, and, on the other hand, differentiations may be lost. The threshold for current stimulation is reduced. Such an excitation state can last for several minutes or for several days and is again replaced by an inhibited state.

The complement titer was determined in 12 rabbits first before working with conditioned reflexes, and then at the time of the elaboration of the conditioned reflexes, and in seven rabbits after the disruption of higher nervous activity had occurred (see Table, page 93).

First of all, we directed attention to the fact that the elaboration of conditioned reflexes in itself, which is usually of protective significance to the body, is a factor which increases its reactivity.

In the normal, control rabbits the complement titer ranges from 0.04 to 0.05 cubic centimeters of serum. Because of the elaboration of conditioned reflexes to the action of the stimulus which produced a protective, defensive reaction in the body (in our experiments, the shaking reflex in response to stimulation by an induced current), the complement titer was increased in nine out of twelve rabbits. The increase was particularly well marked at the time of elaboration of the conditioned reflexes, at that time reaching 0.03 cubic centimeters of serum. In three rabbits the complement titer did not change in response to the elaboration of the conditioned reflexes.

It may be supposed that this fact discovered by us is of essential importance in the understanding of the mechanism of protective reactions of the body to the effects of various environmental stimuli.

Participation of the brain, particularly in the formation of the conditioned reflexes to the effects of stimuli which produce defensive reflexes in the body, induces, so to speak, a mobilization of the adaptative protective capacities of the body as a whole with respect to its various indices.

As a matter of fact, the ear-shaking reaction of the rabbits in response to stimulation by an induced current would seem in itself to be adequate for the accomplishment of the necessary protection; nevertheless, we see that as time associations are formed to this reflex, the body mobilizes also its other protective capacities, which is expressed specifically in an increase of the natural immunity.

With the disruption of the higher nervous activity a fall in complement titer was seen, whereby in four rabbits it fell below the initial level. On the average, the complement titer dropped to 0.06 cubic centimeters of serum, that is, it was determined in an eight-fold dilution.

Disruptions of conditioned-reflex activity affect markedly not only the condition of the natural immunity but also the state of the entire body. If the disruption is long maintained, dystrophic disturbances are noted in some rabbits: the fur loses its previous lustre, becomes dishevelled, and tufts of hair fall out in places. In one rabbit with a disorder of conditioned-reflex activity, trophic ulcers developed on the anterior extremities, and the nails fell out. The ulcers persisted for several months and crippled the paws to such an extent that the rabbit could not use them for locomotion (Fig. 1.). It had also chronic rhinitis, which also persisted for several month. The nervous system disruption was produced in this rabbit at the beginning of May 1952; the ulcer developed after a month, first on one paw, and then on the other. Soon afterwards, the rhinitis was added to the picture. The ulcer and the rhinitis persisted for six months; there was no tendency to recovery shown (for this reason the rabbit was killed).

Fig. 1. Trophic Ulcers on the Front Paws of a Rabbit as a Result of a Disruption of Higher Nervous Activity.

COMPLEMENT TITERS IN RABBITS

Time of investigation	No of rabbit	Quantity of serum in cubic centimeters					
		0.12	0.06	0.05	0.04	0.03	0.02
Before elaboration of conditioned reflexes	1	+++	+++	+++	+++	+++	–
	2	+++	+++	+++	++	+	–
	3	+++	+++	–	–	–	–
	4	+++	+++	+++	++	–	–
	5	+++	+++	++	++	–	–
	6	+++	+++	+++	++	–	–
	7	+++	+++	+++	+++	+++	–
	8	+++	+++	+++	+++	++	–
	9	+++	+++	–	–	–	–
	10	+++	+++	+++	+++	++	–
	11	+++	+++	+	–	–	–
	12	+++	+++	–	–	–	–
In the process of elaboration of the conditioned reflexes	13	+++	+++	+++	+++	++	–
	14	+++	+++	+++	+++	++	–
	15	+++	+++	+++	+++	++	–
	16	+++	+++	+++	++	++	–
	17	+++	+++	+++	+++	+++	–
	18	+++	+++	+++	++	+	–

COMPLEMENT TITERS IN RABBITS
(Con't)

Time of investigation	No of rabbit	Quantity of serum in cubic centimeters					
		0.12	0.06	0.05	0.04	0.03	0.02
With disruption of higher nervous activity	19	+++	+++	+++	+++	+++	[illegible]
	20	+++	+++	+++	+++	+++	++
	21	+++	+++	+++	+++	++	[illegible]
	22	+++	+++	+++	+++	+++	[illegible]
	23	+++	+++	+++	+++	+++	−
	24	+++	+++	−	−	−	−
	25	+++	+++	+++	+++	+++	++
	26	+++	+++	−	−	−	−
	27	+++	+++	−	−	−	−
	28	+++	+++	++	++	−	−
	29	+++	+++	+++	+++	++	−
	30	+++	+++	+++	++	−	−
	31	+++	+++	+++	+++	++	−

In the majority of rabbits, the development of various pathological processes were observed as a result of the disruptions of higher nervous activity; tropic ulcers of the extremity, rhinitides, pneumonies, skin abscesses, etc. (Fig. 2).

Fig. 2. Chronic Rhinitis in the Rabbit as a Result of a Disruption of Higher Nervous Activity.

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The data obtained stimulated us to perform special experiments in which the resistance of the rabbits was determined to the subcutaneous administration of staphylococcus toxin in connection with the elaboration of conditioned defense reflexes in them, and also in the presence of disruptions of higher nervous activity.

If an adult rabbit is given a lethal dose of toxin subcutaneously, a typical inflammatory reaction occurs thereby which counteracts the action of the toxin and prevents the death of the animal. The adult rabbit does not die even when two, and in individual cases even three, lethal doses are given to it subcutaneously (I. A. Arshavskiy, 1947).

Conditioned reflexes were elaborated in three rabbits. In the process of elaboration of the conditioned reflexes each of them was given staphylococcus toxin subcutaneously in the ear in equal doses. A well expressed inflammatory reaction was seen in all the rabbits at the site of injection of the toxin; this reaction was expressed to a more marked extent than the reaction in the control rabbits. Of the three rabbits, only one died, on the second day. In four rabbits, conditioned reflexes were elaborated, after which a disruption of nervous activity was produced. Against the background of the disruption, each of them was given staphylococcus toxin in equal doses under the skin of the ear. The animals of this group responded with less definite signs of inflammatory reaction at the site of injection of the toxin compared with the controls. Three of these rabbits died at one, five and seven days, respectively, after the injection of the toxin; one rabbit survived.

The data obtained permit us to conclude that the cerebral cortex plays the leading part in the mechanisms which determine the conditions of reactivity and resistance of the body to the effects of various pathogenic stimulations of the environment. Individual stimuli, which act on the body, cannot produce disease-type reactions in cases of well-balanced nervous systems.

M. K. Petrova (1945) writes: "Pathogenic microbes always and everywhere surround us, but pathology occurs only when the nervous system, its physiological and physico-chemical processes, are altered; this occurs sometimes so unnoticeably that often, according to the statement of I. P. Pavlov, 'it is difficult to say where physiology ends and pathology begins'". (M. K. Petrova, On the Role of the Functionally Weakened Cerebral Cortex in the Occurrence of Various Pathological Processes in the Body, Medgiz, 1945).

We have found that disruptions of higher nervous activity of gravid female rabbits retard the growth and development of the fetus. The corresponding material is being presented in another special work.

In conclusion, we believe it essential to note certain advantages of rabbits as experimental animals in connection with the resolution of problems connected with various subjects of physiology and pathology of higher nervous activity. The rabbits are more easily kept under laboratory conditions, and less time is spent per rabbit (using our method) in working with conditioned reflexes than on the dog when working with food conditioned reflexes. This permits the performance of experiments on several rabbits during the course of the day. We believe that in pathology, where a large number of animals is often required for the experimental resolution of certain pathophysiological problems of higher nervous activity, rabbits are very suitable as experimental animals.

ON THE ROLE OF REFLEX MECHANISMS IN THE PROCESS OF CARBOHYDRATE METABOLISM DURING A DISTURBANCE OF LIVER FUNCTION

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A study of the role of the nervous system in the metabolic process in the body is one of the essential links in the investigation of the problem of nerve trophism.

Normal existance of the body is possible only when its metabolic processes correspond to the functional state of the body. This is why the study of nerve trophism and of its disturbances of of first-rank significance for the understanding of many concrete mechanisms of adaptation of the body to the conditions of its existence.

In the numerous works of I. P. Pavlov and his students it has been shown that reflex and conditioned-reflex stimulations can become the sources of alteration of various metabolic processes. Particularly abundant data have been obtained in this connection in the laboratories of K. M. Bykov and A.D. Speranskiy.

The present work is devoted to a clarification of the significance of reflex mechanisms in carbohydrate metabolism during a disturbance of liver function. The dynamics of the blood sugar were studied as one of the indices of carbohydrate metabolism.

The liver function disturbance was produced by thermal stimulation of a localized section of the liver, which was achieved by the injection of three cubic centimeters of water at a temperature of 80-90°C into the hepatic tissue.

This procedure has undoubted advantages over the other forms of stimulation (infectious or chemical) for the study of reflex mechanisms of compensation for the disturbed liver functions. With this method, the liver as a whole is not excluded, and other systems participation in the carbohydrate metabolism are not injured.

