LOSS AND RESTORATION OF REGENERATIVE CAPACITY IN TISSUES AND ORGANS OF ANIMALS

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L. V. Polezhaev

With a Preface by Bruce M. Carlson

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PREFACE

Throughout most of the postwar years Soviet research in the field of regeneration has undergone a pronounced shift in emphasis from basic biological studies conducted primarily upon amphibians to more practically oriented problems in mammalian systems. The research of Professor Polezhaev underwent a corresponding shift with respect to experimental systems, but the overall emphasis on restoration of lost regenerative capacity has been retained since his work on limb regeneration in frogs in the 1930s. Although much of the author's early work on regeneration in amphibian systems is well known, most of the more recent research from his laboratory is virtually unknown to many Western biologists. This is due in large part to the factor of language as well as to the limited circulation of many of the reports from his laboratory.

This monograph summarizes the bulk of the author's research during the postwar years as well as some of his earlier work on limb regeneration in frogs. A good summary of his earlier work on experimental morphology and limb regeneration in urodeles can be found in an earlier monograph (Polezhaev, L.V. Fundamentals of the Mechanics of Development of Vertebrates [in Russian]. Moskva, Izdatel'stvo Akademii Nauk SSSR. 1945. 290 pp.).

There are three sections in the present monograph. The first is primarily a theoretical discussion. The bulk of the monograph is taken up by the second section — a summary of the author's own research. The final section is a general conclusion. Many of the implications of the introductory theoretical section are likely to escape the reader who is not familiar with the postwar development of Soviet biological thought or to the remarkably abrupt change in the direction of research in regeneration in the late 1940s. The historical development and the main theoretical positions of present-day Soviet investigators in regeneration are briefly reviewed in a recent article of mine (Carlson, B.M. Regeneration Research in the Soviet Union. — Anat. Rec., 160:665-674. 1968). This article gives a number of references to the literature which forms the basis for the discussions in the introductory section.

As translation editor, I have found it necessary to make relatively few changes in the original translation. The style of the English translation follows quite closely that of the Russian text. I have not seen translations of the legends to the illustrations and the list of references, but the material in these is not likely to present much difficulty to the reader.

Bruce M. Carlson,
Translation Editor

FOREWORD

The present monograph deals with an analysis of the problem of loss and restoration of the capacity for reparative regeneration of organs and tissues in animals. This monograph summarizes the studies of the author and his co-workers in the Laboratory of Experimental Morphology of Animals at the Institute of Animal Morphology imeni A. M. Severtsov of the Academy of Sciences of the USSR over the last 35 years (1932—1967). When work in this direction was begun, the problem had just been defined, while at present, after a sufficiently long and intensive study, the problem has not only been fully defined but has also resulted in the formation of a special new direction of studies on regeneration, with its own subject, purposes, tasks, and methodology. The emergence and formulation of this problem, and later of this particular trend of studies, of necessity led to a revision of all the basic notions and principles of the study of regeneration, resulting in a new solution of a number of principal problems (e. g., the problem of sources of regenerative material, means of regeneration, and many others) and finally brought about a new formulation of the main laws of regeneration, the basis of which, although previously unknown, was established by the author, namely that the cause of regeneration of organs depends on the condition of destruction and dedifferentiation of the tissues composing the organ.

The study of regeneration is one of the chapters of experimental morphology and develops in close relationship with the state and general level of development of the latter, while, on the other hand, by its development it changes this state. These facts are sufficiently clearly evident in the given case. For several decades the general problems of experimental morphology, e. g., the problem of determination of the processes of morphogenesis directly affected the problems and state of knowledge of regeneration. However, the special area of problems elaborated by the latter study, in particular in connection with the elaboration of the problem of loss and restoration of the regenerative capacity of animal organs and the results obtained, in their turn greatly influenced experimental morphology, its problems, and its methodology. Moreover, these factors also influenced adjacent chapters of biology and medicine which are closely related to the study of regeneration: embryology, histology, cytology, histochemistry, biochemistry, physiology, data on heredity, theory of evolution, pathological anatomy, surgery, therapy, and even stomatology, neuropathology, and psychiatry. Touching upon and changing our ideas of some important general biological problems, such as the problems of development and growth of organs and their directed changes in animals, the theory of regeneration made possible the elaboration of certain problems from the domain of agriculture, e. g., problems of the development and growth of organs and tissues.

Earlier the theory of regeneration studied in the realm of experimental morphology was concerned mainly with purely theoretical problems, while at present, thanks to the new direction which concerns itself with problems of directed changes in processes of regeneration, loss, and restoration of the regenerative capacity of organs and tissues, it became directed toward problems which are of great importance for medical practice and for agriculture.

The present monograph illuminates the problems and course of studies only of the aforementioned single direction in the study of regeneration. Another direction of these studies defending the assumption that all animals, and in particular mammals, are capable of regenerating all the organs in their body, does not enter into the scope of this book, and is touched upon only to the extent that it is necessary for the elucidation and solution of the task posed here. The basis of this second direction is given in great detail in a number of papers by its representatives (Vorontsova, 1949, 1953; Vorontsova and Liozner, 1955, 1957; Liozner, 1959, 1960, 1962; Studitskii, 1948, 1954, 1959, and others). Problems concerning the theory of regeneration as a whole, and illuminating mainly its previous state and old ideas, are discussed in detail in monographs and review articles by foreign scientists (Morgan, 1907; Przibram, 1909; Korschelt, 1927; Weiss, 1930, 1939; Abeloos, 1932; J. Needham, 1936, 1942; A. Needham, 1952, 1960; Hay, 1966, and others).

The present study was carried out with the direct participation in the experimental work of a number of permanent and temporary co-workers of the author: I. I. Morozov, V. N. Favorina, G. I. Gintsburg, L. I. Skorobogataya, I. G. Rogal', N. F. Barakina, A. I. Matveeva, N. A. Zakharova-Muzlaeva, L. V. Akhabadze, V. L. Mant'eva, M. P. Yavich, N. I. Ermakova, N. A. Teplits, S. Ya. Tuchkovaya, E. N. Karnaukhova, V. A. Mitrofanova, K. Yu. Reznikov, S. P. Kolchin, L. V. Cherkasova, I. E. Sadokova, S. P. Kilina, and others. Some important data were obtained in studies in which the author cooperated with G. P. Ramenskaya and A. E. Gurvich. The author expresses his sincere gratitude to all the above comrades for the effort invested by them in the study of this problem.

The author will be very grateful to all persons who will find it possible to make any critical comments on this monograph in order to improve and develop its main ideas.

The author will be grateful if this book will awaken the interest of young scientists in this new direction, and will induce them to propose and develop new experimental problems.

The author considers it his duty to mention a number of outstanding Soviet biologists whose ideas forced him to ponder deeply and who, in one way or another, helped in the development of this study: Prof. D. P. Filatov, Prof. M. M. Zavadovskii, Academician M. V. Nasonov. The author would also like to point out the significance of the work of those Soviet scientists who did much to elaborate a number of problems in the theory of regeneration and experimental morphology, although he does not agree with some of their ideas: Professors G. E. Koritskii, L. Ya. Blyakher, M. I. Efimov, E. E. Umanskii, V. I. Astrakhan, M. A. Vorontsova, L. D. Liozner, A. A. Voitkevich, B. P. Tokin, A. N. Studitskii, L. N. Zhinkin, V. P. Mikhailov, G. D. Tumanishvili, and others. Finally, the author cannot avoid mentioning the names of prominent contemporary foreign scientists who greatly contributed

to the study of regeneration and experimental morphology: Professors P. Weiss, E. Guyénot, J. Needham, S. M. Rose, M. Singer, J. Ebert, A. Needham, Dr. A. Scharf, and others.

Introduction and Presentation of Problem

The fact that in some animals organs do regenerate after amputation, while in others they do not, has been known for a long time. For example, in man, dogs, rabbits, rats, and mice, after amputation of the extremities or toes, regeneration never occurs and the amputated wound surfaces of the stumps heal smoothly; in axolotls, tritons, and salamanders complete regeneration of these organs takes place. Extremities, tails, ears, parts of the snout, teeth, eyes, and many other organs of mammals do not regenerate. Extremities, tails, fins, gills, parts of the eye, the mandibles, and many other organs of caudate amphibians regenerate very well. Analysis of data from published materials shows that one could divide the animals into two groups: those which are capable, and those which are not capable of regeneration of certain organs.

The question necessarily arises as to why some animals are capable of organ regeneration while others are not. How could such phenomena develop in the history of development of animals? Is it not possible to cause experimentally the regeneration of organs which, under normal conditions of amputation, are not capable of regeneration? Is it not possible to cause regeneration of an extremity, or even of a toe, in man or rat? A positive solution of these questions would be theoretically very interesting and important, but would also be of great practical value primarily for man, since one could restore various lost, damaged or diseased, vitally important organs, e. g., the heart, brain, etc.

For a long time this problem seemed to be one which was practically insoluble, or soluble in the very distant future, perhaps after many decades, not only in the opinion of physicians and biologists, but also in the mind of any thinking human being. In any case, physicians in their modern practice start from the assumption that to cause regeneration of organs which ordinarily do not regenerate (arms, legs, heart, brain, etc.) in man is an unrealizable task. However, as the experience of the last 35 years shows, this problem can be scientifically posed, and one can suggest concrete ways to solve it. Already some very important positive data have been obtained and accumulated from experiments which show that the problem is a real one, and already has been partially solved and is, thus, a soluble one in the near future and not in some far-removed time.

In order to solve this problem a number of questions must be answered. First of all, what is regeneration? How does it come about? What are its causes and basis? What is regenerative capacity? How did it appear and develop? What are the fundamental laws of regeneration? How can regenerative capacity be changed? What is the best way to be chosen for successful elaboration of the problem? What will be gained from the solution of the problem? And finally, what are the tasks and prospects following the solution of this problem, or even of some aspects of its solution?

Part One

THEORETICAL BASIS

Chapter I

THEORY OF REGENERATION IN BIOLOGY AND MEDICINE

Until recently the theory of regeneration developed along two different and separate directions in biology and medicine, so different that frequently biologists were not aware of studies performed by physicians, while physicians were unfamiliar with investigations carried out by biologists. The study of regeneration has been marked by a distinct dualism, which even now prevails in the studies of Western European and American scientists.

The biological studies of problems of regeneration carried out mainly on such animals capable of repair regeneration of organs as axolotls, tritons, Hydra, Planaria, hydroid polyps, Infusoria, Amoeba, Arthropoda, and others, were of a theoretical nature. They were essentially detached from the requirements of practice and were devoted to such problems as the rules of regeneration during onto- and phylogenesis of animals; the distribution of regenerative capacity in the animal kingdom; the importance of external and internal conditions in the course of the regenerative process; the role of endocrine factors in regeneration; the influence of the nervous system on phenomena of regeneration; problems of histogenesis of the regenerated material; the biochemical changes taking place in the course of regeneration; the means and mechanics of the regeneration process. Data on all these problems have been published in many hundreds and thousands of different journal articles and in appropriate biological reviews and monographs (Morgan, 1901; Weissman, 1902; Przibram, 1909; Korschelt, 1927; Weiss, 1930, 1939; Abeloos, 1932, and others; Shimkevich, 1923; Koritskii, 1926; Astrakhan, 1929; Zavadovskii, 1931; Filipchenko, 1932; Nasonov, 1941; Polezhaev, 1945, 1947, 1948, 1950, 1958, 1959; Vorontsova, 1949, 1953; Vorontsova and Liozner, 1955, 1957; Liozner, 1959, 1960, 1962; Studitskii, 1948, 1959; Studitskii and Striganova, 1951, and others).

The medical studies on problems of regeneration were mainly carried out on mammals (rabbits, dogs, rats, mice, cats, guinea pigs, and others) which are characterized by a low capacity for reparative regeneration of organs, and the studies were mainly connected with problems of therapeutic practice of wounds of various organs and tissues. Problems of healing of bone fractures and of stimulating regeneration of damaged tissues were studied; the course and peculiarities of the regenerative process, the role of inflammatory processes in regeneration, the pathological-anatomical peculiarities of the processes of scar-tissue formation, and the healing of wounds in diseases of various organs and tissues of man and mammals, etc., were also studied. Such problems as, for example, the role of the

nervous and endocrine systems in processes of tissue regeneration, the stimulation of regenerative processes, histogenesis in processes of regeneration, the role of the age factor in regeneration, etc., were common to biological and medical studies; nevertheless, they differed from each other due to the great difference in the definition of the problems, tasks, methods, and objects of study (axolotl — dog; planaria — rats, etc.). For this very reason it was not always possible to compare the results of these studies and to make generalizations in order to apply them to phenomena of regeneration observed in man in clinical practice or dissection. The results of studies by physicians and pathologist-anatomists were published in the form of many hundreds and thousands of articles in medical, but not in biological, journals and were generalized in appropriate chapters on regeneration in textbooks of pathological anatomy (Abrikosov, 1947, 1949; Abrikosov and Strukov, 1954), or in the form of reviews and monographs (Marchand, 1901; Goldzieher and Makai, 1913; Needham, 1952; Peer, 1955; Nemilov, 1940; Liozner and co-workers, 1960; Studitskii, 1959; Matveeva, 1962, and others).

One must point out that this dualism in the study of regeneration was also reflected in the Bol'shaya meditsinskaya entsiklopediya (Great Medical Encyclopedia) (1932), in which the data on the biological study of restoration of organs were given in one article, while data on the regeneration of tissues of man and mammals were given in another separate article. This dualism was particularly pointed out in published materials (Polezhaev, 1956, 1958; Liozner, 1959, 1962; Voitkevich, 1960).

In current studies on regeneration carried out in the USSR, attempts are being made to overcome this dualism. In the study of regeneration two new directions appeared, both opposing the old one and each approaching the problem in a different way. The common denominator for the two directions is the study of processes of regeneration in mammals and an attempt to bring the subject closer to the tasks of medical practice. At the same time, the two directions differ considerably in their approach both to the subject of study and in a number of leading assumptions. In order to understand all this we must briefly discuss the main stages of development of the theory of regeneration.

The oldest was the medical direction in the study of regeneration. Problems of regeneration arose at the very beginning of medical practice in connection with the requirements of the healing practice, the necessity of healing wounds in various, primarily external, organs; bone fractures; etc., inasmuch as healing of damaged tissues is a phenomenon of regeneration. Thus, problems of regeneration arose on an empirical basis before medicine had been formulated on a scientific basis. With the development of scientific medicine, these problems gradually acquired a scientific basis. For example, in connection with the development of endocrinology, the problem has arisen concerning the effect of the functions of various endocrine glands on processes of regeneration; in connection with the development of the idea of nerve theory, the problem arose of the control of the nervous system over processes of regeneration of tissues in various damaged organs, etc. However, it must be admitted that problems of regeneration have so far been treated empirically and often randomly, and attempts were made to elucidate whether one or another substance would affect the process of regeneration. With the development of medical experimentation, and with the design of model experiments which reproduced, to some extent, some phenomena of human pathology, the task of studying

the processes of regeneration under experimental conditions on organisms very close to man (i. e., mainly on such mammals as dogs, rabbits, apes, rats, cats, and other species) has naturally arisen. The empirical and utilitarian approach greatly limited the possibilities and range of studies on processes of regeneration in medicine. Medicine is based on biology and made advances mainly due to the development of its biological foundations. It suffices to point out the rapid and vigorous development of the study of antibiotics which is based on the biological principle of the inter-species struggle for existence among the simplest organisms or on the discovery of microorganisms which are the causative agents of a number of infectious diseases, etc. At the same time physicians interested in problems of regeneration were, to a great extent, removed from the theoretical problems of biology. As a rule, they did not carry out comparative studies and had to turn to biologists on questions of biological theory.

In biology the study of regeneration is of relatively recent origin. Two hundred and fifty years have passed since the famous experiments of Réaumur (1712) on regeneration in starfish and crayfish, and of Trembley (1740) on the regeneration of hydra. In the first 150 years, the biologists studied phenomena of regeneration mainly by descriptive and comparative methods and in the last 80—100 years by experimental methods as well. At first they investigated the problem of whether parts of a body, an organ, or parts of organs of various species of animals regenerated; the occurrence of phenomena of regeneration in the animal kingdom; the dependence of regeneration on age. Subsequently, they began to study problems of histogenesis during regeneration, its dependence on external and internal conditions, the means of regeneration, the importance of different components and systems of organs in regeneration of parts of the body and organs, the determination of regeneration, etc. Phenomena of regeneration were studied in general zoology, and the capacity for regeneration was compared to the means of multiplication of animals and particularly to asexual multiplication (Kholodkovskii, 1933). Zoologists began to look on regeneration also as one of the attributes characterizing animals as belonging to a given taxonomic group. Later, regeneration was studied intensively by histologists, and textbooks on histology added to the structural characteristics of tissues and the capacity of these tissues for regeneration. Thus, e. g., nervous tissue of the brain and spinal cord in mammals was characterized as incapable of repair and even physiological regeneration. Later, in connection with the appearance and development of experimental morphology, regeneration was studied from the point of view of the principles of this discipline in order to establish the immediate causes determining the appearance of certain phenomena in the process of regeneration. For example, the role of the decomposition products of tissues, the so-called wound hormones, in the processes of cell division in damaged and regenerating organs was studied; the importance of different components (the skeleton, muscles, skin, nervous system, etc.) in the regeneration of organs. In connection with the emergence in experimental morphology of the problem of determination, which served for many decades as the basis for this discipline, similar studies were also conducted on regeneration: the question of interest was whether the regeneration anlage was determined at an early stage of ontogenesis and what factors determine its quality, axes, etc.

A characteristic feature of the biological investigations was the fact that, having elucidated the degree of the regenerating capacity of various species of animals, the investigators concentrated their attention on objects with a high capacity for regeneration of organs and disregarded processes of restoration in animals with a low capacity for regeneration. For example, dozens, and even hundreds, of works devoted to the study of regeneration of extremities in axolotls, tritons and salamanders have been performed, but, on the other hand, there are only a few studies on the processes of healing of stumps after amputation of extremities in mammals. The study of processes which are characterized by some restorative phenomena is also a characteristic feature of medical investigations. Physicians and pathologist-anatomists did not study phenomena observed after amputation of mammalian organs not capable of regeneration: extremities, ears, eyes, etc. They were much more interested in the course of healing of skin wounds and bone fractures, how to restore damaged peripheral nerves or large blood vessels, and how to replace extensive defects after burns, trauma, and processes of atrophy and hypertrophy of organs after removal of their parts, etc. This situation in the study of regeneration was due to several factors, primarily to the general level of development of biology and of morphology in particular. The study of life by descriptive, comparative, and experimental methods and not by an active directed change, by transformation of the nature of the animal organisms, their hereditary properties, and, in particular, the capacity for repair and regeneration of organs was characteristic for the biology, and especially for the morphology of animals. Secondly, it is always easier to study spontaneous processes of regeneration of organs or tissues than to try to force regeneration of organs which are not capable of regeneration. Thirdly, the rules of change and of loss and restoration of the capacity for reparative regeneration of organs in phylogenesis and ontogenesis of animals (and also plants) have not been established. All this explains why biologists and physicians did not make serious attempts to deal with the problem of restoration of lost organs which are not capable of repair regeneration under ordinary conditions of amputation.

Problems of ontogenesis, heredity, and evolution are of basic interest in all the chapters of biology. The correct solution of these problems, separately and in their interrelationships, determines the possibility of elaboration of a correct approach to the formulation of a unitary theory of development of the organic world, and provides a key for the solution of a number of main problems of biology, in particular the problem of directed changes in the regenerating capacity of organs and animals.

In the post-Darwinian period for many decades the main topics of biology were the problem of heredity and the problem of whether directed changes of heredity were possible. According to the laws of Mendel and Morgan, hereditary phenomena are determined by genes, by the chromosomal apparatus of the nucleus of the sexual cells, which develop according to special laws independent of changes in the external environment and in the soma, i. e., independent of all the other organs and tissues of the organism. Changes in the external environment may cause changes in the soma, but the latter do not cause adequate changes in the germinal layer in the chromosomes or genes. Hereditary changes in the nuclear apparatus of the sexual cells occur at random in different directions and, as a rule,

they turn out not to be useful, but are subject to natural selection which acts as a sieve. Genes determine the development of the different characteristics of the organism, e. g., the color of the eyes, the shape of the wings in Drosophila, pigmentation in rats or rabbits, etc. Such attributes are subject to the laws of Mendel, undergoing Mendelian segregation. However, such other basic hereditarily determined phenomena of ontogenetic morphogenesis of animals as gastrulation, neurulation, organogenesis, and regeneration do not behave according to the laws of Mendel and are not governed by the action of individual genes. It is not clear, therefore, how they can be altered in a directed manner. On the other hand, the connection between phylogenesis and ontogenesis, or between phylogenesis and heredity, is not clear. The biogenetic law postulates a relationship between phylogenesis and ontogenesis inasmuch as in ontogenesis some essential phenomena, or stages of phylogenesis, are repeated in an abbreviated form. However, how and under the influence of what factors does ontogenesis change hereditarily, resulting in phylogenesis? Changes in phylogenesis are subject to laws, but at the same time they must be based on merely accidental changes in the genome and not on adequate changes in the external environment. The role of the latter in the biology of development of the organism is not clear. The external environment is considered to play the role of a background, an essential one, but at the same time serving as a nonspecific factor in the development of organisms. The development of the organism depends on the external environment but heredity does not depend on it. This approach cannot satisfactorily explain the basic biological phenomena such as heredity, ontogenesis, and evolution and their interrelationships. It does not allow one to determine how one can change hereditarily determined properties of organisms, e.g., their capacity for reparative regeneration of organs. It did not enable biologists who studied problems of regeneration to approach practical problems and, thus, caused a dissociation between theory and practice. Starting from this theory, it was practically impossible to elaborate theoretically well-founded methods for directed changes of hereditarily determined properties of organisms. In order to elaborate a desired hereditarily determined property, according to this theory, one had to find a change that appeared in nature at random and develop it in subsequent generations by crossing and selection of animals. For example, in order to elaborate methods for the restoration of the lost regenerating capacity of extremities in frogs, it would be necessary to do the following: catch a great number (for instance, 1,000) of adult frogs; amputate the extremities in all of them, e. g., the hind ones, and establish whether formation of atypical regenerating appendages could be observed in one, two, or three frogs instead of a smooth healing of the wounds on the stumps. This having been established, selection of these individuals would have to be made and they would have to be crossed with each other. From the eggs the experimenter would have to grow tadpoles and then maintain the young frogs for three years until sexual maturity, amputate their extremities, and in the following year select the individuals with signs of regeneration. Subsequently, the latter would have to be crossed again to obtain a new generation, and the entire procedure repeated. This approach meets with serious criticism. First of all, even if it were successful, a positive result,—regeneration of extremities in adult frogs—would be obtained not in any individual at will taken from nature, but in only a few out of a multitude. Consequently, this method could not be used for the restoration of lost

regenerative capacity of damaged and regenerating organs and tissues in man. Furthermore, the foregoing approach is extremely complicated and requires much time in order to obtain even a relatively successful result: one must have a multitude (thousands) of individuals, obtain several generations, and even then, the investigator will be confronted with a random, not a regular event: i. e., accidentally find a result showing regeneration, accidentally find to a certain extent a regenerative process, etc. It is doubtful whether by using such a method one could succeed in developing a regenerative capacity in organs and tissues of animals. However, even if one would succeed, by selection and crossing, in obtaining a line of animals with a high capacity for reparative regeneration of organs, what caused the loss of the regenerative capacity in the processes of phylogenesis and ontogenesis of animals would still remain unknown. Therefore, one could not elaborate biological methods for the restoration of the latter. These considerations forced us to reject this approach to the problem of loss and restoration of the regenerative capacity of organs and tissues in animals, and to look for other general biological approaches to its solution.

According to the principle of unity between the organism and the environment, introduced by I. M. Sechenov, any living body (a plant or animal organism, microorganisms, an isolated organ or tissue) exists and develops only under certain conditions of the external environment, forming an inseparable unity with them. A fundamental property of life is metabolism, which changes constantly depending on the changes in the conditions of the external environment and as a result of which the structure, nature of the living body, and its properties change in a corresponding manner. The knowledge of this principle allows one to change in a directed manner the heredity, the individual, and historical development of any organism. The change in the external environment is a primary cause for the changes in the physiology, structure, nature, and development of the organism. Changes in the external environment acting for a long time in a certain direction necessarily alter the entire organism, its metabolism, its different properties and attributes, and these changes are transmitted hereditarily to the offspring. Heredity, ontogenesis, and evolution of an organism change under the influence of changes in the environmental conditions.

This assumption permits a new approach to the basic phenomena of regeneration and to the problem of directed changes in the regenerative capacity.

Old ideas in the study of regeneration gave a fully determined characteristic to regenerative capacity in the animal world. The accumulated factual data led to the conclusion that, as a rule, the capacity for reparative regeneration decreases in comparative and ontogenetic series of animals corresponding to the increase in complexity of their organization. In mammals and man the regenerative capacity of organs is lower than in other species of animals. On the other hand, investigators studied the regenerative capacity as such, considering it as something stable, hereditarily determined, and independent of external conditions, and they did not even raise the problem of the possibility of changing it. Criticism of the old ideas developed along the aforementioned two lines, and two new directions appeared in the study of regeneration.

According to the first new direction, developed by Professors M. A. Vorontsova, L. D. Liozner, and A. N. Studitskii, the main error of the old ideas on regeneration was the conclusion that the capacity for reparative

regeneration decreases with increase in the level of organization of the animal, since from this it follows that the regenerative capacity of man is the lowest; this assumption according to these investigators is a reactionary one, since it disarms practicing physicians. In the opinion of these scientists, the old notions are incorrect, erroneous, and fictitious. Vorontsova (1953) says: "One must finally shed the chains of those false constructions and imaginary laws which were created by the bourgeois idealistic science. Among these are the reactionary statement by Weismann that the higher the animal organism the weaker its capacity for restoration of lost organs... one must decisively fight such false theories which inhibit the development of science" (p. 5). "Weismann's ideas were particularly harmful for the theory of regeneration in mammals and man. Studies by physicians and biologists who attempted to investigate the manifestations of regeneration in mammals and man were declared to be fruitless a priori and doomed to failure" (p. 17). Studitskii (1954) says: "The now widespread theory of regeneration developed for many decades under the influence of the reactionary concepts of Weismann-Morgan on the decline of the restorative properties of organisms with the increase in the complexity of their organization taking place in evolution" (p. 11). Rejecting the old notions on the regularities of regeneration, these investigators reach entirely different, diametrically opposed conclusions. Vorontsova (1953) says: "the capacity for regeneration, both physiological and reparative regeneration, is a universal phenomenon characteristic to all animals and also all plants" (p. 17). She concludes that mammals are capable of regenerating external and internal organs. A similar conclusion is also reached by Vorontsova and Liozner (1955, 1959), and by Liozner (1959, 1960, 1962).

I do not intend to discuss these statements here, although I shall do so later; I want only to emphasize the fact that Vorontsova, Liozner, and Studitskii who developed this direction, consider as their main thesis the assumption that higher animals, and primarily mammals and man, possess a high capacity for regeneration of organs. This thesis is in contradiction to the old first law of regeneration.

These investigators and their co-workers carried out an extensive study on the capacity for regeneration and hypertrophy of various organs and tissues in mammals, and they drew the attention of many other investigators to these phenomena. This was no doubt a step forward in the study of regeneration. A sharp transition, a jump from studying regeneration in axolotl to studying it in mammals was undertaken. New interesting observations, which are described by these scientists in great detail in their papers and in the aforementioned reviews, were made in the process. The main achievement of their work was the proof that the capacity for reparative regeneration of organs and tissues of mammals is higher than had been considered earlier, and is not merely scar tissue formation and healing of wounds and bone fractures.

However, it is difficult to agree with a number of assumptions made by these researchers, for example, with the statement which equates regeneration with hypertrophy of organs after damage. It must also be pointed out that their studies were concerned mainly with the phenomenology and not with the depth, means, and essence of the processes of regeneration. In making the statement that in all animals, including mammals, regeneration is possible in all organs, these investigators essentially deny a problem

of biology which is most important theoretically and practically, namely, the problem of loss and restoration of the capacity for repair regeneration of organs in animals in the processes of their phylogenesis and ontogenesis. However, this problem exists, is being elaborated, and in the course of its study new important data are accumulated and new important generalizations made.

The second new direction which has appeared in the study of regeneration, as a counterbalance to the old views, and developed by us and other Soviet and non-Soviet scientists (Umanskii and Kudokotsev, 1951, 1952: Rise, 1944, 1945; Singer, 1954; Scharf, 1961, and others), is characterized by an attempt to achieve directed experimental changes in the regenerative capacity and cause regeneration of organs which do not regenerate under ordinary conditions. The problem of which animals (higher or lower) regenerate better is of secondary importance. The fact that in higher animals and in man, as a rule, the capacity for reparative regeneration is lower than in lower animals does not and cannot be of negative value. This fact is not due to a methodological error. The main fault of the old ideas concerning the study of regeneration was that while studying phenomena of regeneration of an organism, the latter was not looked upon as part of the unity of the organism and external conditions. The regenerative capacity was studied as such, as something given, conservative, unchangeable. There were no serious systematic studies attempting to change regeneration, to restore the lost regenerative capacity. This fact was responsible for the birth of a basic methodological error to which numerous investigators succumbed, namely, autogenesis, i. e., the idea that the regenerative capacity does not depend on external conditions and it cannot be changed in a directed manner.

The aforementioned second new direction in the study of regeneration made an attempt to change the basis of regeneration, the course of the process of ontogenesis and its hereditary basis, changing the conditions of regeneration, the physiological state of the tissues of the organ under study and the organism as a whole. From this position we reexamined all the basic phenomena and notions on regeneration. This enabled us to propose new concrete methods of investigation, and to show that it is possible to restore the capacity for reparative regeneration of organs and tissues lost in phylogenesis and ontogenesis of vertebrates and, in particular, mammals. We succeeded in showing that it is possible to change experimentally the regenerative capacity in a directed manner, no matter how low and conservative it may have been, in any animal species, at any age, and in any organ or tissue. The importance of these data can hardly be overestimated. Developing new theoretical assumptions, this new direction points to a direct avenue into medical practice and agriculture.

The two new directions have in common their striving to overcome the limitations of the old ideas concerning regeneration; they both make mammals their main object of study, thus coming closer to man; they attempt to liquidate the gap which existed for centuries between theory and practice, between biology and medicine, in the study of regeneration. The new approach also brings closer together the positions of biologists and physicians.

At the same time there is a great difference between the two new directions. According to the first direction, all the organs of any animal are capable of regeneration. According to the second direction, different organs of different animals differ greatly in their capacity for regeneration.

Starting from the thesis of the first direction, it is not possible to change the law of loss of the capacity for repair regeneration of organs and tissues in phylogenesis and ontogenesis of animals because no such loss of this capacity takes place. However, this conclusion is contradicted by fact, and, therefore, I cannot accept it. The analysis of this problem will be given later.

The following is a summary of the main points of this chapter:

1. Until recently, for about 250 years, two main directions prevailed and developed separately in the study of regeneration: the medical and biological directions. The former had as its major purpose practical tasks and its work, mainly healing of wounds, was performed on mammals; the latter direction elaborated mainly theoretical problems and its work was largely performed on animals with high capacity for regeneration. This dualism was formed historically by the course of development of biology and medicine. The problem of loss and restoration of the regenerative capacity of organs, if one disregards a very few episodes, was not elaborated by either physicians or biologists, in that the previous level of knowledge allowed for no approach by which it could be solved.

2. In the late 1940's, new ideas concerning regeneration replaced the old ones; recently, two new directions emerged. Both of them were concerned with work on regeneration in mammals, but on different assumptions. The representatives of one direction reached the conclusion that all the organs and tissues of mammals are capable of regeneration. The representatives of the second direction reached the conclusion that in some animal groups, and in particular in mammals, the capacity for reparative regeneration of organs can always be changed in a directed manner, no matter how low and conservative it may have been. The basis and the development of this latter assumption served as the theoretical basis for the elaboration of a new direction in the study of regeneration striving toward the establishment of the rules governing the loss and restoration of the regenerative capacity of organs in animals.

Chapter II

CHANGES IN REGENERATIVE CAPACITY DURING EVOLUTION AND ONTOGENESIS

The first few decades of studies on regenerative capacity already showed that in some species of animals such capacity is present, i. e., after amputation organs regenerate nicely, while in other species it is nonexistent, i. e., after amputation homologous organs do not regenerate. For example, in axolotls, tritons, and salamanders the extremities regenerate nicely, while in frogs, lizards, mice, and rats they do not regenerate (Korschelt, 1927; Weiss, 1930, and others). However, further studies have shown that, in addition to these two groups of animals, there is a third group, an intermediate one, in which the organs are capable of regenerating until a given stage of ontogenesis, and later lose this capacity. The order of tailless Amphibia turned out to be such an intermediate group. In the species examined, after amputation of the extremities, there was no regeneration in adult frogs. At early stages of the process of metamorphosis, the extremities regenerate after amputation in tadpoles, while they do not regenerate in the later stages.* (Spallanzani, 1769; Barfurth, 1889; Kochs, 1897; Byrnes, 1904; Kammerer, 1905; Bauer, 1905; Marcucci, 1916; Guyenot, 1927; Liozner, 1931; Polezhaev, 1933; Borsuk, 1935; Ginsburg, 1941; Schotte and Harland, 1943, and others). The regenerative capacity of organs is lost at a given stage of metamorphosis, which differs for different organs: for the forelegs, hind extremities, tail, gills, etc. The common point for all these organs is the fact that their regenerative capacity is regularly lost in connection with some changes, general for the entire organism, taking place during metamorphosis. Comparative study shows that in all the species of tailless Amphibia which are considered by zoologists to belong to a lower level of organization, and which spend the major part of their life in an aquatic environment (*Bombina bombina*, *Bombinator pachypus*, South African *Xenopus laevis*, etc.), the capacity for regeneration of extremities is better expressed than in species of higher organization which spend a considerable part of their life on land (frogs belonging to *Rana temporaria*, *R. esculenta*, *R. ridibunda*, toads *Bufo bufo*, *B. viridis*, etc.).

A similar phenomenon of loss of regenerative capacity of the extremities is also observed in birds and mammals. In birds, as shown by operations performed on embryos, the extremities lose very early the capacity for regeneration at a stage when the germinal layer of the extremity does not

* In *Xenopus laevis* the capacity for typical regeneration of the extremities is preserved even after metamorphosis of the tadpole (Skowron and Komala, 1957; Dent, 1962).

yet show signs of histological differentiation in its mesodermal part (Shorey, 1909, Spurling, 1923, and others).

In work on mammals (mice, rats, rabbits, and guinea pigs) a method was worked out for intrauterine operations (Bors, 1925), which made possible amputation of developing extremities and tails. Due to technical difficulties the amputation could be performed only at later stages of embryogenesis, in the second half of pregnancy. In a majority of the cases the amputation wound surfaces healed smoothly and no regeneration was observed (Nicholas, 1925—1934; Mickewicz, 1934, 1936; Aizupet, 1935). Some hints of regeneration of rudiments of phalanges in the embryonic toes were obtained in experiments on guinea pigs and rabbits (Mickewicz, 1936; Aizupet, 1935). Only the small toes grew, while there was smooth healing of the wounds at the site of amputation of the middle toes. Amputation was performed at an early stage when there were very short rudiments instead of toes, and the middle toes stuck out. It is quite possible, therefore, that the small toes of the embryos were not amputated at all but simply grew subsequently. No histological examination of the extremities after amputation was performed. In one of the experiments on mice simultaneous amputation of the extremities and tails was carried out, and in two cases it was observed that the tails were of normal length and structure after birth of the mice (Mickewich, 1934). The investigator is of the opinion that the tails regenerated. However, against this assumption is the fact that the regeneration of tails should have been completed within 4—5 days after the operation, a fact which has not been observed even in tadpoles. It is more probable that the tails were not successfully amputated, since amputation was carried out blindly, the uterus being stuck with needles and no organs taken out. Later studies on mice (Polezhaev, 1949, unpublished) showed that after intrauterine amputation of extremities and tails in embryos, the wounds healed smoothly and no regeneration of organs took place.

Nevertheless, there is a good basis for the assumption that in mammals and birds, and probably also in lizards, the capacity for regeneration of extremities is lost at some early stage in ontogenesis, as in the case of tailless Amphibia. The capacity for regeneration of extremities decreases in the process of ontogenesis in all vertebrates, but in some (caudate Amphibia, fish) it changes qualitatively very little or does not change at all, while in others (acaudate Amphibia, Mammalia, Aves) it changes qualitatively at quite early stages of ontogenesis, and the higher the organization of a given taxonomic group of animals and the shorter their period of embryogenesis, the more rapidly ontogenesis is accomplished, the earlier the capacity for reparative regeneration is lost.

The discovery of the intermediary group bridges the gap between animals capable and not capable of regeneration. In the analysis of this problem, it turns out that there exists one order of animals which differ in their capacity for regeneration of extremities and other organs in that regeneration is lost at different times during ontogenesis. On the whole, it may be said that, as a rule, the higher the organization of a given species of animals, the earlier in ontogenesis is the capacity for regeneration of extremities and other organs lost. It must be emphasized that this can be spoken of only as a rule and not as a law, inasmuch as there are exceptions to the rule which can be explained by the peculiarities of the conditions of development of the different animals. Nevertheless, a rule exists and is applicable not

only to extremities, but also to many other organs. As shall be seen later it is also true of the cranial bones, the cardiac muscle, the nerve tissue of the brain, and of other animal organs.

Establishment of the presence or absence of the capacity for reparative regeneration of organs in these or other animal species in comparative series, or at one or another stage of ontogenesis in their ontogenetic series, is achieved by simple description of facts. Part of the animal organ is amputated and the results objectively described. Subsequently, objective comparison of these data is made and a conclusion reached as to what takes place in what case. Studying the question of whether animals of a given species at a given age are capable of regenerating extremities or other organs, the investigator wants to establish a fact and not prove that a given species of animals is or is not capable of regeneration. The investigator cannot have, and should not have, a wish to force facts to comply with some a priori notions. It may be said, therefore, that the descriptive and comparative methods allow for the establishment of an objective truth. The results of such studies are truthful, although some facts always must be checked additionally and the picture be made more precise, of course. That means that despite the opinions of some investigators, there is no basis to assume that our forerunners intentionally distorted or falsified reality when studying regeneration in comparative and ontogenetic series of animals. Analysis of data on regeneration in comparative and ontogenetic series of animals leads to the conclusion that, as a rule, the development in phylogenesis and ontogenesis of animals decreases the capacity for repair regeneration of organs.

What then are the reasons for the loss of the regenerative capacity in phylogenesis and ontogenesis of animals? Is it not possible to restore the lost capacity for regeneration of organs? Biological and medical reports made a few attempts to answer these questions, but these were not successful.

The well-known American experimental zoologist, Morgan (1908), put forward the assumption that the loss of the regenerative capacity of extremities in adult frogs is caused by enzymatic changes in the tissues. In order to restore these enzymatic properties and to cause regeneration of extremities in frogs, he amputated their extremities and transplanted onto the wound surfaces tissues taken from extremities and tails of tadpoles, capable of regenerating these organs. The result of the experiment was negative, the extremities of the frogs did not regenerate.

The prominent German surgeon, Bier (1923), suggested that all animals and man possessed the capacity for reparative regeneration of organs, but that appropriate conditions must be found for its manifestation. To cause regeneration of a completely removed fibula of a child, he transplanted in its place a ground bone from a dog on the assumption that the decomposition products of the tissues would cause regeneration of bone. However, the removed bone did not regenerate.

The well-known Austrian experimental morphologist, Weiss (1930), started from entirely different theoretical assumptions. In his experiments he performed a homoplastic transplant of young, undetermined blastemes from tails capable of regeneration onto amputation wound surfaces of extremities of lizards incapable of regeneration. The extremities did not regenerate. When more highly developed, determined blastemas were transplanted, tails grew out. Weiss is of the opinion that the loss of regenerative capacity of extremities in lizards is due to the disappearance of "the morphogenetic field," i. e., of that hypothetical factor which, according to his ideas

(Weiss, 1926), determines the processes of morphogenesis in embryogenesis and in regeneration, and which is associated with the organ as a whole and develops independently of the material (tissues and components) of the organ, acting on the indifferent, "nullipotent" material of the blastema. The field theory of Weiss has been discussed repeatedly and criticized in Soviet biological publications (Polezhaev, 1933, 1936, 1937, 1945; Vorontsova, 1940, 1949; Liozner, 1940; Vorontsova and Liozner, 1936, 1938, and others). The notion of the "field" is idealistic in that the "field" is a nonmaterial factor independent of organic matter, developing according to its own laws, of an unknown nature. "The morphogenetic field" of Weiss basically coincides with the "embryonic field" or "biological field" of A. G. Gurvich (1922, 1930, 1944) and is of a transcendental nature. Despite the fact that the idea of the "field" is widespread in non-Soviet biological writings, until now, after 45 years of existence, it has not brought about any positive results; it did not lead to the discovery of any concrete properties or phenomena in the processes of embryogenesis or regeneration. It also contributed nothing to the explanation of the phenomenon of loss of the capacity for regeneration of organs.

An interesting attempt to explain the loss of the capacity for regeneration of extremities was made by M. I. Efimov (1937). This investigator reached the conclusion that the loss of regenerative capacity of extremities is due to the absence of regenerative material, and that the latter is at the beginning indifferent and is determined by the tissues of the stump. Working on tadpoles of Rana ridibunda, Efimov removed the skin from the hind extremities, amputated the foot and pulled the extremity through the thickness of the tail so that the amputation wound surface was at the level of the wound of the tail and in contact with cells from the tail tissues. During metamorphosis the tail was resorbed and an atypical regenerate grew out on the experimental extremity. Efimov concluded that the nondetermined cellular material from the tail was transformed into the regenerate of the extremity. However, repeating this experiment on tadpoles of Rana temporaria, I could not confirm his data: the extremities did not regenerate (Polezhaev, 1947). Evidently in Efimov's experiments, animals were used which had preserved the capacity for regeneration at the stages and levels at which the extremities (foot) were amputated.

Recently Vorontsova (1949, 1953), Vorontsova and Liozner (1955, 1957), and Liozner (1960, 1962) have developed an idea similar to the aforementioned one of Bier. These authors think that all the organs of mammals and man are capable of regeneration, but that certain conditions are essential for the manifestation of the regenerative capacity. However, as in the case of Bier, this assumption remains without proof. Accepting this idea would lead to saying that no matter whether organs do or do not regenerate in various animal species, they are all similarly capable of regeneration. This conclusion is contradicted, however, by fact and does not allow for finding a way for elaborating the problem of restoration of the lost regenerative capacity. The investigators point out that on creation of known conditions, regeneration of organs and tissues may be obtained in animals which are not capable of regeneration. This means that restoration of lost capacity for regeneration takes place, but not its manifestation. I shall discuss this problem in greater detail in the subsequent chapter when I shall analyze the basic phenomena and notions on regeneration.

The notion that the lack (loss) of capacity for regeneration of organs in animals is due to rapid development of scar tissue in the damaged area, is widespread among physicians and biologists. This conclusion is made on the basis of the observation that scar tissue appears in the area of the defect of damaged skin and muscles in cardiac infarctions and in infarctions of the liver and other organs of mammals and man. I. I. Grekov (1901), for example made the outright statement that the reason for the absence of regeneration of the bones of the cranial arch in man is the formation of scar tissue in the region of damage. Rose (1944, 1945) is of the opinion that the reason for the loss of regenerative capacity of the extremities of frogs or lizards is the rapid coverage of the wound by scar tissue and skin. However, my experimental data force me to disagree with this opinion. Cutting away the skin on a stump of an extremity of metamorphosizing tadpoles did not result in regeneration of organs, and the wounds healed smoothly (Polezhaev, 1947). Upon removal of the parietal bone in adult dogs, under special conditions, instead of scar tissue, the formation of a typical cranial bone can be obtained from the connective tissue in the region of the defect (Polezhaev, 1957; Matveeva, 1962). Scar tissue indeed interferes with regeneration of organs and tissues. However, it is not the cause but, rather, the result of loss of regenerative capacity. Development of scar tissue in foci of damage is based on some deeper causes of the development of organs and tissues in certain animal species.

The notion that the absence of regenerative capacity is due to the inability of the cells in the tissues of the organs to multiply is also quite widespread. Ide-Rozas (1936) draws such a conclusion from studying the process of loss of capacity for regeneration of extremities during metamorphosis of frog tadpoles. Histologists and embryologists draw this conclusion for the nerve tissue of the cortex of the cerebral hemispheres in mammals and man (Zavarzin and Rumyantsev, 1946; Zavarzin and Shchelkunov, 1954, and others). I cannot readily agree with this view either. The experiment has shown that the number of dividing cells is approximately equal in regenerating and nonregenerating extremities of tadpoles (Polezhaev, 1935, 1939, 1948). In respect to the cortex of the cerebral hemispheres of adult mammals, it has been shown in recent years that the nerve cells may automatically divide, while repair regeneration of nerve tissue in the focus of damage does not take place. Consequently, the reason for this phenomenon must be something else.

The lack of a sufficient experimental basis and of a preliminary systematic investigation is characteristic of all these hypotheses. The foundation for systematic studies was laid down by Guyénot (1926, 1927). Working on tailless Amphibia, this investigator elucidated experimentally the point whether the regenerative capacity of extremities in tadpoles is lost under the influence of the internal humoral medium of the animals or is independent of the latter. He xenoplastically transplanted tails and an extremity from tadpoles of frogs to salamanders, and he showed that organs which lost the regenerative capacity do not restore the latter when under the influence of the internal humoral environment of animals capable of regeneration. However, organs which are capable of regeneration do not lose this capacity, despite changes in the internal humoral environment. Liozner (1931) confirmed these data in experiments on homoplastic transplantations of extremities using tadpoles of the grass frog at various stages

of metamorphosis. Developed extremities of tadpoles at later stages did not regenerate after transplantation to young tadpoles which were capable of regeneration. Extremities of young tadpoles maintained the capacity to regenerate after being transplanted to tadpoles at late stages which were not capable of regeneration. Later, however, we have shown that in such cases the internal humoral environment of the tadpoles which lost the regenerative capacity facilitates the loss of this capacity in transplanted extremities of young animals which are capable of regeneration (Polezhaev and Gintsburg, 1939).