The sugar content in the blood was determined, in the majority of experiments, at five, 15, and 30 minutes, and also at one and two hours.

In the first series of experiments, 14 rabbits were used. Seven of them were subjected to liver damage; as a control, the remaining animals were injected with three cubic centimeters of hot water subcutaneously each in the area of the left hypochondrium.

Hyperglycemia was seen in all the experimental rabbits after the injury to the liver; this was determined as early as five minutes after the injury and most often was best expressed after 30 minutes. The degree of hyperglycemia thereby reached 78 milligrams percent in some cases; on the average it equaled 44 milligrams percent, and only in one case was it less than 25 milligrams percent.

At the end of two hours after the liver injury the level of the sugar content in the blood approached normal in the majority of animals (Fig. 1).

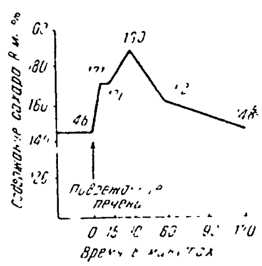

Fig. 1. Blood Sugar Content with Inuury to Liver of a Normal Rabbit.

In a portion of the control rabbits (five animals) an increase in the sugar content in the blood was also seen; however, it did not in any case achieve the same degree as in the experimental rabbits.

For purposes of studying the mechanisms of this hyperglycemia, the blood sugar content in the next series of experiments was investigated after injury to the liver following the denervation of it (maximal disruption of innervation). Denervation of the liver was attained by means of the transection of the hepatogastric ligament and also, as much as possible, of the hepatoduodenal ligament. The hepatic artery, portal vein and also the common bile duct passing through the hepatoduodenal ligament were carefully dissected out, freed from the surrounding tissues and treated with 96 percent alcohol. In addition, the suspensory ligament and the triangular ligament were transected.

Eight rabbits were subjected to the experiment. The liver injury was produced one to eight days after the disruption of the liver innervation.

It should be noted that in itself the disruption of the liver innervation in a number of cases led to hyperglycemia, which continued for several days. N. B. Medvedeva also observed hyperglycemia in such cases.

Of the eight experimental rabbits of this series, the blood sugar content in six rabbits was normal at the time of injury to the liver; in two rabbits, it was increased (157 and 166 milligrams percent).

Injury of the liver under conditions of maximal disruption of innervation produced only a brief increase of the blood sugar, which was replaced by hyperglycemia after 15-30 minutes (Fig 2). The decrease in blood sugar

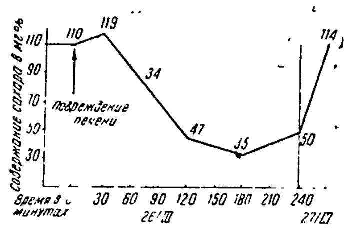

Fig. 2. Blood Sugar Content with Injury to Denervated Liver Lobule.

attained considerable magnitudes in this process; on the average, the quantity of sugar dropped to 57 milligrams percent and, in one case, even by 89 milligrams percent.

In this series, only one rabbit was an exception; in it, hyperglycemia was seen, possibly in consequence of the less complete disruption of innervation than in all the other rabbits of this series.

Thus, the results obtained in this series of experiments speak for a reflex mechanism of the hyperglycemia which was observed in the first series of experiments after injury to the liver under normal conditions, because when the injury to the liver was accomplished in the presence of a disrupted innervation, the blood sugar curve assumed a completely different appearance. The hyperglycemia which occurs here may be associated with stimulation of the parasympathetic nerve plexi found in the liver tissue.

It might be supposed that stimulation of the sympathetic division of the nervous system might have definite importance in the mechanism of hyperglycemia which was observed following liver injury. As is well know, there are many indications of this in the literature.

Experiments with the use of ergotamine were performed for purposes of experimental verification of this theory.

The ergotamine was injected intravenously in a dose of one milligram per kilogram of weight. Injury to the liver section was produced by the usual means at 40, 45, 60 and 70 minutes after the injection of the ergotamine.

The investigation of the blood sugar content showed that in the presence of liver damage 40 and 45 minutes after the ergotamine injection hyperglycemia is still seen in the rabbits, which reaches approximately the same level which was seen in the animals of the first series of experiments.

In the presence of liver injury, 60-70 minutes after the injection of ergotamine hyperglycemia is no longer observed (Fig 3) which may be explained by the action of ergotamine on the sympathetic nervous system.

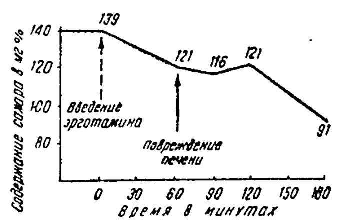

Fig. 3. Blood Sugar Content with Injury to Lobule of Liver of Normal Rabbit under conditions of the Use of Ergotamine

Thus, the data of this experiment indicate that the changes of the condition of the sympathetic division of the nervous system can influence the blood sugar content after liver injury.

However, the data of modern physiology do not permit us to regard the changes of blood sugar content observed as the results of the isolated effect of the sympathetic nervous system on the processes of carbohydrate metabolism. The sympathetic nervous system itself is under the regulating and correcting influence of the higher centers of the nervous system--the cerebral cortex.

The next series of experiments was devoted to the study of the role of the cerebral cortex in the accomplishment of the compensatory mechanisms under conditions of a disturbed carbohydrate metabolism.

Twelve animals were subjected to experiment. The liver injury was produced at various intervals following the bilateral removal of the cerebral cortex. It should be noted that in connection with the methodological difficulties of operation in removing the cortex its basal sections were preserved. In all these cases, it was noted that the operation itself leads to an increase of the blood sugar, reaching, sometimes, 180 milligrams percents.

Injury to the liver one to three days after the removal of the cortex in four out of six rabbits led to a further increase of the blood sugar level (which in one rabbit reached 296 milligrams percent), and in two rabbits, to decreases of 34 and 45 milligrams percent, respectively.

In view of the fact that the treatment of the data obtained was made difficult by the presence of a high blood sugar after decortication, we afterwards injured the liver at later periods.

It turned out that six days after the decortication the blood sugar content in all six rabbits exceeded the normal, reaching 161 milligrams percent. The liver injury led to an increase in the blood sugar content by 102 milligrams percent. Three hours after this, the sugar content had still not returned to the initial level.

In later experiments, we used liver injuries performed three weeks after the removal of the cortex. At this time, the blood sugar content had become normal.

In all five rabbits following the localized injury of the liver, hyperglycemia was observed which reached much higher degrees than it did with liver injuries in normal animals (Fig 4). Here, in individual cases, the blood sugar increased by 151 milligrams percent; on the average, the blood sugar content increased by 119 milligrams percent.

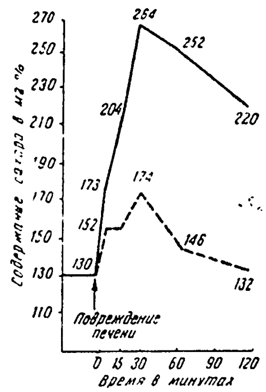

Fig. 4. Blood Sugar Content with Injury of Liver of Decorticate Rabbit and in Rabbit with an Intact Cerebral Cortex.

——————— decorticate rabbit:
- - - - - - - - rabbit with intact cortex.

Let us recall that with liver injuries in normal rabbits the blood sugar content never rose more than 78 milligrams percent, and on the average it increased by 44 milligrams percent.

It should be noted also that in animals with intact cerebral cortices, two hours after the liver injury the blood sugar content had already returned to normal; in decorticate animals, the quantity of sugar in the blood at this time never returned to the initial level.

Thus, in decorticate rabbits the disturbance of carbohydrate metabolism from localized liver injuries reached higher levels than in normal rabbits.

This permits us to draw the conclusion that the cerebral cortex is important in the compensation for disturbed carbohydrate metabolism.

It should be remembered that, aside from the liver other systems of the body also participate in the metabolic processes; the functions of these systems are also regulated by the higher centers of the nervous system. Disturbances in the regulation of these systems unconditionally also find their reflections in more profound blood sugar content changes which were observed in our experiments.

The material obtained in the present work attests to the importance of reflex mechanisms in the processes of carbohydrate metabolism and of the important role of the cerebral cortex in compensating for the disturbances of this metabolism.

ON THE MECHANISM OF ACTION OF PENICILLIN ON ASEPTIC INFLAMMATION

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Until now, the mechanism of the therapeutic action of penicillin in various diseases has been studied without taking into account the date of Pavlovian physiology. Therefore, the opinion has been offered that antibiotics affect only the functional states of the microbes up to the destruction of their structures. This opinion is based on various experiments which have been performed in vitro. Physiological reactions occurring in the complex intact animals body in response to the injections of antibiotics have as yet not been studied. It is believed that the antibiotics "can in principle disturb also the processes which occur in the host organism" (M. D. Mashkovskiy). The so-called side-effects of the antibiotics comes to attention here.

The clarification of the problem of the effect of penicillin on non-microbial, aseptic inflammation assumes greater importance.

The data in the literature are inadequate on this subject. Only the work of N. V. Lazarev and L. S. Salyamon and the articles of M. P. Yermolayeva have been published on the study of the effects of sulfonamides and penicillin on aseptic inflammation.

The experiments of L. S. Salyamon show that aseptic inflammation of the aural conchae of rabbits produced by burning, freezing, smearing with croton oil and allergic inflammations can be markedly weakened or even completely suppressed by means of treating the animals with sulfonamides. Penicillin therapy did not lead to any positive therapeutic results.

It should be noted that the criteria of the course of the inflammatory reactions in the given case were only the temperature of the aural concha and edema of the ear. No histological examinations were performed. The animals were observed only 72-96 hours.

All this stimulated us to undertake the study of the mechanism of action of penicillin under such methodological conditions which would permit us to observe the animals for a long time and to perform not only a clinical but also a histological analysis of the inflammatory focus. The experiments were performed on 51 white rats weighing 150-180 grams. With the aim of producing the aseptic in-

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flammatory focus, 0.3 cubic centimeter of turpentine was injected in the muscles of the right shin.

After three days (not counting the day of injection of the turpentine) and following the formation of local inflammatory infiltrate, all the animals were divided into five groups.

First group -- the control (11 rats). The animals were not subjected to any procedures.

Second group (10 rats). The animals were injected with penicillin (sodium salt) in physiological solution into the muscles of the left shank.

Third group (10 rats). The animals were injected with physiological solution only into the muscles of the left shank.

Fourth group (10 rats). The animals were injected with penicillin diluted with 0.5 percent novocaine solution in the muscles of the left shank.

Fifth group (10 rats). The animals were injected with 0.5 percent novocaine solution into the muscles of the left shank.

The animals of the second, third, fourth and fifth groups received the injections on the fifth, sixth, seventh, eighth and ninth days of the disease.

The blood count was investigated in the animals before and after treatment. Systematic observations were performed on the inflammatory focus and the function of the extremity.

On the 10th, 21st, 32nd and 42nd days, the ailing rats were killed according to the calculation that at each of these times several animals of each of the various groups were killed.

At autopsy, the muscles of the right and left shanks were taken for histological examination. Staining of the preparations was accomplished by the Van Gisson and hematoxylin-eosin methods.

The control animals (11 rats) were injected with 0.3 cubic centimeters of turpentine into the depth of the right gastrocnemius muscle.

On the second day after the injection of turpentine, the right hind paw was somewhat swollen, hyperemia of the skin appeared, and on the third day, a tender thickening was felt in the depth of the gastrocnemius muscle of the right hind paw -- the inflammatory infiltrate. In connection with this, a limitation of active and passive movements of this extremity was noted (in consequence of the formation of an inflammatory contracture of the muscle). On the 10th day, the thickening of the muscles reached from one to two centimeters in diameter. Three days after the turpentine injection a significant change in the blood count was noted -- an increase in the percentage of transitional forms and of mononuclear cells (from the normal 2-5 percent to 20-25 percent).

An autopsy of the rats at various periods of the disease, inflammatory changes, extensive infiltrates and also abscesses (from one to two centimeters in diameter) were found in the area of the muscles of the right shank with scar formation in the surrounding tissue and the formation of a capsule.

In the rats of the second group, the state and function of the right hind paw during the first five days after the injection of turpentine corresponded to that described in the control group of animals. Edema, an infiltrate, and limitation of movements (active and passive) appeared in connection with the appearance of an inflammatory contracture of the muscles. Changes of the blood counts in these animals were no different from those which obtained in the controls.

Beginning with the fifth day, this group of rats was injected intramuscularly with the sodium salt of penicillin which had been diluted in physiological solution (1,200,000 units per each injection) three times a day (at 9 a.m., 12 noon and 5 p.m.). The penicillin was injected into the muscles of the left hind extremity which corresponded to the inflammatory focus [on the right side] in 0.6 cubic centimeter volumes.

After a day, a certain decrease in the infiltrate was noted and a definite increase in the range of movements of the right paw.

The pencillin injections were given for five days. As the total course of therapy each rat received 18,000,000 units of penicillin.

As a result, active and passive movements of the extremities were recovered by seven rats on the 10th day of the disease. A significant decrease was noted in the size of the thickening (on the average, it decreased to 0.5 centimeter in diameter).

Autopsies of rats killed on the 10th day showed a considerable hyperplasia of scar tissue. An extensive capsule was noted, within which there was a small (compared with the control) amount of granular pus.

Afterwards, on the 21st day, only a small hard thickening could be palpated in the depth of the muscles. If there had been an abscess, it usually was walled off by a strong connective-tissue capsule.

Simultaneously with the recovery of the function of the extremity, and as early as at 14 days, normalization of the blood picture was seen.

Rats of the third group, beginning with the fifth day after the turpentine injection, were injected intramuscularly for five days with 0.6 cubic centimeter each of physiological solution. The furthere clinical course of the focal inflammation did not differ from that described in the control group of animals. Under the conditions of this experiment, the foci of inflammation were even distinguished by somewhat larger sizes, while the functional disturbance of the extremity was prolonged.

Five days after the turpentine injections, the animals of the fourth group were injected intramuscularly with the sodium salt of penicillin in 0.5 percent novocaine solution in a volume of 0.6 cubic centimeter (each dose containing 1,200,000 units three times a day). The course of treatment, as in the other groups of animals, lasted five days. In this time, each rat received 18,000,000 units of penicillin.

It should be noted that this form of treatment brought about the elimination of the muscular contractures with particular rapidity. They disappeared completely in six rats as early as one to two days after the onset of therapy; the inflammatory infiltrates were also decreased. The figures of the blood count rapidly became normal.

On the 10th day, three rats were killed. In these animals, only cicatricial changes of the muscles could be noted in the area where turpentine had been injected, and less often, there were small abscesses (0.3 to 0.5 centimeters in diameter) with well-developed capsules and cicatricial changes of the surrounding muscles.

The same picture was found also at later periods.

The last, the fifth group, of animals was injected (three times a day) with 0.5 percent novocaine solution five days after the injection of turpentine.

As early as the second day of treatment the contractures of the muscles disappeared in the majority of animals, and free movements of the extremities were recovered, although the inflammatory infiltrates decreased in size in only three rats.

Autopsies of the animals showed that under these conditions the recovery of the inflammatory focus progresses better and quicker than in the control group of rats, but not so well as in the second and, especially, the fourth group.

The results of our experiments give evidence of the influence of penicillin on aseptic focal inflammation, which effects a certain decrease of the inflammatory phenomena, decrease in the inflammatory contractures and particularly the stimulation of hyperplasia of the connective tissue around the abscess formed.

It is particularly important to note the good therapeutic effect in animals which were injected with penicillin diluted in 0.5 percent novocaine solution. This coincides with the observations of A. A. Vishnevskiy and his co-workers. However, our data give evidence to the effect that the combination of penicillin with novocaine is effective not only because the penicillin acts etiotropically on microbes and because novocaine acts pathogenetically in the inflammatory process (as A. A. Vishnevskiy believes). Apparently, the action of pencillin for a normalization of the neural trophism of the tissues of the inflammatory focus is also important here.

Our experiments give us the basis to believe that in suppurative processes, penicillin intensified the proliferation of connective tissue, and consequently also the encapsulation of the suppurative focus, which can lead to the formation of latent suppurative foci. These data are confirmed by the experiences of surgical clinics (Shlapoberskiy and others) and are still the only basis for the use of the combination of penicillin therapy with the obligatory incision and drainage of a suppurative focus.

Certainly, here, account should be taken of the fact that, depending on the dosage, penicillin can stimulate or suppress the proliferation of connective tissue. The latter has been shown by Ye. A. Timofeyevskaya under tissue-culture conditions. The data of M. P. Yermolayeva can also be explained by this; by using ordinary penicillin, she showed its suppressive effect on connective tissue during the formation of a capsule around a foreign body which had been injected into the tissues of a white rat.

ON THE ROLE OF THE NERVOUS SYSTEM IN THE AUTOTRANSPLANTATION OF THE THYROID AND ADRENAL GLANDS

I. M. Shapiro

The role and significance of hormones, vitamins and other humoral factors of body activity are being at present evaluated from two points of view which are different in principle.

One of them is based on the principles of the cellular pathology of Virchow and the cellular physiology of Fervorn [a transliteration. Fairborne?], according to whom the effects of various humoral agents are the result of the direct contacts of the given agents with the tissue substrates. The glands of internal secretion are looked upon as a kind of isolated system independent of the influence of the environment and of neural influences.

Another point of view is based on the teaching of I. M. Sechenov and I. P. Pavlov on the integrity of the complex organism and the leading significance of the nervous syste in its activity.

This makes it possible to analyze and evaluate the effects of various factors of the external and internal milieux as the results of their indirect actions through a number of nerve links. The teaching of I. P. Pavlov regards all processes occurring in the complex organisms of the higher animals and man from the point of view of reflex nervous activity.

Investigations, begun in his time, in the laboratory of A. D. Speranskiy dealt with the role of the nervous system in the endocrine regulations of the bodily functions.

Numerous data which have been accumulated at the present time permit us to believe that no gland of internal secretion is autonomic in its physiological functions in the body and that they are all under the direct regulating influence of the nervous system.