Working with transplants, Liozner (1931) established a highly significant fact, namely, that the transplantation operation itself stimulates regeneration of extremities and, when performed at a critical stage (stage IIa), may lead to restoration of the recently lost capacity for regeneration of extremities in tadpoles.

Further studies were performed in a systematic manner and were long-lasting and purposeful. They were characterized by a new approach to the subject and by the establishment of new, highly important facts, some of which will be described in the following paragraphs.

Our approach to the problem is characterized by a number of features. First of all, emphasis must be placed on the fact that the problem of loss and restoration of regenerative capacity cannot be worked out empirically. A theoretical basis for such a study is essential. The initial hypothesis and the starting theory may change in the course of investigation, as indeed took place in my case, but at the beginning, as well as at any stage of the study, there should be a theoretical basis. The study necessitates an hypothesis and is always beset with some risk. Not any hypothesis, however, should be put forward but only such which follows from the preliminary investigation of the process by descriptive, comparative, or experimental methods. Furthermore, the investigation should disclose actual phenomena and not substitute them with general considerations, a priori schemes, and logical constructions into which the investigator tries to force his facts and show that they are not contradicted by these facts. It is easy to explain the loss of regenerative capacity by a loss of a "morphodynamic field"; however, the "field" itself is an hypothesis, an unknown by which some investigators attempt to explain another unknown. An investigation should lead to the establishment of earlier unknown, objectively existent regularities, i. e., it should lead to true discoveries and not to other interpretations of already known facts. In the latter case, it is easy to slip onto a path of substituting casuistry for discoveries.

A problem may be approached from various positions on the basis of various methods: description, comparison, or experiment. In our study we started from experiment. The characteristic peculiarity of our approach was not a passive but an active investigation of the causes of loss of regenerative capacity of animal organs in experiments in which we attempted to achieve artificial restoration of this capacity (Polezhaev, 1933—1948, 1956, 1958). Such an approach provides more reliable data than any other approach because only an experiment enables one to directly check the phenomenon under investigation. Several hypotheses were put forward concerning the causes of the loss of regenerative capacity of organs but only a direct experiment showed the actual status in reality. It turned out that the loss of regenerative capacity of organs is due not to the loss of the capacity of the cells to divide or of their capacity for morphogenesis,

nor to scar tissue formation in the wound, but to the tissues' loss of their capacity for disintegration and dedifferentiation, as shown experimentally.

Elaboration of the problem of loss and restoration of regenerative capacity constitutes a special direction in the study of regeneration. The subject of this direction is not the phenomenon of regeneration as such, but its change (disappearance or restoration — appearance) in the natural course of phylogenesis and ontogenesis, or under pathological conditions in certain diseases, or under the action of ionizing radiation. Therefore, this phenomenon must necessarily be studied in its development, formation, comparing phenomena of regeneration and its loss or restoration in the course of a given studied process, e. g., in the case of developing extremities, cranial bones, etc. The study of loss and restoration of regenerative capacity is much more complex and difficult than the study of regeneration as such. It requires knowledge of the cause and mechanics of the normal process of regeneration; it requires knowledge of embryogenesis, of normal development of the given organs; it requires knowledge of general biological rules of ontogenesis, heredity and evolution; and finally, it necessitates the knowledge of some phenomena of general and special pathology. The purpose and task of this direction is not to study the phenomena and laws of regeneration as such, but to study the rules of change in the regenerative capacity in the course of individual and historic development of animals under pathological and experimental conditions. The elucidation of these rules may and should allow for elaboration of new biological methods of directed changes in the regenerative capacity of various organs and animal tissues (suppression of existing and restoration of lost), reparative and physiological regeneration, restoration of form (structure of organs and tissues), and function. It is clear, therefore, that such a study has great prospects, especially when it concerns vital organs and functions, and it should always yield results which cannot be obtained by another direction of study of regeneration. The method, the means of investigation in this direction, is not description, comparison, or an experiment directed toward the investigation of the mechanics of the spontaneous processes of regeneration, but an active interference altering the regenerative capacity, at the basis of the phenomenon of regeneration, the internal, hereditarily determined conditions of development of the organism and its organs and tissues, the molecular basis of cells and tissues. In studying the mechanics of the regenerative process, the role of external factors of development are investigated, as are the role of the internal factors of the different components of the organ, of the internal humoral environment, endocrine glands, the nervous system, etc. In studying the laws of loss and restoration of the regenerative capacity of organs, their condition or the condition of the organism as a whole are changed.

Working in this direction it is advisable at first to narrow down the task, to make it more concrete, and limit the investigation to one given object which should be studied in great detail. Subsequently, having established the basic phenomenon and having experimentally elaborated on it, the investigator must go deeper and extend the investigation. The approach itself should be deepened, the means of investigation widened. It should be carried out in a complex manner, studying not one organ but a number of them, not in one group of animals but in a series of higher

animals, mammals. In our 35-year study (1932—1967) the first 16 years (1932—1948) were spent in working mainly on the order of tailless Amphibia, concentrating on the paired extremities and only on the plane of experimental morphology, mainly using methods of surgery and histology. In the subsequent 19 years (1948—1967) we went on to a comparative study and to work mainly on mammals. The objects of our investigation were not only extremities of vertebrates but also other organs: the cranial bones, dental tissues, cardiac muscle, the nerve tissue of the cortex of the hemispheres of mammals, and also organs in which the capacity for repair regeneration has been suppressed by X-ray irradiation. In this investigation we succeeded in deepening and widening the scope of data concerning the laws of loss and restoration of the regenerative capacity of animal organs, earlier established in studies of the regeneration of extremities in tailless Amphibia. During this period, the investigation was carried out on the plane not only of experimental morphology, not only of the laws of individual development of animals, but also of the laws of heredity and historic development; this was a complex study based on methods of experimental morphology, histology, histochemistry, biochemistry, and physiology. The elaboration and establishment of the rules of loss and restoration of the regenerative capacity of animal organs allowed us to establish actual objectively existing, but previously unknown laws of regeneration: 1) the ways in which the initial stages of regeneration are determined — accumulation of cellular regenerative material basically without mitoses, due to dedifferentiation of tissues in the residue of the organ; 2) the phenomenon of regeneration by induction; 3) the phenomenon of regeneration of organs independent of the structure of the main component tissues, etc. In addition, we elaborated concrete methods for the establishment of lost regenerative capacity based on one and the same law, but differing considerably in nature, and specific for each organ. All these methods were directed toward activation of processes which do not take place under ordinary conditions of amputation of organs. In the majority of cases, this activation was achieved by a drastic enhancement of phenomena of destruction and dedifferentiation of tissues in the residue of the organ. However, another means of activation is possible, enhancement of the assimilatory or creative phase of the process of regeneration by other means: directed changes in metabolism, e. g., enhancement of mineral metabolism during regeneration of cranial bones (Rogal', 1952, 1957), attachment of an additional nerve to the damaged organ (Singer, 1954). In these cases the same result is achieved by different methods, a fact which confirms the known generalization of D. P. Filatov (1937, 1939) that the final result of morphogenesis is always more conservative than the means of its formation. However, on the other hand, it turns out that factors determining this morphogenesis, which arises in different ways, are identical, a fact which confirms the principle of equifinality we formulated earlier (Polezhaev, 1936, 1938). At that time this principle had been suggested on the basis of analysis of data obtained by experimental biology and by study of regeneration. We later became convinced that it is applicable to different biological phenomena and evidently is of universal significance. For example, development of an extremity (final) takes place by different routes in normal ontogenesis and in regeneration, but the same factors are involved at the basis of these processes. Hereditary change in plants may be obtained by sexual or vegetative hybridization or by the action of factors

from the external environment. Restoration of lost regenerative capacity of extremities in frogs or lizards may be achieved by enhancement of phenomena of destruction and dedifferentiation, and by additional innervation. In both cases the assimilatory phase of the process of restoration and processes of protein and nucleic acid biosynthesis are enhanced. Such cases do not contradict the established rules of loss of regenerative capacity but indicate new ways for a more penetrating investigation.

In summarizing the data discussed in this chapter, the following are noted:

1. All animals may be divided into three groups on the basis of capacity for reparative regeneration of organs: those which are capable of it, those which are not capable, and those which lose this capacity during ontogenesis. A deeper study shows that all three of these taxonomic groups of animals should be arranged in a single series in which the organs in all of them are capable of regeneration until a given stage of ontogenesis, and are not capable of it after this stage has been reached. In other words, the regenerative capacity is regularly lost during the process of ontogenesis in all the animals.

2. In the study of regeneration, physicians and biologists put forward several hypotheses explaining the reasons for the loss of the capacity for regeneration of animal organs (loss of the capacity of the cells to divide or loss of their capacity for morphogenesis, rapid development of scar tissue, disappearance of the "morphodynamic field," etc.). However, none of these hypotheses correctly explained the phenomena under study, mainly because there was no orderly, systematic, and purposeful approach to the study of this regularity, since phenomena of regeneration were investigated as such in a passive manner without trying to change their nature or their course.

3. An active approach to the problem, the use of methods changing the physiology, the state of organs, the basis of regeneration, and the regenerative capacity, allowed the latter to change, and established the fact that the direct cause for its loss was a decrease in the capacity of the tissues of the organ for destruction and dedifferentiation.

4. Investigation of the regularity of loss and restoration of the capacity for regeneration of organs in the phylogenesis and ontogenesis of animals constitutes a new special direction in the study of regeneration which has its own subject, its own purposes and tasks, and its own method of investigation. This direction permits the posing and development of new problems, suggestion of new biological methods, and establishment of new, heretofore unknown, objectively existent, laws of regeneration.

Chapter III

BASIC PHENOMENA AND NOTIONS IN THE STUDY OF REGENERATION

In studies of regeneration and the problem of loss and restoration of the regenerative capacity of animal organs, certain phenomena and notions must necessarily be dealt with, although their definitions have changed and continue to change in the history and development of the theory of regeneration, and various authors ascribe different meanings to the phenomena and notions. Therefore, at least the more important ones should be discussed. Some notions, e. g., those concerning the basis of regeneration and others, have not been developed at all.

What is regeneration?

Many definitions of the phenomenon of regeneration are available, different investigators turning their attention to its different aspects and peculiarities. However, there is always in mind something common, constant, and basic, and this should be reflected in the main definition of regeneration. Regeneration is rebirth or restoration of a lost or damaged part (large segments of the body, organs, parts of organs, tissues, cells, parts of cells) of the body, and in our case, of the animal body.

Two main forms of regeneration, physiological and reparative regeneration, are customarily distinguished from each other. Physiological regeneration is the restoration of lost elements of a living organism during its normal activity, physiology, and work. The best known common examples of regeneration phenomena are restoration of calluses and epithelial peeling off of the skin, or epithelial peeling off of mucous membranes of the buccal cavity, the alimentary tract, uterus, etc. Strictly speaking, however, physiological regeneration characterizes in some form and to some degree all organs and tissues, as all of them work, are subject to wear and tear, and are restored. In these processes loss and restoration of structural parts of cells and tissues take place and not only molecular turnover, which, as has now been convincingly proven by biochemistry using methods of radioactive labeling, is characteristic of all living cells and particles of the living body. Physiological regeneration is a universal phenomenon characteristic of all animals, plants, and microorganism, of all organs, tissues, and cells. Physiological regeneration is characterized by being spread out, regular, and unaccompanied by coarse or accidental destruction of normal body structures. Cells undergo gradual wear and are gradually replaced by new ones. The reason for cell damage and for their wear (i. e.,

for the entire process of physiological regeneration) is the work, function, and physiology of organs. The phenomena of physiological regeneration have been discussed in detail in the book by Vorontsova and Liozner (1955).

Reparative regeneration is restoration for loss caused by trauma, damage which does not occur regularly but is, rather, accidental. The reason for reparative regeneration is not the physiology of the organ, but damage which usually grossly affects the structure of the organs and tissues and is only occasionally diffuse and widespread. Damage may be of different kinds: mechanical, chemical, thermal, radiational; it may be caused by infection or invasion, etc. The nature of the damage is of great importance for the subsequent process, but this circumstance does not alter the basic point that in all such cases of repair, post-traumatic, nonphysiological regeneration takes place. This book will deal mainly with reparative regeneration and the laws of its loss and restoration, because physiological regeneration is not lost during phylogenesis and ontogenesis.

In the individual development of animals, self-reproduction of the organism as a whole, or of its different parts, is always present. These forms of reproduction differ and are sometimes dissimilar: sexual reproduction based on fusion of sexual cells; asexual multiplication by division, budding, etc.; growth; regeneration; hypertrophy; hyperplasia; malignant and benign neoplasms. All these forms of self-reproduction have been known for a long time, have been described in detail in textbooks on biology and pathological anatomy, and have in common molecular and structural self-reproduction. But they differ qualitatively and cannot be exchanged with each other, each being based on its own peculiar characteristics. Therefore, they cannot be identified with each other, as done recently by some investigators who attempted to relate such qualitatively different phenomena as, e. g., hypertrophy of organs to regeneration.

Sexual cells which are the basis of the process of sexual multiplication, differ qualitatively from all other cells of the animal body in structure, morphogenetic capacities, means of formation (reduction of chromatin), etc. The zygote differs in its properties, and in particular in its capacity for morphogenesis and self-reproduction, from all other cells. It suffices to say that only the zygote is the source of normal ontogenesis in all animal species.

Many authors (Kholodkovskii, 1933; Vorontsova and Liozner, 1957, and others) point out that asexual multiplication is intimately connected with phenomena of reparative regeneration. Regeneration is better manifested in those cases in which asexual multiplication is most developed (hydra, segmented worms, etc.). Despite the similarity of these forms of self-reproduction, however, their differences should be pointed out, namely, the fact that asexual multiplication is a quite regular phenomenon and takes place independently of damage, while regeneration is based on accidental injuries.

Growth is a universal biological phenomenon. It is characterized by an increase of the living mass (the live weight of the body), an increase of the number of cells and nuclear material, but not by qualitative transformations (transformations of the living mass). For a long time many biologists and physicians considered regeneration as a phenomenon of growth, assuming that it is nothing else but accumulation of cells and growth of tissues as a result of damage to the organ. The theory of regeneration by growth of tissues (Sprossungstheorie) is based on this notion. In one of the first

extensive reviews on problems of regeneration Morgan (1907) wrote: "Regeneration is nothing else but resumption of temporarily arrested growth" (p. 158). However, as shown by numerous studies of many decades, regeneration is not identical to growth. Regeneration is a phenomenon of development, or as nicely put by Liozner (1962), it is a phenomenon of repeated development. Regeneration as a phenomenon of development also includes growth and transformation, differentiation, qualitative change of the restored parts of the organism. Regeneration of extremities in axolotl by formation, growth, and differentiation of the blastema, is a phenomenon of development and not only of growth. Regeneration of an intact hydra or planaria from a small body segment is a phenomenon of development, differentiation, and transformation; there may be no growth or increase in living mass at all. Even in the case of regeneration of mammalian tissues, e. g., of bone and muscle, there is never a simple accumulation of cells but always cell transformation: osteoblasts, cartilage, bone; myoblasts, myosymplasts, and differentiated muscle fibers appear.

Hypertrophy is a phenomenon of increase in the mass of an organ or tissue in response to some damage, such as removal of one of a pair of organs, e. g., a kidney, or part of an organ, e. g., liver or spleen, leading to compensation. The main reason for hypertrophy is an increased functional load of the organ. In hypertrophy the mass and dimensions of the individual cells and structural units of the organ, e. g., the lobes of the liver, the thickness of the renal tubules, etc., usually increase. Phenomena of hyperplasia, i. e., increase in the volume of a rudiment of an organ due to an increase in the number of cells or structural units without an increase in their volume, is sometimes erroneously considered as hypertrophy. Such a phenomenon is observed, e. g., on removal of a part of the thyroid gland and enlargement of the residue. Hypertrophy and hyperplasia are characterized by lack of restoration of the initial shape of the organ, lack of regeneration from the wound surface, formation of a defect, sealed with scar tissue in the region of the wound, with increase in weight, mass, and volume of the organ residue. There are no significant differences in the course and outcome of the process of hypertrophy taking place as a result of removal of one of a pair of organs or following removal of a part of an organ. Hypertrophy and hyperplasia take place in internal organs of vertebrates: the liver, kidneys, spleen, heart, lungs, thyroid gland, and other organs which are not capable of regeneration. Physiological regeneration is well expressed in these organs. Phenomena of hypertrophy are of great significance for the organism, and their elucidation is an achievement of the investigators who dealt with this problem (Vorontsova and Liozner, 1955, 1957, and others). Hypertrophy is usually characteristic of epithelial and paranchymatous organs. At the basis of hypertrophy and hyperplasia are regularities of growth, increase in mass, weight, volume, and cell number, but not development. Hypertrophy and hyperplasia are characterized by the absence of phenomena of dedifferentiation. These phenomena lead to compensation of a disturbed function.

The phenomenon of hypertrophy is opposite to atrophy of organs and tissues, i. e., reduction in volume, weight, and mass of the organ caused by reduction in the functional load (e. g., an immobilized muscle), starvation, denervation. Therefore, these phenomena should be studied together.

Regeneration is a phenomemon of restoration of a lost part of the organism, an organ, tissue, or cell. In regeneration the lost shape and

structure are always restored, but not always the function of the organ. From a boneless extremtiy of axolotl devoid of function, an extremity regenerates after amputation (Weiss, 1925; Brunst, 1932). Upon partial or atypical regeneration of an extremity in frogs or lizards, the function of this organ is not restored. In other cases of regeneration the function may be fully restored. Regeneration is characterized by restoration of the lost elements, while lack of regeneration is characterized by lack of restoration of these elements. For example, in regeneration following the loss of an extremity, bone, or muscle, the extremity, bone, or muscle, respectively, is restored. Lack of regeneration of these organs is characterized by scar tissue formation on the wound or slight proliferation of tissues from the residue of the organ or tissue without restoration of the tissue. In regeneration, the weight, dimensions, mass, volume of the organ, or amount of cellular material may not reach the initial values. Regeneration, as already pointed out in the preceding paragraphs, is a process of development and not of growth. At the basis of regeneration always lie phenomena of destruction and dedifferentiation of the tissues of the organ.

Regeneration may be typical, leading to restoration of the main essential features of the typical normal structure of organs and tissues, e. g., regeneration of the extremities in axolotl or the tubular bones in rats. But regeneration also may be atypical, incomplete, or partial, if not all of the basic properties of the organ or tissue are restored, e. g., regeneration of a conical appendage in the place of an extremity in a lizard, or of scar tissue-muscle tissue upon removal of a considerable segment of skeletal muscle in adult rabbits. Under greatly altered conditions the course of regeneration may be entirely distorted. For example, in decapoda in the absence of the nerve ganglion an antenna regenerates instead of the pediculate eye (Herbst, 1895). However, regeneration never takes place in the complete absence of restoration of the lost organ or tissue, e. g., in the case of formation of scar tissue in a smoothly healing stump of an extremity or tail of a mouse, in the formation of heat infarctions, and infarctions of liver, spleen, or lungs in man and other mammals. This is the phenomenon of absence of regeneration. Pathologist-anatomists diagnose a past infarction on the basis of such scars in internal organs.

On the basis of the aforesaid, we consider wrong and very artificial the attempts of some investigators to identify phenomena of regeneration with those of hypertrophy (Liozner, 1962).

Some investigators quote the foregoing phenomena in order to prove their thesis that mammals are capable of regeneration of organs in the same way as are lower animals, or even better. However, a serious error is thus compounded of identifying with each other qualitatively different phenomena. Regeneration and hypertrophy are distinct forms of self-reproduction of the living tissue, as are sexual and asexual multiplication, growth and development, etc. At the basis of hypertrophy lie the laws of growth, while laws of development are at the basis of regeneration. In regeneration there is dedifferentiation of the main tissues comprising the damaged organs, while in hypertrophy there is not. The criterion of regeneration and of hypertrophy is mainly a morphological and not a functional one. If an organ regenerates but does not function normally, regeneration has, nevertheless, occurred. Restoration of function may be due not to true regeneration of the organ or tissue but to compensation, whereby some other elements of the organism take upon themselves a

given function. Phenomena of compensation are widespread and are particularly well-known to physiologists who are familiar with the central nervous system. Erroneous identification of qualitatively different phenomena of self-reproduction based on different regularities of regeneration and hypertrophy lead investigators to another even more serious error, namely, denial of unitary regularities in phenomena of regeneration (Liozner, 1962), which shall be discussed later in another chapter.

Benign and malignant neoplasms are similar to regeneration in that neoformation takes place in all such cases. These phenomena differ, however, qualitatively. In regeneration a lost shape (organ, tissue) is restored, the process leading to restoration of the normal state. In the case of tumors something new has formed which had not been there before, and the process leads to a pathological state, sometimes a very deep-seated one accompanied by death, as, e. g., in cancer or sarcoma. Consequently, these forms of self-production are also qualitatively different from regeneration.

Successful investigation of regeneration as a phenomenon of repeated growth is possible only on the basis of knowledge of the normal development of organs in ontogenesis. Therefore, we studied the individual development of animals within the framework of descriptive and experimental embryology as reflected in the author's book "Osnovy mekhaniki razvikiya nozvonochnykh" (Principles of the Mechanics of Development of Vertebrates) (1945). As it is not possible, within the scope and volume of the present monograph, to discuss in any detail the regularities, phenomena, and factual data related to this problem, I shall only point out some aspects which will have to be dealt with subsequently.

Normal ontogenesis of animals is characterized by a progressive process of differentiation, an increase in complexity of organization and function, a transition from lower stages of development to higher ones, and—in the final stages—by processes of involution and aging and disturbance of functions. In the course of ontogenesis there is a continuous limitation of the morphogenetic potentials of cellular and tissue material. Thus, for example, the ectoderm of the gastrula of amphibia is capable of transforming into any organ of any germinal layer. The ectoderm of the late gastrula already loses this capacity, but preserves the capacity for transforming into ectodermal and nerve organs. At the stage of the early neurula, various segments of the nerve plate may transform one into another after being transplanted; for example, the presumptive anlage of the eye may be transformed into a spinal column or the presumptive spinal column anlage into an eye, while at the stage of late neurula they lose this capacity, etc. The limitation of the potential is regular but not absolute. It has been established that under certain conditions the embryonic organs, tissues and cells are capable of so-called self-differentiation, i. e., of development in a certain direction despite changes in the normal conditions, e. g., when transplanted to another site in the animal body, they may change the direction of their development (Spemann, 1936; Filatov, 1939; Polezhaev, 1945). For example, the presumptive epidermis of the gills of amphibian embryos transplanted laterally will develop into gills, while, when the eye anlage is transplanted beneath it, it forms a lens (Manuilova and Kislov, 1931).

The process of limitation of the potentials is associated primarily with the process of determination. The notion of determination was for many decades the main and leading idea in experimental embryology. It was given

a different content at different times in the history of development of this discipline, and by different investigators (Filatov, 1934; Polezhaev, 1944, 1944a). Determination was understood as fixation of properties, limitation of potentials or induction, and interaction of parts determining the morphogenesis of each of them and of the anlage as a whole. This latter definition (Polezhaev, 1938, 1944, 1945) was the most complete one and corresponded to reality. It follows from this definition that the entire process of ontogenesis, of development of systems and different anlage parts and developed organs of the animal, primarily in the period of embryogenesis, is based on a chain of determining processes occurring under given external and internal conditions. The nature of the determining influences may differ: contact, physical, chemical, radiation, material, energy, etc. The most frequent form is induction, i. e., an effect of an external factor or of an anlage of an organ which under certain external and internal conditions changes qualitatively the state of another anlage inducing it to a certain morphogenesis. For example, the eye anlage induces the formation of a lens in the epidermis, while the acoustic vesicle induces a cartilaginous capsule in the surrounding mesenchyme; the primary organizer, and the material of the presumptive chordamesoderm of amphibian gastrula induces a neural plate in the ectoderm of early gastrula, etc. As a rule, induction is of a chemical nature and certain inducing substances released even by dead tissues act in the process of induction (Holtfreter, 1933). It is possible to isolate substances which induce ectodermal or mesodermal organs (Saxen and Toivonen, 1963), eyes (Lopashov, 1936), and cartilage (Strudel, 1962). In other words, the inducer and inducing substances may be specific.

The study of the laws of determination has been advanced and developed in the comparative-morphological (Filatov, 1939) and physicochemical (Needham, 1931, 1942) aspects.

Later it was pointed out that experimental embryology — elucidating phenomena of determination which are of great importance in embryogenesis and in ontogenesis, on the whole — does not elucidate their basis, the role of living conditions, the adaptive regularity, the connection between phenomena of heredity, ontogenesis and phylogenesis (Polezhaev, 1950). Having in mind these remarks and studying the causes of ontogenetic morphogenesis, the main question is not posed in the framework of the problem of determination, but in the framework of the problem of developmental biology. The subjects of the study are not the processes of determination, but the living conditions which determine their development in ontogenesis and phylogenesis. The problem is also posed in this plane in regard to phenomena of regeneration as repeated development.

The reports long ago had pointed out that regeneration of organs and tissues takes place on the basis of different laws. Therefore, the investigator distinguishes between organ and tissue regeneration. In a number of cases regeneration of tissues takes place, while regeneration of organs does not. For example, in mammals and birds (rats, rabbits, chickens) the tubular bones after fracture, the skeletal muscles, and sometimes the skin regenerate, while extremities which contain these components do not regenerate. On the other hand, in tritons the extremities with all their skeleton, muscles, skin, etc., regenerate completely after 90—120 days, while regeneration of bone tissue after fracture of the tubular bone occurs very slowly, after 230 days (Wurmbach, 1927). It is not always correct to speak of tissue regeneration; sometimes to be accurate, the term

organ regeneration should be used in reports. For example, when half of the femur of an axolotl is removed and it regenerates, it is spoken of as organ, and not as tissue regeneration. However, if the bone tissue regenerates after fracture of the tubular bone, or the skeletal muscle after removal of a segment of it, this is doubtless tissue regeneration and not organ regeneration. Therefore, we can hardly agree with the opinion voiced by some investigators (Liozner, 1962) that researchers should always speak of regeneration of organs and not of tissues, since there are no tissues in the pure form in an organism, and a larger or smaller sector of the organ (extremity, skin, etc.) is always damaged; that in the pure form a tissue exists only in a tissue culture outside the body. We are of the opinion that even in the latter case it is not always possible to obtain a pure tissue. For example, so far nobody has succeeded in explantation of muscle tissue without connective tissue. However, it is quite possible to speak of organ and tissue regeneration as quite real phenomena based on different laws. Organ regeneration is characterized by regularities similar to those governing embryogenesis, in particular the interaction between parts of the organ, e. g., the epithelium and the tissues of mesodermal origin in regeneration and development of extremities. Tissue regeneration is characterized by conditions which prevail inside the organ housing these tissues. These are entirely different conditions. Researchers should even more strictly distinguish between the regeneration of organs and cells. Multiplication and local accumulation of cells is, by far, not the regeneration of an organ. Therefore, in order to establish the laws of organ regeneration, and in particular of the loss of their capacity for reparative regeneration, investigators cannot merely study the changes in cells taking place in the process, or only the problems of histogenesis. However, certain investigators, e. g., Ide-Rozas (1936), assumed that the causes for the loss of regenerative capacity of organs (extremities in metamorphosing tadpoles) may be established on the basis of cytological investigations only. It is not at all astonishing that these attempts have not been successful.

Finally, we should point out that the phenomenon of regeneration cannot be reduced to the different phenomena characterizing or accompanying it. However, numerous investigators erroneously speak of regeneration where, in reality, no such thing occurs but only different signs appear: mitosis or amitosis, dedifferentiation, inflammation, destruction, etc. Mitosis or amitosis may occur in damaged tissues, but regeneration may be absent. For example, in the well-known experiments of Haberlandt (1922) with wound hormones, upon cutting a kohlrabi tuber there is no regeneration on the wound surface but the number of mitotic cells may increase. Or, in the case of amputation of an extremity in tadpoles at late stages of metamorphosis, there is no organ regeneration but there are mitoses in the tissues of the wound surface (Polezhaev, 1935, 1939). After injury to the mammalian myocardium by burning or the application of a ligature, the nuclei of the muscle fibers may divide amitotically or even mitotically (Anichkov, 1912; Ring, 1950, and others), and sometimes even dedifferentiate (Rumyantsev, 1954, 1955), but they do not regenerate. Phenomena of inflammation after amputation of extremities in mice and rats have been observed, but there was no regeneration of the extremities. Different indications do not mean that regeneration takes place, and the absence of some of the indications characterizing regeneration does not always mean that regeneration is absent. For example, I became convinced that the initial stages of regeneration of extremities in axolotl and in tadpoles occur basically without mitosis, due

to the release of cells from tissue associations by dedifferentiation of the tissues (Polezhaev, 1935, 1939, 1940, 1945).

Thus, reparative regeneration is one of the forms of self-reproduction of living matter which cannot be reduced to its other forms, in particular to the phenomenon of hypertrophy of organs. Regeneration of organs, tissues, and cells has something in common, but at the same time it is subject to different laws. Regeneration is a phenomenon of repeated development and this phenomenon cannot be reduced to its different components.

Essence of regeneration

With the phenomenon of regeneration defined, its different forms, and how it differs from other types of self-reproduction in animals, the question of the essence of regeneration necessarily arises.

Three main ideas concerning the essence of regeneration are now prevalent: 1) regeneration as a primary or original property of living matter, as self-reproduction; 2) regeneration as a secondary acquisition or adaptation; 3) regeneration as one of the forms of adaptive self-reproduction of living bodies.

Darwin (1868) said: "Different forms of budding, multiplication by division, and restoration of damage, and finally development, all these essentially represent the realization of the same capacity. . . All this is part of the same great law." On the other hand, he states: "There is no doubt that the capacity for reproduction, although not always perfect, represents an excellent adaptation suitable for many occasions, even for those which are encountered very seldom. However, this capacity is not more astonishing than growth and development of any living being, and in particular of those which develop by splitting." It can be seen that Darwin considered the essence of regeneration to be based on its contradiction: both as an original property of living matter, capacity for self-reproduction, and as an adaptation, i. e., at the same time as a primary and secondary phenomenon.

K. A. Timiryazev (1922) pointed out that "in connection with simple heredity manifested in the vegetative reproduction of whole organisms from parts of other organisms, one may also consider phenomena of partial reproduction of parts of a whole organism which lost these parts accidentally, the so-called phenomena of regeneration or restoration of organs." Heredity, according to Timiryazev, changes under the influence of external environment, being an adaptive phenomenon. Thus Timiryazev develops the Darwinian idea of the nature of regeneration.

In the post-Darwinian period the majority of biologists were under the influence of the ideas of A. Weismann. For many decades the alternative posed by Weismann (1902) was discussed: "Is the regenerative capacity a basic primary property of any living organism in the sense that it is realized everywhere and with the same force independently of external conditions, to some extent as an inevitable source of the primary properties of living matter? Or is it an adaptive phenomenon even in its oldest appearance which is based on a special mechanism and is not always manifested to the same extent and force?" Weismann gives a definitive

answer. The regenerative capacity is not a primary property but a secondary phenomenon, adaptation. It is characteristic of Weismann to admit the possibility of existence and development of the main properties of living matter independently of external living conditions. In the most distinct form, this idea was expressed in his theory of the germ plasm but it is also reflected in his views on regeneration. According to Morgan (1907), who came out with a critique of Darwin and Weismann, "The regenerative capacity is a primary property of living matter and to its known extent it is already part of the determination of the organic," and not adaptation. The great Austrian zoologist Przibram (1909), who devoted much of his thoughts to regeneration, also says: "Regenerative capacity is a primary general property of animals." Morgan's opinion was supported by a number of biologists (Davydov, 1908; Schulz, 1905, and others). V. M. Shimkevich (1907) was one of the few investigators who, having studied the phenomenon of regeneration, followed in the footsteps of Darwin. He said: "The majority considers the regenerative capacity as an adaptation elaborated by animals in order to restore lost parts (Weismann, 1899). From another point of view it is maybe a residual capacity of the animal for asexual development by division. Both views are essentially not so contradictory as may seem. Nature used the regenerative capacity characteristic of lower forms, and manifested in them during development by division, as a form of adaptation and maintained it for some higher forms which have, however, lost the capacity for asexual multiplication."

The way in which Weismann posed the problem initially drew the attention of many investigators who published a series of studies on this problem, but this interest faded after some time. The question was posed in a scholastic manner and there was no real possibility to solve it. The investigators, not having solved it, went on to other problems of regeneration; later, however, the problem has been raised again.

Studitskii (1948) again poses the alternative: "Is regeneration an adaptation or an original property of living beings?" (p. 8). Criticizing the notions of Morgan concerning regeneration as an original property of living beings, this investigator assumes that regeneration is an adaptation secondarily elaborated in evolution due to frequent damage and loss of organs. This idea, however, is ascribed by Studitskii, not to Weismann who developed it intensively, but to Darwin, on the basis of one aspect of Darwin's utterances, namely, that the regenerative capacity is of adaptive value. Studitskii's assumptions were soon assimilated by some Soviet biologists. Thus, O. A. Kuznetsova (1950), in her dissertation, says: "Evidently one of the acquired properties which has arisen under certain conditions, is the capacity of organisms for restoring lost parts of the body, i. e., the capacity for regeneration. . . namely, as an adaptive property regeneration was considered under the influence of the evolutionary theory in the post-Darwinian period; a similar view is held at present by Studitskii." Both Studitskii and Kuznetsova are of the opinion that the recognition of the capacity for regeneration as an original property of organisms is "basically wrong," "it contradicts the materialistic understanding of purposefulness."

Vorontsova (1949) rejects the idea of regeneration as an adaptation and accepts with Shulz (1905), Davydov (1908), and other investigators, the idea of regeneration as an original property of living matter. She says: "Regeneration is based on the process of reproduction" (p. 233), and she

adds that this is a Darwinian position referring to another aspect of Darwin's utterances, namely, that regeneration as well as development, budding, division, and multiplication are variations of one and the same capacity of the organism for reproduction. Vorontsova emphasizes the connection between regeneration and asexual multiplication.

Studitskii (1948) and Vorontsova (1949) take from Darwin certain ideas without encompassing his views on regeneration as a whole with their contradictions. Despite their different views on the essence of regeneration, they both accept as their starting point Weismann's alternative. Their ideas on physiological regeneration as the basis of reparative regeneration, and on the comparative method as an historical method in the study of regeneration and other phenomena (see below) are also similar. However, in contrast to Weismann and Morgan, these investigators admit that regeneration depends on external conditions.

L. V. Polezhaev (1950) subjected Weismann's alternative to criticism showing that its formulation of "either-or" is wrong. The ideas of both Weismann and Morgan are wrong. The essence of regeneration is contradictory: regeneration as one of the forms of self-reproduction is an original property of living matter, but self-reproduction is adaptation. Regeneration is one of the forms of adaptive self-reproduction of living matter. This opinion is a Darwinian one inasmuch as regeneration is considered as a whole and as a contradiction.

The question of the essence of regeneration is not abstract, but is a concrete problem and of great importance for the solution of a number of serious theoretical and practical tasks of biology and medicine. The concepts of Weismann and Morgan do not allow determination of a correct approach to directed changes in the regenerative capacity and control of the process of regeneration. If living bodies are considered as devoid of the capacity for self-reproduction, there will be no real material basis which could and should be changed in order to change regenerative capacity. According to this Weismannian idea, the unknown basis of regeneration changes ontogenetically, while the conditions retain as a sieve only random changes. If, according to Morgan, living bodies are considered as having an original capacity for reproduction independent of changes in the external environment, the regenerative capacity also cannot be changed in a directed manner. In both cases, autogenesis of adaptation is spoken of as a secondary phenomenon arising accidentally and not in response to changes in the conditions of the external environment.

The method differs entirely if one starts from the Darwinian idea on the essence or generation. From this concept it follows that the capacity for regeneration changes in nature and under experimental conditions under the influence of changes in the conditions of existence of organisms. When the significance of the latter for development and regeneration is known, these processes can be changed in a directed manner.

Concluding the discussion of the problem of the essence of regeneration, it is necessary to point out that recently Vorontsova and Liozner (1957), Liozner (1962), and Studitskii (1959) have considerably changed their ideas and have reached conclusions similar to mine, although they continue to insist on some of their earlier positions. All these investigators now point out the incorrectness of both Weissmann's and Morgan's tenets. They also recognize now that regenerative capacity is an

original property of living matter, one of the forms of its reproduction, and at the same time a phenomenon of adaptation. Thus, the idea we have developed of the contradictory essence of regeneration has now been widely recognized.

Basis of regeneration

What is regeneration based on? This question was not posed by the old investigators. Neither Darwin, Morgan, Weismann, nor Przibram have touched upon this question. Nor was it discussed by Vorontsova (1949) or Studitskii (1948, 1951). This may be explained first by the fact that the prerequisites for posing the question were missing. The question arises in connection with the question of how the capacity for reparative regeneration can be changed in a directed manner and the lost regenerative capacity restored. This question, however, as I have already pointed out, had not been properly posed earlier and was not systematically and purposefully elaborated.

Starting from the concept that the regenerative capacity is one of the hereditarily determined properties, and heredity as a phenomenon of self-reproduction follows from metabolism, we determined the basis of any regeneration, repair or physiological, as metabolism, assimilation-dissimilation, as restoration after a forced or physiological (wear) damage, caused by accident or by conditions of existence (work, direct effect of external conditions) of living bodies (Polezhaev, 1950). Metabolism is an adaptive process which determines the capacity for self-reproduction of living matter. At the basis of life there is metabolism and adaptation. I. P. Pavlov (1913) says: "Living matter has one basic generally accepted property — to adapt, or as I prefer to say it, to equilibrate gradually with the surrounding world, i. e., in the interest of the wholeness and well-being of a given system of living matter, it enters into associations with the new conditions. In other words, it begins to respond by a given activity to earlier indifferent agents" (p. 338).

All such basic orginal properties of life as growth, multiplication, nutrition, movement, respiration, reproduction, and in particular, regeneration, follow from metabolism. Adaptation is a change of a living body in correspondence with changes in the conditions of the external environment. Only the presence of this property allows the living body to maintain itself in the face of constant, and sometimes very considerable, changes in life conditions. When life conditions change, metabolism necessarily changes also. Adaptation is an original property of living matter as is metabolism itself. Moreover, it may exist even during temporary complete cessation of metabolism for any length of time, as in the case of vitrification, for example, overcooling of a fluid without crystallization. This phenomenon is observed not only experimentally under artificial conditions, but also in nature, particularly in plants, microorganisms, and insects, and it is always characteristic in a latent form of any living bodies and in human tissues, in particular.

From the very first studies we always saw two opposed and interrelated phenomena in the process of regeneration — destruction and restoration,

and we have shown experimentally that changing, enhancing, or weakening the processes of destruction permits control of the processes of restoration of organs (Polezhaev, 1933 – 1948). Furthermore, we have shown that metabolism is at the basis of these phenomena and that, therefore, changing metabolism in a directed manner allows the researcher to control any processes of repair or physiological regeneration (Polezhaev, 1950). These assumptions concerning the basis of regeneration are in close agreement with the ideas of Pavlov (1951) who said: "The functional activity of the organs is associated with their partial destruction, as has been presently demonstrated for almost all the organs... how should one understand restoration of an organ?... schematically one may distinguish in each living organ the mechanism and the transformed material. Glandular cells as well as muscle fibers do not disintegrate massively during any work they perform; only a certain transformation of a known part of their content takes place... it is proper to consider on the one hand, restoration of certain cells anew after work, repair of the mechanism itself, and on the other hand, the return of the same working cell to its initial composition, restoration of used up material, or, in short, restoration of the mechanism and restoration of the material. Questions concerning restoration in organs of materials spent during work... will serve as a key to the solution of further and more complicated questions of the trophic process: hypertrophy, atrophy, aging of living tissues, etc." (pp. 142 – 143).

Pavlov considered metabolism as the basis of the processes of regeneration and he determined biochemically the degree of restoration of organs — the artificially stimulated submaxillary glands in cats and dogs — determining nitrogen in the released saliva according to Kjeldahl.

Studitskii (1954) and Vorontsova and Liozner (1957) now also say that metabolism is the basis of regeneration, although they still insist that regeneration should not be considered as adaptation. However, Studitskii (1954), Vorontsova (1953), and Vorontsova and Liozner (1955) assume that the basis of reparative regeneration is physiological regeneration, i.e., taking place in the process of normal activity of an organism, restoration of destroyed cellular and acellular tissue structures. These investigators tried to base their conclusion on Pavlov's idea that any pathology may develop only on the basis of a physiological norm. Pavlov's statement is true but the statement of the foregoing investigators does not follow from Pavlov's assumption. Metabolism, not physiological regeneration, is the physiological norm or the basis of any regeneration, whether it is repair or physiological. At the beginning of evolution there was only one form of self-reproduction of living bodies, i.e., physiological and reparative regeneration were identical. Subsequently, in the course of evolution, of changes in conditions of existence, of adaptive changes in metabolism, increase in complexity and differentiation of living bodies, these forms increased in number. Physiological regeneration as the one securing a constant process of renewal of each tissue and of any living particle of the body in the process of evolution, necessarily became the universal form of self-reproduction. Reparative regeneration, however, was reduced in a number of cases and disappeared, being pushed out by other more successful adaptations: an increase in complexity of differentiation and organization of living matter in the course of progressive evolution, a higher sexual productivity, e. g., in the course of regressive evolution in parasitic worms, etc. Liozner (1962) basically

accepted these assumptions concerning the origin and evolution of regeneration, pointing out that in primitive beings that appeared on earth millions of years ago, phenomena of growth, asexual multiplication, physiological and reparative regeneration, were one and the same (pp. 84–85).

In order to elicit regeneration of organs which do not regenerate under ordinary conditions, it is necessary to change its basis in a directed manner. For this purpose, according to the concepts of Vorontsova and Studitskii, it is necessary to change physiological regeneration; according to my ideas, it is necessary to change the type of metabolism of the organism and of the given organ. The aforementioned investigators studied the course of regeneration of muscles, tubular bones, ovaries, and other organs of mammals capable of regeneration, and also reactions to mechanical damage (compensatory hypertrophy) of liver, spleen, and other internal nonregenerating organs, without posing the question of changing their capacity for reparative regeneration, as the investigators are of the opinion that mammals have a high capacity for regeneration. We, on the other hand, work on the problem of loss and restoration of the regenerative capacity of organs in lower and higher animals and man in the processes of their phylogenesis and ontogenesis, and strive to cause regeneration of organs which do not regenerate under ordinary conditions, by changing the nature of metabolism. Using this approach I have succeeded in causing regeneration of nonregenerating extremities in tailless Amphibia, bones in the cranial vault, dental tissues, and cardiac muscle, and in stimulating physiological regeneration of nerve tissue in the cortex of the cerebral hemispheres in adult mammals.

Critical remarks concerning the basis of regeneration and, in particular, the assumption that physiological regeneration is not the basis for reparative regeneration (Polezhaev, 1956, 1958, 1959), evidently have been of positive value. Liozner (1960, 1962) and Studitskii (1959) in their recent communications no longer state that physiological regeneration is the basis of reparative regeneration. However, this assumption became widespread and is being maintained by some other investigators. Therefore, I must again say something concerning this problem. Following, in the chapter on regeneration of nerve tissue, this problem will be illuminated with concrete examples from experimental studies.

Thus the basis of any regeneration is metabolism. Changes in metabolism affect differently processes of reparative and physiological regeneration.

Causes of regeneration

The question of what is the reason, i. e., the factor which activates or causes the process of regeneration, is highly important both theoretically and practically. However, it is far from the clear and simple question it may seem at first. In order to establish the reason for a phenomenon, showing that a given factor is always associated in time and space with a given phenomenon, is not sufficient. The connection may not be the cause but a coincidence. The causative dependence should be proven experimentally and not simply by description or comparison. Description alone may serve at best as a basis for a hypothesis concerning the cause of a phenomenon. It suffices to remind the reader how many clever theories of gastrulation there were in embryology based on descriptive and comparative

methods; only the experiment, however, the use of the method of vital marking (Vogt, 1925), made possible the establishment of the true course of gastrulation in Amphibia, aves, and other animals. In biochemistry the idea of metabolically inert and active proteins prevailed for a long time, but direct experimentation using radioactive isotopes forced the investigators to change this notion, showing that the living organism contains no protein molecules or other substances which do not participate in metabolism. In the study of regeneration, an opinion was widely held that the reason for reparative regeneration was loss of an organ, because usually regeneration takes place after loss of an organ. It turned out, however, that the matter is much more complicated. Loss is not the reason for regeneration, but is an accompanying factor. This assumption was proven in a direct experiment by N. V. Nasonov (1930), who caused regeneration of extremities in axolotl without amputation of organs, by applying a ligature to these extremities. The original extremity was preserved and a second additional leg grew out above the ligature by regeneration. There was no loss of an organ but, nevertheless, regeneration took place. Histological study of this process showed that it was identical with true regeneration (Kazanzeff, 1930). In the area in which the ligature was applied, decomposition of tissues took place, a typical regeneration blastema formed, grew, differentiated, etc. Nasonov made the unexpected conclusion from his experiments that the reason for regeneration was not the loss of organs but the weakening of a function, since the extremities could not function after the application of the ligature because the bone under the ligature was destroyed. Nasonov's experiment was very interesting and led him to other interesting experiments, in which he attempted to cause artificially supernumerary formations in axolotls (Nasonov, 1941). He succeeded in obtaining these by placing pieces of cartilage under the skin of the extremities of axolotls. However, Nasonov's conclusion concerning the reasons for regeneration was incorrect. The function of the organ may be shut off, for example, by completely removing from the extremity of triton or axolotl the femur or humerus which do not regenerate, but accessory formations or regeneration do not occur (Wendelstadt, 1901, 1904; Weiss, 1925, and others). The reason for the regeneration in the experiments of Nasonov (1930) was not the loss of the organ's function, but severe damage caused by the application of the ligature and, in other experiments, by the introduction of cartilage under the skin. This conclusion concerning damage as the reason for regeneration of organs may be confirmed by reference to my experimental data. If the tail or extremity of triton or axolotl is amputated and the wound is sewed up with skin, regeneration does not occur (Tornier, 1906; Schaxel, 1921; Godlewski, 1928). As shown by Efimov (1931), suppression of regeneration in these organs was due to the fact that there was no epithelium on the wound surface. If the epithelized wound in the tail is sewed up with skin, there is full regeneration and perforation of the skin. In my experiments, after amputation of an extremity of axolotl, the wound surface was sewed up with skin which was removed after one month when the phenomena of damage and destruction of tissues had already disappeared. Despite the presence of epithelium and nerve in the area of the wound, there was no regeneration of the extremity and the wound healed smoothly (Polezhaev, 1933).