At the same time, it has been noted that hormonal factors can exert effects not only directly on tissues but also by means of specific forms of stimulation of the nervous system -- through its sensory apparatus. Our investigations have established that the survival and functional integrity of an autotransplant depend primarily on the site of its transplantation.

The thyroid and adrenal glands served as the objects for our transplantations; these were selected for the following reasons. The conditions of survival and regeneration of autotransplants of the thyroid gland in various parts of the body have been studied most. However, in the experimental investigations and in clinical practice, the results of thyroid gland transplantation in the cases of its functional abortion or inadequacy have been but little studied.

In our experiments, we also used as a starting point certain facts of clinical endocrinology pertaining to the interaction of the functions of various glands of internal secretion in the body. Such a connection has been repeatedly established between the thyroid and adrenal glands.

Survival of the adrenal gland under conditions of autotransplantation with prolonged maintenance of its functions has not been obtained by anyone.

After the removal of both adrenal glands, animals die quickly, as a rule. This permits us to determine more easily the functional integrity of the grafted transplant, and, based on this, to evaluate more readily the conditions of this "taking-root process".

To accomplish as complete as possible an adaptation of the grafted gland to its new circumstances, we had to review the technical methods used and to perfect several of them.

In the present report, we are presenting experiments demonstrating the results of the graftings mentioned in connection with an altered body reactivity.

We performed one series of experiments with the simultaneous removal of both adrenal glands and the autotransplantation of them into the area of the lower poles of the kidneys and two control series of experiments.

From our own observations and from the data in the literature we know that large grafted pieces of the cortical and medullary layers of the adrenal gland often undergo necrosis.

Taking this into account, in all the series of experiments on rabbits we transplanted the entire adrenal gland mass in its finely divided form. The finely divided adrenal gland was collected on a tongue depressor, applied in an evan layer to the area selected, and lightly rubbed into it. The removal of both adrenal glands and the autotransplantation into the lower poles of the kidneys at the edge of the vena cava were performed simultaneously.

Special attention was directed to the post-operative period. Immediately after the operation the rabbits were placed in a special cage with an air temperature of 21-22° C. The food of the animals during the first 10 days after the operation consisted of fresh cabbage, carrots cut in thin slices, beets and hay or clover.

In all, 27 rabbits were operated in this series of experiments; 22 of them survived and were killed at various time intervals after the grafting, while five rabbits died seven to thirteen days after the transplantation.

Our observations show that signs of weakness were not marked in the first few days after the operation; the body temperature increased slightly and after four days it came back to normal. Refusal of food was noted only for the first three days, although a complete diet was not taken until the tenth day. The all in body weight continued until the 21st day after the simultaneous removal of both adrenal glands and the autotransplantation of them.

At the autopsies of the rabbits, adrenal transplants were found of different forms and sizes. Thus, for example, in the first three days after the grafting the transplant had the appearance of individual pieces which were not connected with one another. Thirty days after the grafting, the transplant was found in the form of a lumpy layer, which after one and a half to two months presented an almost smooth surface of yellow-pink color. After three months, lumpiness appeared again on the transplant, which gradually increased and fused into a common mass. At eight months to one year after the grafting, we found transplants at autopsy which had the form of the normal adrenal (Fig. 1.)

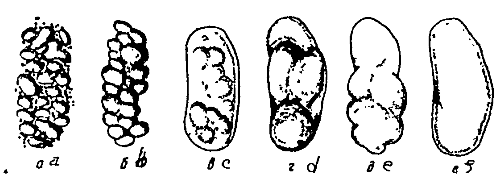

Fig. 1. Development of the Adrenal Gland Transplant after its Grafting into the Area of the Lower Poles of the Kidneys (Diagram).

a - at 1-3 days; b - at 30 days; c - at 3 months; d - at 5 months; e - at 7-8 months; f - at a year

On histological examination of these adrenal transplants it was found that degeneration of the cells of the cortical and medullary layers of the adrenal gland are seen beginning with the first day after the grafting. The medullary layer is seen, three to seven days after the grafting, in the form ofpoorly staining cellular elements, and decomposition of them is seen. Bundles of "old" nerve fibers of different calibers are seen here as well as individual fragments of these fibers, which are encountered most often near the vessels among the tissue detritus of the medullary layer even 21-30 days after the operation. Areas of cortical-layer cells, more or less intact, are found in the first seven to ten days after the grafting. A notable regeneration of them is seen 14 days after the grafting. At this time, cells of the cortical layer are seen in certain sections of the transplant, which are radially disposed along the courses of the blood vessels and which have cytoplasms and nuclei of normal structures.

Twenty one days after the grafting, bundles of "old" nerve fibers of medium and large calibers in the cortex are markedly and unequally stained. The fine neural fibers are, for the most part, not notably changed.

Three months after the autotransplantation, a significant portion of the cells of the cortex of the transplant are distinctly seen. In the layers of connective tissue which connect the individual sections of the transplant, bundles of nerve fibers are found, directed toward the cortical layer.

A year after the grafting, the transplant has an oval shape equal to two thirds of the normal adrenal gland and consists of cortical cells having normal structures and disposed haphazardly in the central part of the transplant (Figs. 2, 3 and 4).

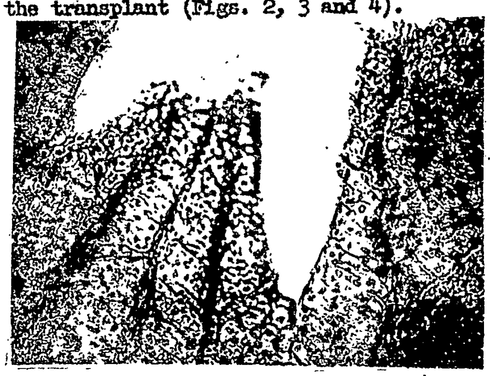

Fig. 2. Transplant of Adrenal Gland at 3 Months after Transplantation into the Area of the Lower Pole of the Right Kidney. Regeneration of Cells of the Cortical Layer. Hematoxylin-Eosin Stain.

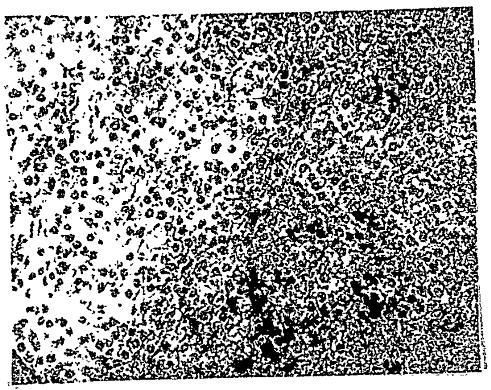

Fig. 3. Cortical Substance of Adrenal Gland at 8 Months after Autotransplantation into the Area of the Lower Pole of the Right Kidney. Hematoxylin-Eosin Stain.

Fig. 4. Transplant of Adrenal Gland at 8 Months After the Transplantation in the Area of the Lower Pole of the Right Kidney. Bundles of Nerve Fibers in the Cortical Layer of the Transplant. Stain by the Bil'shovskiy-Gross Method.

As a control, experiments were performed on rabbits with the simultaneous removal of both adrenal glands without transplanting them.

Thirty rabbits underwent the operation. They all died 24-63 hours after the operation with signs of coma.

In order to clarify the role of the adrenal transplants, experiments were performed on 20 rabbits with the removal of the adrenal transplants at different time intervals following their simultaneous removal and transplantation into the areas of the lower poles of the kidneys. All the animals died 20-30 hours after the removal of the transplants.

In the rabbits of this series, the same clinical symptoms of adrenal insufficiency were found as in the rabbits in which both adrenals had been removed without transplanting them, but these symptoms appeared sooner.

A year and two months after the transplantation, in addition to the definite cortical cells, a narrow strip of cells was found in one section of the adrenal transplant going from the periphery to the center. Their cytoplasms were stained (fixation in the Vizel' method, staining with hematoxylin-eosin) a chestnut brown color, the borders of these cells were not distinguishable, while the nuclei were larger than the cortical cell nuclei, and the cytoplasms did not stain so well (Fig. 5).

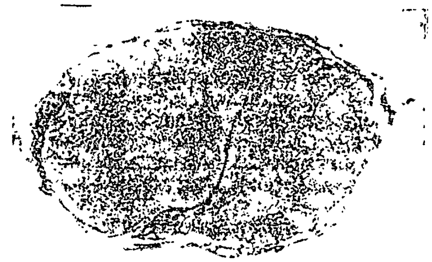

Fig. 5. Transplant of Adrenal Gland at 1 Year and 2 Months after the Transplantation in the Area of the Lower Pole of the Right Kidney. Strip of Medullary Substance. Fixation in the Vizel' Method, Hematoxylin-Eosin Stain.

It was of great interest to elucidate how the operation performed (removal and autotransplantation of the adrenal gland) reflected itself on certain important functions of the body.

For the resolution of this problem experiments were performed on the study of the physiological and biochemical indices of the blood under these conditions (sugar content, adrenalin content and content of adrenalin-like substances).

We shall not present the numerous investigations on this subject here. Let us note only that the most varied opinions exist -- from a complete negation of the role of adrenalin in the activity of the body (Gray, 1930; Stewart and Rogoff, 1917-1920) to the evaluation of it as a factor which determines practically all human behavior (Cannon, 1929-1932).

K. M. Bykov (1947) writes, ". . .We are, for example, well informed on the effect which adrenalin exerts on the blood pressure, heart rate, sugar content in the blood, development of fatigue in the isolated muscle, movements of isolated intestines, etc. These data were obtained after the injection of adrenalin into the blood or by studying its effect on isolated organs. However, we know very little about the actual significance of the elaboration of adrenalin in the adrenal glands for the activity of the entire body." (K. M. Bykov, The Cerebral Cortex and the Internal Organs, Moscow, Medgiz, 1947, p 286).

We made determinations of the blood adrenalin content of rabbits on isolated frogs' hearts. Using perfusions of Ringer's solution and after the cardiac contractions had acquired a stable character with respect to rate and amplitude, we injected three drops of normal rabbit's blood into the cannula.

The force of the cardiac contractions then increased somewhat compared with the initial contractions (Fig. 6). On the background of the original, established contractions, the blood of a rabbit from which both adrenal glands had been removed without transplanting them was injected into the cannula. In these cases, the strength and frequency of the cardiac contractions decreased considerably (Fig. 7).

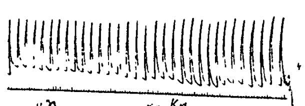

Fig. 6. Action of Normal Rabbit's Blood on the Isolated Frog's Heart.

Arbitrary designations: r - Ringer's solution injected;
kr - blood of normal rabbit injected.

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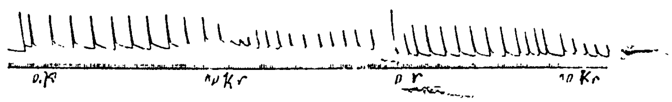

Fig. 7. Contraction of Isolated Frog's Heart during the Injection of the Blood of a Rabbit from which Both Adrenal Galnds were Simultaneously Removed One Day before the Experiment.

Arbitrary designations: r - Ringer's solution injected; kr - blood of rabbit, from which adrenal gland was removed, injected.

If then, after perfusion of the heart, blood of a rabbit from which both adrenals had been removed and an autotransplantation of them performed is injected into it, the strength and particularly the frequency of the cardiac contractions increased markedly. The positive chronotropic and inotropic effects therefrom were considerably better expressed than the effects from blood taken from the normal rabbit. (Fig. 8)

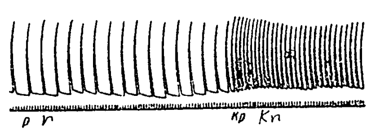

Fig. 8. Contraction of Isolated Heart of Frog during the Injection of the Blood of a Rabbit from which Both Adrenal Glands were Simultaneously Removed One Day before the Experiment and a Transplantation of them Performed in the Area of the Lower Poles of the Kidneys.

Designations: r - Ringer's solution injected; kr - blood of rabbit injected from which adrenal glands were removed but in which transplantation of this tissue was performed.

In subsequent experiments the content of the adrenalin in the blood of rabbits was investigated at various periods of time following the removal of both adrenal glands and transplantation of them.

It was established that the strength of the cardiac contractions (after injection of blood from the rabbit five and seven days after the operation) compared with the original curves is either the same or even a little increased.

Fourteen and twenty one days after the removal and transplantation of both adrenals, the strength of the contractions continues to be a little increased compared with the reaction of the heart to the blood of the normal rabbit.

Positive chronotropic and inotropic effects on the isolated frog's heart also occur after the injection of blood taken from a rabbit 41 days after the removal of both adrenal glands from it and autotransplantation of them.

It must be supposed that a day after the removal of both adrenal glands not only a disappearance of the adrenalin from the blood occurs, but apparently also an accumulation of acetylcholine-like substances which evidently also give increased chronotropic and inotropic effects on the frog's heart.

We also need to discuss the following question.

The rabbit dies a day after the removal of both adrenal glands. As our experiments have shown, deaths of the rabbits can be averted in the majority of cases if, at the same time as the operation of removing the adrenal glands an autotransplantation of them is at the same time performed in a definite area. As the material of our anatomical investigations show, before the rooting adrenal glands establish a connection with the nervous system, a progressive destruction of the medullary substances and the cortical layer occurs in them.

Only the data obtained a day after the removal of both adrenal glands and transplantation of them might explain the destruction of the medullary layers of the transplants of the adrenal glands and the associated excessive excretion of adrenalin into the blood from these adrenal glands.

How can we explain the fact that as early as several days after the operation a content of adrenalin and adrenalin-like substances is established in the blood of the rabbits operated on by the method described above, which content approaches normal and even frequently exceeds it?

It would be natural to believe that the compensation for the function lost by the adrenal glands, particularly by their medullary layers, is achieved by an intensification of the function of the chromaffin system which remains.

However, as follows from our data, this compensation occurs only in the event that following the removal of the adrenal glands they are transplanted in a certain spot only (in the area of the lower poles of the kidneys). It must simply be granted here that compen-

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sation can occur quickly, because the transplanted adrenal gland continues, for a certain time, to secrete its hormones.

In the literature, there is mention of the fact that at autopsy sometimes various pathological changes of the chromaffin cells are accidentally found in the medullary layer of the adrenal glands, which goes as far as the complete loss of the chromaffin reaction by these cells.

It is characteristic that some of those who die with this picture never suffered from Addison's disease during life nor from any other symptoms of adrenal insufficiency which ordinarily accompany destructions of the adrenal glands. In such cases, reference is usually made to the compensatory hypertrophy of the chromaffin tissue in the residual healthy paraganglia of the chromaffin system.

If the residual chromaffin system compensates for the adrenal function which has been lost by the adrenal glands, then why do the animals still die after the removal of these organs?

From the investigations of the I. P. Pavlov school it follows that the neural regulation of the activity of the various systems of organs is accomplished through the direct control of the cerebral cortex and the adjacent subcortex. The function of the adrenal or chromaffin system can hardly be an exception.

In the further course of the investigation the problem was posed to establish in what way a change of function of the cerebral cortex, produced by means of pharmacologic agents, affects the adrenalin content and the content of adrenalin-like agents in the blood of normal and experimental rabbits.

Suppression of the activity of the cerebral cortex and of the adjacent subcortex was achieved by us by means of chloral hydrate. We attempted to produce increases of the cerebral cortical activity by means of caffeine and bromides. Control experiments for the determination of the effect of chloral hydrate, bromides and caffeine were carried on with the addition of these agents to a sample of blood already taken or to Ringer's solution.

Chloral hydrate, by suppressing the activity of the cerebral cortex by the same token decreases its effect on the chromaffin system. As a result, a decrease is seen in the adrenalin content and in the content of adrenalin-like agents in the blood, which was shown by a biological test.

At the same time, caffeine and bromides, when injected in a definite dose into the body, increase the activity of the cerebral cortex, thanks to which, possibly, the stimulant effect on the chromaffin system is also increased. As a result, the content of adrenalin and of adrenalin-like agents in the blood is increased.

Any different treatment of these results would be simply incomprehensible, because the stimuli used by us in themselves exert a weak or even an opposite effect on the isolated frog's heart compared with their effects in the body.

Therefore, it is seen from the experiments that in the vast majority of cases when both adrenal glands are removed from rabbits without their transplantation, compensation for their lost function does not occur. This is confirmed by a decrease in the content of adrenalin in the blood, by an increase in the hypoglycemia, emaciation, fall in temperature, and high mortality rate of the animals.

After the removal of both adrenal glands and autotransplantations of them into the areas of the lower poles of the kidneys (along the border of the vena cava) the normal content of adrenalin and adrenalin-like agents is rapidly established in the blood of the experimental rabbits.

It may be granted that during the first one to two days after the removal of both adrenal glands and transplantation of them, the continuing excretion of the adrenalin into the blood might be explained by an excess content of it in the tissues of the adrenal glands. However, this explanation is not very probable. As a matter of fact, injections of adrenalin into animals from which the adrenal glands have been removed do not protect them from death at definite intervals. Furthermore, transplantation of the adrenals in various other sites rarely gives any success, despite the fact that the destruction and resorption of the medullary layer of the adrenal gland in both places proceeds almost equally. The idea arises involuntarily that at the basis of all this lies a specific neural excitation which is conditioned by the site of the transplantation.

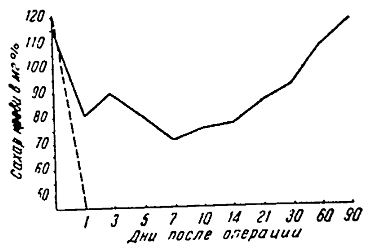
The decrease in the blood sugar content is also referable to the clinical symptoms of removal of the adrenal glands. In cats, guinea pigs and rabbits the concentration of sugar in the blood decreases by half.

We have not come across any observations dealing with the state of the blood sugar in rabbits following the simultaneous removal of both adrenal glands and autotransplantation of them.

Our experiments on the determination of blood sugar were performed on 22 rabbits; five of them were controls with the simultaneous removal of both adrenal glands, and 17 were experimental with the simultaneous removal of both adrenal glands and autotransplantation of them into the area of the lower poles of the kidneys. The examination of of the blood was made by the Hagedorn-Jensen method.