The role of damage in phenomena of regeneration , and development of regenerative capacity in phylogenesis has been pointed out long ago, but this problem was and remains disputable. Darwin (1868) thought that the primary capacity of living matter for self-reproduction, and in particular regeneration, was a beautiful adaptation during evolution, and he associated the elaboration of the capacity for reparative regeneration in the process of natural selection with the frequency of damage of organisms and their organs. He said: "Triton possesses this capacity to a remarkable extent; in this animal, very often other tritons bite off the extremities, the tail, eye, and maxillae." Weismann (1902) took this latter assumption from Darwin without paying attention to the fact that, according to Darwin, regeneration is, nevertheless, an original property of living matter, one of the forms of self-reproduction, and he states that the capacity for regeneration is elaborated in the course of natural selection in a secondary manner under the influence of purely mechanical damage. According to Weismann, rainworms regenerate well because they are bitten by moles and pricked by birds, but that protected, mechanically undamaged, internal organs, e.g., liver and spleen of mammals, do not regenerate. Morgan (1907), criticizing Darwin and Weismann, concludes that "the principles of natural selection cannot be invoked to explain the regenerative capacity. On the contrary, this capacity belongs to the primary properties of living matter" (p. 158). According to Morgan, damage does not explain the origin and development of regenerative capacity. This investigator bases his conclusion on some examples: in hermit-crabs which hide in the shells of mollusks, the extremities are protected from damage but they regenerate well.

In connection with the development of the experimental trend in morphology and the study of regeneration, the problem of damage as a cause of regeneration, raised by Darwin and discussed by Weismann and Morgan in an abstract manner, had been left by investigators for a long time. Later it was taken up again. Thus, Vorontsova (1949, 1953) is of the opinion that mechanical damage is not the cause of regenerative capacity which is a primary property of living matter. Studitskii (1948, 1954) criticizes the opinions of Morgan and Vorontsova, and he thinks that mechanical damage of organs is the reason of regeneration. According to Studitskii (1954), the capacity for regeneration of external organs in tritons and axolotls has developed because these animals tend to "bite off the tails and extremities of each other" (p. 37). On the same subject he writes later (Studitskii, 1955): "The property of regeneration of complex organs is a result of adaptation to the harmful effects of the surrounding world. This dependence was proven already by Darwin who pointed out that tritons, in which the property of regeneration of extremities, tails, eyes, and jaws is greatly developed, in the absence of food ordinarily damage each other greatly in all the external parts of their body" (p. 214). Vorontsova (1953) criticizes this idea of Studitskii, pointing out that he did not understand Darwin. It should be said that data from experiments and practice do not confirm Studitskii's statement. Weismann cut off the tails of mice in 19 generations; nevertheless, no regenerative capacity developed in these animals. Studitskii (1948) thinks that wolves and foxes often catch escaping rabbits by their backs and, therefore, the rabbits should readily regenerate the skin on the back. However, M. E. Aspiz, Studitskii's collaborator, could not confirm

this hypothesis in experiments on rabbit skin. The ancient custom of circumcision, prevailing in some peoples, shows that the regenerative capacity of the organ does not appear because of it. Studitskii himself (1955) correctly points out that in the case of man, skin is very often damaged. It is widely known, however, that regeneration does not occur following damage of skin on the face and body of man, and that disfiguring scars appear which must often be repaired by plastic surgery. Frequent damage of human skin did not lead to the development of regenerative capacity. The idea that frequent damage is a reason for regeneration has also been developed by A. Needham (1964), who discussed cases of regeneration in a comparative study on animals, and concluded that organs which are most frequently damaged are those which most frequently regenerate.

Vorontsova and Liozner (1957) think that reparative regeneration is related to asexual multiplication which, they conclude, brings about reparative regeneration, or that reparative regeneration is a manifestation of rudiments of asexual multiplication transformed in the course of evolution. This idea is very interesting and is similar to those of Darwin, Morgan, Kholodkovski, Ivanov, and other zoologists. In contrast to asexual multiplication, reparative regeneration is caused by an external stimulus. The notion that the reason for regeneration is damage and that regenerative capacity is elaborated as a result of frequent damage of organs is rejected by Vorontsova and Liozner who think that regeneration is an original property of living matter.

I believe that the reason for both reparative and physiological regeneration is damage, but that the regenerative capacity is elaborated under the influence of regular, prolonged, continuously recurring damages, and not of individual, random, short-lived injuries, although these may have occurred repeatedly in subsequent generations. Only such constant and regular damages could affect metabolism, change it, and lead to inheritance of the acquired property of restoration. Only such damage, necessarily connected by metabolism and environmental conditions with the basis of regeneration, could regularly and hereditarily change the regenerative capacity, and not accidental and mechanical damage as that used in the experiments of Weismann who cut off the tails of mice, or that which occurs upon circumcision or accidental damage of skin and other animal organs. Rainworms could hardly survive at all if they were pricked by birds and bitten by moles, and rabbits could hardly survive if wolves and foxes damaged the skin on their back frequently. At any rate, it is hardly probable that animals which survive such ordeals could develop regenerative capacity. Furthermore, direct experiments do not confirm the statement that they do. The alternative explanation, namely, that the high regenerative capacity of rainworms developed in connection with their living conditions in the ground because of constant rubbing and damaging of their body by the earth, by the sharp edges of sand grains and pebbles; that the high capacity for regeneration of extremities in tritons and axolotls developed historically in these animals in connection with the aquatic conditions of their existence and the high destructibility of tissues, which is the main prerequisite of regeneration, is more likely. In the case of physiological regeneration the cause is destruction of tissues due to constant work of organs, i. e., physiology. This opinion on regeneration was developed by Pavlov and we fully agree with, and accept it. This opinion also explains the origin of pathology which, according to Pavlov,

is a result of excessive work leading to deep or irreversible destruction, emaciation of organs. Thus, pathology is based on an excessive stimulation of normal physiological mechanisms, and not on coarse mechanical damage. Disease and injury are not one and the same.

Studitskii (1954, 1955) accepts Pavlov's idea only as it applies to internal organs. He assumes that the reason for regeneration of external organs is purely mechanical damage, as we just described it, while the reason for regeneration of internal organs is their functional destruction during normal activity. We assume that there is no basis for the belief that physiological regeneration of external organs is determined by other causes from those causing the regeneration of internal organs.

Summing up all the foregoing may lead to the conclusion that the capacity for physiological and reparative regeneration developed in evolution from the capacity of living bodies for self-reproduction, on the basis of metabolism under the influence of environmental conditions, in which direct effects of the conditions of the external environment and the functions of the organism, and its different organs which damaged and destroyed living matter was a long and continuous process. The reason for regeneration is damage, and is not loss or absence of an organ or the disappearance or weakening of its function.

Ways of regeneration

In his book, Morgan (1901), generalizing the available data on regeneration, introduced the idea of two basic ways of regeneration to the theory of regeneration: morphallaxis and epimorphosis. Since then these assumptions have been checked many times and confirmed by many other investigators; they are seen constantly in all major reviews and monographs on regeneration. Morphallaxis is a phenomenon, or a way of regeneration, realized by rearrangement of existing structures, organs, tissues, and systems, dedifferentiation followed by redifferentiation, neoformation of lost structures. Examples of morphallaxis are phenomena of regeneration in hydra, planaria, hydroid polyps, and other animals. Pieces of the bodies cut out of these animals rearrange themselves completely, and regenerate whole animals of smaller dimensions. Epimorphosis is regeneration by superstructure, proliferation of a regenerate from the wound surface of the residue of an organ in which the old structures were, on the whole, preserved. Examples of epimorphosis are cases of regeneration of extremities, tails, and gills in axolotl, regeneration of muscle from a muscle stump, regeneration of bone from periosteum after extirpation of the diaphysis of the tubular bone of chicken, rabbit, or rat, and many other phenomena. These phenomena may be encountered simultaneously and to different extents during regeneration of various organs or parts of the body in various animal species. In principle, however, they retain their meaning. Morphallaxis is encountered in lower animals, while epimorphosis is found in more highly developed animals.

Vorontsova (1949), Vorontsova and Liozner (1955, 1957), and Liozner (1962) suggest considering as regenerative phenomena the hypertrophy of internal organs of vertebrates after partial resection, e. g., liver, spleen,

kidneys, and lungs, calling them "regenerative hypertrophy," as mentioned in the previous chapter. We think that these phenomena are very important and must be carefully studied, but they must be considered as belonging to another category of phenomena, i. e., hypertrophy and not regeneration.

However, no matter whether or not phenomena of hypertrophy of organs are considered as regeneration, a new, previously unknown, objectively existing way of regeneration of organs by induction can now be established. This way has been established by us in the studies on restoration of lost regenerative capacity of organs in mammals (Polezhaev, 1957—1962). Its discovery has been so late because earlier investigators did not concern themselves with the problem of loss and restoration of regenerative capacity of organs of animals, and when they became interested in it later, they studied mainly extremities of vertebrates where the phenomena of regeneration by induction had not been established. We established this phenomenon in studies on induction of regeneration in the bones of the cranial arch, tissues of teeth, and cardiac muscles of mammals.

Let us first point out that in experimental morphology, induction means a qualitative morphogenetic change of some part of the organism under the formative influence of another part or of a factor from the external environment, as a result of which a new anlage of an organ or tissue is formed. In induction, an inducer and reacting material must always be present, and the result of induction is the appearance of a qualitatively new form. The phenomenon of induction occurs under certain environmental conditions (salt composition, temperature, etc.). Most frequently chemical processes lie at the basis of induction, the inducer releases chemical substances (Holtfretter, 1933, and others). In addition to phenomena of embryonic induction of organs, the feasibility of induction of tissues during the postnatal period of ontogenesis of animals may also be pointed out. Levander (1938—1956, 1964) conducted a large series of studies on adult rabbits under aseptic conditions, in which he implanted homoplastically into the subcutaneous connective tissue, pieces of tissues used as inducers: cartilage, bone, skeletal muscles, mucosa of the stomach, endometrium. The tissues were kept for 24—48 hours before implantation in a 1% aqueous solution of trypan blue, which resulted in their death; after 6—10 hours respiration stopped, followed by disappearance of cell nuclei and changes in the structure of the cytoplasm. It is characteristic that necrosis of the tissue took place without being accompanied by denaturation of proteins which occurs upon heating, treatment with alcohol, and in some other cases. The implants released substances under the influence of which a specific induction took place in the adjacent activated connective tissue. Under the influence of the appropriate aforementioned inducers, the following tissues appeared: cartilage, bone, muscles, epithelium, stomach mucosa, decidual cells of endometrium. After some time the induced tissues, with the exception of cartilage and bone, gradually decomposed and disappeared. However, the fact that there was induction, and in an adult mammal at that, is of interest: a qualitative change, transformation, it would seem, of a completely determined and differentiated connective tissue into tissues of another type.

Levander followed the ideas of Spemann (1936) on embryonic induction. His data did not arouse due attention of experimental embryologists who, in their experiments on embryonic induction, mainly in amphibian embryos,

were used to observing induction of whole systems of organs and of individual organs: chordomesoderm, the nerve tube, eyes, lens, acoustic organs, pronephros, and many others. Therefore, the data these investigators obtained concerning different tissues on induction, although obtained in an adult mammal, seemed to be almost self-evident and of little interest. Histologists, however, who do not work much on problems of causal morphology, and who use the experimental approach much less frequently than do experimental morphologists, are used mainly to descriptive and comparative methods which do not allow them to change tissues and cells experimentally in a directed manner. Histologists consider cells and tissues as stable, as incapable of extensive transformations beyond the limits of one type of tissue. Therefore histologists (Clarke LeGross, 1946) treated Levander's data with suspicion and disbelief, or passed them by without trying to repeat them. They were willing to believe A. A. Maximov (1923) who showed transformation of lymphocytes in a tissue culture, but not Levander whose data shook the foundations of histology. For unprejudiced investigators, however, these data are quite acceptable and surgeons, Peer (1955), for example, quote these studies in detail. The data on induction of tissues explain well the phenomenon of heterotropic formation of bone in animals and man, i. e., the appearance of bone and tissues of various organs in such unusual places as muscles, kidneys, heart, eye, and other areas (Pozharisskii, 1904; Al'bitskii, 1959; Fridenshtein, 1963). Evidently, in connection with hitherto unknown conditions, substances appear in these organs under the influence of which local connective tissue is transformed into bone. In favor of this assumption are the data on experimental osteogenesis upon transplantation of epithelial mucosa of the urinary bladder of mammals into muscle (Huggins, 1931; Kramarenko, 1934; Fridenshtein, 1963, and others).

In postnatal ontogenesis of Amphibia, formation of additional organs may be caused by deviation of a nerve (Locatelli, 1923; Guyénot and Schotté, 1926), or by a silk thread [sic] (Milojevic, Grbic, and Vlatkovic, 1928); by fracture and wounding of an organ (Bruchdreifachbildung, Przibram, 1921); in application of a ligature to an extremity (DellaValle, 1913); by transplantation under the skin of a piece of cartilage (Nasonov, 1941). At the site of removal of the nerve and of the silk thread, or at the site of implantation of cartilage, local tissues are destroyed and proliferate, and the formation of an organ by epimorphosis takes place.

In addition to phenomena of induction of tissues, another phenomenon has been described in adult tritons, the so-called Wolffian regeneration of lens (Colucci, 1891; Wolff, 1895). Upon removal of the lens from the eye of an adult triton, a thickening appears at the edges of the iris which separates later and is transformed into lens. This is a phenomenon of both regeneration and induction. In experimental embryology it is looked upon as a phenomenon of embryonic induction persisting and manifesting itself under special conditions in postnatal ontogenesis in adult tritons (in other species of animals this has not been observed). It has been shown experimentally that the eyes of adult tritons (Lopashov and Stroeva, 1963) preserve their capacity for induction of lens, a fact which can be demonstrated by removal of the lens and implantation of pieces of embryonic epidermis in its place. At any rate, the phenomenon of Wolffian regeneration of lens was considered by experimental biologists as induction, and induction and regeneration were

considered unrelated to each other. Other phenomena of regeneration by means of induction have not been observed. In all the reviews and monographs on regeneration, morphallaxis and epimorphosis are always described, but there has never been a description of regeneration by induction. In the 1920's a number of foreign investigators described the phenomenon of determination of "nullipotent" regeneration blastemas of extremities and tails in tritons and axolotls under the influence of a "field" (Milojević, 1924; Weiss, 1925, 1926, and others). Studies by Soviet scientists, however, have shown that even a young, nondetermined blastema is not "nullipotent," but possesses well-defined organizing potentialities (Polezhaev, 1934, 1936, 1937, 1937a, 1945) and that skin (Efimov, 1931, 1933; Polezhaev, 1935, 1936, 1937, 1940; Gol'tsman, 1939, 1940, 1947; Gintsburg, 1946, 1946a, 1947, and others), muscles (Vorontsova and Liozner, 1935; Vorontsova, 1940, 1949), and skeleton (Umanskii, 1937; Liozner, 1939) possess specific morphogenetic properties and actively participate in the formation of regenerates of organs. Thus, the unsubstantiated data of the aforementioned foreign investigators have been disproved, and a powerful blow was dealt to the idealistic field theory.

On the other hand, the experimental studies, performed in recent years on the restoration of organs and tissues in mammals which under ordinary conditions do not regenerate after amputation or damage, on the basis of the laws concerning the loss and restoration of regenerative capacity of animals, made possible the discovery of a new phenomenon in the study of regeneration, or a new way of regeneration by induction (Polezhaev, 1964, 1966).

1. In some of our experiments on restoration of the bones of the cranial vault, which usually do not regenerate in mice, rats, and dogs, we used the method of destruction. Striving to enhance phenomena of destruction and dedifferentiation of tissues, we ground fresh bone to powder and transplanted it autoplastically and homoplastically, wetting it with blood, into the area of a defect in the cranium. As a result, there was regeneration of bone in 100% of cases. The bone powder dissolved and certain substances were released which acted on the connective tissue and stimulated its transformation into bone which completely covered the damaged area of the cranium. The bone powder was an inducer, while the local connective tissue was the reacting material, and the result was regeneration by induction. Induction also occurred in the absence of periosteum in the cranium, but the presence of the dura matter was essential (Polezhaev, 1957; Polezhaev, Matveeva and Zakharova, 1957; Matveeva, 1962).

2. A similar result was obtained in experiments on the restoration of dental tissues in dogs. We bored a hole in the crown of the canine teeth to the tooth canal in an adult dog, and implanted in it a piece of connective tissue from the gums (amphodont) and a few dentine shavings. The latter played the role of the inducer stimulating the amphodont, which was the reacting material, to transform into bone. As a result regeneration of dental tissues took place by induction (Polezhaev, 1961).

3. Upon the introduction of a foreign body, a steel or gelatine needle, into the cardiac muscle of a rabbit, the muscle fibers are destroyed and granulation appears in the focus of damage, transforming into scar tissue (Oppel, 1901; Anichkov, 1912). Upon implantation of a piece of skeletal muscle, treated by Levander's (1956) method with a 1% aqueous solution of trypan blue for 48 hours, into the cardiac muscle of a rabbit, in the connective

tissue of the granulations, in the vicinity of the implant (the inducer), sectors of tissue were induced. These were approximately equal in volume to the implant and consisted of giant muscle fibers of a syncytial structure with intensely, amitotically, dividing nuclei of the embryonic type (Polezhaev, 1962).

In these three cases there was regeneration of tissues by induction in adult mammals, regeneration of tissues which do not usually regenerate under ordinary conditions of damage.

The hitherto unknown, objectively existing phenomenon or way of regeneration by induction, found in our experiments on restoration of lost capacity for reparative regeneration of organs and tissues in mammals, consists of the fact that the newly formed part of a damaged organ (bone of the cranial vault, dental tissue, cardiac muscle) arises as a result of the action of a specific inducer (bone shavings, dentine shavings, decomposing muscle, respectively) on the reacting material, which changes qualitatively under its influence (connective tissue, amphodont, granulations of myocardium) in the presence of certain external and internal conditions.

The main theoretical significance of this assumption consists of opening a new way for the regeneration of organs and tissues in animals and man. Its main practical importance is that knowledge of it allows for suggestions for working out principally new methods of restoration of a number of vitally important organs and tissues which do not normally regenerate under ordinary conditions of damage in higher animals and man. This method may be of great importance in a number of fields in biology (experimental morphology, theory of regeneration, histology, cytology, and embryology) and medicine (pathological anatomy, in particular theory of metaplasia, restorative surgery, therapy, stomatology, etc.).

Regenerative capacity

In dealing with regeneration it is necessary to distinguish between the phenomena or processes of regeneration on the one hand, and the regenerative capacity, on the other. These different aspects should not be confused or identified with each other. Between the phenomena and capacity for regeneration there is such a difference as between the categories of possibility and reality in philosophy, expressing that which is real and that which could be. If an organ regenerates after amputation, clearly the phenomenon and the capacity for regeneration are present. If, however, an organ does not regenerate after amputation, it does not yet mean that it is not capable of regeneration. For example, an extremity or a tail of axolotl is capable of regeneration but does not regenerate if the wound surfaces are sewed with skin after amputation (Tornier, 1906; Schaxel, 1921; Godlewsky, 1928, and others). In this case some conditions necessary for the realization of the process of regeneration are missing. Concretely speaking, there are no conditions available for the epithelialization of the wound surface (Efimov, 1931). A similar phenomenon, where there is no regeneration of these organs, may be observed upon denervation (Weiss, 1925; Schotté, 1926; Borsuk, 1940, and others). The question arises, what is regenerative capacity? How can one distinguish between the phenomenon of loss or absence of regenerative capacity, and the phenomenon of absence of regeneration in the presence of the capacity for it?

The phenomenon of regeneration is established in a direct experiment: an organ is amputated and the animal is kept under ordinary conditions which are not changed. However, if the conditions are changed, for example, if the wound surface is sewn with skin, the course of the process changes but the capacity for regeneration does not. If under the same conditions the organ does not regenerate after amputation, that means that its capacity for regeneration changed. For example, after amputation of the hind limbs of tadpoles of the grass frog at stage I (according to Blyakher, 1928) these organs regenerate fully, while after amputation at stage IIb they do not regenerate (Liozner, 1931; Polezhaev, 1933). That means that at stage IIb the regenerative capacity was lost. A similar statement may be made concerning regeneration and regenerative capacity of cranial bones in dog, rats, and other species of mammals, and in other cases. After removal of a piece of bone from the cranial vault of a young puppy the bone regenerates, while in an adult dog it does not. That means that with age the dog loses the capacity for regeneration of cranial bones. Consequently, if the minimum of factors necessary for regeneration exist and the process of regeneration does not take place, this means that the regenerative capacity changed, was lost. Loss of regenerative capacity is caused by a change in the state of the organ and possibly of the organism as a whole, and not by the absence of some components of the organ or of some known external conditions of regeneration (temperature, water, air, etc.).

The capacity for reparative regeneration is a sum total of external and internal conditions determining the physiological state and the capacity of a damaged or amputated organ to regenerate. The definition of regenerative capacity must include conditions of external environment, the life conditions of the animal, since regeneration is one of the phenomena of life which cannot be separated from external environmental conditions. This circumstance, as well as the fact that regeneration as one of the forms of self-reproduction of living beings is based on metabolism, and therefore always depends on the life conditions of the organism, the conditions of the environment, shows that the regenerative capacity is not something unalterable, predetermined, frozen, but something which may change a sufficiently mobile and labile property. Nevertheless, it is much more difficult to alter the regenerative capacity than the process of regeneration, because the former is endowed with great constancy and conservatism so great that until recently the majority of biologists and physicians adhered to the opinion that it is practically impossible to change it.

Thus the regenerative capacity is characterized by a contradiction: conservatism and variability, stability and lability.

Formerly, the problem of regenerative capacity seemed to be well defined: this capacity is either present or absent, or lost at a given moment of ontogenesis, in one or other animal species. Nobody doubted the fact, however, that it changes in the course of evolution or ontogenesis, although many people did not believe that it can be restored after having been lost. Later, a new direction has appeared in the study of regeneration according to which all the organs of all the animals, including mammals and man, are capable of regeneration (Vorontsova, Liozner, and Studitskii). In trying to prove this assumption some investigators began to voice the opinion that in the course of evolution and ontogenesis, not the regenerative capacity itself, but only its manifestation changes, and that the regenerative capacity may change only quantitatively, may be enhanced or weakened. The ideas

of Liozner, one of the leaders of this direction, are characteristic in this respect. In his paper "Regeneration" (1962), which appeared in the "Bol'shaya Sovetskaya Entsiklopediya", he states: "The regenerative capacity is not an absolutely determined and constant property; depending on conditions, it may be manifested in different ways" (p. 145). He repeats this in his other papers (Liozner, 1962a). He does not deny the possibility of changing the course of regeneration and obtaining it where it is absent. Moreover, he points out that this is a serious problem, but he denies the possibility of changing the regenerative capacity itself; only its manifestation changes by changes in conditions. He states: "The use of means which allow one to enhance regeneration, or to obtain it in those cases where it is absent or poorly manifested, is one of the main tasks of studies in this field" (Ibid, 1962, p. 145). In another place he says: "One must distinguish between two types of regenerative capacity: that which is manifested under ordinary conditions of amputation and that which can be detected only following additional experimental interference" (Liozner, 1962a, p. 87). "The problem of restoration of the regenerative capacity of organs, which under ordinary conditions do not manifest it, constitutes a large and important chapter in the study of regeneration. Its theoretical and practical significance is quite obvious" (pp. 87 – 88). "The expression 'restoration of regenerative capacity' is not at all precise, since the possibility of obtaining regeneration indicates that the capacity for regeneration has not been lost" (p. 88). If, for example, one succeeds experimentally under special conditions to obtain regeneration of extremities of adult frogs or toes of rats, which under ordinary conditions do not regenerate after amputation, this means, according to Liozner, that the regenerative capacity of these animals has not been lost. Somewhat later (p. 101), he develops his idea further: ". . . The absence of regeneration is a special form of its manifestation."

Thus Liozner's main idea is as follows: regenerative capacity is something unalterable, characterizing all regenerating and nonregenerating organs of all animals; only its manifestation changes depending on changes in conditions.

It is difficult to agree with this point of view: 1) it contradicts facts — if an organ, e. g., an extremity or tail of a rat, does not regenerate after amputation there is no basis to say that it is capable of regeneration; 2) the researcher's point of view is dualistic; it recognizes the importance of conditions for regeneration but it does not accept the fact that regenerative capacity depends on conditions. Thus regenerative capacity is characterized as something absolute, independent of environmental conditions; in essence this statement rejects the unity of the organism and its inherent property of autoreproduction, regeneration, and the conditions of its existence; 3) the investigator's statement identifies without proper foundation the categories of possibility and reality; restoration of something lost is an important problem of life, and a very real one; a biologist has the right to speak of true restoration of the lost regenerative capacity of organs and not only of its manifestation, as similarly, a physician has the right to speak of restoration of potency upon the disappearance of a function, for example, the sexual function (impotence). One deals with different categories — possibility and reality. Possibility, under certain conditions, may be manifested in reality but this does not mean that the two are identical, that the point is only in the manifestation of something which already exists. This is a preformistic position and, in essence, denies

development. Disappearance or loss of regenerative capacity means that in the course of normal phylogenesis or ontogenesis, or upon the appearance of a pathological condition, something has changed, an organ became something else from what it had been before. This is a facet of development, of transformation, the reasons for which are, in the final account, some changes in the evolution of external conditions of life and not the facet of manifestation of something preformed, constant, and absolute, common for all beings. If the thing which has changed will change again, that which has been will appear again, i. e., the lost regenerative capacity will be restored. From the logical point of view it is incorrect to say that two kinds of regenerative capacity or two types of possibilities exist. From the fact that, under certain experimental conditions, investigators succeeded in obtaining regeneration of organs which ordinarily are not restored, it does not follow that these organs in the given species and age of animal have the capacity for regeneration. The aforementioned discussions of ideas of various investigators are highly artificial and sophisticated, and complicate a simple and clear problem. Different interpretations of the problem should not be dealt with but a concrete solution should be found for it, namely, establishment of the regularity of loss and restoration of regenerative capacity of organs. For this purpose the first necessity is the clear and precise determination of what regenerative capacity is, including in its definition the environmental conditions, or more precisely, the conditions of existence of the animal, a thing which has not been done by the aforementioned investigators. Only in this manner can this problem really and factually be worked out and solved.

At the beginning of the process of evolution, in the first living beings which were closer to substance than to beings, the form of self-reproduction was undifferentiated. Upon changes in conditions (temperature, salt composition, pressure, etc.) they decomposed and reassembled again; they synthesized; the functions of growth, asexual multiplication, and physiological and reparative regeneration were united. Later, with differentiation of the body, differentiation into sexual and asexual multiplication appeared, dissociating into homogeneous and heterogeneous elements, the former being able to exist to some degree and synthesize a new body; the latter could not accomplish this, but when recombining again, a process which was accompanied by combination of various heterogeneous parts and not of the previous elements, the result in many cases was a more successful, progressive one. Those which were more capable than others of further synthesis, preservation, and development, survived. Survival acted here. Heredity, variation, and survival determined natural selection. At this stage began the divergence of regeneration into physiological regeneration which is characteristic of all living bodies, and reparative regeneration characteristic only of those which could develop by combination of diverse elements, i. e., by a sexual process. With the development of organs and specialization of functions, destruction of some organs was accompanied (and of others, unaccompanied) by restoration; nevertheless, such organisms survived and became perfected in those cases when the process of biological progress went along the path of control of the general viability of the organisms. Destruction was caused by the action of changing life conditions (temperature, energy, effect of chemicals and physical bodies, etc.) and of internal chemical reactions, and interrelationships between the parts of living bodies. Due to the great diversity of conditions, development went on in

different forms, the main direction being progressive, although there was also a regressive direction, development occurred at the same level in various directions. At even later stages of evolution, the capacity for reparative regeneration of organs became more and more limited due to the specialization of the organs and functions of living bodies. Naturally this capacity became more and more limited due to the development of the sexual mechanism of multiplication, and it was maintained to a greater extent with asexual multiplication. The entire process of evolution went along the path of adaptation to the changing environmental conditions, and this also happened with the capacity for regeneration. In the course of this process some forms of adaptation were continuously replaced by others, more suitable for the organisms, i. e., better facilitating their development and survival. In this form the process of evolution reached our time.

Is it possible to realize some of the biological, namely, chemical and physical, transformations of properties of organisms in our days within a short period, while in evolution the process was a very long one lasting millions of years? — Yes. For this purpose it is necessary to know what transformations and reactions have to be invoked. Theoretically, therefore, the task of controlled study of restoration of the capacity for reparative regeneration of organs, which has been lost in phylogenesis and ontogenesis, is achievable, a real and present task and not something in the distant future.

Thus: 1. The regenerative capacity is the totality of external and internal conditions determining the physiological state and the possibility of the organs to regenerate.

2. The regenerative capacity, and not its manifestation, necessarily depends on conditions and changes as a function of changes in conditions.

3. Theoretically it is quite feasible to change conditions in such a manner that the regenerative capacity may change fundamentally within several months, weeks, days, or even hours, while such changes took many millions of years in the natural course of evolution.

Conclusions

Having discussed some of the basic phenomena and notions of regeneration in the present chapter, we summarize the discussion briefly.

Regeneration is the restoration of segments of an animal body after damage or loss. It is possible to distinguish between two basic forms of regeneration: physiological and reparative. Physiological regeneration arises from wear and tear and loss of different structural (cellular and tissue) elements of the animal body. This is a universal phenomenon relating to all organs and tissues of all animal organisms, and also of plants and microorganisms. Reparative regeneration is restoration caused by trauma and damage. This phenomenon is not universal and is by no means encountered in all taxonomic groups of animals. Physiological regeneration is a diffuse phenomenon occurring without any disturbance in the normal working structure of organs, tissues, and organisms. Reparative regeneration is a local phenomenon accompanying substantial disturbances of the normal structures of organs and tissues deeply affecting the work of the organs.

Regeneration is one of the forms of self reproduction of living beings. It differs from all the other forms of self-reproduction: development,

growth, multiplication (sexual and asexual), hypertrophy, hyperplasia, and benign and malignant neoplasia having in common with the latter the fact that all these are different forms of the same basic, biological phenomenon, namely, self-reproduction. Regeneration, as well as any other form of self-reproduction of living beings, behaves according to its own rules.

Regeneration is development and, more precisely, repeated development. Regeneration of organs and tissues is realized in different ways: the former by the embryonic organogenesis type, and the latter by the tissue development type. The process of regeneration of an organ is not simply a sum of processes of regeneration of tissues or cells. It is characterized by a number of signs: destruction, dedifferentiation of tissues, changes in the relationships between tissues, release of cells from tissues, their accumulation, organization, amitotic and mitotic division, growth, differentiation, changes of metabolic indices, etc. The presence of different signs of regeneration, e. g., mitotic division of cells or growth, does not yet indicate regeneration of an organ or even a tissue.

Regenerative capacity is that which determines the capacity of the organism or tissue for regeneration. This is a totality of external and internal conditions determining the physiological state and the capacity of the organ for regeneration. The difference between regenerative capacity and regeneration is the same as that between the categories of possibility and reality. In the definition of regenerative capacity, the conditions of external environment or conditions of existence from which regeneration cannot be separated, must be included. Changes in regenerative capacity occur under the influence of changes in environmental conditions, depending on the latter. Regenerative capacity itself, and not only its manifestation, changes in this case. The idea that not the regenerative capacity itself, but only its manifestation changes during evolution, presupposes that regenerative capacity is something unalterable, absolute, and common to all forms of living beings, i. e., something preformed. This idea is wrong. Regenerative capacity developed in evolution from the capacity of living bodies for self-reproduction together with its other forms. Its development was determined by natural selection, i. e., by heredity, variation, and survival. Regenerative capacity is a contradictory phenomenon being both stable and labile, constant and variable.

The essence of regeneration is also contradictory: regeneration is one of the forms of adaptive self-reproduction of living beings, but it is also a primary property of living beings and a secondary phenomenon, being a result of adaptation of living beings to changing conditions of existence.

The basis of regeneration, both reparative and physiological, is metabolism, i. e., an adaptive process. The main property of life, metabolism, is an adaptation of the living matter to the conditions of its existence. The main properties of all living beings are metabolism and adaptation, and these properties determine self-reproduction in all its forms, and regeneration in particular. The concept that some investigators have, that the basis of reparative regeneration is physiological regeneration, is wrong. Knowledge of the essence and basis of regeneration is essential for controlling the processes of regeneration. One can change reparative regeneration in a controlled manner by changing metabolism, but one cannot change physiological regeneration.

The cause for regeneration is damage and not loss or absence of an organ or tissue or weakening or disappearance of a function. Not all kinds of damage may be a reason for the development of regenerative capacity during ontogenesis, however. Accidental, mechanical damage of the organs cannot result in the elaboration of the capacity for regeneration, as confirmed by a number of facts and observations. The reason for the development of regenerative capacity in evolution was frequent, systematic damage, essentially connected with the living conditions of a given animal. The reason for physiological regeneration is the work of organs resulting in destruction of different structures, cells, and tissues.

The means of regeneration are morphallaxis, epimorphosis, and regeneration by induction. The latter was discovered recently in a study of the regularities of loss and restoration of the regenerative capacity of animal organs in studies of restoration of the bones of the cranial vault, dental tissues and cardiac muscle of mammals which do not regenerate normally. Through this means of regeneration, the lost part of an organ is restored by typical embryonic induction with an inducer acting chemically (bone powder, dentine fillings, decomposing cardiac muscle), a reacting material (connective tissue, amphodont, granulation in the myocardium), which changes qualitatively under the influence of the inducer; the result of induction is regeneration of the cranial bone, of the tissues of the tooth, and of the cardiac muscle. This phenomenon is of practical interest and not only of theoretical interest, in that it allows for suggesting and elaborating new biological methods for the restoration of organs in mammals, and probably in man, which do not normally regenerate under ordinary conditions.

Chapter IV

FUNDAMENTAL RULES IN THE STUDY OF REGENERATION

As mentioned earlier, biological studies of the problem of regeneration had already begun in the beginning and middle of the 18th century by Reaumur and Trumble. Medical observations of regeneration, however, were made also in ancient times. The data accumulated since then have been the basis for 3 major generalizations, the first 2 of which were made about 100 years ago by Charles Darwin: 1) the capacity for reparative regeneration and the level of organization of animals are inversely related; 2) the younger the organism, or the lower the degree of differentiation of its tissues and organs, the higher the capacity for regeneration; 3) during regeneration the tissues of the organs preserve their typical specificity and do not transform into each other. These generalizations, expressed as rules and not as laws, are still valid and, on the whole, they are accepted by the majority of biologists and pathologists. They cannot, however, satisfy completely the requirements of a number of scientists, and they have been repeatedly subjected to criticism and revision (Astrakhan, 1929; Ivanov, 1937; Veitsman, 1934, 1938; Studitskii, 1948, 1951, 1954, 1959; Vorontsova, 1949, 1953; Vorontsova and Liozner, 1955, 1957; Liozner, 1960, 1962; Polezhaev, 1950, 1956, 1958, 1959, and others). Some of the critics deny these rules entirely, while others approach them differently. We merely point out that, in principle, criticism developed because these rules have numerous exceptions which undermine their force and meaning, and that they are methodologically inconsistent and, according to some critics, fictitious or speculative. In order to elucidate how matters really are, it is necessary to analyze each rule and the corresponding criticism.

1. **The first rule of regeneration** was first formulated by Charles Darwin (1868) who said: "Animals which are at a low level may, according to the general rule, reproduce removed parts much better than do highly organized animals... In higher vertebrates, e. g., birds and mammals, this capacity is limited to a great extent." Here Darwin makes the following critical remarks: "However, a few strange exceptions to this rule exist: thus, for example, nemertean worms, as people say, show little capacity for restoration although they are of low organization."

In the wake of Darwin, this rule was accepted by the majority of zoologists and pathologists and entered into all, or almost all, textbooks on biology, zoology, and pathological anatomy. It was accepted by Weismann (1902) who also pointed out a number of exceptions to the rule, and said that "neither the level of organization nor kinship alone can solve the intensity of the force of regeneration" (p. 13). M. A. Kholodkovskii (1933) in his

"Textbook of Zoology," criticizes Weismann's germ plasm theory, saying: "The capacity for asexual multiplication is in close association with the capacity for regeneration, i. e., for restoration of lost parts of the body. Both these capacities are most strongly developed in lower animals. The higher the organization the more limited is the capacity for regeneration and for asexual multiplication." The well-known Soviet embryologist, P. P. Ivanov (1937) says: "The more simply organized animals are capable of regenerating larger parts and more diversified complexes of organs than are animals with higher organization." In addition, he points out that one cannot say that "all the simpler organized animals are capable of regeneration, while animals with a more complex organization are less capable or not at all capable of regeneration." The prominent Danish biologist A. Fischer (1941) says: "While lower animals are capable of regenerating large and complex parts, this property is very limited in higher animals." Professor M. M. Zavadovskii (1931) says: "With the increase in complexity of organization, the regenerative capacity of organs decreases and reaches almost zero level in the so-called higher vertebrates. . . " At the same time, however, he makes the following reservation: "The statement that regenerative capacity is inversely proportional to the level of organization is acceptable only in the most general form. Upon detailed study, one may detect many exceptions to this rule" (p. 227). Academician A. I. Abrikosov (1949) says in his textbook: "The less perfect the organization of the animal, the simpler its structure, the higher is its capacity for regeneration; conversely, the higher the development of the animal and the more complex its organization, the less are its tissues capable of regeneration" (p. 318). In the general paper "Regeneration," published in the British Encyclopaedia, the well known experimental morphologist, Hamburger (1961), says: "Lower organisms have a high capacity for regeneration and this capacity diminishes with increase in the complexity of organization. Mammals, including man, with the acquisition of high organization, almost completely lost regenerative capacity" (pp. 4—5).

The first detailed criticism of all 3 rules of regeneration, and particularly of the first and most important rule, was given in the monograph by V. I. Astrakhan (1929). He points out that there are numerous exceptions to this rule which, in his opinion, make it inconsistent. Certain species of animals belonging to one order or class differ greatly in their regenerative capacity. For example, among the various species of nemerteans and ascidia some have a high capacity, while others have a low capacity for regeneration There are systematic groups of animals with high regenerative capacity, and there are other groups with a much lower organization, but with a much lower capacity for regeneration. For example, lancelets regenerate much more poorly than do the considerably higher organized tritons. Thus Astrakhan (1929) says: "There is no direct relationship between the level of organization and regenerative capacity" (p. 13). He came out very strongly against the third rule of regeneration, in particular, pointing out the possibility of extensive metaplasia, although he criticized all 3 rules. Although his criticism was not ignored by specialists, it did not achieve wide recognition.

In the opinion of Studitskii (1948, 1951, 1954, 1959) and Vorontsova (1949, 1953, and others), the Darwinian rule concerning the decrease in the regenerative capacity with increase in level of organization of an animal

is based on incorrect facts and constitutes a methodological error, a "Weismannism," in the study of regeneration, disarming physicians in their practical activity, and becoming widespread and well known in biology and medicine in our country in recent years. Vorontsova (1953) says: "This conclusion can be reached only on the basis of wrong treatment of the facts accumulated by science. This material clearly demonstrates that the capacity for regeneration, both physiological and reparative, is a universal phenomenon characteristic of all animals and also of all plants" (p. 17). Studitskii (1954) says: "Comparative studies of some restorative processes in lower and higher vertebrates undertaken in our laboratory, beginning from 1947, convinced us of the falsity of the widely accepted concept that the restorative properties of organisms decrease in the course of their phylogenetic development" (Studitskii, 1954, p. 14). "The level of the restorative reaction in response to damaging factors in higher animals not only does not decrease but, on the contrary, increases as compared with lower animals, and is in complete accord with the high level of metabolism and the metabolic energy of higher animals" (Studitskii, 1954, p. 37).

Summarizing prolonged discussions of this problem, and introducing a number of corrections in his ideas and the ideas of a number of other investigators, Liozner (1962) comes to the following quite pessimistic conclusion: "We finally reached the quite sad conclusion that, at present, one cannot find any rule which governs the evolution of regenerative capacity in animals" (p. 108).

Is this really so? How do matters stand in reality?

First of all, why are the facts on which the first rule of regeneration is based, and their treatment, wrong? There were two reasons to conclude that these facts were wrong: 1) The exceptions to the rule; 2) the contradiction between the new and old data. Let us analyze these two points separately.

Vorontsova (1949, 1953) and Studitskii (1954, 1959) brought forward a great number of exceptions to the rule of regeneration being discussed. However, does this mean that the rule loses its validity? Exceptions show that the evolutionary regularity of regeneration cannot be unequivocally expressed by this rule; nevertheless, it exists as do the facts which are the basis of the rule. The fact remains that an intact organism may be restored from a part only in lower animals: Infusoria, Hydra, sea-urchins, ascidians, and mainly in those which are capable of asexual multiplication, but not in arthropods or vertebrates. The latter may regenerate only whole organs or their parts. The fact remains that in axolotls, tritons, and Salamandra, extremities and tails regenerate, while they do not regenerate in rats and mice. Exceptions do not disprove the rule but show that it may not be absolute, that its value is relative, and that more general rules in phenomena of regeneration must be sought.

We have already pointed out earlier (Polezhaev, 1958, 1959) that, together with A. N. Severtsov (1934), we can say that the main direction in evolution is progress, increasing complexity of organization, and differentiation of organs and tissues. Development, however, may also be regressive, e. g., in parasitism (flatworms, etc.), or in secondary reversal of animals to previous conditions of existence, e. g., in ascidians. The capacity for reparative regeneration which is associated with asexual multiplication will change in different ways in these cases. In progress, increasing complexity of organization in the course of evolution, the capacity for reparative regeneration will, as a rule, become limited. In regression, decrease in complexity of

organization, it may develop strongly as in ascidians, or become limited and be replaced by another more convenient adaptation, an enhanced sexual reproduction characteristic of the majority of parasitic worms. In continuing to develop these ideas Liozner (1962) points out the significance of Severtsov's so-called idioadaptations, i. e., evolution proceeding along different paths but at the same level, when the organization of the animals differs but the level is the same. In idioadaptation the regenerative capacity will change in different manners, depending on changes in the conditions of existence of the given animal species. It can be said that evolution of animal organisms (as well as plants and microorganisms) is realized in different ways, depending on changes in the conditions of their existence. In this connection the capacity for self-reproduction of the animal also changes, particularly that of reparative regeneration. The regularity of the evolution of the latter will be determined largely by the dependence of regeneration on the conditions of the animals' existence. This general principle also explains the well known general regularity of changes in regenerative capacity expressed by the first Darwinian rule of regeneration, and the diversity of changes in this capacity in the animal world, the exceptions to this rule. The Darwinian rule relates mainly to cases of progressive evolution where there is an increase in complexity of organization of the animal. The exceptions to this rule of regeneration relate to other paths of evolution (regression, idioadaptation). The conclusion is reached, therefore, that we cannot be satisfied by the first rule of regeneration but we must not reject it. Both the rule and the exceptions to it are true. A general regularity in the evolution of regenerative capacity exists, however, and it is expressed in a very definitive way (see above).

The adherents of the idea that the first rule of regeneration does not agree with the true facts and that it lost its meaning, as for instance, Liozner (1962), point out that the decisive basis for this conclusion are not the exceptions to the rule which can be explained by the diversity of paths of evolution, but those phenomena which directly contradict it. Therefore, these phenomena shall now be discussed.