The blood sugar content in the blood of rabbits with simultaneous removal of both adrenal glands without transplantation of them falls to less than half on the day following operation. In the experimental rabbits a day after the simultaneous removal of both adrenal glands and autotransplantation of them into the area of the lower poles of the kidneys, the change of the blood sugar content was expressed to a smaller extent than in the control rabbits (Fig. 9). In the next three days, a small rise is seen in the experimental animals, and then a fall in the blood sugar content, which is continued for seven to ten days. Beginning with the 21st day the blood sugar content begins to increase appreciably and reaches the initial level three months after the operation.

Blood sugar in mg. %



Days after operation

Fig. 9. Sugar Content in Blood of Rabbits After Simultaneous Removal of both Adrenal Glands and Autotransplantation of them in the Area of the Lower Poles of the Kidneys.

Designations: __________ experiment; - - - - - control (simultaneous removal of both adrenal glands).

Thus, the adrenal transplants during the first one to two days after the operation create in the body the conditions of a smaller degree of drop in blood sugar than in the control rabbits in which the adrenal glands were removed without autotransplantation.

One cannot help but direct attention to the fact that the beginning of the significant increase in blood sugar content coincides in time (21st day after the operation) with the cessation of weight loss by the animals and the ingrowth of neural structures into the transplant capsule from the surrounding tissues.

Observations show that the restoration of the blood sugar to its initial level occurs three months after the transplantation of the adrenal glands, that is, at the time of formation of the new neural apparatus and of regeneration of the cortical layer of the transplant.

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THE OCCURRENCE AND DEVELOPMENT OF INDUCED TUMORS UNDER CONDITIONS OF BODILY REACTIVITY WHICH HAS BEEN ALTERED BY ACCESSORY STIMULI

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For the past few years the problem of our investigations has been the elucidation of the significance of nerve reflex mechanisms in the occurrence and development of malignant tumors.

The application of accessory stimulations served as the method of analysis in our investigations, which, by changing the reactivity of the body, exert influences on the formation of tumors experimentally. By this route it is possible to set about the elucidation of the rules and regulations of the occurr nce and development of malignant tumors.

The elucidation of these rules and regulations can in their turn indicate the routes of the most effective methods of influencing the tumor process.

Certain foreign scientists (Berenblum and his co-workers), who have been engaged in investigations of the action of accessory stimuli on carcinogenesis, regard the actions of these stimuli as exclusively direct ones, associating them with their permanent properties of intensification or inhibition of tumor development, and of exerting "co-carcinogenic" or "anti-carcinogenic" effects. Thus, the question of the significance of the reactivity of the body in the tumor process has been completely ignored by these authors.

Our first investigations, however, (A. A. Solov'yev, S. I. Lebedinskaya and A.A. Solov'yev), conducted in the laboratories of A. D. Speranskiy, showed that the effect of accessory stimuli on the development of tumors is not only connected with their qualities but also depends on the conditions of their use.

The experiments showed that one and the same stimulus can, depending on the site of application, either intensify or inhibit tumor development.

These data, along with others, permitted us to express a hypothesis concerning the nerve-reflex mehcanism of action of the accessory stimuli, which alter the functional state of the nervous system and which therefore affect the tumor process.

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Such a hypothesis is in accordance with data obtained by I. P. Pavlov and his students in the study of higher nervous activity on the effect of external stimuli on the conditioned-reflex activity of animals and also with the experimental material of the laboratories of A. D. Speranskiy on the effect of accessory stimuli on various pathological processes.

From the very beginning of the work on conditioned reflexes in the laboratories of I. P. Pavlov it was noted that an extraneous (accessory) stimulus can, under certain conditions, disrupt the reaction, and under other conditions, contrariwise, intensify this reaction. The observed phenomenon can be explained, based on the teaching of I. P. Pavlov, by the interrelationships between the excitatory and inhititory processes which make up the activity of the body under various concrete conditions.

A. D. Speranskiy and his co-workers showed with the use of extensive experimental material that various accessory stimulations can also influence the pathological process, intensifying it under certain conditions and inhibiting it under other conditions. Stimulations produced by means of the use of accessory stimuli received the special name of "counter-stimulations". This conception has as its purpose the emphasis of the collision of the two processes which occur simultaneously in the nervous system.

In the present report, our new material is being presented on the study of the effect of accessory stimuli, under various conditions of using them, on the occurrence of induced tumors.

It was noted in our previous experiments on rats that with repeated application of accessory stimulations to the skin, the intensifying or weakening effect of these stimulations on the tumor process is manifested maximally at a definite time after the injection of carcinogenic stimulus, namely, between the 16th and 22nd weeks after the start of the experiment.

In connection with these data, naturally, the necessity arose of performing a new series of experiments purporting to study the effects of accessory stimulations under conditions of their being applied at various times after the time of the subcutaneous injection of the substance which induces the tumor.

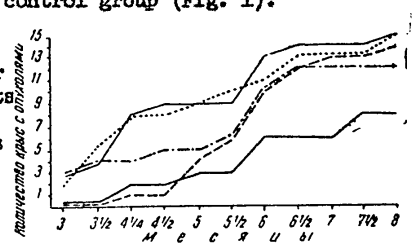
The experiments were performed on 150 white female-rats weighing 115-130 grams each. In this series of experiments as well as in the subsequent ones the tumor was produced by the subcutaneous injection of 0.5 milligrams of 9,10-dimethyl-1,2-benzanthracene in 0.2 cubic centimeters of apricot oil into one of the hind extremities. Cantharidin, which had been tested by us previously was used in the capacity of an accessory stimulus; as in preceding experiments, it was applied once a week to the skin of the opposite extremity.

The animals were divided into five equal groups. In the first and second groups the acessory stimulus was applied for eight weeks before the injection of the 9,10-dimethyl-1,2-benzanthracene, after which the smearing was stopped in the first group but continued in the second group until the end of the experiment, throughout the next eight months of the observation. In the third group, the application of the accessory stimulus was begun after eight weeks; in the fourth group, 16 weeks after the subcutaneous administration of a carcinogenic stimulus. The fifth group of animals served as controls. In each group there were 30 rats, which were kept in the experiment until the end of the observations.

The results of the experiment showed that under the conditions mentioned the application of an accessory stimulation exerts an intensifying effect on tumor growth regardless of the time of its application; this effect is dintinguihhed only by the time of its appearance.

In the first three groups of experimental animals the tumors appeared one month and seven days earlier than in the control rats. In the fourth group (where use of the accessory stimulus was begun later), its intensifying effect could be determined beginning with $5\frac{1}{2}$ months, at which time the number of tumors in this group was two times greater than in the control group (Fig. 1).

Number of rats with tumors



Months
After the injection of the carcinogen

Fig. 1. The Development of Tumors in Rats with the Repeated Application of an Accessory Stimulus (Cantharidin) at Various Periods after the Subcutaneous Injection of 9,10-dimethyl-1,2-benzanthracene.

-·-·-·-·-·-	preliminary use of cantharidin;
━━━━━	use of cantharidin before and after the injection of the carcinogenic agent;
....	use of cantharidin eight weeks after the injection of the carcinogenic agent;
-----------	use of cantharidin 16 weeks after the injection of the carcinogenic agent;
_______	control.

Results obtained in animals of the first group of this series of experiments are of special interest. They indicate that the intensifying effect of the accessory stimulation maintains its effect for a long time after it has stopped being used./

This brings up the question as to whether the prolonged effect of the accessory stimulation in this case might not be associated with the occurrence of a stable focus of excitation in the central nervous system.

The residual effect of the accessory stimulation which has been established by us has given us the idea that under conditions of abbreviated time intervals between the individual applications of the accessory stimulations the presence of residua of preceding stimulations can manifest itself in various effects on the tumor.

In connection with this, the following series of experiments was performed by us in which an accessory stimulation was used with different frequencies.

One hundred and twenty female rats weighing 115-120 grams each were used in the experiment. All the rats were injected with 9,10-dimethyl-1,2-benzanthracene subcutaneously in one of the hind extremities. The animals were divided into four equal groups, with 30 rats in each group.

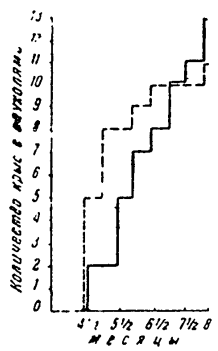
In the three experimental groups cantharidin was used in the capacity of an accessory stimulus beginning from the time of the carcinogenic agent; cantharidin was applied to the skin of the opposite extremity at unequal frequencies in the different groups of rats, namely, one, two and three times a week, respectively. The fourth group of animals served as controls.

At the end of the observation, of the experimental animals 29 rats remained in the first group, 30 rats in the second, 28, in the third; and 28 rats in the control group.

The results of this experiment showed that one and the same stimulus when applied to the skin of the opposite extremity exerts different effects on the development of the induced sarcoma, as the course of the experiment progresses, depending on the time intervals between its separate applications.

Whereas a single weekly accessory stimulation exerted an intensifying effect on the tumor development, more frequent applications of the stimulation retarded this process (Figs. 2 and 3). This may be seen particularly clearly in the experimental group of animals in which the accessory stimulus was applied three times a week. After

Number of rats with tumors

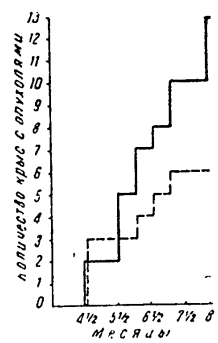


Months

Fig. 2. Development of Tumors in Rats with the Subcutaneous Injection of 9,10-Dimethyl-1,2-Benzanthracene and the Weekly Single Application of Cantharidin to the Skin of the Opposite Extremity.

Arbitrary designations:
broken line - the experiment;
solid line - the control.

Number of rats with tumors



Months

Fig. 3. The Development of Tumors in Rats with the Subcutaneous Injection of 9,10-Dimethyl-1,2-Benzanthracene and the Application of Cantharidin to the Skin of the Opposite Extremity Three Times a Week.

Arbitrary designations:
broken line - the experiment;
solid line - the control.

eight months of observation, the number of rats with tumors was, in this group, half of that in the group of control animals.

The group of experimental rats in which the accessory stimulation was given twice a week occupied a middle position between the first and third experimental groups with respect to the rate of formation of the tumors.

The series of experiments presented, therefore, in accordance with our data previously published also speak for the reflex action of accessory stimuli.

In search of more direct proof of the reflex mechanism of action of the accessory stimuli used by us, experiments were undertaken with deafferentation of the extremity, to the skin of which the accessory stimulus was applied.

The experiment was performed on 63 rats, weight 170-270 grams, males and females. In half of the animals (31 rats) deafferentation of the left hind extremity was produced by means of the transection of the corresponding six posterior roots of the spinal cord. After a month, these and the other 32 rats were injected with one and the same dose of 9,10-dimethyl-1,2-benzanthracene subcutaneously in the right posterior extremity.

All the animals were divided into four groups selected respectively according to sex and weight.

In the first group there were 16 rats, in which no procedures were performed aside from the subcutaneous injection of 9,10-dimethyl-1,2-benzanthracene.

Beginning with the next day after the injection of the carcinogenic stimulus, rats of the second group (16 animals) received one drop of cantharidin weekly throughout the entire subsequent observation on the skin of the opposite normal extremity.

The third group consisted of 16 rats in which deafferentation had first been performed. One drop of cantharidin was applied to the skin of the opposite, deafferented extremity weekly throughout the entire period of observation of these rats beginning with the day after the subcutaneous injection of the carcinogenic stimulus.

The remaining 15 rats with deafferented extremities constituted the fourth group. No procedures were performed in these rats after the subcutaneous injection of 9,10-dimethyl-1,2-benzanthracene.

The observation lasted six months after the administration of the carcinogenic stimulus. At the end of the experiment, 16 rats remained alive in the first group; 14, in the second, 14 in the third, and nine rats in the fourth.

At the end of the experiment, in the first group, in which no additional procedures had been performed, there were four rats with tumors; in the second group, in which the accessory stimulus had been applied to the skin of the normal extremity, there were seven rats with tumors; in the third group, where accessory stimulation had been applied to the skin of the deaferented extremity, there were a total of two rats; and, finally, in the fourth group, with deafferentation of the extremity without the application of the accessory stimulation, there were also two rats with tumors (Table 1).

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Table 1

DEVELOPMENT OF TUMORS IN RATS AFTER THE SUBCUTANEOUS INJECTION OF 9, 10-DIMETHYL-1,2-BENZANTHRACENE UNDER THE CONDITIONS OF THE APPLICATION OF CANTHARIDIN TO THE SKIN OF THE NORMAL AND THE DEAFFERENTED EXTREMITY

<table>
<tr><th rowspan="3">Conditions of injection of carcinogenic agent and of application of the accessory stimulus</th><th rowspan="3">9,10-dimethyl-1,2-benzanthracene injected under the skin of the right extremity</th><th colspan="3">9,10-dimethyl-1,2-benzanthracene injected under the skin of the right extremity</th></tr>
<tr><th colspan="2">application of cantharidin to the skin</th><th rowspan="2">deafferentation of left extremity</th></tr>
<tr><th>of normal left extremity</th><th>of deafferented left extremity</th></tr>
<tr><td>Number of rats with tumors after six months. . . . . . .</td><td>4</td><td>7</td><td>2</td><td>2</td></tr>
</table>

Thus, when an accessory stimulus is applied to the skin of an extremity whichhas been deprived of sensitivity, the intensifying effect of the simulus, which is manifested under the conditions of normal afferentation, is removed.

The results of this experiment may serve as direct proofs of the reflex mechanism of action of the accessory stimulus on tumor development.

In these experiments, the fact draws attention that deafferentation of the extremity not only removes the intensifying effect of the accessory stimulus but also diminishes the "yield" of tumors compared with the control.

Lack of the necessary data permit us to give only a suppositional explanation of thsi phenomenon. Pertinent to this, the widely known factsmay be adduced which show that after trauma to neural apparatus in one extremity reflected changes of functional and anatomical characters occur also in the opposite extremity (A. S. Vishnevskiy, M. L. Borovskiy and others).

It may be supposed that these changes exert definite effects on the development of the induced tumor.

We have performed experiments, the purpose of which was to clarify

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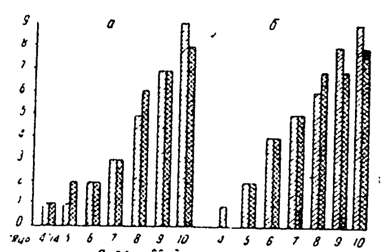
the possibility of a reflex action of 9,10-dimethyl-1,2-benzanthracene, when it is used in the capacity of an accessory stimulus.

Interest in the use of tumor-inducing agents in the capacity of accessory stimuli is intensified by the fact that according to data in the literature such agents can exert therapeutic effects on human malignant skin tumors.

Experiments were performed in the following way: 100 female rats, weighing 135-180 grams, were injected under the skin of the left posterior extremity with the dose of 9,10-dimethyl-1,2-benzanthracene adopted by us (0.5 milligrams in 0.2 cubic centimeter of apricot oil). The animals were divided into four equal groups with 25 rats in each. Beginning with the first day of the experiment and for the eight months of the subsequent observation 0.1 percent solution of 9,10-dimethyl-1,2-benzanthracene in benzol was applied dropwise to the experimental rats twice a week; in one group, on the skin of the opposite extremity (from the site of the subcutaneous injection of the carcinogen); in the other group, on the skin of the same extremity. Benzol was applied, correspondingly, to the skin of the same or opposite extremity in the two control groups.

The results of the experiment showed that the application of 9,10-dimethyl-1,2-benzanthracene to the skin of rats over the course of a long period of time, regardless of whether the skin of the same extremity was smeared as was injected with this substance or the skin of the opposite extremity, did not exert any essential effect on the development of the induced sarcomas. The development of tumors in the experimental groups of rats and the corresponding control groups began approximately at the same times in the course of the observation (Fig. 4). In connection with the results of these experi-

Number of rats with tumors



Months

After injection of carcinogen

Fig. 4. Development of Tumors in Rats with the Injection of 9,10-Dimethyl-1,2-Benzanthracene under the Skin of an Extremity and the Repeated Application of 9,10-Dimethyl-1,2-Benzanthracene to the Skin of the Opposite (Diagram on Left) or of the Same (Diagram on the Right) Extremity.

Key: light columns - experiment; dark columns - control.

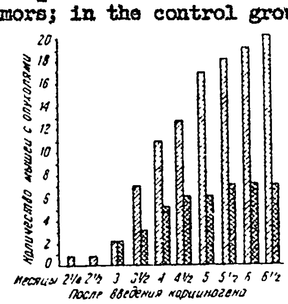
ments, the question arose: are not the negative results of these experiments the consequences of poor sensitivity of the skin of the rats to the given carcinogenic stimulus? Experiments which have been performed under the same conditions on mice might aid in the resolution of this problem, on the one hand; on the other, they might possibly explain the differences in skin reactions of mice and rats to the carcinogenic stimulus when applied to the skin; this difference has, up to now, not been clear. It is known that the occurrence of skin cancer in rats under the influence of a carcinogenic stimulus is of exceptional rarity, whereas in mice under the same conditions the cancer occurs readily.

These considerations led us to the performance of appropriate experiments on mice.

The experiment was performed on 45 mice of the CC57 line of brown females and males weighing 20-22 grams. All the mice were injected simultaneously with 0.1 milligrams of 9,10-dimethyl-1,2-benzanthracene in 0.2 cubic centimeter of apricot oil under the skin of the left hind extremities. The animals were divided into two groups, selected respectively according to sex and weight. Twenty three mice of the experimental group (six females and 17 males) received, dropwise, 0.05 percent solution of 9,10-dimethyl-1,2-benzanthracene in benzol twice a week, which was applied to the skin of the opposite extremities. Twenty two mice of the control group (six females and 16 males) were given applications of benzol twice a week.

The tumors in the mice of the experimental group appeared three weeks earlier that in the mice of the control group. For the duration of the entire observation the number of tumors in the group of experimental mice considerably exceeded the number of tumors in the control group. After 6½ months of observation, 20 mice in the experimental group had tumors; in the control group, seven mice (Fig. 5).