Studitskii (1954) considers as one such phenomenon the regeneration of tubular bones, skeletal muscles, skin, peripheral nerves, and liver, while Vorontsova (1953) and Liozner (1960, 1962) consider the regeneration of internal organs which, according to their data, regenerate better in mammals than in Amphibia. Careful analysis of these phenomena shows that the investigators' conclusions are based on a misunderstanding. Studitskii (1954) concludes, on the basis of the following experiments, that the skeleton in birds and mammals regenerates better than in axolotls. He removed the diaphysis of the humerus of a chick or rat, leaving intact the periosteum and epiphyses, thus repeating the old experiments on puppies and calves (Heine, 1834—1836) and the clinical data on man (Pirogov, 1866; Bockenheimer, 1905; Berlatskii, 1917; Blacklock and Rankin, 1935; Bruskin, 1945)* and he removed the entire humerus of axolotls together with the periosteum and the epiphyses, repeating the well known experiments of Wendelstadt (1901, 1904). As could have been expected in the former case, the bone regenerated from the periosteum, while in the second case where there was no periosteum, there was no regeneration. The conditions in these two experiments differed greatly, however; in one case there was a source

* See review by A.G. Babaeva (1960).

of bone regeneration (periosteum), while there was none in the other. If the experiments are done in similar conditions, however, complete removal of a part of the humerus makes it regenerate rapidly and fully in the axolotl, while it does not regenerate in birds and mammals. The same may be said also of skeletal muscles which regenerate much better in axolotl than in mammals. In young mice, rats, and rabbits (rodents) they regenerate, while in dog and man they do not. Liozner (1960) concludes, for good reasons, that one cannot agree with Studitskii (1954) that the processes of regeneration in mammals are more favorable than in Amphibia. He points out that skin, bones, muscles, extremities, and tails of Amphibia regenerate better than in mammals (pp. 360—361). The data of R. P. Zhenevskaya (1954), a co-worker of Studitskii, showing that in birds, and even in rats, the liver can regenerate from the wound surface, could not be repeated, and turned out to be wrong (Vorontsova and Liozner, 1955, 1957; Liozner, 1960, 1962). Liozner (1960), therefore, is correct in saying: "In mammals we do not observe growth of removed parts of liver from the wound surface" (p. 361). According to T. I. Zelikina (1954), another of Studitskii's co-workers, peripheral nerves in rats regenerate more rapidly than in frogs. In rats, however, the process occurs at a high temperature, while it occurs at a low temperature in frogs, a fact which greatly influences the rate of regeneration. On the whole, the vast amount of data show beyond any doubt that both the peripheral and central nervous system of higher vertebrates regenerate incomparably less well than in lower vertebrates (Wendell, 1959). As far as internal organs are concerned (liver, spleen, kidney, lungs, etc.), it has already been shown (Chapter 3, part 1) that after amputation these organs do not regenerate but undergo hypertrophy, and that processes of regeneration and hypertrophy obey different rules: the rules of development and the rules of growth. Therefore, the fact that hypertrophy of internal organs occurs in warm-blooded animals more rapidly than in cold-blooded vertebrates, as pointed out by Korschelt (1927) and Studitskii (1954), in no way speaks against Darwin's first rule of regeneration.

The great merit of the work of Vorontsova, Liozner, and Studitskii was the fact that their studies drew the attention of numerous investigators to work on regeneration, and to the very important problem of hypertrophy of organs in mammals, and that they demonstrated a somewhat higher capacity for regeneration of organs and tissues in mammals than had been supposed earlier, reducing it to healing and scar formation on wounds. However, the basic factual material accumulated by hundreds and thousands of investigators for decades and centuries, and which served as the basis for the foregoing rule of regeneration, remains undeniable. It is not wrong but right. The data of the preceding investigators force us to broaden our ideas on the regenerative capacity of higher animals and man, and to this extent we agree with Liozner (1957, 1962); these data do not contradict in any way the fact that in these animals regeneration of the majority of organs is poorer than in such lower animals as axolotls, Hydra, and the like.

Vorontsova (1953) says: "Regeneration may occur in higher vertebrates, and in order to obtain it one needs only to create favorable conditions" (p. 109). This is double talk. Such a formulation and the word "only" give the impression that regeneration in higher animals is easy and simple. The problem of restoration of lost regenerative capacity in phylogenesis and ontogenesis, however, is a most difficult and complicated one, and its elaboration constitutes a special trend in the study of regeneration.

From the fact that under certain experimental conditions investigators sometimes succeed in obtaining regeneration of organs which usually do not regenerate, Vorontsova concludes that these animals possess regenerative capacity. Liozner (1962) says: "The fact that one succeeds in causing regeneration of an extremity in frogs after experimental treatment with a solution of table salt, contraindicates the idea of weakening of regenerative capacity with increase in organization" (p. 105). These conclusions, however, contradict facts. The assumption of Studitskii (1952, p. 36) that "the higher (in the evolutionary sense — L. P.) the animal, the higher the level of self-renewal of its tissues, and that means also the level of the restorative reaction to damage," has not been proven, and also contradicts facts. It is not true that higher animals regenerate better than lower; that metabolism in higher animals (e.g., in man) is higher than in lower animals (e.g., in mice); nor is it true that the extent of regeneration depends on the level of metabolism. The degree of regenerative capacity depends mainly on molecular and structural capacities of cells, tissues, and organs, but not on the level of metabolism under conditions of normal life activity of the organism. The level of metabolism in Hydra, Planaria, and axolotls is not higher than in man, but the degree of their regenerative capacity is much higher than that of man.

What then is the methodological error, the "Weismannism" so to say, of this rule of regeneration we discussed concerning the decrease of capacity for reparative regeneration with increase in organization of the animal? It is characteristic that neither Vorontsova nor Studitskii, who severely criticized this rule, nowhere points out what is wrong with it methodologically. They always show that this rule belongs to Weismann, but we have already seen that this is wrong. This rule was first formulated by Darwin (1868), and Weismann (1899, 1902) only repeated it, as did many other zoologists in the wake of Darwin. Darwin made some errors but he cannot be called a "Weismannist." Nor is there any "Weismannism" in the statement that some species of animals regenerate organs better than others. These facts have not been contested by anybody. Thus, for instance, Liozner (1962) says that even "... animal species close to each other may possess different regenerative capacities" (p. 110). Why then is the statement that hydra and axolotls regenerate better than man, a "Weismannism," idealism and metaphysics, while the statement that man regenerates better than hydra or axolotls, is materialism? Is it then correct to call this Darwinian rule of regeneration reactionary, because according to this rule man has a lower capacity for reparative regeneration of organs? It remains a fact that man cannot regenerate an extremity, while tritons can, or that sponges and Hydra regenerate even after they are ground through a sieve, while a mouse is not capable of this feat. There is nothing bad or reactionary in the fact that man's capacity for regeneration is lower than that of tritons, axolotls, and other lower animals. Evolution of man went along another path. Man became the crown of nature because the nervous system and brain developed to a much greater extent, although during the millions of years of his evolutionary development he did not acquire the capacity to regenerate a hand, kidney, or even a finger. In the process of evolution there is a continuous change in adaptations but not an increase of one on top of the other. Acquiring one evolutionary adaptation, organisms lose another one which used to be excellent in the past. This is a law of nature and there is nothing, and can be nothing, bad in it.

Vorontsova and Studitskii want to uproot "Weismannism" from the theory of regeneration starting from the erroneous alternative of Weismann on the essence of regeneration (see Chapter 3).

There is "Weismannism" in the study of regeneration; it does not consist at all of the notorious first rule of Darwin, however, but of the autogenetic notion of life, development, and heredity, which are supposedly independent of conditions of the external environment, the conditions of existence of organisms; in not understanding the fact that the basic property of life, metabolism, is adaptation; that adaptation is a primary property of living matter; in the inability to understand the contradictory nature of regeneration as one of the adaptive forms of self-reproduction of living beings; in the misunderstanding of the fact that regenerative capacity is not something frozen and not dependent on conditions, but that it is a changing phenomenon always depending on environmental conditions, the conditions of life. It is clear, therefore, that regenerative capacity can always be changed in a controlled manner, even in man, despite the fact that it may be conservative. The statement that regenerative capacity is conservative or very low in these or other organisms, and in particular in man, is not what is reactionary and what disarms investigators, physicians, and biologists, but the idea that it cannot be changed in a directed manner is the one which is reactionary and disarming.

After discussing the problem and the critical remarks concerning the foregoing rules of regeneration, the authors of these remarks greatly changed their positions. Studitskii (1959) no longer says that tubular bones of mammals regenerate better than in Amphibia.* Liozner (1962) says: "The level of organization (as well as its other characteristics) affect regenerative capacity... there is a level of organization incompatible with the capacity of the animals for certain types of regeneration, for example, for restoration of a whole organism from a body fragment... such capacity is observed only in animals with a relatively low level of organization... the level of organization is reflected in the forms of the regenerative process, since increasing complexity of organization diminishes the morphogenetic plasticity of organisms" (p. 109). But even here he tries to maintain, to some extent, the position which he and Vorontsova so valiantly defended earlier, and he says: "The weakening of regenerative capacity is not a general regularity..., one cannot say that in highly organized animals the regenerative capacity must necessarily disappear" (p. 110). Nevertheless, in another place he admits: "Regenerative capacity of mammals is, in a number of respects, inferior to that of some vertebrates with low organization without even mentioning that of invertebrates" (Liozner, 1961, p. 361).

Now, neither Studitskii, Vorontsova, Liozner (1957), nor Liozner (1960, 1962) says that the first Darwinian rule of regeneration is a "Weismannism," and that it is reactionary. Now even they point out that this rule was formulated by Darwin; nevertheless, they continue somehow to associate it with the name of Weismann, although they point out that the latter was not very much interested in regeneration. In other words, these investigators admit the error of their previous positions.

* Recently, however, Studitskii (1966) repeated again his previous conclusions, claiming that tubular bones of rats regenerate better than in axolotl.

To summarize what has been said in the present chapter, the first Darwinian rule of regeneration, that regenerative capacity decreases with increase in the level of organization, is not fiction, is not false, but reflects a regularity of some objectively existing phenomena of regeneration. There are no data indicating an opposed phenomenon, an increase in capacity for reparative regeneration of organs with increase in complexity of organization of animals. Among such phenomena some investigators erroneously consider phenomena of hypertrophy of liver, kidneys, and other internal organs, which do not represent regeneration (repeated development) but its absence and growth of organs under conditions of damage.

There is no "Weismannism" or any methodological error in the aforementioned first rule of regeneration. It represents a relative, and not an absolute truth, and it will necessarily have to change with the development of our knowledge of natural sciences.

The foregoing rule of regeneration is related to the main trend of evolution, progress, increase in complexity of organization; the exceptions to this rule belong to other paths of evolution (regression, idioadaptation of Severtsov). The general regularity of changes in regenerative capacity in the process of evolution may be formulated as follows: in the process of evolution and in a comparative series of animals, the capacity for reparative regeneration changes regularly, depending on its regular adaptive change in response to changing conditions of existence; as a rule, it decreases with increase in complexity of organization of the animal; in other cases, it changes in relation to other forms of adaptation.

2. **The second rule of regeneration** also was formulated by Darwin (1868), who said: "The capacity for restoration is much stronger in young animals, or at early stages of their development, than in the adult age." This rule also has been accepted by the majority of zoologists and entered into all the textbooks and handbooks on biology, embryology, histology, and pathological anatomy. Thus Zavadovskii (1931) writes in his book: "As a rule, the younger the organism the easier it regenerates lost parts" (pp. 228–229). The pathologist-anatomist, Academician Abrikosov (1949), writes in his textbook: "The higher the development of the tissue the more complex its organization and differentiation, the more highly specialized its function, the lower the capacity of the tissue for regeneration, and conversely, the less complex a tissue is constructed and differentiated, the higher the degree of its regenerative manifestations" (p. 318). This rule acquired a practical significance in medicine. Physicians always think that healing of wounds and bone fractures in children is much better than in adults, and particularly in old people. Furthermore in medicine, for therapeutic purposes, in order to accelerate processes of regeneration, one uses embryonic extracts, ointments, and other preparations. This rule, however, also has exceptions. For example, in ascidians the larvae do not regenerate, while the adult forms regenerate beautifully. In Planaria the larvae and young individuals regenerate more poorly than do adults. In tadpoles of frogs and toads, and in adult caudate Amphibia, the tails regenerate beautifully, while in embryos of these species the tail anlage does not regenerate. On the basis of these exceptions to the rule, Vorontsova (1949) states that the change in the rule. Astrakhan (1929) concludes that the second rule of regeneration regularity. Astrakhan (1929) concludes that the second rule of regeneration "is an arbitrary reflection of the first rule", and is not sufficiently well founded (p. 15). Studitskii (1954) calls the phenomenon of decrease in

regenerative capacity with increase of differentiation of tissues during ontogenesis, an "imaginary rule" and he concludes that this rule also "represents a variant of the idioplasm concept" (p. 13).

Analyzing the second rule of regeneration and impartially evaluating the available facts, should lead to saying that a change (mainly reduction) of regenerative capacity during ontogenesis does occur objectively and is not a fictitious rule, but that this characteristic is not similar in different animal species. There is no reason to doubt the facts which serve as the basis of this second rule of regeneration, or the facts which are exceptions to the rule. Both the rule and the exceptions must be explained by a general regularity of ontogenetic change in the regenerative capacity of animals.

First it can be pointed out that the second rule of regeneration is very closely connected with the first, inasmuch as the phenomena which they reflect are closely connected with each other. In the process of ontogenesis, or with age, as well as in the process of phylogenesis, organization increases in complexity, as a rule, and there is an increase in the level of differentiation of organs and tissues. This process of ontogenetic change, according to the biogenetic law, repeats in its main features the phylogenetic changes of the animals of the given species. Naturally, the same characteristics of the phylogenetic changes in regenerative capacity are also repeated in ontogenesis. As a rule, in ontogenesis, or with increasing age of the animal, as in phylogenesis, there is a decrease in regenerative capacity of the organs. It may be said that this is the way in which regenerative capacity changes in the case of progressive evolution. In the case of regressive evolution, as well as in idioadaptation, the adaptation of animals to their life conditions is not achieved by increase in complexity of organization and increase in the level of differentiation, but is reached by another path, and correspondingly, regenerative capacity also changes in a different manner. These phenomena, however, also obey the biogenetic law. To consider several examples: in progressive evolution, transition of the tailless Amphibia from aquatic conditions of life to terrestrial conditions, brought an increase in the complexity of their organization and a decrease in, or loss of, the capacity for reparative regeneration of the extremities, tail, and other organs. This progress can be observed in a comparative series of this order of animals: Bombina and Xenopus, which spend the major part of their lives in water, have a more primitive organization than the grass-frogs or toads which spend a considerable part of their lives on land. Accordingly, the regenerative capacity of the extremities in Bombina and Xenopus is higher than in the grass frog or toad. A similar picture can be observed in the ontogenesis of these animals. Decrease or loss of capacity for regeneration of extremities in the former occurs at later stages of metamorphosis than in the latter. In the case of regressive evolution of the tunicates, e. g., in the ascidian Clavellina lepadiformis and others, larvae and adult forms live in water, but their life pattern changes: the larvae are free-swimming forms, while the adults are sedentary. In the larvae the organization and level of differentiation of tissues is much higher than in the adult forms. The larva has a head and a tail, a cord, spinal column, metameric muscles. In the adult forms these organs are reduced and disappear during metamorphosis, and their organization becomes secondarily simplified. In larvae the capacity for reparative regeneration is very limited and low, while in the adult forms it is high; a part of

their body can regenerate into an entire individual. Upon changes in life conditions, upon transition from a free-swimming to a sedentary form of life, the organization of the body of ascidians changes regressively and their capacity for reparative regeneration increases. In another case of regressive evolution, e. g., in parasitic worms (flatworms and roundworms), the organization of the animals also becomes simpler, but as a more convenient adaptation for the species. What develops is not a capacity for reparative regeneration, but another form of self-reproduction, namely sexual productivity. As is well known, the latter is extremely high in these animals. The reasons for the temporary loss of the capacity for regeneration of the caudal bud in amphibia have not yet been elucidated. Probably this loss is due to some changes in the life conditions of the organism, but what these changes are is still difficult to say. The hypothesis of Svetlov (1934), that the intensity of morphogenetic processes at a given moment of ontogenetic development of the caudal anlage is the reason, has not yet been confirmed by facts or objective qualitative data. However, the fact that not all cases may reveal immediately an explanation for certain phenomena which are exceptions to this second rule of regeneration, does not contradict the existence of some general regularity in all these cases.

The reasons for ontogenetic changes in the development of animals cannot always be established directly. It is more correct to approach them from the position of phylogenesis, using the comparative method and the biogenetic law. In this case it can be said quite definitely that the main reason for ontogenetic changes, including changes in regenerative capacity of organs, in the metamorphosis of tailless Amphibia, is their passage from aquatic conditions of life to terrestrial conditions. In the case of ascidians, the reason for the changes in the organization of the animals and in the regenerative capacity is the transition of individuals from swimming to a sedentary form of life. The reason for the changes in their regenerative capacity is the change in life conditions. This statement encompasses and explains all the cases of ontogenetic changes in regenerative capacity of organs, both decrease and increase, or temporary loss during ontogenesis. Incidentally, attention should be turned to one other circumstance. In the process of ontogenesis there is a continuous adaptational change in the organism in response to conditions, and in the case of a progressive direction of evolution, the structures and functions of the animal increase in complexity, and the animal becomes more and more independent (this does not occur in parasitism, although adaptation may be quite extensive and deep), but because of this it never becomes less dependent on environmental conditions. Any living being, at any stage of life and at any stage of its development, will always depend on the living conditions. Therefore, I can in no way agree with those investigators who reach the conclusion that, as a result of adaptation and improvement in the course of historical and individual development, organisms become more and more emancipated and more and more independent of living conditions. If this were so, evolutionary development of the animal would stop and life would cease. The principle of unity of organism and environment, or conditions of its life, is universal and is true of any organism at any time of its development.

Conclusion. In the process of ontogenesis which repeats the phylogenesis of the given species (genus), the regenerative capacity of organs changes regularly depending on the changes in the living conditions of the animals.

As a rule, in the case of progressive evolution, there is a decrease in the capacity for regeneration of organs with age.

3. **The third law of regeneration**, or as it is often incorrectly called, the law of specific productivity of tissues, was formulated by histologists, probably for the first time by Kölliker, then by Waldyer, and spread rapidly among pathohistologists and zoologists. According to this rule each tissue forms one like itself during regeneration: epithelium forms epithelium, muscles form muscles, nerves form nerves, connective tissue forms connective tissue. Transformation of one type of tissue into another does not occur during regeneration as in embryogenesis, in which development of organs and tissues is determined by the specificity of the germinal layers.

First it must be pointed out that this rule cannot be called a law because it has a number of exceptions, and is the subject of heated discussions both among biologists and in particular among pathologists, who know very well the phenomenon of metaplasia, i. e., transformation of one kind or type of tissue into another. Proof for metaplasia was supplied 70—80 years ago by zoologists and, even earlier, by pathohistologists. In the 1920's a severe blow was dealt to the notion of the specificity of germinal layers by experimental embryologists (Spemann, 1936; Mangold, 1923; Holtfreter, 1933, 1933a, and others), who have shown the possibility of transformation of one germinal layer into another. Nevertheless, probably in connection with the well known dissociation of studies in the field of embryology, histology, and pathology, and the investigators' lack of knowledge of the basic meaning of facts established by experimental embryology, the phenomenon of metaplasia is still vigorously disputed by a number of histologists. Some histologists make attempts to deny even the hard established facts in favor of old ideas claiming that the type of tissue does not change. In this connection, at least some of the basic facts relating to this problem must be considered. The essence of the question is whether, under certain conditions, one can change the kind or type of a given tissue, e. g., transform connective tissue into bone or muscle, muscle into connective tissue. The answer to this question requires precise data which would allow for solution of this problem in a clear-cut manner, either positively or negatively. Its solution is of great theoretical and practical importance. Because a vast quantity of published material has been devoted to the possibility of metaplasia, this part of the present chapter discusses only some of the most pertinent facts.

In his first review on biological studies in the field of regeneration, Morgan (1901) stated: "In my opinion a single clear-cut exception is sufficient to shake the traditional dogma on germinal layers to its foundation; together with it falls the basic hypothesis that like is always formed from like during regeneration. The time has come to turn to a better theory concerning the effect of an old tissue on the appearance of a new one." At present a great amount of data have accumulated which are clear exceptions to the old traditional ideas on the specificity of germinal layers, and the specific productivity during regeneration in lower and higher animals. New data and theories appeared which allowed establishment of a quite definite regularity in the appearance of tissues, unlike those from which they originate, during embryogenesis and in the postnatal period.

It has been established that after removal of the lens from the eye of an adult triton, a lens regenerates from the upper edge of the iris (Colucci, 1891; Wolff, 1895). The edge of the iris is depigmented, thickens, the

thickening separates and turns into a pouch and a well formed lens. In normal embryogenesis the lens appears from the ectodermal epithelium, while in this experiment of so-called Wolffian regeneration of lens, it is formed from the iris which is an ectodermal tissue.

K. N. Davydov (1910) removed the anterior prebuccal segment of the body or proboscis of nemerteans, completely devoid of an ectodermal gut. From the proboscis an entire individual was regenerated, the gut of which appeared from mesodermal and not from ectodermal tissues.

Nussbaum and Oxner (1910—1913), studying regeneration in other species of nemerteans, showed that the brain ganglion formed from the tegumentary epithelium, i. e., nerve tissue, mesenchymal, and muscle cells.

According to Nussbaum (1908) and Ivanov (1912), musculature and the nerve system formed from the ectodermal epithelium during regeneration in parakeets. From the rudiments of the old nerve system, only few nerve fibers appeared, and from the rudiments of old muscles, only few myoblasts.

During regeneration of Oligochaeta the circular muscles appeared from the ectodermal epithelium (Rabes, 1902; Weitzmann, 1927, and others).

It has been shown experimentally that in Hydra the entoderm cells may transform into cells of ectoderm (Haynes and Burnett, 1963).

Similar examples of metaplasia during regeneration of organs in invertebrate animals can be quoted in great numbers. In this connection a number of Soviet and non-Soviet zoologists admitted the existence of a phenomenon of metaplasia in lower, invertebrate animals. Nevertheless, they expressed doubts as to whether it occurs in vertebrates and man, although they cannot deny the fact confirmed many times by numerous investigators of Wolffian regeneration of lens in tritons. Liozner (1962) states that in invertebrates "some cells are capable of metaplasia. However... this phenomenon is not very widespread... in mammals no transformation of cells of one tissue into cells of another has been described" (p. 81). Investigators willingly admit transformation of cells within one type of tissue, e. g., transformation of cylindrical epithelium into flat epithelium (Golovin, 1958), or immature connective tissue into cartilage or bone. The latter may be illustrated by the phenomenon of heterotopic formation of bone, i. e., cases in which bone appears in an unusual spot outside the skeleton-forming periosteum. The bone appears in skeletal muscles, in heart, kidneys, inside the eye, liver, in the brain, i. e., in all organs of man and mammals (Pozharisskii, 1904; Al'bitskii, 1959). There is reason to believe that in at least some cases of heterotopic development of bone, the bone is formed by induction. Under the influence of autoplastic transplantation of a piece of mucosa from the urinary bladder into the smooth muscle of the stomach in dogs, bone is induced from the local connective tissue (Huggins, 1931, and others). This phenomenon has been studied by histochemical methods (Fridenshtein, 1963).

Direct proof of transformation of loose connective tissue into cartilage was given in experiments with tissue cultures outside the body. When a culture was made of fibroblasts from the heart of a chick embryo together with a piece of hyaline cartilage, it transformed into hyaline cartilage (Fischer, 1931). Nasonov (1934) cultivated subcutaneous loose connective tissue of axolotl outside the body and observed its transformation into hyaline cartilage on addition to the nutrient medium of an aqueous extract obtained from cartilage of the epiphyses of tubular bones of axolotl.

There are data showing that under conditions of prolonged induction-current stimulation of the urinary bladder, the smooth muscles may transform into striated ones (Koshtoyants, 1950, 1957).

The main questions in the problem of metaplasia are: within what range, and in what organisms is transformation of tissues possible, and what are the conditions for such a transformation?

Politzer (1955) shows in a number of examples not only at early stages of embryogenesis, but also at late stages of development of the foetus and even in the post-foetal life of man, that epithelial tissues are transformed into mesenchyme and the latter into epithelial tissues. The dorsal wall of the cavity from which the adrenals, sexual glands, and other organs are formed, participates in these processes of transformation. A similar situation prevails in transformations in other germinal layers. A number of other instances may be added to these data. Thus, from the epithelial type of tissue of the nerve stalk (ganglial strip) arise: ectomesenchyme, nerve ganglia, visceral cartilage, pigment cells (Stone, 1922, 1926, 1933; Raven, 1931, 1933, 1935, 1937, and others). From the mesothelium of the somatopleura arises the mesenchyme of the anlage of the extremities from which skeleton and connective tissue of the extremities further develop. In the process of neurulation in caudate Amphibia, the most posterior part of the nerve plate, which consists of cells of cylindrical epithelium, invaginates and transforms into axial muscles (Bijtel, 1931). As is well known, somites form myotomes which produce the axial muscles of vertebrates. However, the first somites decompose and form the mesenchyme, part of which is transformed into the cartilage capsules of the acoustic pouches. The ectoderm of the gastrula forms the nerve plate and the nerve tube; from the latter arise not only nerve cells, but also ganglial cells, i. e., connective tissue cells.

In embryogenesis mutual transformations of one type of tissue into another are always dealt with; this fact long ago ceased to astonish embryologists. Many histologists, however, who are used to dealing with a developed organism and who do not usually create special conditions for changing the development of formed tissues, believe that these tissues are stable and unvarying. However, this conclusion is not well founded. Special experiments are needed which should thoroughly check this problem.

The Swedish experimental morphologist and surgeon, G. Levander (1964), recently summarized his interesting 30-year research work on the induction of tissues in an adult mammalian organism. On the basis of the concept of embryonic induction, he used as inducers pieces of adult-rabbit tissue which had been kept for 24—48 hours in a 1% aqueous solution of trypan blue. The inducers were transplanted under the skin of adult rabbits. As a result, in the subcutaneous connective tissue, bone was induced under the influence of bone, induction of myoblasts and muscle fibers under the influence of skeletal muscle, and under the influence of endometrium was formed epithelium of the mucosa of the uterus with characteristic decidual cells. Epithelium from the mucosa of the stomach formed under the influence of gastric epithelium; epidermal epithelium formed under the influence of skin epidermis; tumor cells were formed under the influence of Erlich carcinoma. In these experiments connective tissue cells were transformed into muscle and epithelial tissue.

The Belgian cytologist, Chevremont (1938, 1940, 1945), showed in a large series of studies that striated skeletal muscle fibers from a chick embryo

transformed into macrophages in a tissue culture outside the organism. Addition of choline considerably stimulated this process and transformation occurred in 50% of cases. In studies on the human muscle, it has also been shown that cellular elements of muscle fibers may transform into macrophages (Firket and Cornil, 1944). We observed similar phenomena of transformation of decomposing cardiac muscle fibers into cells of the macrophage type in experiments on stimulation of regeneration of the myocardium (Polezhaev and co-workers, 1958, 1959).

Some investigators state that under pathological conditions, and during regeneration, skin epidermis is transformed into blastema cells of the embryonic type (Koritskii, 1926; Astrakhan, 1929; Godlewsky, 1928; Rose, 1948, 1965). Koritskii (1926) and Astrakhan (1929) are also of the opinion that in this case epithelial cells may transform into erythrocytes. Transformation of striated muscle fibers into erythrocytes in frogs (Weitzman, 1934, 1938; Gintsburg, 1951) and mammals (Wajda, 1946; Lavrov, 1949, 1959) in special experiments has been described by some investigators. These data were contested by a number of histologists (Dmitrieva, 1957, and others), but they were not seriously checked. Meanwhile, data appeared showing that in monolayer cultures of tissues from the ovary, skin, kidney, and stomach of a 5-month-old human foetus, and also from the pulmonary and cerebral layer of the rabbit bud, phenomena of metaplasia are observed: formation of blood vessels, normoblasts, erythrocytes, and segmented leukocytes from the explants (Schneider, Muirhead, and Zydeck, 1963). In leukoses in man extramedullar hemopoesis occurs in the connective tissue at the site of introduction of penicillin: subcutaneous connective tissue transforms into erythrocytes, lymphocytes, and segmented leukocytes (Ageev, 1961).

Upon implantation of necrotized pieces of liver treated, according to Levander, into the lateral skin of adult rabbits or rats, extramedullar myeloid hemopoesis occurs in the subcutaneous connective tissue: granulopoesis, erythropoesis, and de novo formation of blood vessels. The cellular elements of connective tissue are transformed into immature and mature formed elements of the granulocyte and erythrocyte series (Polezhaev and Cherkasova, 1967).

Very interesting data concerning the possibility of transformation of leukocytes into different types of tissue have been described. This kind of transformation was earlier described by I. I. Mechnikov (1892). Colucci (1886) reached the conclusion that polynuclears participated in the formation of regenerative blastema. These data, however, were later subjected to criticism, and investigators concluded that polynuclears can only decompose and are not capable of transformation. The pathologist-anatomist, M. N. Nikiforov (1890), concluded that in the case of scar tissue formation in wounds, the connective tissue formed is of local origin, and leukocytes do not participate in its formation. This opinion is still held by the majority of pathologist-anatomists. However, Maximov (1926—1928), in his classical studies on tissue culture, had already shown that lymphocytes are capable of transforming into polyblasts, macrophages, and fibroblasts. His data were confirmed by many investigators. At present a dispute goes on concerning whether lymphocytes and connective tissue in the region of a wound transform in situ. Introduction into the blood of carmine granules which are phagocytized by lymphocytes, has shown that the latter indeed participate

in the formation of connective tissue cells during regeneration after a defect has been introduced into the tail of a triton (Vierling, 1926). A similar conclusion was made by Hellmich (1930) and others in studies of regeneration in Amphibia. In recent years new data were obtained with the use of diffusion chambers, the membrane of which does not allow cellular elements to pass through, but is permeable to the liquid part of the blood and lymph. It turned out that agranular mononucleate leukocytes of man placed in diffusion chambers which were implanted under the skin of man, transformed into polyblasts, macrophages, fat cells, and fibroblasts producing reticulin and collagen (Petrakis, Davis, and Lucia, 1961). Human leukocytes kept in diffusion chambers which were placed on the chorioallantois of 7-day-old chick embryos, transformed into epithelium-like cells (Bell, 1960). Nowak (1962, 1962a) placed rabbit leukocytes in diffusion chambers and implanted the chambers under the skin into the skeletal muscle or cartilage of rabbits. There was simultaneous formation of connective tissue cells, muscle fibers, and chondroblasts from the segmented leukocytes placed in the chambers. Thus leukocytes, being a plastic material, were transformed into the appropriate cellular forms when under the inductive influence of adjacent tissues.

A number of investigators, mainly Soviet ones, showed that not only lymphocytes , but also granulated leukocytes and polynuclears, are capable of transforming into connective tissue cells (Makarov, 1948—1966; Esslova, 1955; Ermolaeva, 1956; Rinchino, 1956; Polezhaev, 1959, 1960; Polezhaev and co-workers, 1959, 1961, 1966; Polezhaev and Cherkasova, 1966). In a wound or in experiments outside the body, some of the polynuclears decompose and die, while others transform into mononucleate cells of the lymphocyte or polyblast type. The latter may arise from different segments of the nuclei of polynuclears or from several coalescing nuclear segments (agglomeration). This process has been especially well studied and described by Makarov (1957—1966). This investigator developed a special method by which a pure culture of polynuclears can be obtained from dog or man, and a special method for their cultivation outside the body, continuously observing the individual cells for 7 days under the microscope. Thus he showed that granulated leukocytes of small dimensions gradually transformed into macrophages and large branched fibroblasts by agglomeration and differentiation.

K. A. Lavrov (1959, 1960) implanted homoplastically an embryonic skeletal muscle into the brains of adult rabbits, causing formation of malignant tumors developing from the nerve tissue. He observed a remarkable mass phenomenon of induction: dendrites of nerve cells transformed into striated muscle fibers. "Nerve-muscle hybrids" appeared. A similar phenomenon was described in experiments with induction on amphibian embryos: striated muscle fibers were induced in the bodies of nerve cells (Wilde, 1961).

In experiments on tissue cultures outside the body, it has been shown that fibroblasts of mice and man under the influence of a microsomal fraction or RNA obtained from liver or kidneys, transformed into nervelike cells with long branching dendrites (Benitez, Murray, and Chargaff, 1960).

In our experiments, the results of which will be described in the experimental part of this book, we observed a tissue-specific induction of bone, dentinelike tissue, skeletal muscle, and even nervelike tissue, from cells of young immature connective tissue in adult dogs, rats, rabbits, and kittens.

Summarizing the data discussed in this chapter, the following conclusions can be made.

1. Metaplasia, i. e., transformation of cells of one type of tissue into cells of another type of tissue has been described, not only for lower invertebrates but also for higher vertebrates, and in particular for mammals and man.

2. Metaplasia is observed not only in embryonic development but also in the postnatal period.

3. In embryogenesis of vertebrates, transformation of tissues is a normal widespread phenomenon. In postnatal development, metaplasia is observed under special pathological conditions or experimentally. Transformation of tissue into a tissue unlike itself is a regular phenomenon.

4. Evidently, at the basis of metaplasia, there is the phenomenon of induction, i. e., certain specific effects on the plastic reactive material which can be at a low stage of differentiation (ectoderm of the gastrula of amphibia, lymphocytes, or polyblasts of vertebrates) or highly differentiated (striated muscle fibers, nerve cells of adult mammals).

5. The presence of metaplasia in processes of regeneration in adult animals had been demonstrated long ago in experiments with invertebrates, e. g., nemerteans (Davydov, 1910); and in certain vertebrates, e. g., the Wolffian regeneration of lens in tritons. In many other cases of regeneration the occurrence of metaplasia requires special proof.*

Thus analysis of the third rule of regeneration, and the data connected with it, allows the following conclusion to be drawn: upon regeneration of organs in animals, each tissue may form one like itself, or one unlike itself, depending on its conditions of development or existence.

Comparison of our conclusions from the analysis of the 3 rules of regeneration, allows us to make the following generalization: the capacity for reparative regeneration of organs and tissues in comparative and ontogenetic series under normal and pathological conditions and in experiments, always depends on the living conditions of the animals. This generalization, and the conclusions from the analysis of our definitions of the phenomena, essence, causes, and basis of regeneration, enable us to formulate a statement which we call the basic law of regeneration: regenerative capacity being one of the forms of adaptive self-reproduction of living beings, is based on metabolism and is caused by continuous damage of living matter, always regularly changes during evolution, ontogenesis, pathology, and experimental conditions, depending on the changes of the living conditions (development) of the animals (or organisms in general).

The importance of the basic law of regeneration consists of the fact that firstly, it includes all three old rules of regeneration and all the exceptions to these rules. Secondly, it is effective in that it not only explains the different regenerative capacities of animal organs, but it also contains general indications as to how it can be experimentally changed in a controlled manner, a thing which the old rules did not do.

From the point of view of the concept we developed, the advantages and shortcomings both of the old rules of regeneration and of their negative criticism is obvious. The old rules of regeneration were not based on a mistake in facts or in methodology, nor were they fiction or a tale, or laws,

* In particular, a special check is necessary of the experimental data by Levander (1964) and of some other investigators.

or even absolute truths, but a relative truth changing with the enrichment of our knowledge of the subject. The old rules truly reflect some of the processes of regeneration which occur under certain conditions of development, and do not reflect another part of these processes which takes place under different conditions.

Thus the negative criticism of these rules is not justified: 1) it denies their real value; 2) it confuses investigators, inducing them to believe that the facts and methodology of the old theory of regeneration are false and fictitious; 3) it leads some investigators to wrong generalizations, to the denial of the existence of a general rule of regeneration, or to statements which do not correspond to reality (such as, that regenerative capacity increases with the increase of the level of organization in animals).

This criticism, however, has its positive aspects: 1) it hastened the weakening of old, incomplete generalizations; 2) it allowed for obtaining new facts which introduced corrections into the old ideas (it turned out that in mammals the regenerative capacity is somewhat higher than was thought before, that it cannot be reduced merely to scar tissue formation); 3) it inspired work by numerous investigators on the regeneration of organs in mammals.

We think that our generalization, the basic law of regeneration, is better than the old rules of regeneration in that it reflects to a greater extent, and more deeply, the phenomena and essence of regeneration, but even this generalization is not an absolute but a relative truth, which having done its duty will necessarily have to be replaced by another, more perfect, generalization.

Chapter V

BASIC PRINCIPLES IN THE STUDY OF REGENERATION

The basic law of regeneration defines some general approaches to the study of phenomena of regeneration and controlled changes of regenerative capacity of organs. It points out that the latter may be affected by changing the basis of regeneration, namely metabolism, because of the dependence of the latter on the living conditions of animals. These data, however, do not yet warrant undertaking attempts to restore lost regenerative capacity. In order to change conditions of existence and metabolism, the investigator can proceed in different directions: to "choose" conditions at random for the restoration of lost regenerative capacity; that means to take the path of empiricism. A scientific approach to the problem requires the knowledge of how and in which direction metabolism ought to be changed in order to bring about the desirable result. Some of the principles of regeneration we formulated help in answering this question (Polezhaev, 1950, 1958, 1959).

1. Principle of stages. It is most appropriate to study the process of regeneration according to its successive stages. This approach allows one to give a correct description not only of the process, but also of the causes for the transition of one stage into another. Speidel (1928) and Efimov (1931), studying the process of regeneration of amphibian tails, proposed singling out 3 morphological stages in the course of the regeneration process:

1) epithelialization of the amputated wound surface of the organ;

2) accumulation of histologically undifferentiated regenerative material; and

3) growth and differentiation of the latter.

Efimov (1931), on the basis of these considerations, successfully studied the role of the edge of the skin in regeneration of the tail in axolotl.

This morphological approach is insufficient for studying the physiology of regeneration, however. The appearance of new forms is determined by the physiology of the old forms and not by their morphology. Taking this into account, and starting from our understanding of metabolism being the basis of regeneration, we formulated the principle of physiological stages of regeneration. In each process of regeneration, one can discern at least two (in reality more) stages differing in type of metabolism, on the basis of which morphological changes occur: 1) a stage of destruction and dedifferentiation, characterized biochemically by proteolysis, by glycolysis, by decrease in nucleic acid metabolism, and by the active reaction of the internal environment in the regenerating tissues; morphologically it is characterized by accumulation of histologically undifferentiated regenerating material in

the focus of damage; 2) a stage of growth and differentiation characterized biochemically by a prevalence of protein synthesis over its decomposition, return to aerobic respiration, return of the RNA content and pH; it is characterized morphologically by growth and differentiation of the regenerating material in the focus of damage; 3) a stage of growth which has been less well studied biochemically.

We gave the physiological characteristics of the stages of regeneration on the basis of analysis from biochemical studies of the process of regeneration (Polezhaev, 1947, 1950). Later, a similar analysis and similar conclusions were made by Needham (A. Needham, 1952, 1960).

We have found that in animals capable of organ regeneration, the tissues of mesodermal origin are greatly destroyed and dedifferentiated, while in animals which are not capable of regeneration, these tissues are destroyed and dedifferentiated to a slight extent only. This rule has been observed in an ontogenetic and comparative series of animals (Polezhaev, 1940, 1948, 1950, 1958, 1959). In other words, there is a reduction of the first physiological stage of regeneration when regenerative capacity of organs is lost. In order to restore the lost regenerative capacity, it is necessary to restore the reduced first physiological stage of regeneration, to enhance strongly phenomena of destruction and dedifferentiation of the basic tissues comprising the organ. This can be done by acting on some biochemical links, for example, protein or nucleic acid metabolism in the first physiological stage of regeneration.

2. The principle of organ-specificity or regionality is the second important principle of regeneration. It allows one to understand what has to be done in order to affect physiologically, in a selective manner, the regeneration of a given organ, in particular a remote or internal organ. This question has been discussed by us in detail (Polezhaev, 1940, 1945, 1950, 1958). According to the principle of regionality or organ specificity, the regenerative capacity differs in different regions or organs of the animal body, according to the specificity of their metabolism and conditions of development * (existence).

Experimental embryology showed that in the process of embryonic development of animals, various rudiments of organs have different morphogenetic potencies and inducing properties (Spemann, 1936; Filatov, 1939; Holtfreter, 1933, and others). On the other hand, the body of an adult animal consists of a number of regions each of which possesses a capacity for the morphogenesis of a given organ. Thus, when the sciatic nerve is introduced into the region of an extremity, an additional extremity appears; when the nerve is introduced into the region of the tail, a tail appears; and in the region of the dorsal fin, a fin appears, etc. (Guyénot and Schotté, 1927) Each of the organs is characterized by a biochemical (Abderhalden, 1921) and immunological (see Vyazov, 1963) specificity.

The same or homologous organs, for example liver or extremities of mammals and amphibia, possess biochemical relatedness; certain organospecific but not species-specific substances, similar metabolism. One can stimulate or inhibit normal development of spleen, liver, kidneys, heart, and brain in embryos of chicks and amphibia using transplants, homogenates, or extracts of the same organs, introducing them by various methods into the recipient body (Murphy, 1916; Dantschakoff, 1918; Weiss,

* Concerning conditions of development or existence for individual organs, there might be influences, emanating from other organs of the animal.

1947; Ebert, 1954; Rose, 1955, and others). Homogenates and transplants of certain organs, labeled with colloid dyes or radioactive isotopes, are absorbed primarily by identical or homologous organs of recipient mice or chick embryos (Belonovsky and Miller, 1926; Ebert, 1954; Mahler, Walter, and Bulbenko, 1956). Similarly, one can stimulate regeneration of extremities, skeleton, or muscles in Bombina and frogs (Polezhaev and Ramenskaya, 1950; Tumanishvili, 1958; Kudokotsev, 1960), or of liver in rats or guinea pigs (Polezhaev, 1956; Tumanishvili, 1958, 1964). This means that the regenerative capacity of any organ can be affected by substances obtained from a similar organ. It is necessary only to determine experimentally the best way for the preparation of organ- or tissue-specific preparations, their dose, means of introduction. etc.

Starting from the principles of stages and organ-specificity, it is possible to affect in a controlled manner the course of the processes of regeneration of animal organs. This possibility could not have been detected in studies carried out on the basis of the old rules of regeneration.

3. Principle of inheritance. Regenerative capacity is a heritable property of organisms. The fact that in some taxonomic groups of animals, organs (for example, extremities of tritons) are capable of regeneration, while in others (for example, extremities of rats) they are not, is determined by their heredity. Therefore, in order to change the regenerative capacity of organs, for example, in order to restore the lost regenerative capacity of extremities of rats or skeletal bones of dogs, it is necessary to change in a controlled manner the basis of this hereditary process of development. This is based on a certain metabolism of a given species of animals, a known molecular and morphological structure of the cell in its interaction with the environmental conditions, determining specific biosyntheses of proteins and nucleoproteins, i.e., the process of self-reproduction of the living being. Modern biochemistry believes that biosynthesis of proteins depends on DNA and RNA molecules, enzymes, and certain environmental conditions, for example temperature, processes of oxidation, etc. This means that the hereditarily determined process of regeneration can always be changed in a controlled manner by changing the foregoing and other links of the biosynthesis of protein, e.g., by changing enzymes, RNA, or conditions of the external environment. An attempt to act directly on DNA molecules is evidently less successful as this stable, and at the same time fragile, molecule is not easy to break or change by a direct effect. An example of this can be seen in the action of ionizing radiation on the body, leading to deep changes in the nuclear apparatus, chromosomes, and DNA molecules. There is no doubt that the latter change continuously during ontogenesis and phylogenesis under the influence of factors (enzymes, conditions of internal and external environment, etc.) on which it depends, but the effect of these factors on DNA is, so to speak, mild, and it has to be taken advantage of in the experiment.

In order to restore the lost regenerative capacity of organs and tissues in animals, the metabolism of animals incapable of regeneration ought to be brought closer to that of animals which possess this capacity. For example, the duration of the processes of proteolysis and destructibility of tissues during the first physiological stage of regeneration must be increased.

Of course, a change in regenerative capacity during ontogenesis of animals does not yet mean that it is inherited by the offspring of a given pair

of individuals. For this property to be inherited, further effects in this direction in a number of generations are necessary. However, this is not the point in our study. For us, it is important to change the regenerative capacity in a given individual.

It is also necessary to distinguish between a change in regenerative capacity and a change (inhibition or stimulation) in the process of regeneration of organs. If a tail of a triton or axolotl is amputated and the wound surface is sewed with skin, regeneration of the tail will be suppressed. However, the regenerative capacity of the tail does not change in the process; only the mechanism of the process changes. If a tail is amputated at later stages of metamorphosis of frog tadpoles, regeneration does not occur. In this case, the capacity for regeneration of organs is indeed changed. In the former case, the metabolism of the animal does not change qualitatively, while in the latter, the metabolism of the animal and of the given organ do change. However, the metabolism and regenerative capacity of an organ can be changed experimentally. For example, the method of repeated amputation can lead to obtaining regeneration of the tip of the tail by resorbtion of its proximal part in a metamorphosizing tadpole (Speidel, 1929).

4. Principle of unity of theory and practice. The final, albeit long-term but basic, purpose of any scientific research is life, practice. The same may be said of the study of regeneration. This is why the latter started in ancient times in medical practice. Its purpose was healing of wounds, bone fractures, and other damage in man. The biological direction of the study of regeneration arose much later, only 200—300 years ago. Dealing with the study of the problem of occurrence of phenomena of regeneration in the animal world, it immediately posed the question why the regenerative capacity is high in some species of animals, while it is low in others, and why one cannot bring about regeneration of organs in man and other animals, in which it does not occur. These were practical questions, but the investigators were not able to answer them. Biologists, therefore, studied other, mainly theoretical questions, on the whole, far removed from practice.

At present, the study of regeneration has reached such a level of development that it has become possible to formulate tasks of great theoretical and practical importance. Medical and biological directions of study converged. But as soon as this happened, the leading role in the studies of regeneration was immediately taken over by the demands of practice. If the investigators can now occupy themselves with solving existing important practical problems, there is no point in wasting effort and time on secondary tasks, removed from life. It should be pointed out that the principle of unity of theory and practice in the study of regeneration, with the leading role played by practice, presupposes the formulation of wide-ranging serious problems which are of great significance for humanity, and not of petty problems of application.

If investigators found a way to elaborate such an important problem in biology and medicine as the restoration of lost regenerative capacity, such other problems must be posed as the restoration of lost, or enhancement of weakened, regenerative capacity of extremities, bones of the cranial vault, dental tissues, cardiac muscle, nerve tissue of the brain, and restoration of tissues after local radiation injury, etc. Answers to these and other questions may and should have a serious theoretical and

practical significance for the curing of many grave diseases which, on the basis of the old ways of development of biology and medicine, have been considered incurable so far.

Thus, a brief discussion of the basic law of regeneration and of the new basic principles of the theory of regeneration shows that by using these tenets, a scientific basis can be found, and it is not necessary to pose them in an empirical manner. Tasks which are of great theoretical and practical importance can be solved, a thing which could not have been done before on the basis of the old rules of regeneration.

Chapter VI

HISTORICAL METHODS IN THE STUDY OF REGENERATION

Processes of regeneration may be studied on the basis of antihistoric and historic methods. In the former case the investigator completely ignores the question of origin and changes in the regenerative capacity of animals. As a result he loses the possibility of finding a correct approach to the study of the phenomena of regeneration. Moreover, the antihistoric approach leads to one-sidedness and idealism. The materialistic approach always requires the use of the historic method. The historic method is an approach, the basis of which is a previous knowledge that a given process of development, for example regeneration, is historically conditioned. It is very important to establish the ways in which this historical conditionality can be disclosed. Most frequently biologists attempt to find the latter by the use of comparative methods. This is the approach of anatomists and embryologists, for example, Severtsov (1939), and comparative physiologists (Koshtoyants, 1950, 1957). This was the way in which A. A. Zavarzin (1941, 1947), who is considered the founder of evolutionary histology, carried out his studies on regeneration and inflammation. Vorontsova (1953) says: "In order to analyze phenomena of regeneration in the historical aspect, it is necessary to compare with each other data obtained on different representatives of the animal world. This will allow one to disclose the basic regularities of the phenomena of regeneration and their changes in the course of evolution" (p. 23). A similar conclusion was reached by Studitskii (1954), who carried out a comparative study of processes of regeneration in vertebrates.

Nasonov (1941) and Ivanov (1937) successfully used the comparative method in the study of regeneration. Nasonov, for example, explained the absence (loss) of regenerative capacity of extremities in frogs by the fact that it is more convenient for animals of this genus, when damaged by infection or losing an organ, to heal the wound rapidly by scar tissue formation than by forming a gentle, fragile, and easily damaged regenerative blastema, which slowly transforms into the regenerating extremity. The great importance of the comparative method for morphology and physiology has been nicely pointed out by the famous Soviet embryologist and experimental morphologist, Filatov (1934): "By comparison, we bring the immobile into motion and thus we elucidate its latent properties". Nevertheless, we consider the comparative method as insufficient to study phenomena of regeneration in the historical aspect: 1) a comparative series of contemporary animals is not identical to the true evolutionary series; 2) in a comparative series of animals, the increase in complexity or organization and the increase in regenerative capacity do not always coincide; 3) the comparative

method does not necessarily include data concerning the conditions of existence of the animals, without which knowledge it is impossible to understand the regularities governing the changes of regenerative capacity. Without taking into account the foregoing factors, the investigator will invariably make mistakes which will not enable him to find the correct path to control processes of regeneration.

Zavarzin, using his comparative-histological method, did not even pose the question of controlled changes of regenerative capacity. Vorontsova (1949, 1953), using the comparative method as the historical one, reached incorrect conclusions, namely that there is no relationship between regenerative capacity and the level of complexity of organization, and that all the animals and plants have a capacity for not only physiological, but also reparative regeneration. She assumes that in order to obtain regeneration one only has to make a correct "choice" of conditions. What that means and how one can do it, she does not say, although this is, in fact, the essence of the problem. She does not say it because the historical method understood as a comparative method can give nothing in this respect. "Choice of conditions", is an empirical approach. Studitskii (1948, 1954, 1959), using the comparative method as the historical one, also could not determine the correct approach for achieving controlled changes in regenerative capacity. According to his statements, liver, muscles, and other organs and tissues of higher animals (mammals and birds) regenerate spontaneously and beautifully after simple amputation, and the lower the animals the better. Thus, the investigator does not have to pose the question of restoration of lost or absent regenerative capacity.

We think that the problem of controlled changes in the regenerative capacity can be solved only by using the historical method, but understanding it not in the way these investigators did. By the historical method of study of processes of regeneration, we mean a triad, a simultaneous use of three methods: comparative, ontogenetic, and experimental, each of which we consider completely separate. We use the comparative method, taking into account the aforementioned points which make it valuable: 1) we consider the series of contemporary animals as a series which is not identical with the true historical series. For example, contemporary fish are not identical with the ancient ancestors of Amphibia, but are similar to them; 2) to study the loss of capacity for regeneration of an organ, for example, extremities in vertebrates, we do not take the whole series of the latter, but only those groups in which this change is clearly expressed, for example, in axolotl, frog, mouse; 3) this choice should be based on our knowledge of the changes in the conditions of existence of animals, which are necessarily reflected in their development and capacity for regeneration; axolotl lives only in water, the frog in water and on land; the mouse only on land; in this series there is a transition from aquatic to terrestrial conditions of life.

By the ontogenetic method, we understand morphological and physiological study of processes of morphogenesis at consecutive stages of individual development, the basis of which is the biogenetic law or the idea of a regular morphological and physiological change and abridged repetition (recapitulation) during ontogenesis of the main stages of phylogenesis of the given genus of organisms. In the course of a given ontogenesis, it is very important to establish the transition from one condition of existence to others.

For example, in tailless Amphibia during metamorphosis a transition may be observed from aquatic life conditions to terrestrial, a deep change of the entire physiology of the organism (the processes of aerobic respiration, the oxidative processes, increase 2-fold, there is an intense proteolysis and also synthesis of new proteins, and the active reaction of the internal milieu shifts in the acidic direction, etc.); a transformation of all the organs, all forms and functions of the animal, takes place. In this process, the capacity for reparative regeneration of organs, in particular extremities, is drastically reduced or lost altogether, while the capacity for physiological regeneration remains high. All these processes, including the change of regenerative capacity, represent a recapitulation.

By the experimental method, we mean an artificial change of the process studied which, when compared with the normal events, allows one to elucidate the acting forces, the external and internal conditions of normal development. The experimental method should be directed toward changing the type of metabolism of the organism and the given organ in the direction determined by the data obtained on the basis of comparative and ontogenetic methods. To restore the regenerative capacity of extremities lost during ontogenesis in the tailless Amphibia, and which is absent in newborn mammals, it is necessary to simulate the type of metabolism of the given organ in those ontogenetic and phylogenetic stages at which this capacity still exists. This can be reached by different methods, directed toward obtaining the first physiological stage of regeneration, destruction, and dedifferentiation, the presence of which secures complete regeneration of organs (Polezhaev, 1933, 1948, 1950, 1956, 1958).

Each of the three different methods, the comparative, the ontogenetic and the experimental, may give some idea of the historic changes in regenerative capacity, but the fullest picture can be obtained only by the simultaneous use of all three methods. In our example with the extremities of vertebrates, the comparative method shows that destruction and dedifferentiation and later regeneration occur in axolotl, and are absent in the lizard and mouse. The ontogenetic method shows that these phenomena are present in tailless amphibia (frog) at early stages of ontogenesis and are lost at later stages. The experimental method allows creation of these phenomena artificially and obtaining complete or partial regeneration of extremities which do not usually regenerate in tadpoles at late stages of metamorphosis, and in adult frogs (Polezhaev, 1933–1948); Rose, 1942, 1944, and others), in lizards (Umanskii and Kudokotsev, 1951), and even in young rats (Rogal', 1951; Umanskii and Kudokotsev, 1952; Scharf, 1961, 1963). When used with mammals, this method allows one to obtain regeneration of nonregenerating bones of the cranial vault, dental tissues, cardiac muscle, and to stimulate physiological regeneration of nerve tissue of the cortex of the large hemispheres of the brain (Polezhaev, 1957, 1958; Polezhaev and Karnaukhova, 1963).

It is not difficult to see that the historical method as we understand it gives much more to the investigator than when understood only as a comparative method.

On the basis of our understanding of the historical method, we see in a new way the process of changes in regenerative capacity in the course of evolution. The conditions of existence of animals change, for example, water animals emerge onto land; according to the law of development adaptation takes place: their physiology changes, their entire metabolism, and

subsequently all the forms and all the organs of the animals, change. In this process some adaptations are replaced by others and in the case of progress, of increasing complexity of organization, primarily of the nervous system and differentiation of all the tissues and organs, as a rule there is a decrease of the capacity for repair regeneration of organs. In other cases the process may differ, depending on changes in other conditions of existence. Continuous damage of the body in rainworms, arising as a result of friction with the ground, may also serve as a reason for the development of a higher regenerative capacity in these worms. It is necessary to emphasize here an important fact: conditions of existence of organs and animals mean not only the external environmental conditions, but also the physiological effect of other organs (induction, metabolic, hormonal, and others). The scheme is as follows: conditions of the external environment change the metabolism, the physiology, and the shape of any organism, and in animals, an active mechanical and physiological function, excitability, and the nervous system, participate in this process. The capacity for reparative regeneration depends on changes in the physiology and metabolism of the organism, which change in response to the conditions of existence of the animals and the functions of their organs.

Chapter VII

REGENERATION IN ANIMALS WHICH POSSESS REGENERATIVE CAPACITY

In order to study why regeneration of organs does not occur in those animals which do not possess regenerative capacity, it is necessary to know how the process of regeneration occurs in animals which do possess regenerative capacity. A number of studies on regeneration in the latter is available (Shimkevich, 1924; Zavadovskii, 1931; Filipchenko, 1932; Polezhaev, 1945, 1947, 1947a; Vorontsova, 1949, 1953; Vorontsova and Liozner, 1955, 1957; Liozner, 1960, 1962; Shmal'gauzen, 1963; Huxley and de Beer, 1936; Morgan, 1901; Przibram, 1909; Schaxel, 1921; Korschelt, 1927; Weiss, 1930, 1939; Abeloos, 1932; Needham, 1952; Kiortsis and Trampusch, 1965; Hay, 1966; Schmidt, 1966, and others). The present chapter points out briefly only some of the more important aspects of the process of regeneration and its dependence on a number of factors which must be known in order to further analyze the problem.

The subject of studies of the spontaneous process of regeneration is the process itself, its causes, conditions and mechanism of action. The purpose of the study is to elucidate the normal course of regeneration and its immediate causes. The methods are histological, histochemical and biochemical description, and comparison and experiment directed toward the elucidation of the causes and conditions of regeneration. Where this approach is employed, no attempts are made to change in a controlled manner the basis of regeneration.

The processes of spontaneous regeneration of extremities and tails in caudate amphibia were best studied. In this process, the following main stages are observed taking place after amputation or damage of the organ: 1) epithelization; 2) formation of an histologically undifferentiated, regenerative anlage or blastema; 3) growth and differentiation of the regenerative anlage; 4) growth of the formed organ.

According to the old ideas, during regeneration each tissue and each component of an organ gives rise to similar tissues and components of the regenerate (theory of regrowth). For example, epithelium gives rise to epithelium, derma to derma, muscles to muscles, skeleton to skeleton, etc. However, it turned out that skeleton, muscles, and derma of the extremities of tritons and axolotls may arise following complete or partial removal of these tissues from the residue of the organ. They are formed from regenerative blastemata consisting of epithelium and a subepithelial part (blastemata proper) in the composition of which there are morphologically homogeneous cells of the embryonic mesenchyme type. The problem of the origin of regenerative blastemata was not clear for a long time. Studies

performed in recent years in which radioactive isotopes were used (Hay and Fishman, 1961; Anton, 1961; Bodemer, 1962; O'Steen and Walker, 1961, and others) confirmed earlier data concerning the origin of the cells of blastemata proper from muscles, connective tissue, cartilage, perichondrium, periosteum, and dermis, dedifferentiated after amputation (Butler, 1933; Polezhaev, 1935, 1945, 1948; Barakina, 1951, 1952; Yakovleva, 1943, 1953, and others).

Originally an opinion prevailed that at its early stages of development, the blastema is undetermined, does not have its own organizing potencies and, therefore, is not capable of self-differentiation upon transplantation to another organ (Schaxel, 1921, 1934; Milojević, 1924; Weiss, 1927, and others). Later, however, it was shown that even at the early stages of development, the blastema is determined and that its inability to develop after transplantation to another site in the animal is due to the fact that being amputated, it is cut off from the stream of cellular regenerative material. If the amount of the latter is increased, then the blastema develops according to its origin, even after transplantation in the place of another organ (Polezhaev, 1934, 1937, 1937a, 1945).

These data necessitated a special study concerning the mechanism of blastemata formation. According to previous theories, regenerative material is formed at the amputation wound surface due to active mitotic cell division under the influence of wound hormones or mitogenetic rays arising as a result of decomposition of damaged tissues (Haberlandt, 1922; Bromley, 1930; Blyakher, 1932). However, it turned out that this is not the case. Mitotic count and the mitotic index in consecutive stages of regeneration of extremities in axolotl and tadpoles showed that within the first 10 days, the mitotic index of the blastema is very low, increasing continuously up to 20 days and remaining at a high level up to 30 days after amputation (Polezhaev, 1935, 1935a, 1939, 1941; Polezhaev and Gintsburg, 1944). These data were confirmed later (Basina, 1940; Litwiller, 1939; Chalkley, 1954). Moreover, it turned out that extremities of grassfrog tadpoles regenerate completely upon total suppression of mitotic activity in their cells under the influence of colchicine (Polezhaev and Gurvich, 1948). These data showed that the regeneration blastema is formed mainly due to the release of cells from their tissue connections during the process of destruction and dedifferentiation of the old tissues of mesodermal origin in the stump of the organ. Mitotic division occurs only after a certain minimum amount of cells necessary for the growth of the regeneration anlage accumulated in the blastema (Polezhaev, 1945).

Thus, the importance of dedifferentiation in phenomena of regeneration has been established. Dedifferentiation was discovered during regeneration in hydranths and Planaria as early as the late 19th century. It was shown later that it can also be observed upon regeneration in ascidians and in a number of invertebrates (Driesch, 1902; Schulz, 1902, 1907; Davydov, 1908, 1910, 1915, and others). A methodological error was made, however: some investigators started to consider dedifferentiation as a reverse development (Schulz, 1907) which does not exist in nature. Therefore, assuming that acceptance of dedifferentiation almost means acceptance of vitalism, many investigators later started to deny dedifferentiation altogether. Only in the 1930's, after dedifferentiation and its significance were demonstrated in processes of regeneration of organs in vertebrates, as mentioned here

earlier, they again began to speak of dedifferentiation in studies on regeneration, and even experimental morphologists (Vorontsova, 1949, 1953; Vorontsova and Liozner, 1957) and histologists (Grigor'eva, 1959) began to accept it. The most important data on the role of dedifferentiation however, were obtained in the study of the problem of loss and restoration of regenerative capacity in organs of vertebrates (see Part Two).

Investigators have devoted great attention to the role of the different components (skeleton, epidermis, dermis, muscle, nervous system, internal humoral environment), participating in the regeneration of complex organs (extremity, tail, etc.) in Amphibia and other animals. As a result of this study the following was found.

For regeneration of organs, a certain quality of epithelium, tissue of mesodermal origin, nerve tissue, and an internal humoral environment are essential. Without epithelium, complex organs do not regenerate; epithelium on a given territory of the animal's body is necessary for regeneration, e.g., regeneration of the extremity does not occur with the participation of epithelium of the skin of the head or back (Efimov, 1933; Polezhaev, 1935, 1936; Gintsburg, 1947, 1948; Gol'tsman, 1947, and others). When the tissues of mesodermal origin in the extremity of axolotl are fully replaced by similar tissues from the tail, regeneration of a taillike organ takes place (Polezhaev, 1934). Among tissues of mesodermal origin, an important role is due to muscles (together with connective tissue), as shown by similar experiments in which muscles from the tail were transplanted into the extremity of axolotl (Vorontsova and Liozner, 1936).

The role of the different components of an organ was demonstrated in experiments on transplantation of nonirradiated components (skin, skeleton, muscles) into an X-irradiated extremity of axolotl (Umanskii, 1937; Thornton, 1942; Trampusch, 1957, 1960, 1965).

When the peripheral nerves were cut, there was no regeneration of extremities in Amphibia (Weiss, 1925; Schotté, 1926; Singer, 1952). Data are available, however, showing that on transplantation of segments of extremities to tadpoles and axolotls, regeneration occurs in the absence of innervation, at least at the initial stage of the process (Polezhaev, 1939, 1946, 1948; Yntema, 1959, 1959a; Shneider, 1940). Evidently in these cases, transplantation compensates somehow for the absence of innervation. Bringing in a nerve to the regions of the extremity, tail, or fin causes the formation of an extremity, taillike or finlike type of organ, respectively (Guyénot, and Schotté, 1926). Destruction of the spinal cord leads to suppression of tail regeneration in axolotl (Godlewski, 1905; Borsuk, 1940). Conversely, transplantation of segments of the spinal column into a fin of axolotl leads to regeneration of the tail with all its axial organs (Efimov, 1951).

Regeneration of an extremity or tail in tritons and axolotls can be suppressed if the wound is sewed with skin after amputation of the organ (Tornier, 1906; Schaxel, 1921; Godlewski, 1928). Suppression of regeneration is due to the absence of epithelium in the region of the wound; this is shown by the fact that the organ (tail of axolotl) regenerates if an epithelialized wound is sewed together with skin (Efimov, 1931). However, as further study showed, in addition to epithelialization, regeneration of the organ also requires a certain physiological state caused by damage of its tissues during amputation (Polezhaev, 1933). In axolotl the extremity was amputated and the wound sewn with skin; then, 2 weeks later the skin was removed. The processes of destruction in the damaged tissues disappeared, and this

led to lack of regeneration of the extremity in spite of epithelization of the wound. This fact emphasizes the necessity for destruction of tissues in order for regeneration of organs to take place in animals which possess the capacity for regeneration.

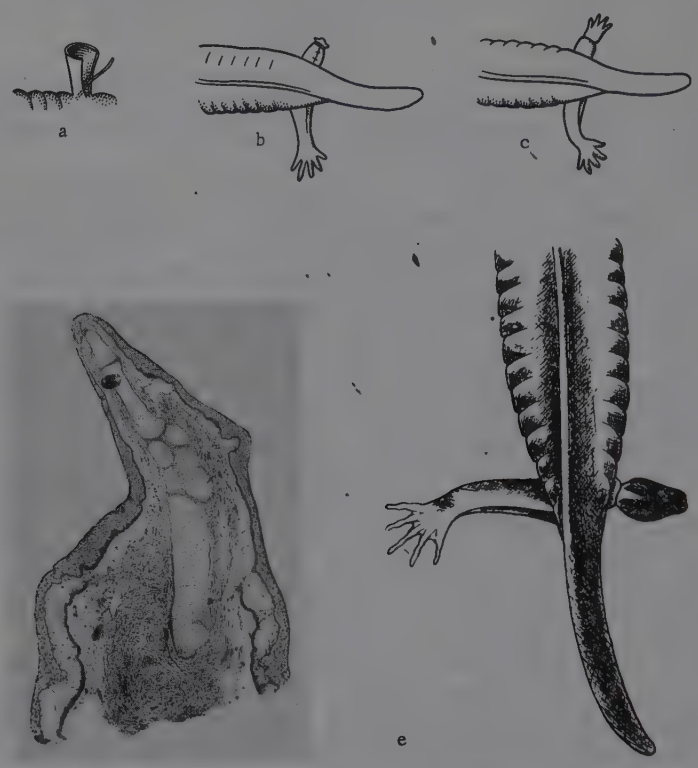


FIGURE 1. Scheme of the operation and its result. Regeneration of organs from destructurized tissues in axolotl:

a – a skin cuff and nerve were left at the site of the amputated extremity; b – ground muscles or muscles, skeleton, and connective tissue were transplanted into the skin cuff; the nerve was made to pass through the implant; c – a typical extremity regenerates after amputation of a part of the cuff with the adhering destroyed tissues; d – longitudinal histological section through the regenerated extremity; e – regeneration of a taillike organ, formed from destroyed tissues of mesodermal origin of the tail, transplanted into the skin cuff of the extremity.

According to the theory of outgrowth, each tissue and each component of an organ form like structures during regeneration. In this connection the question arises whether a normal topographic structure of the main component tissues is necessary for regeneration. It turned out that after removal of the skeletal elements from the shin of a triton or salamander and amputation of the extremity, the latter fully regenerates with the skeleton (Fritsch, 1911; Weiss, 1925; Bischler, 1926). If one half of the shin of a triton or axolotl is split and amputated then a whole extremity regenerates from the other half of the cross section of the extremity (Weiss, 1926).

Subsequent studies showed that the regenerate often may have an atypical structure: there may be a smaller than normal number of toes, bones, etc. (Lomovskaya, 1940). The atypical structure of the regenerate may be ascribed to at least 2 factors: reduction in the amputation wound surface of the extremity, and change of the topographic structure of the residual tissues.

It is known that after sponges (Microciona prolifera) were ground and passed through a sieve, tiny individuals regenerated fully (Wilson, 1907; Müller, 1911, and others). A similar finding was made in experiments with hydra (Aizupet, 1935). These lower invertebrates are capable of regenerating whole individuals from small parts. However, it has been shown that regeneration of some organs in vertebrates also can take place, in case of complete destruction of the basic tissues composing the organs. If the extremity of an axolotl is amputated very close to the trunk, leaving a previously prepared skin cuff, sciatic nerve, and femoral artery, and into this cuff are implanted removed parts of an extremity (muscle, skeleton, and connective tissue), or only muscle which has been cut to pieces by scissors and ground in a mortar; and if the nerve and blood vessel are let pass through the implant and the distal part of the organ is amputated two weeks later, complete regeneration of the extremity takes place — Figure 1, a, b, c, d (Polezhaev, 1934, 1937, 1937a, 1945). If destroyed mesodermal tissues from the tail are placed into the skin cuff of the extremity, a taillike or finlike type of organ regenerates — Figure 1e (Polezhaev, 1934, 1936, 1937; Efimov, 1935). In order for regeneration of a typical tail to take place, a special structure of the residual organ, primarily the spinal column is required (Efimov, 1951).

Our data have been questioned by some investigators (Tokin, 1935, 1959; Vorontsova and Liozner, 1936, 1938, 1938a). However, these data, i. e., proof that it is possible to obtain regeneration of organs independently of the structure of the basic tissues comprising them, were confirmed by a number of investigators, and were greatly developed. E. E. Umanskii (1938) and Vorontsova (1949) confirmed these results in experiments on regeneration of extremities and tails in axolotls. We (Polezhaev, 1936) have shown that the eye anlage in axolotl embryos can regenerate after its complete destruction. These data have been confirmed (Del Pianti, 1942). Studitskii (1952, 1959) and co-workers used the method of destruction extensively, and showed that regeneration of skeletal muscles is possible after they are ground and autotransplanted with adequate innervation into young chicks, rats, or rabbits. Moreover, the possibility of regeneration after destruction of the thyroid gland, pancreas, and lymphatic nodes in rats and rabbits was later studied (Gorbunova, 1955; Khristolyubova, 1955; Bogoyavlenskii, 1955).

Summarizing briefly the data discussed in the present chapter, the following is pointed out. The study of the process of regeneration in animals which have a high capacity for regeneration allows elucidation of a number of points concerning the course of the process, its mechanics and ways, histogenesis, role of different components, etc., but does not contribute anything to the understanding of the reasons for the absence of regeneration in animals which have lost the regenerative capacity of organs.

Chapter VIII

APPROACH TO THE STUDY OF LOSS AND RESTORATION OF REGENERATIVE CAPACITY OF ORGANS IN ANIMALS

Problems of regeneration, as pointed out in the previous chapter, were the subject of many comprehensive reviews in which thousands of individual studies were quoted. But only a single monograph was devoted to the problem of loss and restoration of regenerative capacity of organs in animals (Polezhaev, 1948), a monograph which summarized the data of several dozens of studies. This situation is due to the fact that this is the most difficult problem in the study of regeneration, and only relatively recently was an experimental approach to the problem found. It has now been elaborated and studied in further depth, and new experimental data indicating a new direction in the study of regeneration will be given in the second part of this book. Here, we shall mention the main properties which we used as the basis of our approach to the problem.

In the study of the loss and restoration of regenerative capacity of animal organs, the subjects are the changes in regenerative capacity which lead to its loss and restoration during ontogenesis and phylogenesis in pathological conditions and in experiments, but not the process of regeneration or regenerative capacity as such. The purpose of the study is to elucidate the rules of the loss and restoration of regenerative capacity during ontogenesis and phylogenesis, to elucidate the reasons for these phenomena, and to elaborate methods for the restoration of lost regenerative capacity. The method is description, comparison, and mainly experiments directed toward the elucidation of the aforementioned regularities, causes, and mechanisms. In contrast to the study of spontaneous regeneration, in this aspect of study the main task consists of a controlled change of the basis of the capacity for reparative regeneration of organs. Thus, this trend in the study of regeneration is characterized by its special investigative units, i. e., the subject, purpose, and method. The leading and main part of the study is the subject. Only after the latter has been defined can the purpose, tasks, and method be formulated.

The approach to the problem should be well founded scientifically, as it cannot be solved empirically by "choosing" by trial and error these or other conditions in order to affect regenerative capacity.

As pointed out before (see Chapter II), certain attempts had already been made to restore lost regenerative capacity of organs of animals and man, but they were not well enough founded and were not successful

(Morgan, 1908; Bier, 1923; Weiss, 1930). Those studies were based merely on assumptions which seemed probable to the investigators, or were empirical, but they did not follow from an analysis of extensive descriptive, comparative, or experimental data.

In contrast to this approach, we propose our own which will be briefly described in the following material. We think that the studies first of all should be based on objective data, well established facts, the deepest possible knowledge of the subject, sufficiently deep knowledge of the mechanics of the spontaneous process of regeneration, and on the existence of valid phenomena and not a priori schemes or ill-founded assumptions, especially when there is no possibility of checking them in reality. Furthermore, in striving to disclose the rule of loss and restoration of regenerative capacity of animal organs, objectively existing and experimentally detectable phenomena must be dealt with, and not a simple, new interpretation of old, already known, facts. For example, hypertrophy can be called regeneration, and on this basis the conclusion made that mammals have a high capacity for regeneration of organs, as has been done by certain investigators (Vorontsova and Liozner, 1957; Liozner, 1962; Studitskii, 1954, 1959). Such conclusions, however, can only solve the problem with words, but cannot define new perspectives for study.

It is best to begin the study from investigating the loss of regenerative capacity of an organ during a critical transition period of ontogenesis and phylogenesis, making an attempt to arrest the process at that moment, but not to study the process of regenerative capacity that had already been lost long ago in ontogenesis and phylogenesis. Furthermore, the correctness of the assumptions concerning the reasons for the loss of regenerative capacity should be constantly checked experimentally, and attempts should be made to restore it. In general, phenomena of loss and restoration of regenerative capacity must be studied in their interrelationships. In the beginning, the process should be studied using one organ of one animal species, and only later, when basic data are at hand, should a comparative study of the process in a comparative series of animals be made. The knowledge obtained will allow the investigator to pass on to the study of changes in the regenerative capacity in different organs of different animal species at different stages of ontogenesis. We expressed these considerations earlier (Polezhaev, 1935, 1940, 1948) and invariably and consistently adhered to this plan of investigation. During the first 16 years of this study (1932—1948), we investigated the process of loss and restoration of regenerative capacity of extremities in tailless amphibia, at the beginning, in tadpoles at the moment when they lost this capacity, and then in adult frogs. At first, the work was conducted with grass frogs (Rana temporaria) and later also in other species of tailless Amphibia, lizards, and mammals. The work was preceded by a study of the mechanics of spontaneous regeneration and normal development of extremities and other organs, which allowed us to establish a new, objective and real phenomenon, and later, the rule of loss and restoration of regenerative capacity of organs during ontogenesis and phylogenesis of animals. In the following stage (1948—1967) the study was conducted on the basis of this rule, using various organs (bones of the cranial vault, dental tissue, cardiac muscle, nerve tissue from the cortex of the cerebral hemispheres) of mammals which

do not regenerate under ordinary conditions of damage, under the action of ionizing radiation, and in some pathological cases. The results of this entire period of study, in which we and some other Soviet and non-Soviet biologists participated, will be described in the following pages.

The investigation of the problem of loss and restoration of regenerative capacity of animal organs was carried out in the plane of experimental morphology, at first mainly dealing with determination, and later also with the problem of external and hereditary factors of development. In the process of the study, methods of experimental morphology, histology, histochemistry, biochemistry, physiology and surgery were used as one methodological complex.

Part Two

EXPERIMENTAL STUDY

In the second part of this book, some data are given pertaining to the problem of loss and restoration of regenerative capacity of animal organs obtained experimentally, mainly in our laboratory during the last 35 years (1932—1967). The data are given in the form of an outline, in a general form, in order to provide a general idea of the factual basis of the new trend developing in the study of regeneration. Details concerning all our own factual data and those from publications, the technology of the experiments, the methodology used in the preparation of histological, histochemical, and biochemical preparations will not be given here. These details have been described in monographs, dissertations, and special papers by my co-workers and me (Polezhaev, 1933, 1940, 1945, 1948, 1961; Polezhaev and Karnaukhova, 1962; Matveeva, 1962; Yavich, 1963; Muzlaeva, 1964; Akhabadze, 1965; Polezhaev, Akhabadze, Muzlaeva, and Yavich, 1965, and others).

The objects of this study were organs which do not regenerate under ordinary conditions of amputation or damage: extremities of tadpoles at late stages of metamorphosis or adult frogs, Bombina, lizards, mice, and rats; bones from the cranial vault of dogs, rats, mice, and other animal species; dental tissues from dogs; cardiac muscle from rats, rabbits, dogs, and cats; nerve tissue from the cortex of the cerebral hemispheres of rats and cats; and, finally, extremities of axolotl after X-irradiation and suppression of their capacity for reparative regeneration. The number of operations in experiments on the extremities exceeded 4,000, with cranial bones — 1,000, with cardiac muscle — 2,000, with nerve tissue — 300, and with irradiated organs —2,000.

We point out once again that the study was conducted by the use of a complex of methods from the fields of experimental morphology, histology, histochemistry, biochemistry, physiology, and surgery.

Chapter I

LOSS AND RESTORATION OF REGENERATIVE CAPACITY OF EXTREMITIES IN VERTEBRATES

Dependence of regenerative capacity on the stage of ontogenesis and the level of amputation of the organ

The phenomenon and, later, the rule of loss and restoration of regenerative capacity of animal organs were discovered in a study of the regenerative capacity of tailless Amphibia. These animals are an intermediary group between groups of animals capable and groups incapable of regeneration of organs. In these animals, the capacity for reparative regeneration of organs is lost at a known stage of ontogenesis. This stage, or the ontogenetic border of regenerative capacity, is strictly defined for each animal species, and differs for different organs. For example, hind extremities of tadpoles lose their regenerative capacity earlier than the forelegs, while the tail loses that capacity later then they do. The fact that metamorphosis of tailless Amphibia is rapid — for example, in tadpoles of the grass frog it occurs within $1^1/_2$—2 months after the eggs are laid — and the fact that these animals are easily accessible makes them a very convenient subject for experimental investigation of the problem of loss and restoration of regenerative capacity of animal organs.

Henceforth, in speaking of the subdivision of the metamorphosis of tadpoles into stages, we shall use the table of L. Ya. Blyakher (1928), to which some additions were made by Liozner (1931) and by us (Polezhaev, 1933), Figure 2.

Stage I — the hind extremities of the tadpole do not have a compound joint between the hip and the shin. The foot has the shape of a spade (Stage Ia), or a spade with hardly noticeable indentations of toes (Stage Ib), or with more clearly manifested toes (Stage Ic).

Stage II — the hip and shin form an obtuse angle, due to the formation of the knee joint and development of muscles; rudiments of toes are quite distinct. The extremities are very small and weakly developed (Stage IIa), or better developed and of larger dimensions (Stage IIb).

Stage III — the hip and shin form an acute angle. In the tadpoles, the abdomen is round, the forelegs are not seen from the outside (Stage IIIa), or the abdomen is lean due to the resorption and shortening of the intestine; the elbows of the forelegs stick out strongly from the opercular membrane; the hind extremities are strongly developed; on the back a characteristic violin-shaped drawing is formed on the skin (Stage IIIb).

Stage IV — one of the forelegs has cut through, the hind extremities increase in dimensions, the body becomes better and better formed, and the horny mouthed parts fall off.

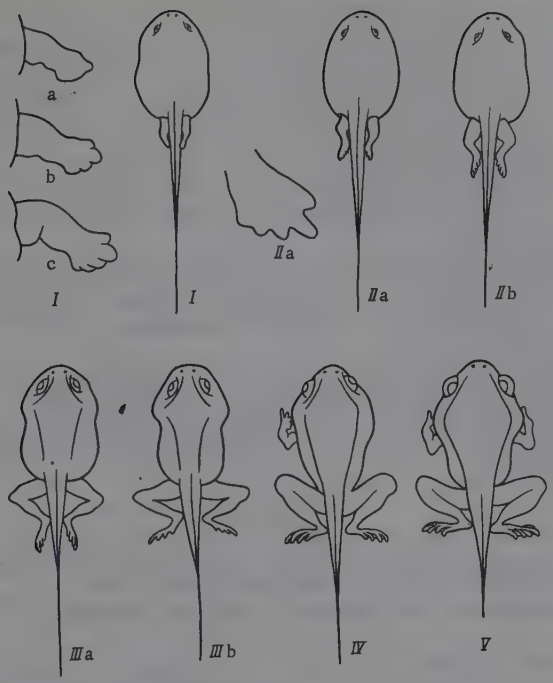


FIGURE 2. Successive stages in the metamorphosis of tadpoles of the grass frog

Stage V —both hind extremities have cut through. The tail is resorbed and shortens, the gills are resorbed, and the tadpole breathes with lungs.

Stage VI — the tail is completely resorbed and the tadpole is transformed into a froglet.

Our histological investigation shows (Polezhaev, 1940, 1948), that at Stage I the tissues of the hind extremities of the tadpole are not yet completely differentiated. The diaphyses of the skeletal elements of the hip and shin are cartilaginous but the epiphyses have not yet chondrified. The muscles appear only in the form of anlagen. The chorial anlage is very thin. At Stage II, differentiation proceeds further: around the cartilaginous diaphyses of the skeletal elements of the hip and shin, a bone with a periosteum appears, the epiphyses are cartilaginous, and the muscle anlagen are transformed into muscle fibers, the chorium develops and thickens. At Stage III further differentiation of tissues and of all the components of the extremities takes place. Under the skin, lymphatic cavities are formed. At all stages, the differentiation of skeletal, muscular and other elements of the extremities proceeds in a proximodistal direction.

Regeneration of extremities in various species of tadpoles had been observed long ago (Spallanzani, 1769; Barfurth, 1895; Kochs, 1897, and others). There was a dispute as to the ontogenetic borders of regenerative capacity of extremities, however, mainly because the investigators did not devote due attention to the characteristics of the stages of metamorphosis and the level of amputation of the extremities. Further studies showed that the capacity for regeneration depends to a great extent on these factors.

It turned out that on amputation of hind extremities of tadpoles of the grass frog (Rana temporaria) at the level of the proximal part of the shin distal to the knee, the extremities regenerate in 100% of cases at Stage I. At Stage IIa, they regenerate in 50% of cases, and at Stage IIb, in 4.6% of cases. If the extremity is amputated at Stage IIa in the proximal part of the hip, complete regeneration occurs in 8.6% of cases; after amputation in the proximal part of the shin, regeneration occurs in 42% of cases; and after amputation in the distal part of the shin, regeneration occurs in 82% of cases (Polezhaev, 1948).

Comparative investigation shows that the regenerative capacity is lost at the same stage in all genera of frogs, somewhat later in Pelobatidae, and even later in Bombina (Kamerer, 1905; Ginsburg, 1941). These data require further checking, however.

It has been shown that the dependence of regeneration of extremities on the amputation level or the gradient dependence also prevails in adult frogs and is due to gradients in the skeleton of these organs (Polezhaev, 1947, 1947a, 1948, 1948a).

Experimental investigation of the phenomenon of loss of regenerative capacity of extremities in tailless Amphibia

This study was initiated by Guyénot (1926, 1927). He investigated the regenerative capacity of extremities of tadpoles in relation to whether it is lost under the influence of the internal humoral environment of the animals or independently of it. He xenoplastically transplanted segments of tails from frog tadpoles capable of regeneration, or extremities from tadpoles not capable of regeneration to salamanders which were capable of regeneration. As a result, the tails regenerated, but not the limbs. From this he reached the conclusion that organs (tails of frog tadpoles) may preserve their regenerative capacity, despite change in the internal humoral environment, while organs which lost this capacity (extremities of frog tadpoles) cannot restore it under the influence of the internal humoral environment of animals which are capable of regeneration. Liozner (1931) made a similar experiment with grass-frog tadpoles, transplanting homoplastically extremities from tadpoles which had lost, and from those which had not lost the regenerative capacity, to tadpoles which did or which did not have this capacity. As a result of these studies, he confirmed Guyénot's data. Being interested in the question of whether regenerative capacity does not change under the influence of the operative procedure itself, he also showed that extremities which have lost the capacity for regeneration in a number of cases (at Stage IIa) may regenerate after autotransplantation, while the second partner of the pair simultaneously amputated does not regenerate*. Regretably, this problem has not been pursued any further.

The Chilean investigator, Ide-Rozas (1936) studied regeneration of extremities of tadpoles of frogs and toads (Rana temporaria,

* Experiments with transplantations of extremities to recipients of different age do not allow one to conclude anything concerning the effect of transplantation on regeneration, as the result in these cases may be due to the action on the transplant of the different internal humoral environment of the animals.

Rana esculenta, and Bufo vulgaris) at Stages I, II, and III according to L. Ya. Blyacher. He distinguished 12 types of cells: epithelial, fibrocytes, perichondrial, wandering cells, macrophages, lymphocytes, eosinophil leukocytes, special leukocytes, pigment cells, chondrocytes, muscle cells, and regeneration cells. The epithelium of the regenerate arises from epithelial cells, while the internal part of the regenerate arises from regeneration cells. The latter are formed by transformation from wandering cells. lymphocytoid cells, perichondrial cells, cartilage, and muscle cells, Regeneration cells arise only before Stage IIa. They cannot appear later, according to the investigator, because they lose the capacity for division. As we have seen, and as we shall show later, this conclusion of Ide-Rozas is incorrect.

The time of loss of regenerative capacity of extremities in tadpoles may be shortened if metamorphosis is shortened by giving the animals thyroidin. If they are fed thyroidin after the process of regeneration has begun, however, regeneration of extremities not only is not inhibited but, contrarily, is enhanced (Speidel, 1929; Liozner, 1936). The mechanism of these phenomena was not elucidated by these investigators.

These questions naturally arise. What takes place in the organs and tissues during the loss of their capacity for regeneration, and is it not possible to restore it?

Restoration of lost regenerative capacity of extremities of tadpoles

We first carried out this study on tadpoles of the grass frog (Rana temporaria), at the same time investigating the reasons for the loss of regenerative capacity (Polezhaev, 1933, 1935, 1936, 1939, 1940). We turned our attention to the fact that loss of regenerative capacity occurs suddenly in jumps. If the extremities are amputated at the knee level at Stage I, they regenerate fully, but on amputation at Stage IIa, they do not regenerate, the blastema is not formed, and the amputation wound surface heals smoothly. Why is the blastema formed at Stage I and not at Stage IIa? The formation of blastemata is associated with the accumulation of cells of regeneration blastemata of the embryonic type under the epithelium of the amputation wound surface of the organ. Data from published reports (Guyénot, 1927; Liozner, 1931) pointed out that changes in the internal humoral environment of the animals cannot serve as an insurmountable obstacle for regeneration of organs in tadpoles. Therefore, the most probable assumption seemed to these investigators to be that insufficient accumulation of cells in the regeneration blastema was due to sluggish cell division. At that time (1932) it was not known what happens with the extremities after amputation at various stages of metamorphosis. They did know, however, that in the process of metamorphosis, the tissues of the extremities differentiate progressively, and it was known from published reports that differentiation of tissues interferes with their proliferation. Description of embryogenesis has shown that periods of growth of the organ alternate with periods of its differentiation, and that "the formative activity of cells increases with time in the period of depression and decreases with time in the period of intense

growth" (Schmalhausen, 1926). In tissue culture outside the body, it was shown that by slowing down growth, processes of progressive differentiation may be obtained; for example, from chondroblasts and osteoblasts development of cartilage and bone can be obtained. A conclusion was drawn, therefore, that "active proliferation and differentiation are simultaneously incompatible" (Fischer and Parker, 1929). In a tissue culture under conditions of rapid and intense growth and proliferation of fibroblasts, argyrophil and collagen fibers do not appear. If the growth of the fibroblasts is slowed down, however, these fibers are formed (Maximow, 1923). Thus, there were good reasons to believe that progressive differentiation of tissues of mesodermal origin in the extremities of tadpoles interferes with the proliferation of cells after amputation of the extremities.

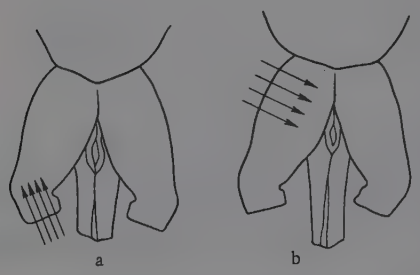


FIGURE 3. Scheme of operation. Amputation of extremities of tadpoles, viewed from the ventral side:

a – traumatization in the distal-proximal direction; b – traumatization in the proximal part of the hip.

We decided to enhance the processes of proliferation in the tissues of the remaining organs after amputation of the extremities in tadpoles which lost the capacity for regeneration, and we suggested using the method of strong additional traumatization for this purpose. It is known that damage of tissues leads to the formation of decomposition products of cells, so-called wound hormones (Bier, 1917; Haberlandt, 1922), and mitogenetic rays (Gurwitsch, 1921, 1934; Blyakher, 1932), which stimulate cell division and regeneration.

In tadpoles at Stages IIa and IIb, both hind extremities were amputated in the distal part of the shin. The left extremity was left without any treatment to serve as a control, while the right extremity was strongly traumatized additionally with a needle in the longitudinal distal-proximal direction (Figure 3a), making 15—20 deep cuts. Traumatization was very strong as the needle was only 2—4 times thinner than the leg of the tadpole. As a result, the residue of the experimental extremity became very strongly hyperemic, bright red, and swollen, while the control extremity showed no such changes. In many cases blastemata appeared on the experimental extremity, grew further, differentiated and transformed into regenerated extremities, while the control extremities healed smoothly (Figure 4, a, b). The regenerates could be typically 5-toed feet, or atypical with one or two toes missing (Figure 4 c).

The positive experimental result confirmed our basic hypothesis that the loss of regenerative capacity of organs is due to the absence of accumulation of cellular regenerative material in the focus of damage. These results also confirm our other hypothesis, later also voiced by Ide-Rozas (1936), that the accumulation of cells in the blastema does not occur because of loss of the capacity for proliferation. Histological examination of the experimental material, however, showed that an entirely different mechanism serves as the basis for the phenomenon of loss and restoration of regenerative capacity.

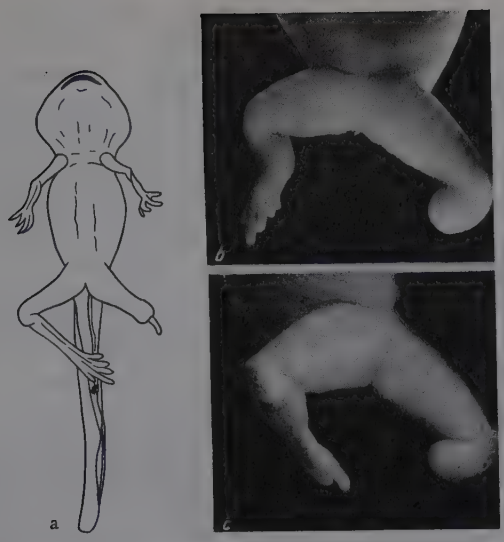


FIGURE 4. Regeneration of experimental, additionally traumatized extremities, and lack of regeneration in the control extremities of tadpoles of the grass frog:

a, b – typical regeneration of extremity; c – atypical regeneration of extremity.

It turned out that the region of the amputation wound surface of the control extremity became epithelized, and tissues of mesodermal origin (cartilage and bone skeleton, muscle bundles, connective tissue) were weakly destroyed and did not dedifferentiate. Subsequently, they became covered with a connective tissue scar, and the amputation wound surface healed smoothly. An entirely different picture was observed in the region of the amputation wound surface of the experimental extremity. The tissues in the region of the wound became epithelized, swollen, impregnated with a serous exudate, and large foci of hemorrhage appeared; the tissues underwent intense destruction and dedifferentiated. The muscle buds dispersed into individual cells, and the basic substance of the cartilage disappeared, the cartilage transforming into prochondral tissue and subsequently into an aggregate of mesenchymal cells which could not be distinguished outwardly from cells arising from dedifferentiated muscles and connective tissue. These dedifferentiated cells moved in a powerful stream in the direction of the epithelium, changed into cells of the embryonic type and formed a regeneration blastema in the region of the amputation wound surface (Figure 5). With growth and differentiation, cartilage and muscle buds appeared in the blastema and the latter was transformed into an extremity with all its typical components. In the regenerating extremity, 3 zones were distinguished: a proximal zone, containing all the tissues and maintaining their differentiation; an intermediate zone containing dedifferentiated and later redifferentiating tissues; and a distal zone containing regenerating tissues. On the whole, the regenerating extremity remained shorter than the

control for a long time, consisted of younger tissues, and contained less differentiated components than did the control.

These data show that the loss of regenerative capacity of extremities during ontogenesis in frog tadpoles is directly associated with disturbance of the accumulation phase, with insufficiency of regenerating material, that is, with the loss of the capacity of mesodermal tissues of the extremities to be destroyed and dedifferentiated. As a result of this, the cells cannot be released from their tissue connections and cannot form accumulations of cellular material in the regeneration blastema, necessary for its growth and development. The immediate reason for the disturbance of the accumulation of cells in the focus of damage is the advancement of differentiation of tissues at the time of amputation of the organ, and not the loss of the capacity of the component cells for division or morphogenesis. To restore the lost regenerative capacity of the extremities of the tadpoles, it is necessary to bring about a strong destruction and dedifferentiation of the main mesodermal tissues of these organs, which would lead to release of cells from their tissue connections, to restoration of the phase of accumulation, and to formation of the regeneration anlage. After amputation of the control extremity in the region of the wound surface, a small amount of mesenchymal type cells arises, but these are not capable of forming an anlage capable of growth. For growth of the blastema, accumulation of a minimum number of cells in it is essential. This is similar to the data which showed that a minimum number of cells is essential for the growth of a tissue culture outside the body (Fischer, 1925).