Number of mice with tumors



months

After injection of carcinogen

Fig. 5. Development of Tumors in Mice with Subcutaneous Injection of Extremity with 9,10-dimethyl-1,2-benzanthracene and Repeated Application of It to skin of Opposite Extremity. Key: Same as for Fig. 4.

The data presented illustrate the definite difference in the results of the experiments on mice and on rats.

In experiments on mice, the use of 9,10-dimethyl-1,2-benzanthracene in the capacity of an accessory stimulus exerted an intensifying effect on the formation of sarcomas induced by the same agent.

These experimental results, without eliminating the necessity for further study of this question, reinforce the hypothesis expressed by us to the effect that the carcinogenic effect obtained from smearing the skin with this stimulus may be determined by the varying degrees of sensitivity of the skin receptors to it.

Therefore, the results of our investigations obtained previously as well as those presented in the present report show that the process of tumor-formation, like other pathological processes, depends on the state of reactivity of the body, depending on which the process of tumor-formation can be intensified or inhibited.

In our investigations the change of reactivity was achieved reflexly, by means of the application of an accessory stimulation to various skin areas. Here, the various effects of stimulation were shown to be dependent not only on the quality of the stimulus and the site of its application but also on the varying frequencies of its application (the intervals between separate applications).

The significance of the after-effect of an accessory stimulus was also shown; it influences the process of tumor-formation for a long time.

The reflex mechanism of action of accessory stimulation has obtained direct proof in experiments with transection of sensory roots, which eliminated the effect of the accessory stimulation.

The fact appears promising that an influence on the reactivity of the body, and, by the same token, on tumor formation, can be shown by the skin, which is an exceptionally extensive and heterogeneous receptor field in the qualitative sense.

As is well known, I. P. Pavlov has made direct references to the significance of the reflex actions of the skin on disease processes.

In the study of the reflex nature of the effect of accessory stimuli we obtained facts which can show the significance of the sensitivity of the cutaneous receptors to carcinogenic stimuli in the pathogenesis of experimental skin cancer.

In conclusion, we should like to note the importance of the study of the tumor process and its dependence on the reactivity of the body in the forms of experiments in which the stimulus produces the appearance of tumors in only a certain percentage of animals, that is, with small doses of carcinogenic stimuli. Only such an experimental form can bring us closer, in some degree, to the natural conditions of tumor formation and facilitate the search for methods of influencing it.

Methods which alter the appropriate form of body reactivity should occupy an important place in this research.

ON THE PROBLEM OF THE SIGNIFICANCE OF FUNCTIONAL CHANGES OF THE HIGHER CENTERS OF THE CENTRAL NERVOUS SYSTEM IN THE PROCESS OF METASTASIS OF THE TRANSPLANTED TUMOR IN THE RABBIT

I. P. Tereshchenko

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In the laboratories directed by A. D. Speranskiy, investigations have been conducted for a number of years devoted to the elucidation of the significance of neurotrophic disturbances in the process of metastasis (S. I. Lebedinskaya, A. S. Solov'yev, O. Ya. Ostryy, V. N. Popov, I. P. Tereshchenko).

This investigational trend is in accord with the view of I. P. Pavlov, who placed tumor growth in relationship with the trophic disturbances produced by the tumor in the tissues around it. It is entirely possible that reflex stimulations arising in connection with the development of the primary tumor can, under conditions where the compensatory function of the nervous system is disturbed, produce trophic changes in the organs and tissues at a distance from the tumor and in this way exert an effect on the development of metastases from the tumor cells transported by the lymph and blood stream currents.

In previous investigations of the A. D. Speranskiy laboratories, special attention was given to the study of the metastatic process under conditions of different kinds of influences on the peripheral segments of the nervous system. In this work, systematic investigations were first carried out on the study of the rules and regulations of metastasis under various experimental conditions, and a number of important facts were obtained which established the possibility of affecting the metastatic process by means of certain procedures performed on the nervous system.

It is quite evident, however, that the decipherment of the mechanisms underlying metastasis cannot be carried out fully without studying the role of the higher nervous system centers in this process.

Recently, a number of investigations have been made which attest to the importance of the state of the higher centers of the nervous system in the process of metastasis. Thus, experiments have been carried out by us (I. P. Tereshchenko, 1951) on the study of the process of metastasis of a transplanted tumor, where the grafting of the tumor onto the testicle of a rabbit was carried out while the rabbit was in a state of deep drug-induced sleep. It was found thereby that inhibition of the higher centers of the central nervous system leads to an intensification of the metastatic process.

N. M. Turkevich and K. P. Balitskiy described the increase in the rate of metastasis under the conditions of intensification of the inhibitory processes in the cerebral cortex, and conversely, a slowing of the rate of metastasis with intensification of the processes of excitation in the cerebral cortex (pharmacologic agents were used for changing the functional state of the cortex).

In the present investigation a study has also been made of the significance of the functional state of the higher centers of the nervous system in the process of metastasis.

The experiments were performed with a transplanted rabbit tumor. The change of the functional state of the higher centers of the nervous system was produced by means of the exclusion of the three distance receptors -- auditory, visual and olfactory.

This method of investigation was first proposed by A. D. Speranskiy, (work of V. S. Balkin) in the study of pathological processes. As is well known, I. P. Pavlov had a high opinion of this form of experiment and considered the observations made by the use of it very important.

Later, special investigations of higher nervous activity using the exclusion of distance receptors (K. S. Abuladze) were undertaken in I. P. Pavlov's laboratory.

In our experiments the exclusion of the receptors was carried out simultaneously at various time intervals before the grafting of the tumor. The investigation was carried out on 27 rabbits.

The exclusion of the three distance receptors was accomplished in one group of animals (14 rabbits) two to 32 days before the grafting of the tumor. The remaining 13 rabbits served as controls. A suspension of tumor cells (25 percent) was injected into the left testicles of experimental and control rabbits in 0.6 cubic centimeter quantities. All the animals were killed 21 days after the grafting of the tumor.

The degree of metastasis was judged according to three indices: the number of metastases in the involved organs, the relative sizes of the metastases and the total number of organs involved. (For more details on the determination of the degree of metastasis see the article of I. P. Tereshchenko published in the collection Disease, Recovery and Therapy, Works of the Academy of Medical Sciences USSR, Moscow, 1952).

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As a result of the operation, a greater degree of involvement by metastases was seen in the group of experimental rabbits in comparison with the control group (Fig. 1). It was established by this that the intensification of the metastasis occurred chiefly on account of an increase in the number and sizes of metastases in the organs involved. Thus, while in the control group of animals a considerable number of metastases was seen in one rabbit and only in the lungs in

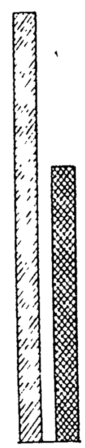

Fig. 1. Correlation of the Degree of Total Involvement of Internal Organs by Metastases in Experimental and Control Groups.

Light column - experimental group;
shaded column - control group.

this one, in four animals of the experimental group there were a considerable number of metastases. In these cases there were metastases in several organs, namely: lungs, liver and kidneys.

No less distinct results were obtained in comparing the sizes of the metastases. Whereas in the control group, the larger metastases, reaching the size of peas, existed in only one rabbit, and thereby only in the liver, appreciable sizes of the metastases were noted in five rabbits of the experimental group, and they existed in the lungs, liver and kidneys.

In Figure 2 is presented the comparison of metastasis by individual organs in the experimental and control groups.

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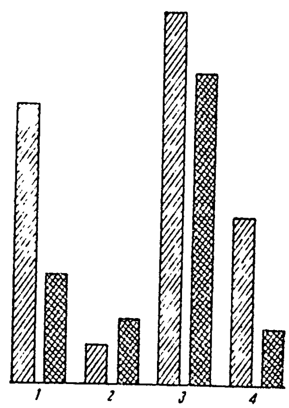

Fig. 2. Correlation of the Degree of Involvement of Individual Organs by Metastases in the Experimental and Control Groups.

1 - kidneys; 2 - adrenal gland; 3 - lungs; 4 - liver.
Light columns -- experimental group;
shaded columns -- control group.

Therefore, the data presented illustrate with adequate clarity the intensification of metastasis in the experimental group on account of the increase in the number of metastases and the sizes of the metastatic nodes. The increase in the total number of organs involved by metastases in the experimental group was insignificant.

The data obtained indicate that the intensification of metastasis which was observed in the experimental group of animals was the result of the acceleration of the development of metastases. This may be associated, on the one hand, with an earlier onset of occurrence of the process of metastasis, and, on the other hand, with an increased rate of growth of the individual metastases.

Proceeding with the explanation of the mechanism of intensification of metastasis it whould be noted that, according to the observations of I. P. Pavlov (experiments of K. S. Abuladze), in the animals, after exclusion of the three distance receptors, a diminution occurs in the tonus of the cerebral cortex which is associated with the inhibition which is developed under these conditions.

In accordance with these observations the intensification of metastasis in animals in which the distance receptors have been excluded can be looked upon as a consequence of the fact that under the conditions of decreased cortical activity the cortical compensation mechanisms are disturbed more quickly than usual. This also finds its expression in our experiments in the shortening of the pre-metastatic period and the more intense growth of metastases.