FIGURE 5. Frontal section of the extremities of a tadpole:

On the left, the experimental extremity: destruction and dedifferentiation of tissues and formation of a regeneration bud on the traumatized extremity; on the right, the control: absence of destruction and dedifferentiation of tissues in the extremity and smooth healing of the wound.

Traumatization causes destruction of tissues and formation of excessive decomposition products of cells and tissues. These substances at first cause dedifferentiation of cells, release of muscle cells from their tissue connections, and formation of blastemata consisting of embryonic-type cells; later these embryonic-type cells begin to proliferate.

Growth of the regeneration bud which arises from traumatization of the residue of an organ which lost its capacity for regeneration determines the onset of differentiation, and the latter determines the new growth of the regeneration anlage.

Mechanism of restoration of lost regenerative capacity

While restoration of the lost phase of accumulation was achieved by destruction and dedifferentiation of tissues of mesodermal origin in the amputation wound surface area of the organ residue, the role of proliferation which could have been strongly stimulated by additional traumatization of tissues remained unclear. In order to clarify this matter we used 2 new methods in this study: 1) traumatization of different zones in the residue of the organ, and 2) count of mitoses in the traumatized and control extremities of the animal.

Distal and proximal traumatization of the residue of the organ after amputation of the extremities of tadpoles of the grass frog at Stage IIa was carried out. Needle prickings were made longitudinally on distal traumatization, and only in the distal part of the shin residue (Figure 3a). In proximal traumatization, the same number of prickings was made transversely in the proximal part of the hip (Figure 3 b). With both distal and proximal traumatization, products of tissue decomposition arose in abundance. These could have freely acted on tissues in the region of the amputation wound surface and stimulated their cells to divide. In addition, the tissues in the region of the amputation wound surface strongly dedifferentiated where distally traumatized while this did not happen in the case of proximal traumatization. Thus, if restoration of the lost regenerative capacity of extremities was due to stimulation of proliferation while dedifferentiation played a secondary role, the extremities would have regenerated in the experiment with proximal traumatization. However, if dedifferentiation is essential for the occurrence of restoration, the extremity would regenerate only in the experiment with distal traumatization.

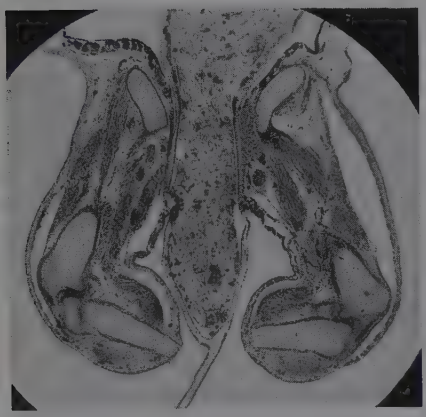


FIGURE 6. Frontal section. Absence of destruction, dedifferentiation, or regeneration in both extremities of tadpoles: on the left, proximal traumatization of the extremity; on the right, control.

The experiment showed that proximal traumatization was not effective, while distal was. In proximal traumatization, strong destruction and dedifferentiation of tissues was observed in the traumatized hip, while tissues were not destroyed or dedifferentiated in the region of the amputation wound surface, and the extremity did not regenerate (Figure 6). There were many mitoses in the zone of traumatization, 80 in 20 serial sections on an average, while in the control extremity hip, there were an average of 10 in the same number of sections. In the tissues of the amputation wound surface and in the thicket of the tissues of mesodermal origin in the residue of the shin, many mitoses were present, 90 per 20 serial sections on the average. The number of mitoses was the same in the experimental and control extremities.

Distal traumatization resulted in strong destruction and dedifferentiation in the tissues of the amputation wound surface and within the thicket of tissues of mesodermal origin in the stump of the shin. The number of mitoses was 90 per 20 serial sections on the average. In the control extremity, phenomena of destruction and dedifferentiation were not seen, and regeneration blastemata did not form. The number of mitoses was, on the average, the same as in the experimental extremity. It is most important that during the period of formation of the regeneration anlage in the experimental extremity, the mitotic coefficient, i. e., the ratio of the number of mitoses to the total number of cells expressed as percent was very low, and there were only a few mitoses in the blastema. That means that the blastema is formed mainly without mitoses, due to the release of cells from the tissue associations during dedifferentiation of tissues.

Thus, for the restoration of lost regenerative capacity of extremities in tadpoles, phenomena of deep destruction and dedifferentiation of tissues in the region of the amputation wound surface are essential and play a most important role. Proliferation, however, is approximately of the same intensity in tissues of the experimental and control extremities. Evidently, additional traumatization cannot enhance cell division significantly, caused by amputation of the organ. The regeneration anlage arises mainly without mitoses. These begin to play a great role at later stages of development of the regeneration anlage. Loss of regenerative capacity of extremities takes place not because the cells in the amputation wound surface lose their capacity for mitotic or amitotic division, but because the cells cannot leave the tissue associations, due to the loss of the tissue's capacity for destruction and dedifferentiation. As will be seen later, this fact is significant not only in the case of regeneration of extremities in tadpoles, but also in a number of other cases, in regeneration of different organs and tissues of animals, and mammals and man in particular. In order to obtain a maximum effect, those tissues which are in the area of damage must be affected directly and not those removed from it. In the latter case, the effect will be weaker.

Prolongation of regenerative capacity of extremities of tadpoles

In the experiment just described, restoration of lost regenerative capacity of extremities of tadpoles was obtained at an early stage of ontogenesis, stage IIa and partially at Stage IIb — of metamorphosis. At later stages of development, the use of the traumatization method was ineffective. Therefore, the problem of restoring lost regenerative capacity at later stages of ontogenesis arose. As shall be seen later, this problem was solved, and regeneration was obtained even in adult frogs. The task was difficult, however, and it was not solved at once. At first (Polezhaev, 1933, 1935, 1936) we did not succeed in restoring regenerative capacity, but we did succeed in prolonging its maintenance in extremities in tadpoles at late stages of metamorphosis by methods of re-regeneration and repeated amputation.

A number of profound changes take place in the animal body during metamorphosis. Changes in cells and tissues may interfere with the restoration of regenerative capacity of the extremities at later stages of metamorphosis, not allowing the appearance of phenomena of destruction and dedifferentiation of tissues in the organ residue, or for example, continuously enhancing the inhibitory effect of the internal humoral environment. If the possibility of destruction and dedifferentiation were associated with a certain degree of differentiation of tissues of mesodermal origin in the organ residue, it could then be expected that regeneration could be obtained by arresting differentiation of the extremities at the late stages of metamorphosis. The method of re-regeneration is based on this principle.

In experiments on re-regeneration in tadpoles of the grass frog, we amputated one hind extremity at the level of the knee at Stage Ib or Ic, while the other extremity was not amputated. The amputated extremities regenerated, and when they reached the same degree of differentiation as that in which they were before amputation (Figure 7 a), a new amputation was carried out: the major part of the regenerate was amputated and the control extremity which was not operated upon was also amputated at the corresponding level. Since ontogenesis and the process of regeneration of extremities developed at different rates in the tadpoles, it was found that the animals were at different stages of metamorphosis, namely, IIa, IIb, IIIa, IIIb, and even IV, at the time of amputation. As a result, the control extremities did not regenerate, and the wounds healed smoothly, while the experimental extremities re-regenerated (Figure 7 b); upon amputation at later stages of metamorphosis, regeneration occurred in young frogs.

Thus, it seems that young, insufficiently differentiated tissues of the regenerate have a high capacity for regeneration, and may regenerate at late stages of metamorphosis despite the effect of the internal humoral milieu which prevails in older animals.

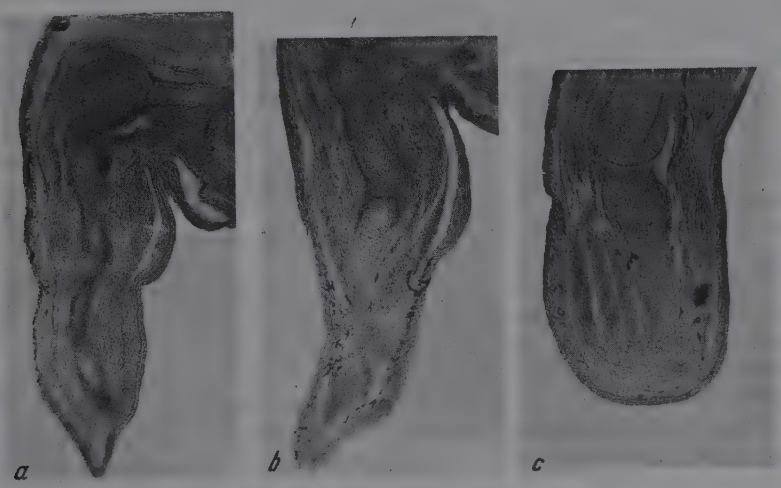


FIGURE 7. Re-regeneration of extremities in tadpoles of grass frogs:

a – regenerate of extremity at time of amputation; b – re-regenerate of extremities; c – control, smooth healing of the wound.

The data obtained are in close agreement with those from published reports. Similar results were obtained by Marcucci (1916)*. T. M. Yakovleva (1938) transplanted hind extremities autoplastically to the back of tadpoles of the grass frog at Stage I. The transplants were arrested in development. After amputation, they regenerated while the second extremity of the pair which remained in place did not regenerate. The tissues of the transplant were considerably younger and less differentiated than the tissues of the control extremities. Speidel (1929), working with some American species of frogs, amputated their tails and placed the animals in water with calcium thyroidin. The tails regenerated and metamorphosis was enhanced. The proximal part of the tail was resorbed while the distal part continued to regenerate. Thus, young regenerating tissues of organs possess a special, higher morphogenetic capacity than do old tissues. Our data and those from published reports were confirmed by the results from some experiments of Studitskii (1963). This investigator reached the conclusion that tubular bones of rats regenerating after extirpation from under the periosteum, or regenerating muscles, are more resistant to X-irradiation than nonregenerating ones. The investigator calls these special properties of regenerating tissues a plastic state.

In the experiment on re-regeneration, the source of regeneration was the young tissues of regenerates of extremities. The question arose whether regeneration could be caused by prolonging the maintenance of the regenerative capacity of the old tissues of the organ. In this connection, we designed experiments with the aim of prolonging regenerative capacity by the repeated-amputation method (Polezhaev and Morozov, 1941).

It has been shown that the main conditions for regeneration of organs are destruction and dedifferentiation of the main component tissues of mesodermal origin. On the other hand, it is known that upon repeated removal of the regeneration bud with the amputation wound surface of the extremities of tails of Amphibia, regeneration is not only not inhibited (Schaxel, 1921; Büttner, 1930), but is even enhanced (Blyakher, 1936). It may be assumed that repeated amputation leads to enhancement of phenomena of dedifferentiation of the old tissues in the residue of the organ and, therefore, to stimulation of regeneration. If this assumption were correct, then repeated amputation of the regeneration bud would rejuvenate the old tissues in the residue of the organ and cause their dedifferentiation in metamorphosing tadpoles in those cases where they would not have been capable of it upon ordinary amputation. As a result, there could be hope to obtain regeneration of extremities which do not regenerate upon simple amputation at the given stage of ontogenesis. We performed such an experiment. In grass frog tadpoles at Stage I, we amputated the right hind extremities in the proximal part of the shin. After several days, we amputated the regeneration buds, or cones, together with a small amount of old tissues. Simultaneously, we also amputated at the same level the left hind controls which had not been operated upon. At the time of amputation the animals were at different stages of development beginning from I to IIIa. The results were quite clear. Upon repeated amputation of the right extremities, when the animals were still in Stage I, both extremities regenerated, whereupon the regeneration in the right extremity was much faster than that in the left extremity (Figure 8 a). This phenomenon was

* We had the opportunity to acquaint ourselves with the work of the Italian, Marcucci (1916) only in 1945, i.e., 2 years after the publication of our own data (Polezhaev, 1933).

evidently due to the fact that as a result of enhancement of destruction and dedifferentiation of old tissues in the residue of the organ after repeated amputation, rapid accumulation of cells took place in the regeneration bud and the rapid increase in the volume of the bud resulted in enhancement of its differentiation. This latter circumstance was convincingly demonstrated in the beautiful work of Filatov (1931) in experiments on the development of buds of extremities in normal ontogenesis of axolotl.

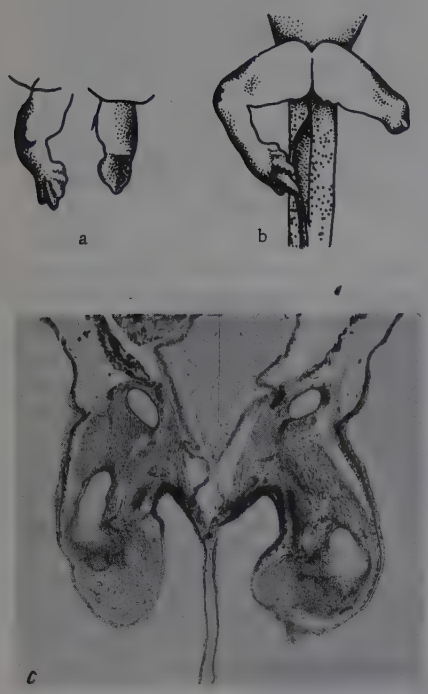


FIGURE 8. Experiment with repeated amputation of extremities in grass frog tadpoles:

a — left side — after repeated amputation; right side — after one amputation at Stage I; b — left — regeneration after repeated amputation; right — absence of regeneration after one amputation at Stage IIb; c — frontal section of hind extremities of tadpoles: left — experimental; after repeated amputation, tissues of mesodermal origin are destroyed and dedifferentiated, regeneration bud is formed; on the right — control; after amputation, tissues not destroyed or dedifferentiated, and regeneration does not occur.

Upon repeated amputation at Stages IIa—IIIa, the result obtained was even more striking: the control extremities did not regenerate, while the experimental ones fully regenerated (Figure 8 b). Positive results of regeneration were obtained in 95.6% of cases. Histological examinations showed that tissues of mesodermal origin in the region of the amputation wound surface were weakly destroyed and weakly dedifferentiated in the control extremity, but they were greatly destroyed and deeply dedifferentiated in the experimental extremity (Figure 8 c). Dedifferentiation encompassed mainly tissues in the distal part of the organ residue, while in the proximal part, the tissues differed slightly from those in the control extremity in respect to differentiation. Mainly muscle tissues were destroyed and dedifferentiated, while cartilage dedifferentiated to a lesser extent.

The experiment with repeated amputation shows that the regenerative capacity may be considerably prolonged; thus, regeneration may be obtained at late stages of metamorphosis (Stage IIIa). The source of regeneration in this case is the old, seemingly rejuvenated, tissues and not the tissues of the regenerate, as was the case in the experiment with re-regeneration. In the experiment with repeated amputation, the basis for regeneration is the same mechanism as in the experiment with traumatization: strong destruction and dedifferentiation of tissues of mesodermal origin in the residue of the organ. Repeated amputation arrests the progress of differentiation in the old tissues of the organ residue.

Changes in regenerative capacity during ontogenesis in tailless Amphibia

The significance we established of destruction and dedifferentiation of tissues for the loss and restoration of regenerative capacity of extremities in tailless Amphibia requires knowledge of how the regeneration process develops after amputation of extremities at various stages of metamorphosis in tailless amphibia. In this connection, we instituted a special histological investigation of the extremities in the process of regeneration or healing of wounds after amputation in the proximal part of the shin at Stages I, IIa, IIb, and IIIa of metamorphosis (Polezhaev, 1935, 1940, 1948).

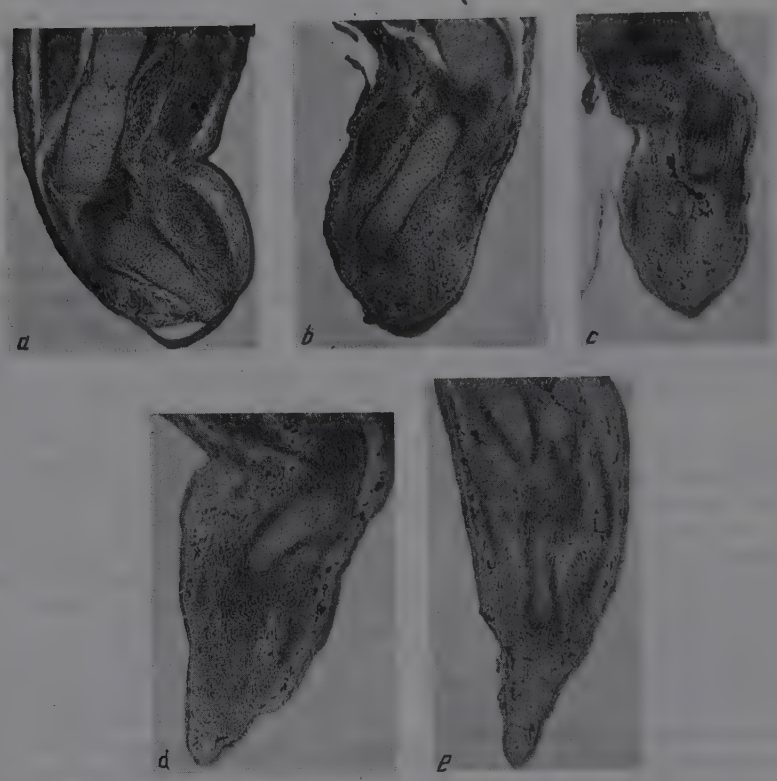


FIGURE 9. Regeneration of extremities in tadpoles of grass frog after amputation at Stage I:

a – after 12 hours; b – after 3 days; c – after 4 days; d – after 7 days; e – after 11 days.

After amputation at Stage I, at which the mesodermal tissues of the extremities were in a stage of differentiation, deep destruction and dedifferentiation of the tissues took place. Epithelization of the wound surface was completed 12 hour after amputation (Figure 9a). After 3 days,

there was intensive dedifferentiation of muscle bundles and the distal ends of skeletal elements, and a regeneration bud appeared in the region of the wound (Figure 9b). At 5 days after amputation, destruction and dedifferentiation were so extensive that a mass of mesenchymal cells with islets of cartilage and prochondral tissue residues of what had been muscle bundles and cartilage skeleton remained in the residues of the organs. These tissues were directly transformed into the cells of the regeneration blastema, which looked like a large nondifferentiated bud covered with epithelium, thickened at its apex (Figure 9c). At 7 days after amputation, a large regeneration cone arose inside which prochondral cartilage anlagen were formed. In the residue of the organ, redifferentiation of the cartiliginous skeleton began (Figure 9d). Eleven days after the operation, the regenerate was similar in shape to the extremity at Stage Ib or Ic. Inside it cartilage skeletal elements with differentiating diaphyses and anlagen of muscles appeared (Figure 9c). Thus, the process of regeneration of extremities in tadpoles of frogs at Stage I, when the regenerative capacity is not yet lost, is characterized by phenomena of destruction and dedifferentiation of the main tissues of mesodermal origin which comprise the residue of the organ. An entirely different picture is observed after amputation of extremities in tadpoles at Stages II — IIIa when the regenerative capacity has already been lost. The tissues of the residue of the organ are epithelized but slightly impaired, and there is practically no dedifferentiation. The skeleton and the muscles basically preserve their structure and continue to differentiate. The small amount of cells formed under the epithelium are transformed into connective scar tissue which covers the edge of the residue of the skeleton, joining the ends of the cut muscle (Figure 10, a, b). As a result the wound heals smoothly.

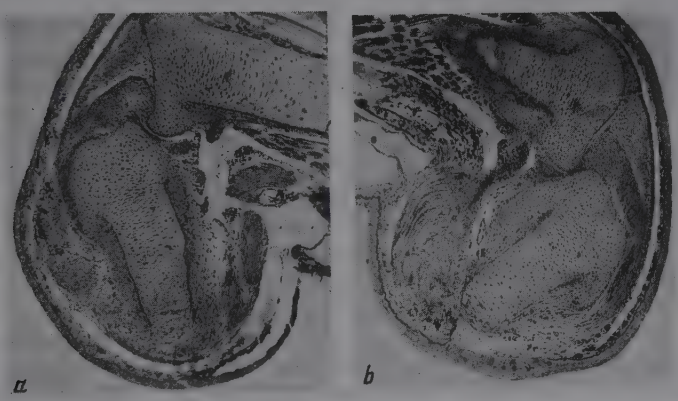


FIGURE 10. Lack of regeneration of extremities after amputation at Stage IIb:

a — after 3 days; b — after 8 days.

Our data agree entirely with those of Ide-Rozas (1936) who studied the cytological changes in extremities after their amputation at various stages of metamorphosis of tadpoles of grass and green frogs, and of the toad, when these are both still capable and no longer capable of regenerating the

extremities. However, as we have already pointed out, this investigator erroneously ascribed the reason for the loss of regenerative capacity of extremities to the loss of the capacity of the cells of these tissues for division. Schotté and Harland (1943) showed that after amputation of extremities in tadpoles of Rana clamitans, the outcome of the process depends on the level of amputation. After amputation at the distal level, the mesodermal tissues dedifferentiate and the extremities regenerate, while after amputation at the proximal level, there is no dedifferentiation, and the extremities do not regenerate. The results that were most similar to ours were obtained by Forsyth (1946), who studied, as we did, regeneration of extremities of Rana sylvatica tadpoles after amputation at various stages of metamorphosis.

Consequently, all these data confirm our conclusion that the loss of regenerative capacity of extremities during ontogenesis of tailless Amphibia is due to the loss of the capacity for destruction and dedifferentiation of tissues of mesodermal origin in these organs.

Changes in the regenerative capacity of extremities in a comparative series of vertebrates

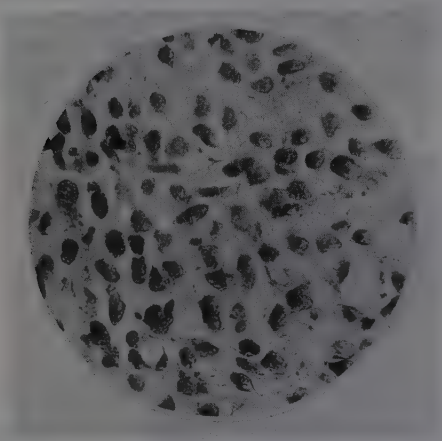


FIGURE 11. Cells of regeneration blastema in the extremity of axolotl

After the regularity of changes in the regenerative capacity of extremities during ontogenesis in tailless Amphibia was established, the question arose as to how does it change in a comparative series of vertebrates. In this connection, we amputated the extremities of animals with different capacities for regeneration of extremities: axolotls, frogs, lizards,* mice, and rats, which constitute a well defined ecological series in which animals pass from aquatic life conditions to terrestrial ones, and we compared the processes of regeneration or healing of wounds (Polezhaev, 1935, 1939, 1940, 1941, 1948; Barakina, 1951, 1951a, 1952, 1952a, 1952b).

It has been found that in axolotl, tissues of mesodermal origin in the region of the amputated wound surface are destroyed and dedifferentiated resulting in the release from these tissues of cells which gradually change and form a continuous series of transitions (transitory cells) from muscle cells, connective tissue, cartilage, periosteum, and cartilage to cells of the embryonic type, which form regeneration blastemata (Figure 11). In the process of transformation, differentiated cells gradually lose their specific properties, becoming morphologically similar, acquire basophil cytoplasm and large nucleolus, with an intense reaction to RNA, according to Brachet. At the

* It is well known that in lizards the tail regenerates readily, while the extremities do not regenerate after amputation.

beginning, typical blastemal cells arise under the epithelium of the amputation wound surface; subsequently, they arise throughout the entire regeneration bud. It is quite important to note that in large axolotls, the volume of dedifferentiated tissues is relatively small. The latter are localized in a zone bordering the amputation wound surface. If amputation of the extremities is carried out in younger, smaller axolotls, the phenomena of dedifferentiation are more strongly manifested than in the case of larger animals.

Regeneration of extremities in Amblystoma by differentiation of tissues of mesodermal origin has also been described by many investigators, and primarily by Americans (Butler, 1933, 1935; Butler and Puckett, 1940; Thornton, 1938, 1938a; Schotté and Harland, 1943, and others). In recent years, these data were confirmed experimentally on the regeneration of extremities in caudate Amphibia by the use of the autodiographic method (Hay and Fischmann, 1961; Anton, 1961; O'Steen and Walker, 1962; Bodemer, 1962, and others). However, these studies carried out on caudate Amphibia do not allow one to establish the importance of phenomena of destruction and dedifferentiation: it is possible that these phenomena only accompany, but do not cause regeneration. Butler (1933), who established the phenomenon of dedifferentiation during regeneration of extremities in larvae of Amblystoma, dealt with very small larvae. He reached the conclusion that amputation trauma causes destruction and dedifferentiation of tissues of mesodermal origin in the extremities, and the blastema which results inhibits dedifferentiation. Thus, his theory, accepted by a number of American investigators (Puckett, 1936; Schotté and Harland, 1943, and others), differs in principle from ours.

In caudate Amphibia, as demonstrated in the preceding material, at various stages of metamorphosis of the frog tadpoles the extremities regenerate after amputation, while they lose this capacity at later stages. In the former case, the tissues of mesodermal origin are destroyed and dedifferentiated, while in the latter case they lose this capacity. N. F. Barakina (1951a) found that in the former case the indicated tissues are very rich in RNA, while in the latter case they are depleted of it. If restoration of lost regenerative capacity of extremities in tadpoles is caused experimentally, RNA accumulates in the cells of the regeneration bud. After amputation of extremities at proximal levels in adult frogs, the tissues of the residues of the organs are partly destroyed, they do not dedifferentiate and organs do not regenerate.

In lizards, extremities do not regenerate after amputation (Marcucci, 1926; 1926a, 1930, 1932; Guyénot et Mattey, 1928; Barber, 1944; Umanskii, 1946; Polezhaev, 1948, and others). Histological studies showed that tissues of mesodermal origin in the residues of organs are not destroyed or dedifferentiated (Polezhaev, 1948).

It is well known that regeneration does not occur after amputation of extremities in adult mice and rats. Barakina (1952) showed that in this case there is no destruction or dedifferentiation of tissues of mesodermal origin in the residues of the organ, and that the cells of these tissues contain a small amount of RNA. A similar histological picture of healing of wounds after amputation of toes in adult mice was described by Schotté and Smith (1959)*.

* Evidently, not knowing the work of Barakina (1952), these investigators state that their paper describes for the first time histological changes taking place after amputation of extremities or toes in adult mammals.

Regeneration of organs did not occur after amputation of extremities and tails by intrauterine operation in embryos of mice, rats, rabbits, and guinea pigs (Nicholas, 1925—1934; Mitskevich, 1934, 1936; Aizupet, 1935). Two cases have been described in which the tails fully regenerated after simultaneous amputation of extremities and tails in embryos of mice (Mitskevich, 1934). However, in this experiment a technical error probably occurred, and the tails were not amputated at all: the operation was carried out in the blind, within the uterus, using needles, the embryos floating in the amniotic fluid. Some hints of regeneration in different cases after amputation of toes in embryos of rabbits and guinea pigs have also been described (Mitskevich, 1936; Aizupet, 1935). There was no histological control, however, and no description of the course of the process was given. When we repeated these experiments on mice (in 1949, unpublished), we could not confirm the above results: in all cases, the amputation wound surfaces of the extremities and tail healed smoothly.

In birds, rudiments of extremities cannot regenerate even after amputation at the stage when they consist of epithelium and a lenslike accumulation of mesenchymal cells (Lillie, 1904; Shorey, 1909; Spurling, 1923, and others). In recent years, it has been shown that in operations at this stage, the anlagen of the extremities have the capacity for regulation (Kieny, 1964).

From the data of the briefly quoted comparison, it can be seen that regenerative capacity of extremities changes regularly within a series of vertebrate animals. It decreases with transition of the animals from aquatic conditions of existence to terrestrial ones, and in air, with regular changes in metabolism, with increase in complexity of structure and function of the animals and their extremities. Histologically, the loss of regenerative capacity of extremities in a comparative ecological series of vertebrates is characterized by a loss of the capacity of tissues of mesodermal origin, comprising these organs, to undergo destruction and dedifferentiation. This capacity is lost in the ontogenesis of each class of vertebrates the earlier the animals switch from aquatic conditions of life to terrestrial ones.

It is necessary to point out that the processes of regeneration of extremities in mammals and birds have been little studied, and should be further investigated. Also, the class of fish requires special attention. In the latter, the capacity for regeneration of the paired fins (extremities) is frequently preserved (Kazantsev, 1935; Korschelt, 1927; Polezhaev, 1947; Vorontsova and Liozner, 1957, and others). However, no histological changes in the aspect of interest were discussed.

From our analysis, the general conclusion may be made that the regenerative capacity of extremities in a comparative series of vertebrates, as well as in the ontogenetic series, is lost in connection with the decrease in the capacity of the main tissues of mesodermal origin, comprising these organs, for undergoing destruction and dedifferentiation. This loss is historically connected with the emergence of the animals from aquatic conditions of existence to those of land, and with the regular adaptational changes of the entire metabolism, of all the functions and structures of all the organs and in particular of the animals' extremities.

The role of destruction, dedifferentiation, and proliferation in regeneration of extremities in caudate and tailless Amphibia

We have shown that upon the restoration of the lost regenerative capacity of extremities in metamorphosing tadpoles of the grass frog, the leading role is due to phenomena of destruction and dedifferentiation and not to mitotic division of the cells of tissues of mesodermal origin in the residue of the organ. The question arises as to what is the importance of these two groups of phenomena in the process of normal regeneration of extremities in caudate and tailless Amphibia, animals which preserve and lose regenerative capacity in ontogenesis, respectively.

It has been pointed out earlier that at the time we began our studies, a theory of regeneration prevailed according to which the decomposition products of damaged tissues containing "wound hormones" (Bier, 1917; Haberlandt, 1922), or emanating mitogenetic rays (Gurvich, 1921), caused intense proliferation of tissues in the region of the amputation wound surface, growth, and regeneration of the organ (Bromley, 1930; Blyakher, 1932). According to this theory, proliferation should be most intensive during the first days after the organ's amputation.

However, our experiments, in which we investigated the potencies of regeneration blastemata of extremities in axolotl, led us to diametrically opposed conclusions. These were: that at early stages of regeneration the blastema forms mainly due to multiplication of the cells of the organ residue. After transplantation of young regeneration blastemata of the tail or extremity of axolotl to the back, or another site, of the animal body, they do not grow. If they are transplanted, however, and if the number of their cells is increased 10-fold in the subepithelial part, growth occurs and regeneration of taillike organs or extremities takes place (Polezhaev, 1934, 1936, 1937, 1937a). Later, these data were confirmed by V. Samarova (1941). From these data, it follows that cells from the tissues of the organ residue accumulate in the blastema without undergoing mitosis. As already pointed out (Polezhaev, 1934, 1935), this problem should have been checked by counts of mitoses and calculation of the mitotic coefficient at consecutive stages of regeneration of the organ. It is worth mentioning that at the same time V. P. Kazantsev (1934) also observed that few mitoses are seen at the beginning of regeneration of extremities in axolotl, but he did not have accurate data.

We carried out a study on the processes of regeneration of extremities in axolotls and tadpoles of the grass frog at Stage I, and calculated the mitotic coefficient (Polezhaev, 1939; 1941; Polezhaev and Gintsburg, 1944). In agreement with our previous data, we found that epithelization of the amputation wound surface occurs without mitosis (Korshelt, 1927; Weiss, 1930, and others). The main attention was devoted to blastema cells. It was found that in both species of animals the initial stages of blastema formation occur with a very small amount of mitoses. The cells enter the blastema mainly without mitoses, by being released from tissues due to dedifferentiation of muscles, connective tissue, cartilage, periosteum, and perichondrium — in the stage of primary accumulation of cells in the blastema (Figure 12 a). The mitotic coefficient increases in the regeneration bud and reaches maximum in the regeneration conus; this is the stage

of secondary accumulation of cells in the regeneration blastema (Figure 12b). It is of interest that during regeneration of extremities in axolotls, the mitotic coefficient is 4- to 10-fold higher and the rate of regeneration 4- to 5-fold slower than in tadpoles. This means that for regeneration of extremities in tadpoles, phenomena of destruction and dedifferentiation of tissues are of greater importance than they are for regeneration in axolotls. Therefore, it can be understood that the inhibition of these phenomena should more strongly affect regeneration of extremities in tadpoles than in axolotls, while inhibition of the mitotic division should more strongly affect regeneration in axolotls than in tadpoles. These conclusions were fully confirmed by experimental data. Thyroidization, enhancing differentiation, leads to acceleration of the loss of regenerative capacity of extremities in tadpoles (Speidel, 1929) but does not inhibit regeneration in Amblystoma.

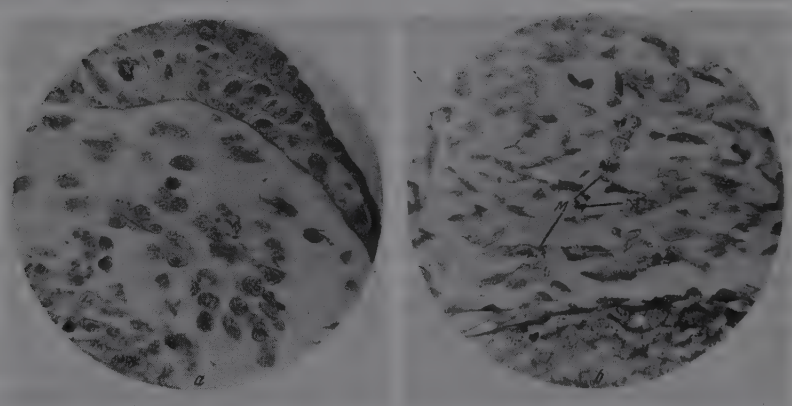


FIGURE 12. Formation of regeneration blastema of extremities in axolotls:

a – stage of primary accumulation of cells without mitoses; b – stage of secondary accumulation; M – mitoses.

Our data concerning changes in the mitotic coefficient at consecutive stages of regeneration have been fully confirmed by data of other investigators (Basina, 1940; Litwiller, 1939; Lüscher, 1946; Chalkley, 1954).

In studies of the role of mitotic division of cells in the process of regeneration, colchicine may be used, as it suppresses mitosis at the stage of metaphase, disturbing the division spindle and does not allow the divided chromosomes to move apart toward the poles. Thornton (1943) showed that colchicine in dilution of 1:1000 and 1:1500 completely suppressed regeneration of extremities in larvae of Amblystoma. We amputated extremities of tadpoles of the grass frog at Stage I, at the level of the knee and the distal third of the tail and placed the animals in a 1:1,000 solution of colchicine. The result was unexpected by us: the tails did not regenerate but the extremities regenerated fully, although with a slight lag, as compared with the control (Figure 13a, b). As a control, we used regenerating extremities of tadpoles also amputated at Stage I. Upon histological and cytological examination, we found that there were few mitoses in the

regeneration buds at early stages of regeneration of extremities, while at later stages there were many. Mitoses were found in all the stages: prophase, metaphase, anaphase, and telophase. They were thin and very well defined (Figure 13 d). In this experiment, the number of mitoses was larger than in the control, and they were mainly at the stage of metaphase, as further cell division was arrested. Chromosomes were found in the same number as in the control (polyploidy did not occur). They were swollen, thick, coarse, and stained lightly with hematoxylin (Figure 13 c). In later stages of regeneration, anaphase and telophase appeared in the developed regeneration bud.

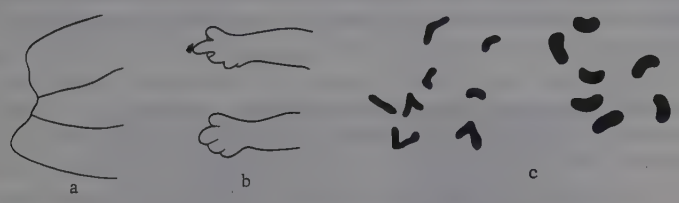


FIGURE 13. Experiments with colchicine using tadpoles of the grass frog:

a – absence of regeneration of the tail; b – complete regeneration of an extremity; c – chromosomes from cells of the blastema of extremities: left – in normal regeneration; right – after treatment with colchicine.

Our results show that mitotic division of cells is of greater importance for regeneration of tails than for regeneration of extremities, a finding which is in agreement with data from published reports (Guyénot, 1927). Comparison of our data with those of Thornton (1943) shows that it plays a greater role in the regeneration of extremities in caudate Amphibia than in the tailless Amphibia. This conclusion is in agreement with our data which show that the mitotic index in the former is much higher, while the degree of dedifferentiation in the old tissues of the residue of the organ is lower than in the latter.

Regeneration of extremities in caudate Amphibia is similar to the epimorphic type while it is more like morphallaxis in tailless Amphibia, as phenomena of dedifferentiation in the latter play a much greater role than in the former. Therefore, it becomes clear that difficulties in dedifferentiation ought to affect more strongly the course of regeneration of extremities in tailless Amphibia than in caudate Amphibia. And that, indeed, is what happens. In ontogenesis the progressive differentiation of tissues in caudate Amphibia does not inhibit, but in tailless Amphibia it inhibits the capacity of tissues of mesodermal origin in the extremities for being destroyed and dedifferentiated and, therefore, for the regeneration of these organs. Differentiation of tissues may affect differently the capacity of the tissues of the organs for being destroyed and dedifferentiated in animals of different species or in the same species at different levels of the organ (gradient dependence). Therefore, we can not agree with the opinion of Schotté and Harland (1943) that differentiation does not affect the capacity of tissues for dedifferentiation and regeneration of organs.

We thus reach the conclusion that the initial stages of regeneration of extremities and of other organs in Amphibia arise not by mitotic division of cells under the influence of decomposition products of tissues in the region of the wound, as has been supposed earlier, but by primary accumulation of blastema cells without mitoses, due to destruction and dedifferentiation of tissues and release of cells from the tissues. Proliferation occurs later at the stage of secondary accumulation of cells in the blastema, when there is a certain minimum number of cells. The decomposition products stimulate cell division but cannot cause growth of the bud if the number of cells in it did not reach a certain minimum. These processes occur in different ways during regeneration of extremities in caudate and in tailless Amphibia. From the aforesaid, it follows that the main factors in the regeneration of extremities in Amphibia, and probably of other organs in other animals, are processes of destruction and dedifferentiation of old tissues in the region of the focus of damage, proliferation at the beginning of the process playing a secondary role, and a foremost role later at the stage of secondary accumulation of cells in the regeneration bud. Consequently, in order to restore lost regenerative capacity, the investigator should strive not toward stimulation of cell division, but toward enhancement of destruction and dedifferentiation of tissues in the damaged organ.

Role of different components in the loss of regenerative capacity in extremities of tailless Amphibia

It is very important to know how the different components which participate in the regeneration of extremities in tailless Amphibia are changed. Among such components are: skin, tissues of mesodermal origin (muscles, skeleton, connective tissue) which, for the sake of brevity, we shall denote as mesodermal tissues, nerve tissue, and the internal humoral environment.

To elucidate the role of the skin and mesodermal tissues in the loss of regenerative capacity of extremities in metamorphosing tadpoles, transplants were performed in which skin and mesodermal tissues of extremities (without feet) were combined, the latter being taken from animals of different stages: I and IIIa. It was found that mesodermal tissues of Stage IIIa, when combined with skin of animals of Stage I, cannot regenerate extremities. Mesodermal tissues taken from animals at Stage I in combination with skin of animals from Stage IIIa also did not regenerate extremities in the majority of cases; nevertheless, they could regenerate in 10—18% of cases (Polezhaev, 1939). That means that in ontogenesis of the tailless Amphibia, the regenerative capacity of both skin and mesodermal tissues of the extremities is lost, the changes being more intensive in the mesodermal tissues than in the skin.

It is known that the presence of the nervous system is essential for the regeneration of extremities in caudate Amphibia (Weiss, 1925; Schotté, 1926; Singer, 1952, and others). Therefore, the assumption that the loss of regenerative capacity of extremities in tailless Amphibia is associated with changes in the properties of the nervous system is quite probable.

However, we have been aware of data which made probable the assumption that extremities in tadpoles which are at Stage I may regenerate without the

participation of the nervous system. We showed that in embryos of tritons extremities could develop from presumptive buds under conditions of explantation in a salt solution (Polezhaev, 1938; Amano, 1956). This means that until a known stage of ontogenesis, cells and tissues have the capacity for morphogenesis without the participation of the nervous system. Later experiments on transplantation of segments of extremities in tadpoles showed that regeneration occurred very rapidly, within 8—12 days. It is probable, therefore, that nerves do not yet manage to proliferate in the transplant, and that the latter regenerates without the participation of the nervous system, at least in the first stages. However, if a segment of extremity is transplanted to the tail of a tadpole, regeneration may occur at late stages of metamorphosis when the tail is resorbed and the transplant regenerates without the participation of the nervous system during the last stage of the process.

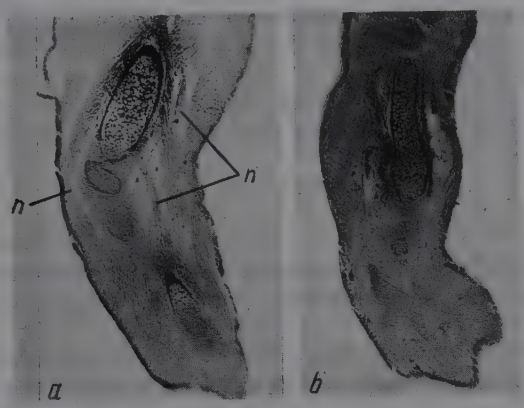


FIGURE 14. Regeneration of extremities in tadpoles at Stage I; silver stained according to Ramon and Kahal:

a — regeneration of control extremity, n — nerves; b — regeneration of transplanted extremity, no nerves present.

In our experiments, segments of extremities were autoplastically transplanted into the region of the abdomen of tadpoles at Stage I and, as control, we amputated at the corresponding level the opposite extremity of the hind pair. Full regeneration of the transplanted and control extremities took place. Impregnation with silver, according to Cajal, showed that the regenerating extremities in the control had well developed nerve fibers (Figure 14 a). There were no nerves in the experimental regenerating extremities (Figure 14 b). Consequently, regeneration of extremities occurred without the participation of the nervous system.

G. G. Shneider (1940) showed that in axolotls, too, segments of extremities transplanted to the back regenerate without the participation of the nervous system.

Schotté and Harland (1943a) denervated and subsequently amputated extremities at the distal level in tadpoles of Rana clamitans, Rana catesbeiana and Rana pipiens. After denervation and amputation at Stage I, the extremities regenerated, while they did not regenerate when amputated at later stages. These investigators maintained that in those

cases in our experiments where we obtained regeneration, the nerves of the recipient grew into the extremities.

Later, however, Yntema (1959, 1959a) repeated our experiments, and he showed that transplanted extremities regenerated after complete denervation. It should also be pointed out that Schotté and Harland (1943a) did not use the method of silver impregnation, which was essential. In addition, they worked with animals in which the ontogenetic borders of the regenerative capacity were not clearly established, and they speak of regeneration even in those cases when only a blastema is formed while further development remains unknown. Their data that the extremities of young tadpoles (Stage I) may regenerate upon denervation confirm our data.

The facts that at Stage I extremities may regenerate without the participation of the nervous system, and that restoration of regenerative capacity is possible at a later stage of metamorphosis, as well as that the properties of the skin and mesodermal tissues change in ontogenesis, make probable the assumption that loss of regenerative capacity is not due to changes in the properties of the nervous system. This problem must be further investigated, however. The possibility cannot be excluded that in phylogenesis the changes in different organs and tissues, and in particular in their regenerative capacity, are due to adaptive changes primarily in the nervous system.

Inhibition of regeneration of extremities under the influence of a changing internal humoral milieu in tailless Amphibia

Experiments with homoplastic reciprocal transplantations of extremities from animals capable of regeneration to animals which have lost this capacity, and vice versa, showed that regenerative capacity is lost due to local changes in tissues and not when tissues do not change or upon the appearance in the blood of factors inhibiting regeneration (Guyénot, 1927; Liozner, 1931). This conclusion is confirmed by data which indicate the possibility of regeneration of extremities in experiments with re-regeneration and repeated amputation, and by the experiments of Yakovleva (1938) on the regeneration of extremities of tadpoles arrested in development due to transplantation.

However, it is not clear whether during metamorphosis of tadpoles, factors inhibiting regeneration of extremities appear in the blood. It has been shown that thyroidin, enhancing metamorphosis, inhibits regeneration of extremities in tadpoles, if these organs were amputated after thyroidin treatment or simultaneously with the treatment of the animals (Speidel, 1928), and that inhibition of regeneration in such a case is not only due to the insufficient time for regeneration (Liozner, 1936). R. A. Borsuk (1935, 1936), who transplanted extremities from Stage I tadpoles to animals at Stage II, reached the conclusion that the internal humoral milieu of the animal changes during metamorphosis and inhibits regeneration of extremities. In these experiments no microscopic examinations of the extremities were performed, the stages used differed only slightly from each other, and the experimental results differed very little from those in the control. In this connection, we carried out experiments in which we transplanted segments of extremities from Stage IIa to Stage III and in the control, to

Stage IIa (Polezhaev and Gintsburg, 1939). In the former case, the regenerative capacity of the extremities was inhibited by changes in the humoral milieu, while it was restored in the latter case. Consequently, in the process of ontogenesis of animals, the internal humoral milieu changes in such a manner that it suppresses the regenerative capacity of organs. Evidently, this facilitates the differentiation of mesodermal tissues and interferes with their dedifferentiation, as shown by data from histological examinations. Further studies in which extremities were transplanted from animals at Stage Ic to very young animals (Stage 0) showed that the regenerative capacity of the tissues in these organs increased in a relatively autonomous manner, i. e., independently of the changes in the internal humoral milieu and also as a function of these changes (Polezhaev and Gintsburg, 1941).

Various methods for the restoration of lost regenerative capacity of extremities in tadpoles

As pointed out in the preceding material, the lost regenerative capacity of extremities in tadpoles of the grass frog can be restored by the method of transplantation (Liozner, 1931) and traumatization at Stage IIa. The following questions arise: 1. What happens with the extremities in the experiments with transplantation? 2. Is it possible to restore the lost regenerative capacity by other methods? 3. Can the lost regenerative capacity of extremities in tadpoles be restored at stages later than Stage IIa?

Before elucidating these questions, however it is necessary to investigate the contradiction which became apparent between our experiments and those of Yakovleva (1938). This investigator repeated the experiments of Liozner (1931) on autotransplantation of extremities in tadpoles of the grass frog at Stage IIa, and she reached the conclusion that "traumatization is not a factor which could force an extremity to regenerate at this stage, when the capacity had already been lost". This statement forced us (Polezhaev and Gintsburg, 1939) to repeat the experiments with autotransplantation of extremities on the same subjects and at the same stage of development. We obtained a positive result of regeneration in 51.8% of cases. Yakovleva's failure can probably be explained by the fact that she did not determine precisely the stages of metamorphosis, and used Stage IIb and not Stage IIa in her experiment, or that she amputated the transplant at a very proximal level. In experiments with traumatization at Stage IIa, the positive result of regeneration constituted 52.1%, i. e., the percentage of regeneration in experiments on traumatization and transplantation was the same.

Histological studies showed that in transplantation, the tissues of the extremities are intensely destroyed and dedifferentiated (Figure 15a), a fact which leads to the formation of a large regeneration bud (Figure 15a), while in the control these phenomena are not observed, and the wound heals smoothly.

In the experiments on traumatization just described, the pricking with the needle was performed immediately after amputation of the extremity. We designed an experiment in which repeated traumatization (4-fold replication) was carried out within four days after amputation at Stage IIb, and we obtained regeneration in 54.17% of cases (Polezhaev, 1945, 1946c). Single

traumatization at Stage IIb resulted in regeneration in only 17.1 % of cases. Histological changes were limited mainly to enhancement of destruction and dedifferentiation of tissues.

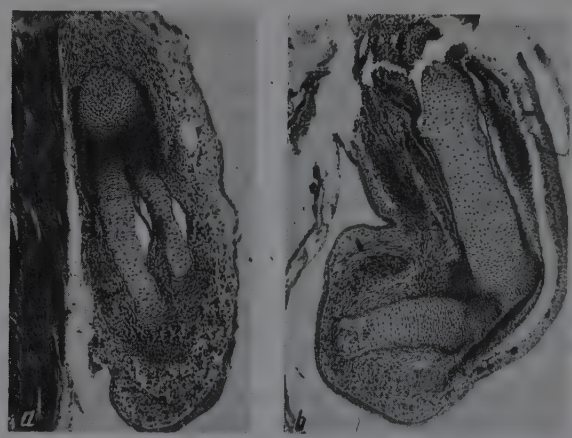


FIGURE 15. Restoration of regenerative capacity of extremities of tadpoles by the transplantation method:

a – destruction and dedifferentiation of tissues in transplanted extremity at Stage IIa, formation of the regenerative bud; b – lack of regeneration in control extremity.

In the meantime, interesting papers by Rose (1942, 1944, 1945) appeared which showed that it was possible to cause regeneration of extremities in adult frogs. Rose treated the amputation wound surfaces of the extremities with a hypertonic solution of table salt. These experiments will be discussed in the following paragraphs.

Rose's experiments prompted us to perform experiments on restoration of lost regenerative capacity of extremities in tadpoles of the grass frog, using a number of chemical factors: table salt (hypertonic solution), glucose (5% solution), iodine (10% alcoholic solution), nitric acid (10% solution), and sodium bicarbonate (0.8% solution). In the case of individual treatment, the amputation wound surfaces were subjected to a brief action of the chemicals, while in the case of general treatment, the animals were placed in appropriately diluted solutions (salt, glucose, bicarbonate) of the chemicals. Restoration of the lost regenerative capacity of the extremities upon amputation at Stage IIa was obtained in 42 % of cases in the experiment with salt, in 31 % with use of iodine, in 40 % with use of acid, in 35 % of cases in which glucose was used. In the experiment with glucose, a positive result of regeneration upon amputation at Stage IIb was obtained in 50 % of cases, while positive results were obtained in 30 % of cases amputated at Stage IIIa.

Thus, we succeeded in obtaining restoration of lost regenerative capacity of extremities in tadpoles by using various methods (traumatization, repeated traumatization, transplantation, chemical treatment) and not only at Stage IIa, but also at Stages IIb and IIIa. As shown by histological examination, the basis of the process of restoration is one and the same mechanism, the appearance of strong destruction and dedifferentiation of the main mesoderma tissues in the stumps of the extremities.

Induction of regeneration of extremities in adult frogs

Rose (1942) published a very interesting paper in which he showed that it was possible to restore the lost regenerative capacity of extremities in frogs of the species Rana clamitans at an age from several weeks after metamorphosis until full maturity. The forelegs were amputated between the elbow and the wrist. In the control, not one of 15 frogs showed any regeneration. In the experimental group, the amputation wound surfaces were treated with a saturated solution of sodium chloride twice daily, several minutes each time, for two months. The treatment was directed toward prolonging irritation in the tissues of the wound surfaces. In 21 of 22 cases, atypical extremities devoid of toes, but containing a number of skeletal elements of the forearm and wrist, regenerated. Somewhat later, Rose (1944) published the results of an experiment on 150 frogs of Rana clamitans, Rana catesbeiana, Rana palustris, and Rana pipiens of the same age as in his previous experiment. The extremities were amputated, as before, below the forearm. In the control, the wounds healed smoothly and were covered with skin. In the experiment the skin was again cut from the edges of the stumps, to subject the wound to a more prolonged effect of the external environment, or the stump was subjected to salt treatment. In the latter case, regeneration was more complete. The regenerated extremities were devoid of toes and contained skeletal elements of the forearm and wrist, muscles, and connective tissue. In Rana pipiens only cartilage and connective tissue regenerated. Histological examination carried out between 3 and 75 days after the operation showed (Rose, 1945) that in the control group the wounds healed smoothly and were covered rapidly with skin and did not dedifferentiate. In the experiment with salt treatment the tissues were strongly inflamed, they dedifferentiated, and as a result blastemata appeared and regeneration of extremities took place. Rose assumes that the primary reason for the lack of regeneration of extremities in tailless Amphibia is that the rapid formation of skin on the wound interferes with dedifferentiation of the tissues of the stump. On homoplastic transplantation of young skin taken from tails of tadpoles to extremities of frogs of species Rana catesbeiana, a blastema appears and regeneration of extremities occurs.

In our experiments (Polezhaev, 1945, 1946, 1947, 1948), we used Rana temporaria frogs and Bombina bombina, 2—3 years after metamorphosis. We studied the possiblity of obtaining in these animals regeneration of forelimbs amputated in the distal part of the forearm, and the mechanics of the regeneration process.

In addition to the control in which the extremities were subjected to simple amputation, the experiment included several series in which the tissues in the area of amputation were subjected to various treatments in order to enhance phenomena of destruction and dedifferentiation: 1) treatment with a saturated solution of sodium chloride every day for 3—7 minutes, and traumatization with a needle (15—20 prickings) for 22 days; 2) treatment with a 10% solution of iodine in alcohol, 1—2 times daily for 7 days; 3) transplantation of a skin cuff preserved according to Filatov (1937) at 2 to 7°C, with subsequent amputation of the extremities along the transplant; 4) transplantation of preserved muscles and amputation of the extremities along the transplant; 5) application of a tight ligature to the extremity, and amputation of the organ 10—14 days later in the region of damage;

6) application of a ligature and salt treatment for 19 days; 7) general treatment of the animals with a 5% aqueous solution of lactose 12—17 hours every day, for 26 days; 8) treatment with a 0.8% solution of sodium bicarbonate (pH 8) for 26 days, 1—2 times daily, 1—3 hours each treatment; 9) autotransplantation of the skin from the extremity at an angle of 90°. In the latter case, we wanted to prevent growth of skin on the wound, as the polarity of the skin is of considerable importance for regeneration (Samarova, 1940). In addition to these experiments, we also performed experiments in which the forelegs were simply amputated at distal and proximal levels, i. e., beneath the forearm and arm. Finally, we collected from nature, cases in which frogs had lost their extremities.

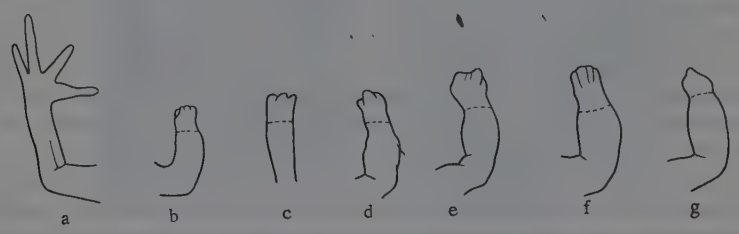


FIGURE 16. Forelegs of grass frogs:

a – normal extremity; b, c, d, e, f, g – atypical regeneration.

The results of the experiments were as follows:

1. After simple amputation of extremities in frogs and Bombina at a distal level, atypical regeneration took place (Figure 16, a, b, c, d-g); spadelike toeless extremities with elevations instead of toes, appeared. They consisted of small cartilage, connective tissue, and skin. Upon proximal amputation, the wounds healed smoothly. Similar results were also obtained by Thornton (1944, 1945). The data showing that it is possible to obtain atypical regeneration of extremities when they have been lost at the distal level are confirmed by findings in nature (Gadow, 1901; Morgan, 1908; Woodland, 1920; our data). In Xenopus laevis frogs extremities regenerate after simple amputation at various levels (Skowron and Komala, 1957).

2. Experimental treatment, especially in experiments with ligatures, bicarbonate, and conserved muscle, resulted in stimulation of the process of regeneration (Figure 17, a, b). The overall dimensions of the regenerate and the dimensions of its different skeletal elements increased, and bundles of muscle fibers appeared. Stimulation resulted in enhancement of phenomena of destruction and dedifferentiation and of growth and development of an atypical regeneration blastema. The cells of the latter looked like cells of young connective tissue and not like those of embryonic mesenchyme.

As shown by our studies (Polezhaev, 1947, 1948), the result of regeneration depends on the presence of skeletal elements inside the extremity. If the skeleton is removed, the extremity does not regenerate even after amputation at a distal level. If the skeleton of the distal part of the extremity is replaced by the skeleton of the proximal part, there is no regeneration of the organ after amputation along the transplant. If the skeleton of the proximal part is replaced by the skeleton of the distal part, the extremity

regenerates atypically after amputation along the transplant. The skeleton is more readily destroyed in the distal part of the extremity than in the proximal part, and produces better regenerative material.

It has been shown later that in adult Bombina, one may induce regeneration of atypical extremities by placing hydrolyzed cartilage of frog under the epithelium of the wound surface (Polezhaev and Ramenskaya, 1950).

Stimulation of regeneration of extremities in adult frogs of Rana pipiens and Rana clamitans was successfully obtained by implanting tissues of embryos and tadpoles of frogs into the extremity (Malinin and Deck, 1958; Malinin, 1960).

Singer (1954) connected the sciatic nerve from the hind extremity to the amputation wound surface of the foreleg of an adult frog and thus caused induction of atypical growth of extremities. Excitation by induction current of the nerves of extremities in frogs stimulates a process of atypical regeneration (Bedemer, 1963; Smith, 1967).

Thus in adult frogs and Bombina, using different methods, it is possible to induce or stimulate regeneration of extremities which do not regenerate or regenerate weakly under ordinary conditions of amputation. The basis of this induction or stimulation is the enhancement of processes of destruction and dedifferentiation or enhancement of innervation of the organ. Evidently, both these mechanisms, according to the principle of equifinality, (Polezhaev, 1938) lead to the same enhancement of biosynthesis of protein in the cells and tissues and to the accumulation in the focus of damage of cellular material with known morphogenetic potencies.

Induction of regeneration of extremities in lizards and young rats

Studies of the phenomena of loss and restoration of regenerative capacity of extremities in tailless Amphibia necessarily led to the investigation of this problem in higher taxonomic groups of vertebrates, namely, lizards and mammals.

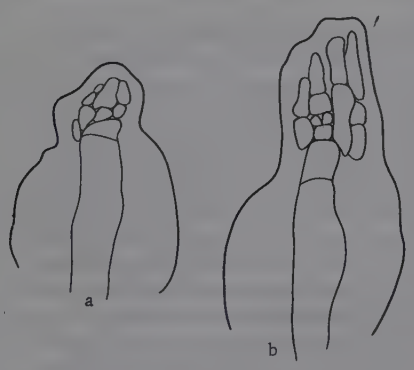


FIGURE 17. Regeneration of atypical extremities in adult frogs, graphic reconstruction:

a – after amputation of the wrist; b – after application of a ligature and subsequent amputation at the same level.

In lizards, the tail regenerates nicely after amputation, and the hind extremities may regenerate long atypical appendages under certain conditions (longitudinal oblique cut along the hip) but the forelegs, as a rule, never regenerate (Marcucci, 1926, 1926a, 1931, 1932; Guyénot and Mattey, 1928). After amputation, the mesodermal tissues of the stump of the tail dedifferentiate and those of extremities do not (Barber, 1944; Umanskii, 1946; Polezhaev, 1948). After amputation of the extremities, the wounds are rapidly covered with the old skin, but cutting off the skin at the edges of the wound does not result in regeneration of the extremities (Barber, 1944, 1945; Polezhaev, 1948). Nor did regeneration result from treating the wounds with ointment

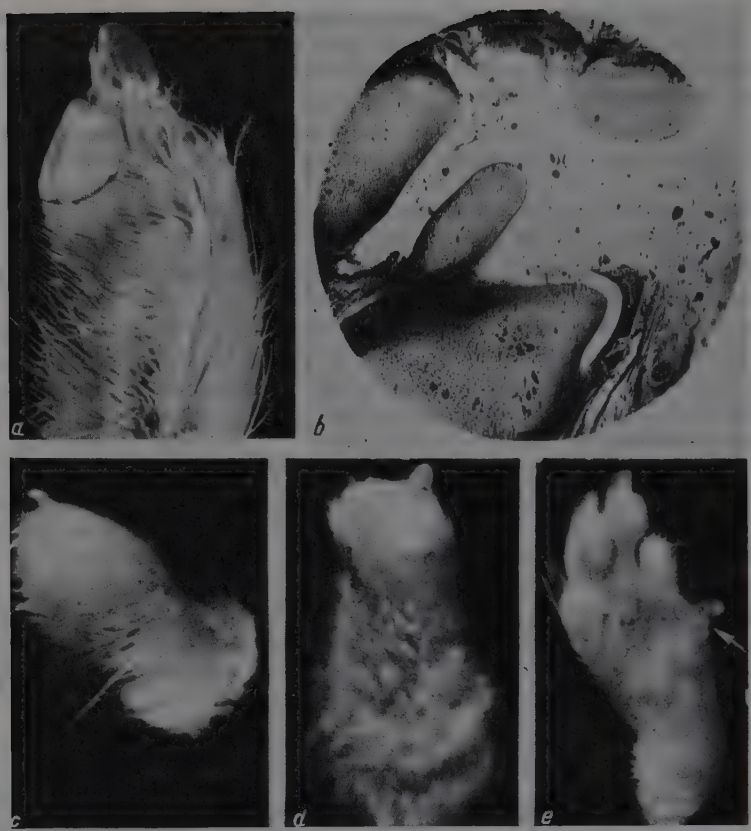


FIGURE 18. Formation of atypical regenerates after amputation of wrists of the forelegs of mammals:

a – atypical regeneration of extremities in newborn rats in an experiment with avitaminosis D and A; b – newly formed cartilage in regenerates (according to Rogal', 1951); c, d – formation of toelike appendages in adult mice after amputation of extremities at the level of the first toe, upon introduction of a vitreous body preparation; e – appendage formed after amputation (shown by arrow) of the first toe of the wrist of an adult mouse; f – longitudinal histological section through the regenerate, which appeared after amputation of the first toe and of the entire wrist of an adult mouse, and injection of a vitreous body preparation: k – old bone; x – newly formed cartilage; ct – connective tissue; z – epidermis with a callused surface layer.

No. 386, which stimulates regeneration of tissues, nor when the skin of the extremities was replaced with skin from the tail (Polezhaev, 1948).

A more successful attempt which resulted in restoration of lost regenerative capacity of extremities in lizards and young rats was achieved by changing the physiology of the organism in order to enhance the capacity for destruction and dedifferentiation of tissues. This was done in our laboratory in experiments with D- and A-avitaminosis (Rogal', 1951). Umanskii and V. P. Kudokotsev (1951, 1952) achieved this in experiments with parathyreocrin. Injections of parathyreocrin resulted in rapid softening of the body skeleton of lizards and young rats at the age of several days. After amputation of the extremities of lizards, long tapered appendages grew out; they contained a number of cartilages, muscle bundles, connective tissue, and skin. In the control, there was smooth healing of wounds after amputation of extremities. Induction of regeneration of atypical appendages in lizards could be obtained by hooking up the sciatic nerves from the paired extremities (Singer, 1962; Kudokotsev, 1964).

Studying the role of the zone of growth of cartilage in the epiphyses of tubular bones, Selye (1934) amputated the epiphyses in young rats aged 12—15 days. When he employed an oblique cut, there was restoration of the epiphysis with the zone of growth. Umanskii and Kudokotsev (1953) injected parathyreocrin in young rats aged 4—12 days and amputated the forearms. In some cases in the regenerating sector of the organ, the epiphyses of the ulna and radius were restored. Later, Kudokotsev (1963) injected extracts of cartilage in young rats with amputated extremities, and he observed stimulation of epiphyseal regeneration.

More interesting results were obtained in the experiments of I. G. Rogal' (1951). Female white rats were kept on a D- and A-avitaminosis diet for a long time. After amputation of the foreleg wrists in newborn rats, quite good structure with toelike appendages regenerated (Figure 18a) in a number of cases. After one year, a nail appeared on the regenerate. Histological examination showed that the regenerate had a number of young cartilages (Figure 18d) which were not found in the smoothly healed control stumps.

In our still unpublished experiments, performed as early as 1949 on adult white mice, after we amputated the wrists of the forelimbs and injected the animals with a preparation of vitreous body, toelike appendages formed on the smoothly healed stumps in the region of the first toe (Figure 18, c, d). A similar result was also obtained in the case of simple amputation of the first toe (Figure 18e). The basis of the regenerate was the old bone and more distally cartilage and connective tissue, and cornified nails grew out from its surface (Figure 18f).

The American investigator, Allan Scharf (1961, 1963), amputated 2 toes of the forelimbs of young rats aged 2 days and treated the wounds with solutions of trypsin and calcium chloride. As a result, no scab appeared on the wound, and instead of the wound's smooth healing as was observed in the control (Figure 19, a, b), 2 short toes with thick ball-like structures at the tips regenerated (Figure 19, c, d) and later nails appeared. These very interesting data can be understood if it is kept in mind that the proteolytic enzyme, trypsin, greatly enhances the destruction and dedifferentiation of tissues.

FIGURE 19. Results of amputation of two toes in newborn rats (according to Sharf, 1961):

a, b — smooth healing of wound in the control; c, d — regeneration of atypical toes after treatment of the amputation wound surfaces with trypsin and calcium chloride.

Stimulation of regeneration of tissues on the amputated wound surfaces of extremities of 2- to 7-day-old rats can be obtained 1) by injection of cartilage extracts; 2) by treatment of the wound surfaces of the amputated tail residues with a solution of trypsin and calcium chloride, and regeneration of the missing half of a vertebra may be induced (Kudokotsev, 1965, 1966).

Thus, it can be seen that a well defined, realistic approach to the induction of regeneration of extremities and toes in mammals, and in principle also in man, begins to emerge.

Reasons for the loss of regenerative capacity of extremities in vertebrates

The suggestion made by Weiss (1925, 1930, 1930a) that the loss of regenerative capacity of extremities in tailless Amphibia is due to the disappearence of a "field," a special organizing factor, in the presence of regenerative material and general conditions for regeneration, has not been proven and is not very likely. On amputated wound surfaces of the extremities of frogs, lizards, and rats, no blastema appears, but if conditions are created for its formation, then, the organs regenerate typically or atypically. Even young blastemata have organizing potentialities (Polezhaev, 1934, 1936, 1937, 1937a, 1945). The presence of morphogenetic "fields," developing independently of cellular material, has not yet been demonstrated by anybody.

Efimov (1937) suggested that the loss of regenerative capacity is due not to the absence of organizing factors, but to the lack of indifferent regenerative material. In one case in his experiments, on the amputated wound surface of an extremity of a tadpole of the lake frog, *Rana ridibunda*, an extremity regenerated from the cellular material of the tail. However, we could not confirm Efimov's data (Polezhaev and Gintsburg, 1939; Polezhaev, 1947b). Probably, in Efimov's experiments, the extremity was amputated very distally, and this level had not yet lost its regenerative capacity.

Guyénot (1926, 1927) and Schotté and Harland (1943) voiced the opinion that the forward movement of histological differentiation cannot be the reason for the loss of regenerative capacity of extremities in tailless Amphibia because in caudate Amphibia, or at a distal level of extremities in tailless Amphibia, the extremities have differentiated tissues and these tissues regenerate. However, the importance of differentiation of tissues may change during evolution and ontogenesis. In some cases, differentiation does not, while in others it does, interfere with dedifferentiation which is the main conditions for regeneration.

Ide-Rozas (1936) thinks that the reason for the loss of regenerative capacity of extremities in tailless Amphibia is the lack of capacity for division in the cells in the region of the wound surface. However, in the loss of regenerative capacity of extremities in tadpoles, the cells are capable of mitotic division but the tissues of the organ lose the capacity for destruction and dedifferentiation. If these latter phenomena are induced, the extremities regenerate and mitotic division plays an insignificant role in the process of accumulation of cellular material in the blastema, and may even be suppressed by colchicine.

According to Morgan (1907), the lack of capacity for regeneration of extremities, tails, and other organs of higher vertebrates is due to the fact that their various components regenerate at different rates. This investigator ascribes special significance to the changes in the bone and nerve tissues. Rose (1944, 1945) thinks that the lack of capacity for

regeneration of extremities in frogs is due to the fact that the corium of the skin and bone is only slightly destroyed. Umanskii (1946) reached the conclusion that the lack of regeneration of extremities in adult frogs and lizards is due to a shift in the course of regeneration, to particularly weak destruction of bone as compared with that in muscles. In his experiments, in which he administered parathyreocrin to lizards and young rats, he observed enhancement of the destruction of bone by osteoclasts (Umanskii and Kudokotsev, 1951, 1952). We think that, in the process of progressive and adaptive evolution, a certain regular process of change in metabolism and progressive differentiation of cells and tissues takes place, during which the capacity of the cells and tissues for destruction and dedifferentiation is altered. The different components of the organs change in different ways. These changes and their role in the process as a whole must be evaluated concretely and with great care. An investigator may ascribe the loss of regenerative capacity of extremities to changes in the bone, or in the skin, or in nerves, or in connective tissue, but errors can easily be made. In the process of loss of regenerative capacity, the destructibility not only of bone but also of muscle and skin changes, as do the properties of the nervous system and the internal humoral milieu. The destructibility of the skeleton of the extremities diminishes during ontogenesis of tailless Amphibia. In atypical regeneration of extremities in frogs, however, the muscles regenerate very poorly, less well than the skeleton. Therefore, it is difficult to ascribe the leading role in the loss of regenerative capacity of extremities to the skeleton.

Rose (1944, 1945) ascribes the main role in this process to the skin because he thinks that the skin of frogs does not dedifferentiate, and that in covering the amputation wound surface, the skin suppresses regeneration of the extremities. However, in our experiments with tadpoles at Stage IIIa, adult frogs, and Bombina, removal of the skin at the edges did not lead to regeneration of the organs, and the amputation wound surface was covered with epithelium and not with skin. The same occurred in experiments with lizards in which the edges of the old skin of stumps of extremities were cut off (Barber, 1944, 1944a). The skin covering the wound surface may inhibit regeneration of the tail and extremities of Amphibia (Tornier, 1906; Schaxel, 1921; Godlewski, 1928; Efimov, 1931; Poležajew and Favorina, 1935), but this is not the reason for the absence of regeneration after amputation of extremities in frogs. The main reason lies in the changes in tissues of mesodermal origin in the stumps of the extremities in adult frogs, in their lack of capacity for destruction and dedifferentiation.

Ontogenetic changes in the internal humoral milieu may also reduce and suppress the regenerative capacity of extremities and other organs in tailless Amphibia (Polezhaev and Gintsburg, 1939, 1941; Schotté and Wilber, 1959) and probably in other animals.

In the ontogenesis of tailless Amphibia and in a comparative series of vertebrates (axolotls, tritons — frogs, Bombina — lizards — rats, mice), with the animals' transition from aquatic life conditions to the terrestrial mode of life, a regular adaptation and a progressive change of the entire physiology, metabolism, functions, structure, and differentiation of all the organs and tissues, the nervous system, and also of the internal humoral milieu take place. As a result of this process of adaptive evolution and ontogenesis, a change (reduction) occurs in the regenerative capacity of extremities and other animal organs. The mechanics or means of realization of the

regenerative process (e.g., conditions leading to the accumulation of cellular regenerative material in the focus of damage) also change, as do the properties and morphogenetic potencies of the cells and tissues. The main change consists of the tissues' loss of their capacity for destruction and dedifferentiation. If the researcher brings about these phenomena, the extremities will regenerate typically or atypically, despite the changes which took place in the humoral milieu, nervous system, and skin of the animals. The regenerative capacity is lost earlier if the changes in metabolism and differentiation associated with the transition from aquatic conditions of existence to terrestrial life occur earlier during ontogenesis.

Restoration of lost regenerative capacity of extremities in vertebrates

The experimental data just discussed show that regeneration of extremities in vertebrates may be obtained in one of two ways at those stages of their ontogenesis when, under ordinary conditions of amputation or damage, no regeneration occurs: 1) by prolongation or maintenance of existing regenerative capacity, and 2) by restoration of lost regenerative capacity of extremities.

Prolongation of the regenerative capacity may be achieved in 3 ways: 1) by the method of re-regeneration (Polezhaev, 1933); 2) by the method of repeated amputation (Polezhaev and Morozov, 1941); and 3) by the method of transplantation (Yakovleva, 1938). In all the 3 methods the first operation is carried out at that stage when the extremities have regenerative capacity (Stage I). In the 1st method, a regenerate which has reached Stage I regenerates; in method 2, the old tissues changed by the formation of blastema on them regenerate; and in the 3rd method, the old tissues changed by prior transplantation of the organ regenerate. These methods allow the investigator to obtain regeneration of extremities in tadpoles of the grass frog after amputation at Stages IIa, IIb, IIIa, IIIb, and even IV. In all these cases, the basis of regeneration is the maintenance by the tissues of their capacity for destruction and dedifferentiation of old tissues of mesodermal origin in the residue of the organ.

Restoration of lost regenerative capacity or induction of regeneration of extremities in tadpoles at Stages IIa, IIb, and IIIa may be obtained by methods of traumatization, repeated traumatization, transplantation, or chemical treatment with a saturated solution of sodium chloride, with a 5% solution of glucose, with a solution of sodium bicarbonate, with a solution of iodine, or with nitric acid. Restoration of lost or weakened regenerative capacity, or induction of regeneration, of extremities in adult frogs and Bombina may also be obtained by different methods: treatment of the amputation wound surfaces with the chemicals listed, with a hydrolysate of cartilage, by transplantation of preserved muscles or skin, by the action of ultraviolet light, by transplantation of embryonic tissues, adrenals, additional innervation, or by induction-current stimulation of nerves. In all these cases, except for the last 2, enhancement of phenomena of destruction and dedifferentiation of tissues of mesodermal origin in the residue of the organ takes place. In the last 2 cases, accumulation of cellular regenerative

material is achieved in another way, which is not yet clearly understood, probably by enhancement of proliferation. The latter was demonstrated in experiments with rerouting of nerves in axolotls (Polezhaev, 1933a, Zamaraev, 1939). According to the principle of equifinality (Polezhaev, 1938), it may be said that the same factors serve as the basis for the different processes leading to the same result (finale). In this case, induction of regeneration of extremities (finale) is achieved by accumulation of cellular regenerative material of tissues in the area of the wound 1) by enhancement of destruction and dedifferentiation of tissues and release of cells from their tissue associations, without proliferation, and 2) by additional innervation without enhancement of these phenomena due to enhancement of proliferation. Thus, destruction and dedifferentiation on the one hand, and additional innervation on the other, causing accumulation of cells in the regeneration bud, are equivalent to some extent. The decomposition products of cells and tissues and the nervous system act in a similar manner. Evidently, the essence of the action of these different factors is enhancement of processes of biosynthesis of proteins and nucleoproteins or nucleic acids. This point is very important for the understanding of a number of important biological phenomena. Innervation is usually essential for regeneration of extremities in Amphibia. However, we saw that transplanted extremities of Amphibia may regenerate without innervation (Polezhaev, 1939; Shneider, 1940; Yntema, 1959, 1959a), and rudiments of extremities of tritons may develop upon explantation into a salt solution (Polezhaev, 1938, 1945). On transplantation of extremities, the effect of the nervous system is probably replaced by the action of decomposition products of tissues, while in explantation of rudiments of extremities, it is replaced by the tissues' young age, and the abundance of RNA in them. Analysis carried out from this point of view may help in understanding the morphogenetic significance of the nervous system.

Induction of regeneration of extremities and toes in lizards and young rats is achieved by D-avitaminosis, by the introduction of parathyreocrin, by trypsin and calcium chloride — substances which cause enhancement of phenomena of destruction and dedifferentiation of tissues of mesodermal origin in the organ residue.

In order to restore lost or weakened regenerative capacity, or to obtain induction of regeneration of extremities in vertebrates, conditions must be created which could determine the course of the regenerative processes in a manner basically similar to that of normal development processes of extremities during ontogenesis or in spontaneous regeneration. Great attention should be devoted to the condition, physiology, or metabolism of the animal's organs and tissues. In order to change the regenerative capacity of organs in a controlled manner, as one of the adaptive forms of self-reproduction of living matter, it is necessary to change in a controlled manner its basis, i. e., metabolism, and first of all, biosynthesis of protein. It is easier and more preferable to achieve this by the action of external factors on protein, and not on nucleic acids or DNA. It is easier to change in a controlled manner the labile enzymatic reactions and, therefore, the condition of the cell, than the stable structures, the DNA molecules, especially since it is not known how to affect these molecules in a controlled manner to obtain induction of regeneration of extremities in vertebrates.

Formulation of the phenomenon and the rules of loss and restoration of regenerative capacity of extremities in vertebrates

Thus, as a result of our experimental studies of the phenomena of loss and restoration of the regenerative capacity of extremities in tailless Amphibia and other groups of vertebrate animals, it is possible to make the following generalizations. In ontogenetic and comparative series of vertebrate animals, in the course of progressive evolution and adaptive transition of animals from life under aquatic conditions to terrestrial conditions, the capacity for reparative regeneration of extremities decreases regularly, and is lost finally. In animals capable of regenerating extremities (caudate Amphibia, tadpoles at early stages of metamorphosis of tailless Amphibia), there is a strong destruction and dedifferentiation of tissues of mesodermal origin (skeleton, muscles, connective tissue) in the residue of the organ after amputation. In animals which have lost this capacity (tadpoles at late stages of metamorphosis, adult tailless Amphibia, lizards, mice, rats), the tissues of mesodermal origin in the residue of the organ lose their capacity for destruction and dedifferentiation after amputation of extremities. Or: the loss of the regenerative capacity of extremities in phylogenesis and ontogenesis of vertebrates is characterized by a reduction of the first physiological stage of regeneration, namely, the stage of destruction and dedifferentiation. Upon experimental restoration of this reduced stage, by strong enhancement of destruction and dedifferentiation of tissues of mesodermal origin, the weakened or lost capacity for reparative regeneration of extremities may be restored and atypical or typical regeneration obtained.

The loss of regenerative capacity of extremities is biochemically characterized by a drastic change in the enzymatic activity (drastic decrease in proteolysis) and by destructibility of their tissues; histochemically this is characterized by a decrease in the intensity of the reaction to RNA, according to Brachet, of the cells and tissues; histologically —by a process of progressive differentiation of tissues (solidification of the skeleton, rapid maturation of a dense scar connective tissue, etc.); physiologically — by a change in the internal humoral milieu which leads to inhibition of regeneration of organs, etc.

The capacity for reparative regeneration, not only of extremities but also of a number of other organs, changes during the processes of ontogenesis and phylogenesis in other animals as well as in vertebrates, as will be shown further on. Therefore the following formula may be suggested: in the processes of phylogenesis and ontogenesis of animals, with progressive evolution and emergence of the animals from aquatic conditions to terrestrial life, the capacity for repair regeneration of organs diminishes steadily and disappears, and is characterized by a reduction in the capacity for destruction and dedifferentiation of the main tissues composing these organs. By strong experimental enhancement of destruction and dedifferentiation, it is possible to restore partially or completely the weakened or lost regenerative capacity.

The knowledge of this rule is very important, since it allows the investigator to approach the solution of the problems of controlled changes in the regenerative capacity of various organs and tissues in various taxonomic groups of animals, and in particular in man.

Chapter II

LOSS AND RESTORATION OF THE REGENERATIVE CAPACITY OF BONES OF THE CRANIAL VAULT

The study of regeneration of extremities in vertebrates led to the discovery of the rule of loss and restoration of the regenerative capacity of animal organs. In this connection new subjects for study appeared. First, it was necessary to elucidate whether the phenomena of regeneration of other organs in other animals also obeyed the aforementioned formulated rule. Thus the necessity arose for increasing the scope of these studies. Second, it was necessary to determine whether it was possible, on the basis of this rule, to pose and solve practical problems. Reality constantly demanded that theory be brought closer to practice and that the gap, which had existed for a long time, between the theoretical-biological and practical-medical directions in the study of regeneration be bridged. At the present level of knowledge, it is difficult to say that biologists will manage in the near future to propose methods which will allow medical personnel to restore the lost regenerative capacity of extremities, or even of human fingers. These are complex organs consisting of a number of differentiated components (cartilaginous and bone skeleton, muscles, connective tissue, skin, nerves, blood and lymph vessels) which are derived from various germinal layers. For practical purposes, the investigator would have to choose other more simple organs, using mammals which constitute the group of higher animals closest to man. Regarding this, we chose as the object of our study the bones of the cranial vault. For a more rapid solution of these problems, we decided to start this study from the requirements of practice and not from a simple interest in theory, which is not related to life.

Problem of replacing defects in the cranial vault

One of the serious problems of contemporary surgery and neurosurgery is that of replacing defects in the cranial bones. In various tumors of the brain and the brain membranes, in tauma to the cranium, in echinococeosis of the brain, and in other cases, the physician must remove substantial pieces of bone from the cranial vault. Here the problem arises of replacing the defect in the cranium. Extensive published materials, both Soviet and others (Marchand, 1901; Grekov, 1901; Ozerov, 1910; Nemilov, 1940; Leibzon, 1959; Matveeva, 1962; Potanina, 1963; Kari Sirola, 1961, and others) have been devoted to this problem.

In contrast to tubular bones, the flat bones of the cranial vault do not regenerate in adult human beings and in a number of mammalian species. It is well-known that the source of regeneration of tubular bones is the cambial layer, the periosteum. Periosteum transplanted into muscles forms a tubular bone in rabbits and other animals (Ollier, 1859; Bredikhin, 1862, and others). If one removes from under the periosteum the entire diaphysis of a tubular bone or segments of ribs or the shoulder blade in puppies and calves, complete regeneration of the bone occurs (Heine, 1834). Complete regeneration of the tubular bone also occurs after its extirpation from under the periosteum in chicks, rats, and young rabbits (Studitskii, 1954). Similarly, tubular bone of man is also restored following its loss from under the periosteum due to osteomyelitis, and in other cases (Pirogov, 1866; Bockenheimer, 1905; Berlatskii, 1917; Blacklock and Rankin, 1935; Bruskin, 1945).* Hollow regions of tubular bone are spontaneously replaced by regenerating bone.

The flat, integumentary or membranous bones of the cranial vault are covered from above by periosteum and from below by the dura mater of the brain, which many anatomists and histologists (Corning, 1931) consider a second periosteum. Upon removal of large pieces of bone from the cranial vault in man, dogs, and other species of mammals, no regeneration occurs despite the preservation of the dura mater and the periosteum. In order to replace defects in bones of the cranial vault in man, surgeons use a number of methods of auto, homo- and heteroplastic surgery, using live bones or cartilage (Matveeva, 1962). One uses pressed together pieces of cranial and tubular bones, ribs, and other material. The best results are obtained from autoplastic surgery since, as a result of the incompatability of tissues, in homo- and heteroplastic surgery the bone transplants are, as a rule, resorbed. Even in autoplastic surgery they are often resorbed and the defects reappear. This induced Soviet and non-Soviet surgeons in recent years to start using the method of alloplastic surgery, i. e., replacing the cranial defects by nonliving material: polymethyl methacrylate, horn, tantalum. This method, however, is not biological. The foreign material is not indifferent to the adjacent healthy tissues of the recipient. This became especially clear when it was shown that transplantation of thin cellophane sheets might easily induce carcinoma and sarcoma (Oppenheimer, B., Oppenheimer, E., and Stout, 1948, 1952; Studitskii, 1964). Therefore, the problem of finding biological methods for replacing bone defects in the cranium continues to be of importance.

New biological approach to the problem

Surgeons strive to cover bone defects in the cranium rapidly and reliably, achieving this by plastic surgery, but, unfortunately, they often meet failure due to resorption of the transplanted bone. A new biological approach to the problem is based on principles from the theory of regeneration. An attempt is being made to cause artificial regeneration of bone which, under ordinary conditions, does not regenerate when it is removed. In this case the

* The first four papers are quoted from A. G. Babaeva (1960).

regenerate is first undeveloped, delicate, and soft, but with time it develops further, becomes more dense, and the area of the defect is reliably covered with live bone tissue. This approach required the elucidation of the following questions:

1. How does the capacity for regeneration of cranial bones change in ontogenetic and comparative series of animals, mainly in mammals?
2. What components are essential for regeneration of bones of the cranial vault?
3. What method may be suggested for use in restoration of the lost regenerative capacity of the bones of the cranial vault?

The results of our experimental studies have been published in a number of papers (Polezhaev, 1951, 1956, 1957; Barakina, Gintsburg, Korchak, Polezhaev, and Rogal', 1952; Gintsburg, 1952, 1954; Rogal', 1952, 1955, 1956, 1957; Korchak, 1959; Matveeva, 1958—1962; Matveeva and Mitrofanova, 1963) and in a monograph by Matveeva (1962).

Changes in the regenerative capacity of the bones of the cranial vault

In our experiments we mainly removed the parietal bone, preserving the dura mater intact, and frequently covering it with a scrap of previously prepared periosteum. The operations were conducted under aseptic conditions. For several days after the operation the animals were given injections of penicillin. The dimensions of the rectangular bone defect differed: in mice they were 6 × 4 mm; in rats, 10 × 5 mm; in rabbits, 15 × 10 mm; in dogs, 40 × 25 mm. In young animals the dimensions of the defects were correspondingly smaller.

The study showed that in adult, sexually mature dogs, rats, mice, sheep, pigs, and goats, the dense, hard bones of the cranial vault of the skull underwent very weak destructive processes and did not regenerate. The region of the bone defect was covered with a thick, connective-tissue scar. The experiment lasted 1—2 years. In only some cases in rats and dogs, a fringe of bone tissue grew out from the edges of the bone in the region of the defect. Thus, in adult mammals of the majority of species studied by us, the bones of the cranial vault do not regenerate exactly as in adult man. On the other hand, under the same experimental conditions, bones of the cranial vault completely regenerate in adult rabbits, chickens, geese, and ducks, and according to some published material (Potanina, 1963), also in guinea pigs.

Complete regeneration of bone occurs after removal of a considerable area of the parietal bone in young rats several days old, and in one-month-old puppies and kittens (Figure 20a, b). The restoration of bone is rapid; one month after the operation the area of the bone defect is already covered with young, but thick and dense, bone tissue, with sinuses and bone marrow cavities (Figure 20c). It is highly important that regeneration, at least in puppies and kittens, starts not from the edges of old bone but throughout the entire area of the defect. Upon removal of bone in puppies aged 3 months, only small islets of bone tissue appear in the region of the defect. In 6-month-old puppies regeneration of cranial bone no longer takes place. In connection with these data, and in our further experiments on the

restoration of lost regenerative capacity of bones of the cranial vault, 2- to 5-year-old dogs were used to be sure that no spontaneous regeneration of bone had occurred.

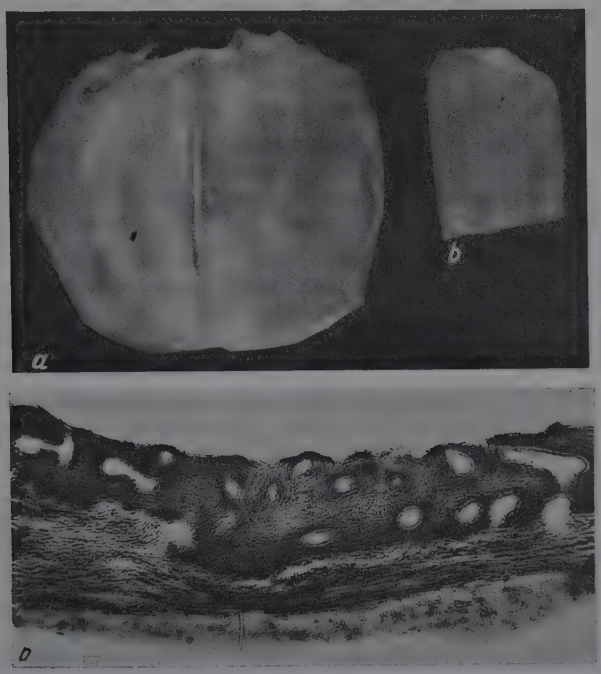


FIGURE 20. Regeneration of bone in the cranial arc of a one-month-old kitten:

a – cranial arc after regeneration; b – removed piece of bone; c – histological cross section of regenerated bone one month after operation.

The data we obtained in experiments on animals are in agreement with clinical data on human beings. In recent years it has been shown, that in trauma to the skulls of children below the age of 3 years, the bones of the cranial vault have some capacity for regeneration (Izvekova, 1959; Potanina, 1963). Therefore, in man and the majority of the mammalian species, the capacity for reparative regeneration of the bones in the cranial vault is lost during ontogenesis, as is similarly the regenerative capacity of extremities in tailless Amphibia. The regenerative capacity of the bones of the skull obeys the second Darwinian rule of regeneration which says that the regenerative capacity of organs decreases with age.

Biochemical investigation has shown that the relatively soft, porous bones of the cranial vault, capable of regeneration in puppies or adult rabbits, are relatively easily destroyed; the calcium salts which are weakly bound to proteins are easily washed out from these bones. Dense, hard bones of the cranial vault of adult dogs, which are not capable of regeneration, are not

readily destroyed; it is difficult to wash out from them the calcium salts which are tightly bound to the tissue proteins (Korchak, 1959). Thus it has been shown experimentally that the loss of regenerative capacity of bones in the cranial vault of mammals is associated with a decrease in the destructibility of the bone tissue.

Significance of the different components for regeneration of bones in the cranial vault

The finding that the bones of the cranial vault of adult rabbits are capable of regeneration allowed us to study the role of the different components in the process of their regeneration: the periosteum, bone, and dura mater.

After effecting defects in the parietal bones of adult rabbits, we removed the periosteum at a considerable distance from the edge of the bone. Despite this the bone regenerated nicely. A wedgelike regeneration proceeded from the periphery to the center of the defect area. In experiments with induction of cranial bone in adult dogs, regeneration of the bone occurred in the absence of periosteum. However, if after removal of large pieces of bone in adult rabbits, or in one-month-old puppies, a piece of dura mater was also removed, regeneration of bone did not occur. The bone was restored only to the edges of the cut in the dura mater. The region of defect in the bone coincided precisely with the region of defect in the dura mater. Similar data were obtained in other experiments on adult dogs in which regeneration of bone was caused by additional treatments (Matveeva, 1962). The bone regenerated only in those places where the dura mater was preserved.

In some experiments on adult rabbits, after preparing a scrap of periosteum, a piece of parietal bone was removed, and a piece of cellophane or a wax plate was put on the dura mater; this was then covered by an implant of the prepared periosteum. Thus, the periosteum and the dura mater were separated from each other by a foreign body. Despite this, the bone regenerated in a wedgelike form from the edges of the region of the bone defect, and regeneration proceeded on the surface of the dura mater. The regenerating bone was separated from the periosteum by the implanted plate.

Thus in these experiments on rabbits, the source of regeneration was the edge of the old bone, and the presence of dura mater was an essential condition; while the presence of periosteum was not obligatory, it somewhat enhanced the course of the bone regeneration. These data are very important for surgeons and neurosurgeons who perform skull and brain operations on human beings.

Replacement of defects in the cranial vault by embryonic anlagen of cranial bones

We performed a series of preliminary experiments on rats and rabbits, making various homoplastic and heteroplastic implants into the region of defect in the parietal bones of the cranium: periosteum from the humerus

and femur of rabbits, dogs, and cocks, and the mucosa from the urinary bladder of a rabbit. The results were negative. No bone appeared in the region of the defect. Transplantation of cartilage taken from the wide xiphoid process gave positive results in experiments with rats; the defects in the cranium were replaced by cartilage which subsequently began to be replaced by bone. Similar results were obtained in experiments with dogs by Leibzon (1959).

We made an attempt to obtain replacement of cranial defects by homoplastic transplantation of embryonic cranial anlagen taken shortly before parturition, or from newborn animals. The experiment was performed on adult mice, rats, and rabbits. In the experiments on mice, we succeeded in obtaining direct takes of transplants and replacement of the cranial defects if the cranial anlagen were taken from newborn animals. If they were taken from 16- to 17-day-old embryos, they were resorbed. In all the other cases, i. e., in experiments with rats, rabbits, and dogs, the transplants were destroyed within the first 2 weeks after the operation and caused regeneration of local tissues. As a result, the areas of the cranial defects were replaced by regenerating bone (Figure 21). The bone was well developed and remained for the entire period of the experiment, which lasted for about 2 years.

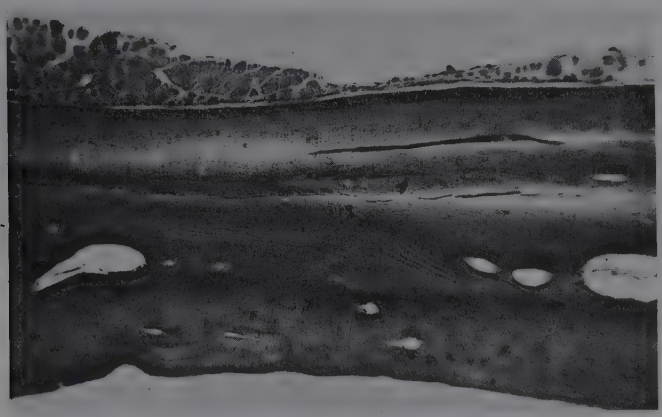


FIGURE 21. Bone regenerating in the region of the cranial defect of an adult dog after transplantation of a cranial anlage from a 45- to 50-day-old dog embryo, 635 days after operation

The results of our studies were published in a number of papers (Polezhaev, 1951, 1956, 1957; Barakina, Gintsburg, Korchak, Polezhaev and Rogal', 1952, and others). Soon thereafter they were confirmed in other laboratories. Momestsarishvili (1953) implanted cranial anlages from human embryos into the regions of cranial defects in rabbits, while A. N. Brudastov (1952, 1954) used cranial anlagen from rabbits. Both investigators state that the defects were replaced by bone. Experiments performed on rabbits are not convincing, however, since the cranial bones of these animals are capable of spontaneous regeneration, while the transplants should have been resorbed as a result of incompatability of tissues in homoplastic and heteroplastic surgery.

Working with embryonic anlagen of cranial bones, we succeeded in making a new observation: in the region of the sagittal suture of the parietal bones of the cranium in mice, we observed formation of cartilage (Figure 22). Evidently these cartilages were a residue of the cartilaginous skeleton of the cranium which is formed in lower vertebrates, and does not appear in higher vertebrates. It is known that the integumentary bones of the cranium are formed directly from connective tissue, bypassing the cartilage stage. Our data were soon confirmed by F. A. Ragol'skaya (1959), who observed formation of cartilages adjacent to the parietal bone sutures during the development of cranial bones in human embryos.

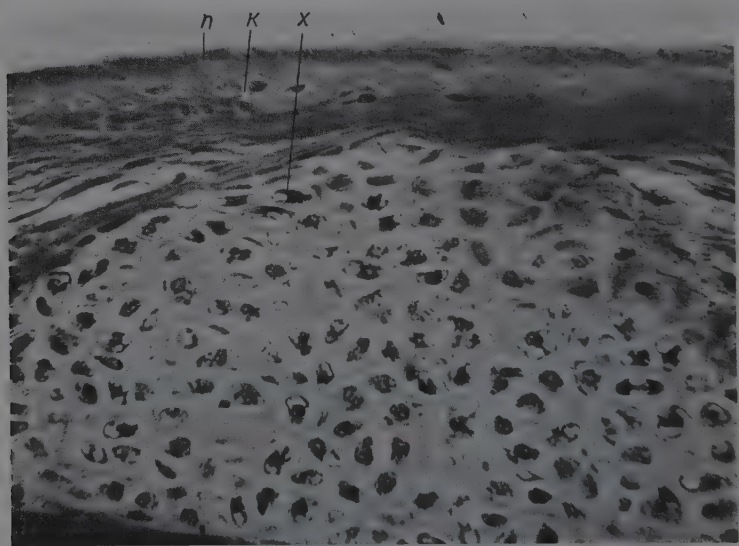


FIGURE 22. Cartilage in the vicinity of the median suture of the parietal bones in a newborn mouse, sagittal section:

n – periosteum; k – bone; x – cartilage.

Replacement of cranial defects by displacement of bone fragments

The second method we proposed for replacing bone defects in the cranium is based on induction of bone regeneration by displacement of a bone fragment. The idea on which this method is based is that under the influence of decomposition products of bone, induction of bone may be caused in an immature connective tissue. Bier (1917) had concluded that decomposition products of tissues stimulate the regeneration of these tissues. Later he (Bier, 1923) proposed to elongate tubular bones in man by cutting them transversely and gradually stretching them in opposite directions, several days later. He suggested that the newly formed bone arises from local connective tissue under the influence of bone decomposition products.

We decided to elaborate a similar method for the displacement of bone defects in the cranium. The experiments were performed on white mice and adult dogs. In these animals we cut out a rectangular piece of the parietal bone without touching the dura mater. After this we cut out another, narrow, rectangular piece along the length of the former rectangle, and we left the 2nd rectangle in place (Figure 23). In 2, 3, or 4 days after the operation, we again dissected the skin in the area of the suture and, using forceps, we slowly pulled the fragment on the bottom of the wound in a direction opposite to the region of the defect. The latter was covered with a connective-tissue film, formed from those cells which were present between the edge of the bone and the fragment, and which were affected by the decomposition products released by the edge of the cut bone.

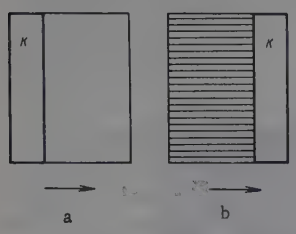


FIGURE 23. Scheme of the operation of displacement of a piece of bone (k) in the region of the cranial defect:

a – before displacement; b – after displacement.

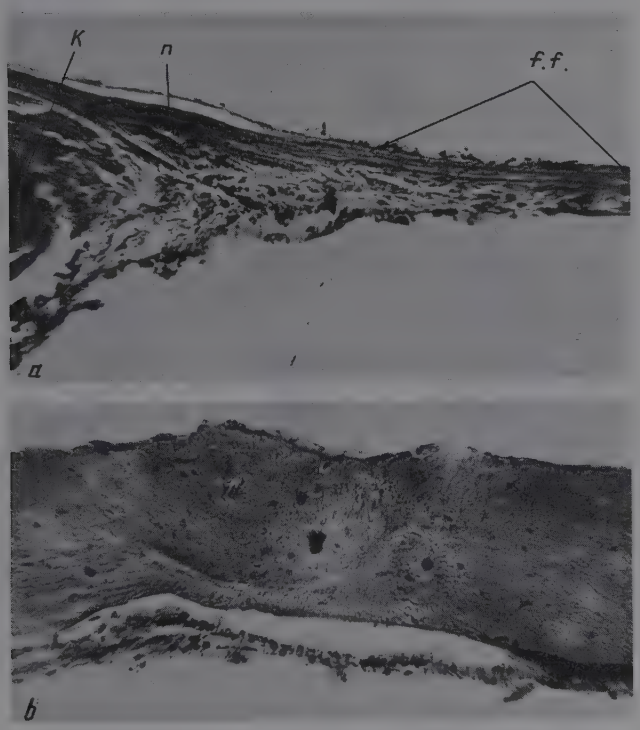


FIGURE 24. Regeneration of cranial bone in mice caused by displacement of a bone fragment:

a – formation of a fibrous film in the region of the defect in the parietal bone of a mouse 125 days after operation: k – bone; n – periosteum; f. f. – fibrous film; b – compact bone regenerated in the region of the cranial defect 115 days after displacement of a bone fragment.

In the control, i. e., after simple removal of a piece of bone 125 days after the operation, the defect area was merely covered with a connective-tissue scar (Figure 24a), while in the experimental animal it was replaced by a well-developed bone (Figure 24b). The best result was obtained in those cases in which the bone fragment was displaoed 4 days after the first operation.

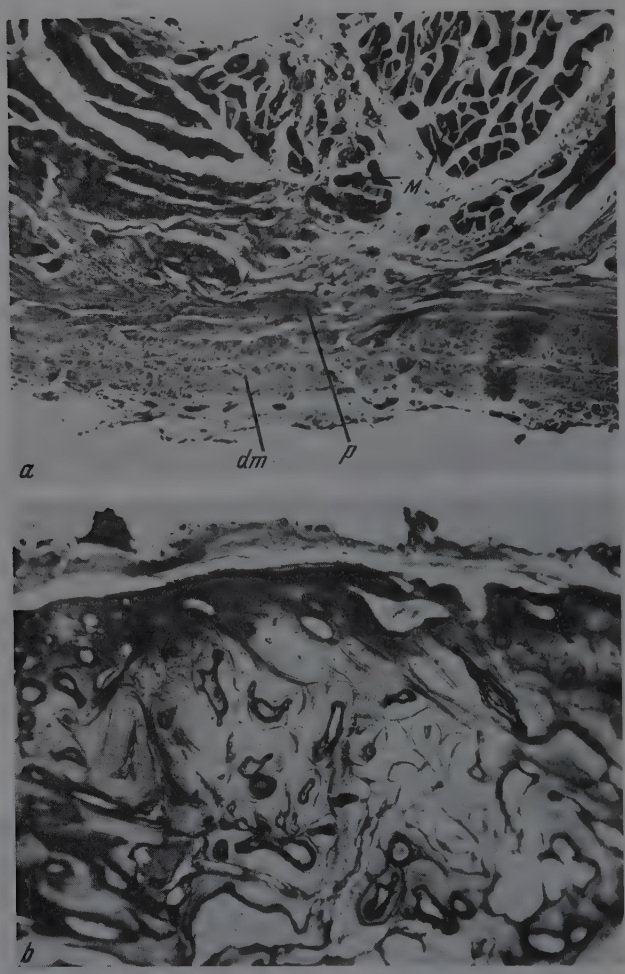


FIGURE 25. Regeneration of bone in the cranium of a dog caused by displacement of a bone fragment:

a – formation of scar (p) in region of the cranial defect of an adult dog; b – bone regenerating in region of the cranial defect in an experiment with displacement of a bone fragment 120 days after operation; m – muscles; dm – dura mater.

A similar experiment was performed on dogs. The bone fragment, however, was displaced without opening the skin wound; this was done

gradually, several millimeters every 3—4 days, by pulling it with 2 silk threads sticking out through the skin to its surface. The operation on dogs was somewhat more complex than that on mice. Nevertheless, instead of causing formation of a connective-tissue scar such as that which appeared in the region of the cranial defect of the control dog (Figure 25a), we succeeded in these experiments, too, in causing formation of regenerating bone (Figure 25b).

Thus, the results obtained confirm the possibility of induction of bone regeneration in the cranial vault in those adult mammals in which bone is not restored under ordinary conditions of removal. However, the source of regeneration in these experiments is not known. It may be activated cells of the periosteum, or local connective tissue, and their potencies may differ.

Replacement of cranial defects by the method of changing metabolism

A maximum of 2 specific conditions is required for normal development or for the regeneration of bone: 1) young, plastic, bone-forming material, and 2) conditions for its calcification and transformation into bone. It may be assumed that the bone-forming material in the region of the cranial defect is present in the form of young, immature connective tissue. When conditions are created in the body for transformation of the tissue into bone, regeneration of bone in the area of the cranial defect could be expected. On the basis of this consideration, Rogal' (1952, 1955, 1957) of our laboratory performed experiments on rats, and continued them outside the laboratory on dogs and sheep, with the purpose of causing regeneration of bone in the cranium by controlled changes in metabolism in the direction of bone formation.

In the control animals a piece of parietal bone was cut out without damaging the dura mater. As a result, the region of the defect was covered with a connective-tissue scar. In the experimental group, after removal of cranial bone fragments, the animals were kept for a long time (from 1 to 4—5 months) on a specific diet. Specific amounts of calcium lactate and vitamins D and A were added to the ordinary food ration because it is known that vitamin D maintains the calcium salts in the tissues. As a result, well-developed bone regenerated in the areas of the cranial defect of rats, dogs, and sheep.

This method is very simple and physiological. However, it requires prolonged application of calcium salts and vitamin D and, therefore, cannot be recommended for the replacement of bone defects in older persons because of the danger of its leading to general sclerosis. Thus, we continued our search for biological methods of replacing cranial defects.

Replacement of cranial defects by the method of destruction

Developing further our major assumption that destruction and dedifferentiation are the main factors in any process of regeneration, and that intensive enhancement of these reductive phenomena may lead to the

restoration of the lost regenerative capacity of animal organs and tissues, we made a further attempt to cause regeneration of cranial tissues by the method of destruction. By this we mean great destruction of tissue not accompanied by death, such destruction causing strong dedifferentiation in tissues. The bones of the cranial vault of adult persons and of the majority of the mammalian species are very dense and hard; they do not regenerate. However, if they are strongly torn down, the researcher may hope to obtain regeneration.

Biologists and medical personnel have used the method of destruction for various purposes in the study of regeneration. I have already described the use of this method in biology (see Part I, Chapter 7). In medicine, surgeons have used the method of destruction. Bier (1923) ground a bone of a dog and transplanted it to a child, but did not obtain regeneration of bone. N. A. Bogoraz (1924) cut a tubular bone into pieces, stretched it, and obtained bone regeneration. Z. I. Kartashev (1930), who wanted to increase the surface of bone fragments, ground a tubular bone into pieces, 5—10 mm long; the bone "filings" coalesced, and regeneration of bone was observed. Experiments with bone "filings" and bone shavings have been successful in stimulating regeneration of tubular and maxillar bones (Bolotin, 1945; Rusanov, 1958; Sitenko, 1935; Ol'shvang, 1956; Zakharzhevskii, 1958, and others).

In contrast to investigators who worked on tubular bones, which are capable of regeneration, we dealt with cranial bones of mice, rats, and dogs which have lost the capacity for regeneration. Our study was based on the following 3 premises:

1. On removal of a piece of bone from the cranial vault of mice, rats, and dogs, signs of regeneration appear in the periosteum at the edge of the dense and hard bone. Probably these are due to the action on the periosteum of decomposition products of bone. These phenomena can be enhanced.
2. Strong destruction and dedifferentiation of tissues leads to the restoration of the regenerative capacity of extremities in tailless Amphibia.
3. On destruction of the basic tissues of the extremities of axolotls, complete regeneration of these organs takes place.

At first we carried out experiments on mice and rats. Subsequently, after we obtained positive results in these animals, we carried out similar experiments on adult dogs. The results of these experiments will be described.

Pieces of parietal bone were removed in the control animals, the wounds healed, and we did not introduce any further experimental treatment. The animals were killed at various times, from one day to two years after the operation. It has been shown that the edges of the solid and hard bone in the region of the defect were only mildly impaired, practically not at all, and the region of the defect was covered with a connective-tissue scar (Figure 26a, b).

At the initial stages of the process the area of the defect underwent strong inflammation. The tissues were edematous, infiltrated with segmented leukocytes, lymphocytes, and polyblasts. Among them were numerous foci of hemorrhage. The periosteum and dura mater which remained in the focus of damage were thickened, inflamed, and the cells of connective tissue were activated. At the edges of the bone numerous osteocytes died, but the basic substance of the bone changed very little. Phenomena of bone regeneration,

which were very limited, were reduced to thickening of the periosteum at the edges of the bone, formation of a narrow ridge of newly formed bone substance, and covering by the latter of the bone-marrow spaces. With the subsidence of the inflammatory processes in the region of the cranial defect, granulation tissue developed, matured, and gradually increased in density, transforming into a solid collagen scar. The periosteum and the dura mater coalesced.

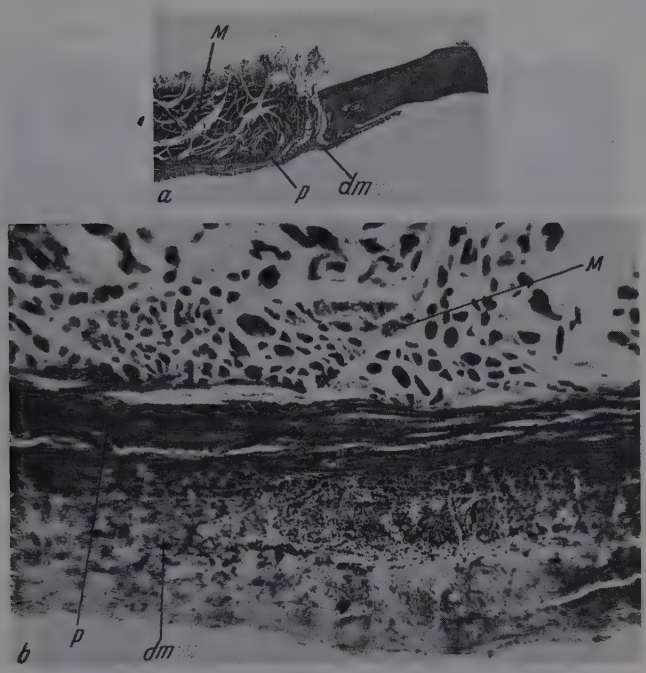


FIGURE 26. Lack of regeneration of bone in an adult dog, and filling of the region of bone defect with a scar:

a – 100 days after operation; b – 227 days after operation; m – muscle; p – scar; dm – dura mater.

We implanted in the experimental animals into the region of the defect in the parietal bone at the level of the bone surface, bone shavings generously soaked in the recipient's blood and in a penicillin solution prepared in a 0.85% physiological solution for warm-blooded animals. The shavings were placed on the dura mater; in some cases they were covered by a previously prepared scrap of periosteum, but in other cases they were not so covered. Later, we sewed up consecutively the dissected and prepared masseter muscle, the aponeurosis (galea aponeurotica), and the skin. In the different series of experiments, we used bone shavings obtained by boring cranial bones and tubular bones with a cutter. We performed autotransplantation and homotransplantation of the bone shavings. The latter were taken in fresh condition, after preservation of the bones according to Filatov (1945)

in cold at 2 — 4°C after lyophilization, and finally, after autoclaving one and a half hours. The results of the experiment differed. The best results were obtained when autoplastic transplants of fresh bone shavings were used. Positive results, were also obtained, however, in homotransplants of bone shavings, fresh and preserved, and somewhat poorer results were obtained with lyophilized shavings. The result was the same when shavings of the spongy substance of the cranial bone and of the metaphyses of tubular bones were used. Autoclaving completely suppressed the activity of the bone shavings.

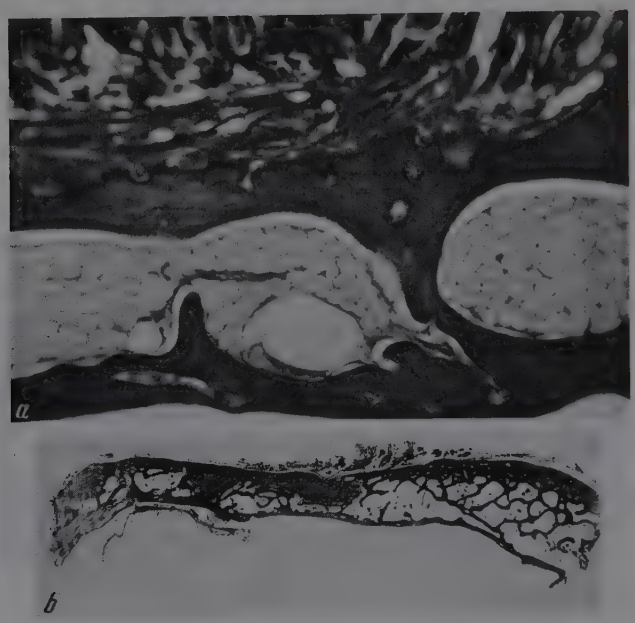


FIGURE 27. Regeneration of anatomically and histologically typical cranial bone with bone marrow in dogs, in an experiment using the destruction method:

a — 365 days after operation; b — 2 years after operation.

In all these applications, with the exception of the autoclaved bone shavings, regeneration of an anatomically and histologically typical cranial bone was obtained in 100% of the cases (Figure 27a, b). The thickness of the regenerating bone depended directly upon the thickness of the layer of implanted shavings. A slight (several days) retardation in regeneration was observed in the experiments with homotransplantation of fresh or preserved shavings, and a longer lag was seen with the use of lyophilized bone shavings. In the latter, a very small slit sometimes appeared between the edge of the old bone and the regenerated bone. Regeneration was also slightly arrested in the absence of a scab of periosteum, which was usually placed on top of the transplanted shavings. However, if a sector of dura mater was removed from the bottom of the area of the bone defect, no regeneration of

bone occurred at this site, and the region of the bone defect remained unsubstituted by bone. An attempt to replace the removed dura mater by transplantation of lyophilized dura mater showed that bone regeneration did not occur despite the presence of transplanted bone shavings (Matveeva and Mitrofanova, 1963). A transplant, however, can be successfully used for replacement of defects in the dura mater, as no adhesions are formed to the brain; these often lead to the appearance of traumatic epilepsy and other pathological phenomena.

It was of great importance to elucidate the question of the role of the bone shavings in regeneration of cranial bone. They may be a structural material, a skeleton on which regeneration takes place from the edges of the bone, or they may be some sort of stimulant or inducer of regeneration. An answer to this question was obtained through histological examination of the processes of regeneration of cranial bone in various experimental series in which the process was examined at frequent intervals: 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 45, 60, and 720 days after the operation. The following data were obtained in experiments on autotransplantation of bone shavings.

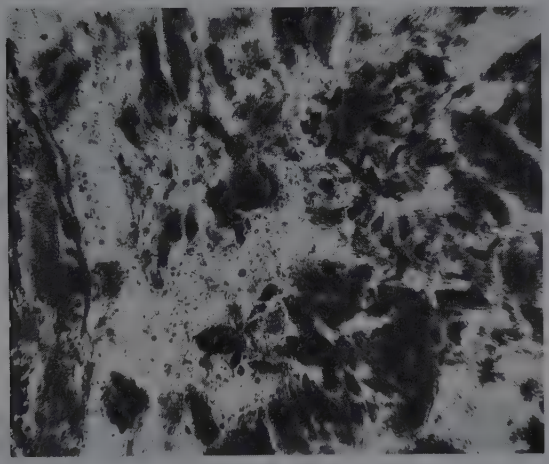


FIGURE 28. Bone fragments and formed elements of blood in the region of the bone defect 3 days after the operation, 7 × 8 (according to Matveeva, 1962)

At 3 days after the operation, the region of the bone defect was filled with bone shavings, formed elements of blood, and cells of immature connective tissue (Figure 28). From below, it was covered with dura mater and from above with periosteum, both slightly swollen due to serous impregnation; from the sides it was covered with the broken edges of the solid, old cranial bone. The bone fragments were of small dimensions and contained only a few osteocytes which died within the first 1 — 2 days after the operation. The major part of the formed elements of the blood consisted of erythrocytes, and a lesser part was of polynuclears. Subsequently the polynuclears decomposed, disappeared, and were replaced by lymphocytes, monocytes, and polyblasts.

The major changes occurred within the first 7 days after the operation, during which time the bone fragments became completely dissolved and the first signs of new formation of bone appeared. The compact matrix of the bone fragments was transformed into fibers, and the fragments split into pieces which gradually lysed and disappeared (Figure 29). Lysis of the bone shavings occurred by an enzymatic reaction without participation of osteoclasts. Simultaneously, the white blood elements were replaced by cells of immature connective tissue, in which numerous small blood vessels filled with blood were formed de novo.

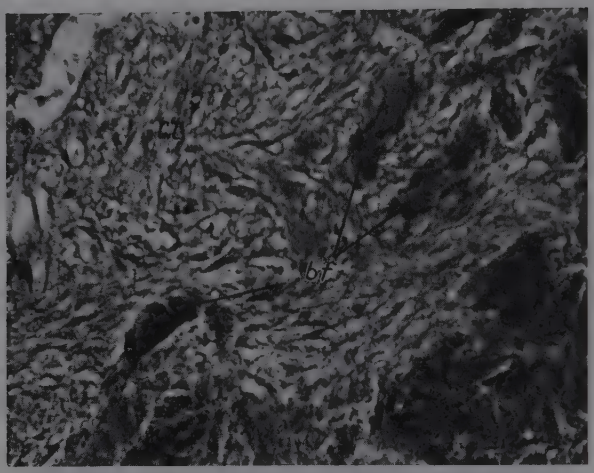


FIGURE 29. Region of bone defect 5 days after operation:

bf – bone fragments; ct – connective tissue, 10 × 20 (according to Matveeva, 1962).

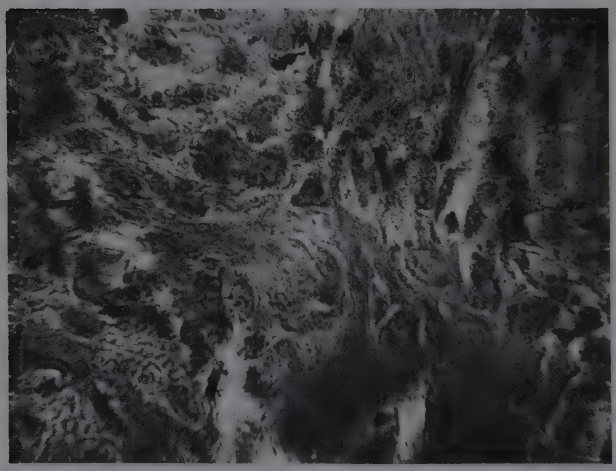


FIGURE 30. Beginning of formation of bone elements in the area of the defect in the cranium of a dog 7 days after operation, 7 × 90

De novo formation of bone took place on the entire surface of the bone defect, as in the case of regeneration of cranial bone in young puppies and kittens, and not by growth from the edges of the old bone, as in adult rabbits. The most intense regeneration occurred in the vicinity of the dura mater and at the edges of the old, damaged bone. New formation of bone in the middle of the defect began with the appearance, side by side with the lysing bone fragments, of a series of fibers among which were large cells of the embryonic type with large light nuclei; these were the induced osteoblasts (Figure 30). Later, in the young osteogenic tissue, convoluted bone elements of irregular shape appeared, covered on the outside by a number of osteoblasts; on the inside, osteocytes were embedded in the bone spaces (Figure 31).

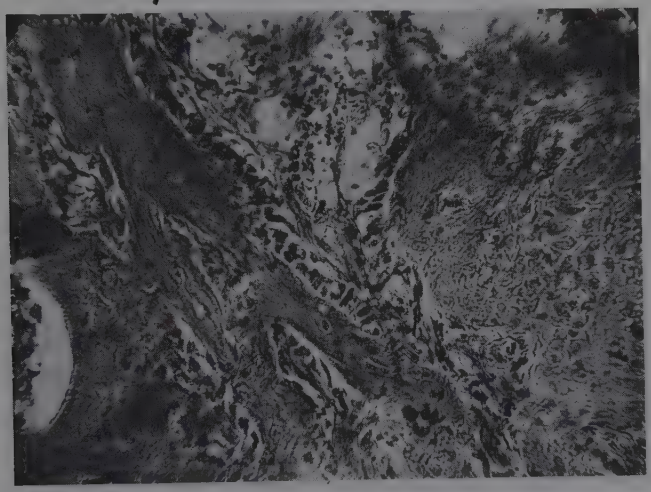


FIGURE 31. De novo formation of bone elements in the region of the defect in the cranium 7 days after operation, 7 × 40 (according to Matveeva, 1962)

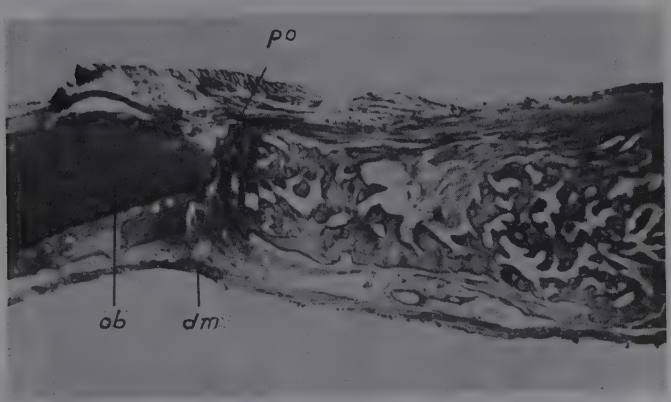


FIGURE 32. Porous bone, filling region of the bone defect in the cranium of a dog 15 days after operation:

ob – old bone; po – periosteum; dm – dura mater. 4 × 4.

Sometimes, in the newly formed bone elements, remnants of incompletely lysed bone fragments could be found; 10—30 days after the operation, the entire area of the bone defect in the cranium was filled with young, spongy bone (Figures 32, 33). The bone elements became thicker, the matrix denser, and among them appeared sinuses containing connective tissue, vessels, and formed blood elements. Gradually the elements turned into bony plates and became denser, and bone marrow was formed between them (Figure 34). The newly formed bone fused smoothly with the old bone of the cranium. The subsequent process of regeneration was characterized by progressive processes of growth and differentiation of bone. One year after the operation the region of the defect differed from the surrounding cranial bone mainly in the thickness of the bone, if an insufficient amount of bone shavings had been transplanted during the operation.

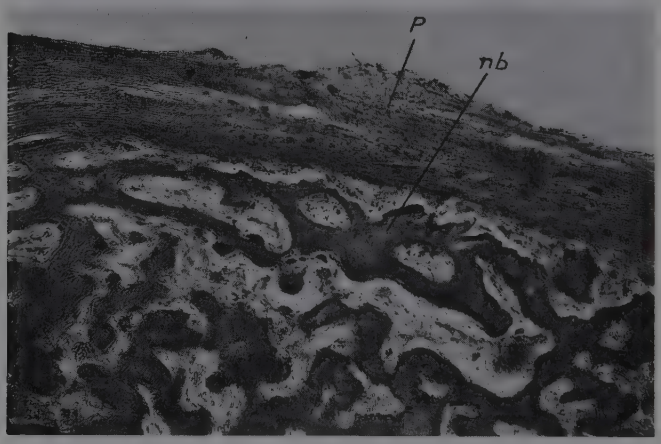


FIGURE 33. Newly formed bone (nb); periosteum (p) forming bone marrow; 15 days after operation; 7 × 8 (according to Matveeva, 1962)

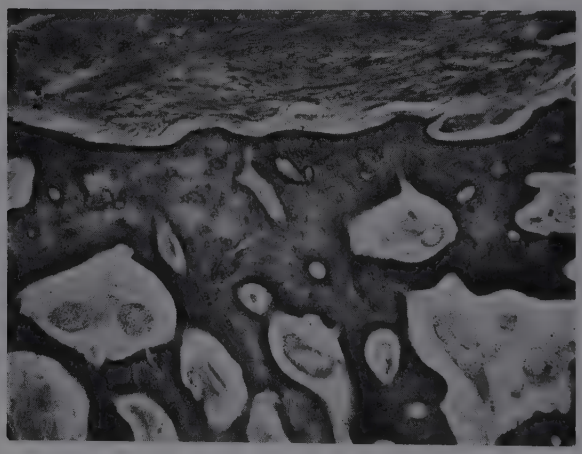


FIGURE 34. Regenerating bone 59 days after operation, 7 ×8 (according to Matveeva, 1962)

If part of the dura mater from the region of the defect in the dog is removed and this entire area is covered with fresh bone shavings, a scar is formed in the zone where dura mater is absent (Figure 35a), while bone regenerates in the zone where dura mater is present (Figure 35b) (Matveeva, 1962). Consequently, dura mater is essential for regeneration of cranial bone.

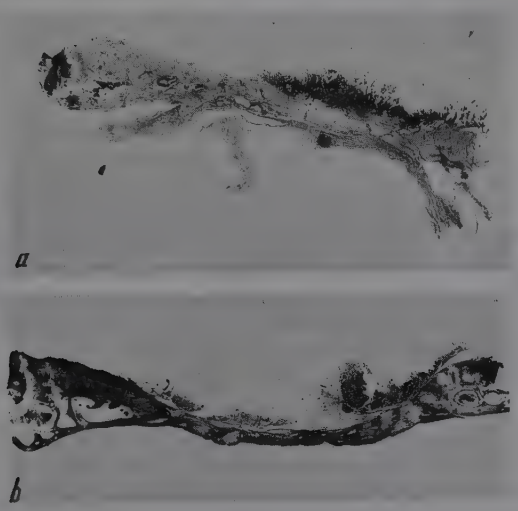


FIGURE 35. Autotransplantation of bone shavings in region of the defect in the cranial vault of a dog 119 days after operation:

a – connective-tissue scar in the zone from which the dura mater was removed; b – regeneration of bone in the zone where dura mater was left (according to Matveeva, 1962).

The preceding, briefly discribed results of histological studies show that regeneration of bones in the cranial vault, under the influence of bone shavings transplanted into the region of the defect, takes place in a special way, i. e., by induction and not by outgrowth from the edges of the bone in the region of the defect. Regeneration occurs by typical embryonic induction in which the following elements may be discerned: inducer-bone shavings; reactive material-cells of young immature connective tissue; conditions for induction, in addition to general physiological conditions, the presence of dura mater. The inducer is specific since bone shavings cannot be replaced by another damaged tissue, e. g., muscles, which are present in the region of the defect in the control animal where bone is not formed. The reacting material is also present in the control, but without the presence of inducer it becomes a connective-tissue scar and is not transformed into bone. The dura mater is essential for regeneration, but it is not the bone forming factor such as the periosteum of a tubular bone, since de novo formation of bone takes place in connective tissue under the influence of bone shavings, and not by production of bone by dura mater.

Regeneration of the cranial bones under the influence of transplanted bone shavings is the first example of regeneration by induction if we do not

count the phenomenon of Wolffian regeneration of lens in the eyes of adult tritons. To prove regeneration by induction requires that the following be shown: 1) that it is indeed regeneration and not simply metaplasia independent of regeneration; 2) that the regeneration process indeed occurs by induction and not in some other way; 3) that the process is true induction in which can be distinguished an inducer, reacting material, and conditions of induction. All these elements of proof are present in the experiments just described in which regeneration of cranial bone was caused by the method of destruction.

Conclusion

Comparison of the old biological methods of replacement of cranial defects (autoplastic, homoplastic, and heteroplastic transplantation of bones and cartilage) and the method of alloplasty, and the new biological methods of replacement suggested by us, shows the cardinal difference between the two. The former allows bone defects of the cranium to be covered immediately with a solid and hard material, but the final result may be unfavorable. The transplanted hard bones are often destroyed and the defect appears again; nonliving implanted material may have an unfavorable effect on the adjacent healthy tissues. The new biological methods do not cover the area of the defect with hard tissue immediately during the first days after the operation, but after 2—4 weeks the area is filled in with newly formed bone, and the later it forms, the better it develops and more reliably covers the area of the operation.

The fact that in transplantation of embryonic analagen of cranium, or when the method of destruction is employed, homoplastic material is used, does not affect unfavorably the course of the process and its final result, since the area of the defect is replaced by regenerating tissue from the recipient tissues and the transplants play the role of stimulants or inducers.

Of the 4 new biological methods we proposed, the simplest and most efficient is the method of destruction. The method of homotransplantation of embryonic anlagen gives good results, but the defects in the cranium are not replaced in 100% of cases. In addition, it requires prior preparation of material for transplantation which is not always a simple matter. The method of displacement of a bone fragment in the area of the defect in the cranium must be applied in stages in order to obtain regeneration of bone there, and to avoid pain during the displacement of the fragment, repeated injections of local anesthetic must be made. The method of controlled changes in metabolism is very simple, but it is risky for use with older people due to the possibility of enhancing sclerosis. The method of destruction is very simple, requires a single surgical interference, and allows replacement of cranial defects by regenerating bone in 100% of cases. If preparations are made beforehand of sterile, lyophilized bone shavings in glass ampules, this method may be very simple and convenient for clinical use in any place wherever a supply of ampules with the material can be maintained.

Despite the differences and specific properties of each of the new biological methods of replacement of cranial defects, they have certain common characteristics. Induction of regenerative osteogenesis, at a site where bone does not usually regenerate when a piece of bone has been removed,

is common to all these methods. In all four methods, this induction of bone regeneration depends on the presence of a plastic reactive material (young, immature connective tissue) in the area of the defect, and on the action on it of some bone-forming factors. In the method of controlled changes in metabolism the factors are calcium lactate and vitamin D, while in the other three methods the factors are the tissue-specific decomposition products of the damaged bone: products of the decomposing homotransplants of the embryonic cranial anlagen, dedifferentiating and dissolving bone shavings, and those substances which are released from the edge of the bone fragment displaced from the defect area. Controlled changes in metabolism in the direction of osteogenesis achieved in various ways are common to all the four methods of de novo formation of bone.

Since, as shown by our thorough histological examinations carried out in experiments using the method of destruction (Matveeva, 1962), regeneration of cranial bone occurred by induction, we assume that the same phenomenon took place in the remaining methods. We cannot, therefore, agree with A. G. Babaeva (1960) who, having no experimental data of her own, voiced the opinion that bone transplants in these cases act as a skeleton, i. e., a mechanical factor. We are convinced, however, that the dissolving bone shavings act chemically and not mechanically, a fact which defines entirely different tasks and prospects for study and recommendations for practice.

Bone shavings are not identical with bone fragments and other large bone transplants. The former dissolve rapidly after transplantation and act chemically as an inducer. The latter remain for a long time after transplantation and may participate in bone formation differently: as a source of bone formation, as a skeleton, as a stimulant, and as a transplant which is preserved. Therefore, only experiments with transplanted, rapidly dissolving bone shavings, and not experiments with transplantation of bone fragments, proved that transplants are inducers and the entire process of bone regeneration occurs by induction.

Concerning the questions of practice, we must mention the results of the interesting studies of V. S. Strebkov (1966). Being a surgeon, he carried out a number of operations on human beings in which he replaced cranial defects by the destruction method. He found that transplantation of bone shavings in the region of the cranial defect in the presence of dura mater resulted in regeneration of bone. In the absence of dura mater, the bone did not regenerate; however, a dense solid scar appeared in the area of the defect. Unfavorable phenomena were not observed during operations. Similar data were obtained by the surgeons G.I. Volkov (1966) and A. S. Kovalevskii (1967).

The study we described allows one to make the following conclusions:

1. The capacity for regeneration of the cranial bones is lost during ontogenesis of mammals, as is the capacity for regeneration of extremities in tailless Amphibia; this loss is associated with the loss by the basic tissues comprising these organs, of the capacity for destruction and dedifferentiation.

2. If bone destruction is strongly enhanced experimentally, the lost regenerative capacity may be restored, and complete regeneration of anatomically and histologically typical bones of the cranial vault is obtained in adult dogs and other mammalian species.

3. This restoration may be achieved in different ways: 1) by homotransplantation of embryonic anlagen of cranium; 2) by displacement of a cranial bone fragment in the area of the defect; 3) by the bone destruction method; 4) by controlled changes in the metabolism of the whole body. Local controlled changes in metabolism leading to local bone formation are common to all these methods.

4. In experiments with destruction, regeneration of bone occurs by embryonic induction in which the transplanted bone shavings are a tissue-specific inducer, while the local connective tissue is the reacting plastic material and the dura mater is the specific condition for regeneration. In all probability, in all the other aforementioned cases, too, the cranial bones of adult dogs and other mammalian species regenerate by induction.

5. Based on the rules of loss and restoration of regenerative capacity of organs in mammals, new biological methods can be proposed allowing for replacement of bone defects in the cranium of regenerating bone, a finding which is of direct practical significance for surgery and neurosurgery. From among these proposed new biological methods of replacement of bone defects in the cranium, the simplest and most effective one is the destruction method which allows regeneration of the bones in the cranial vault in areas of defects of various size and shape, in 100% of cases.

Chapter III

RESTORATION OF LOST REGENERATIVE CAPACITY OF DENTAL TISSUES

The previous chapter discusses how the use of quite simple methods may cause regeneration of large pieces of bone, which do not regenerate under ordinary conditions, in the cranial vault of adult dogs, rats, mice, and sheep. The bones of the cranial vault are characterized by a very high density and hardness; after removal of segments, the edges of the bone are practically not impaired or dedifferentiated. However, if artificial destruction of bone is caused, regeneration occurs. This finding invariably raised the question of the possibility of forcing the regeneration of the densest and hardest of all tissues of the human and mammalian body, the dental tissue, which, as is well known, never regenerates under ordinary conditions.

The dental problems of the vast majority of people are most often due to caries. Everybody knows very well that holes in the crown of a tooth, drilled with a bore, are never filled with regenerating tissue. They are either filled with a phosphate cement or other substances, or — if they are not treated — result in destruction of dental tissues.

Must this be so, however? Is it not possible to cause regeneration of dental tissues? Is it not possible to heal diseased teeth using such a method?

The lack of regeneration of dental tissues in man may be due to at least two causes: 1) the fact that during dental operations, the teeth are subjected to medication which destroys living tissues capable of regeneration, and 2) the fact that during operations no such biological conditions are created as would facilitate regeneration processes. Consequently, the problem of the possibility of obtaining regeneration of dental tissue in animals and man could be solved in an experiment in which no medication which could destroy living tissue is used, and where biological conditions would be created which determine the appearance of regeneration. Tissue, especially dentine and cement, is very similar in its biochemical composition and histological structure to bone tissue. Therefore, there is some basis for the hope that in appropriate experimental conditions, it will be possible to induce regeneration of dental tissue, as has been done with the bony tissue of the cranial vault.

Our experiment was performed on adult dogs aged 2 — 5 years. The objects of study were the canine teeth in the upper or lower jaws. The operations were conducted under morphia-ether narcosis in aseptic conditions. The tooth was thoroughly sterilized with 70% alcohol, and the animals were given injections of 100,000 units of penicillin for 3 days after the operation. The dogs were killed at different times up to 436 days after the operation.

The experimental teeth were cut at the neck, and thick cuttings or histological sections were prepared. The teeth were fixed in a 20% solution of formaldehyde, decalcified, embedded in celloidin or celloidin-paraffin. The sections, 15 — 30 μ thick, were stained with hematoxylin-eosin. The plane of the cuttings and sections crossed the tooth canal and the chamber drilled with the bore. An electric boring machine and a bowl-like No. 3 bore were used for drilling.

In our study we carried out two main experiments in which regeneration of tooth tissue was attempted, by the destruction method and by the induction method.

Regeneration of dental tissues effected by the destruction method

In the first experiment we attempted to cause regeneration of dental tissues by the destruction method. The rationale for this experiment was data which showed that regeneration of extremities in axolotl could be obtained after complete destruction of the basic tissues of mesodermal origin which comprise these organs (Polezhaev, 1934, and others). These data were later successfully developed by Studitskii (1959) and his co-workers in experiments on regeneration of ground skeletal muscles in warm-blooded animals (young chicks, rats, rabbits). Our data on regeneration of bones in the cranial vault caused by the destruction method, also served as a basis for these experiments (Polezhaev, 1957; Matveeva, 1962). In these experiments on dogs and rats, bone shavings implanted into the region of the cranial vault defect were dissolved, inducing bone regeneration in the surrounding connective tissue. In the experiments with mice, the crumbled solid and hard bone implants did not dissolve, but remained inside the induced bone for the duration of the experiment, i. e., for 6 months (for an average lifespan of a mouse of 2 years).

In the present experiments, we decided to attempt to cause regeneration of dental tissues in dogs by implantation of dentine filings in a previously prepared chamber in the tooth. The experiment was performed as follows. The crown of the tooth was drilled to a depth of 5 — 8 mm to the pulp. The chamber formed had a diameter of 3 — 4 mm. The first portions of the tiny enamel filings obtained during the drilling were discarded. The remaining part of the dentine shavings were collected into a sterile rubber cup, which was placed on the base of the tooth through a hole in its bottom. The cup had been made from the fingertip of a rubber glove. The shavings, poured into the bottom of the cup, were slightly wetted with penicillin prepared in a physiological solution, and the tooth chamber was then filled to the tooth canal with the resultant thick paste. The upper part of the chamber was filled with phosphate-cement at a depth of 1 — 1.5 mm to keep the implanted dentine filings in place. The phosphate-cement fell out 4 — 8 weeks after the operation, but sometimes remained for more than a year.

In all cases the results obtained were unequivocal: the biological implant was transformed into bone or a dentinelike tissue which completely covered the tooth chamber (Figure 36). The middle part of the tooth canal, located against the chamber with the implant, was completely or partially

obliterated. The pulp in this zone was transformed into osteoid tissue, and newly formed dentine layers, irregular dentine, appeared on the inner surface of the dentine. These phenomena were particularly distinct in those cases where the material of the implant was removed from the walls of the distended chamber during histological treatment (Figure 37). A bonelike plug of the implant directly touches the pulp, which is transformed into a dense osteoid tissue. The dentine in the pierced wall of the tooth changes very little. It consists of the basic substance with characteristic radial, slightly wavy, dentine channels (Figure 38) through which appendages of odontoblasts traverse. Odontoblasts and the loose connective tissue of the pulp stick to its inner surface. At the inner surface and the lateral walls of the tooth canal, which are located against the implant, is a thick layer of newly formed induced dentine (Figure 39) due to which the tooth canal lumen in the region of the operation is greatly constricted. The layers of newly formed dentine are very thick against the implant and become gradually thinner upward and downward. A similar phenomenon of de novo formation of dentine, so-called irregular dentine, is sometimes observed against irritated spots in a tooth, e. g., in caries. The thickness of the newly formed dentine, together with the osteoid tissue induced in the pulp, may be so great as to lead to complete obliteration of the tooth canal.

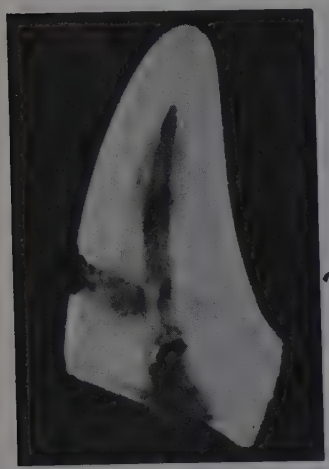


FIGURE 36. A cutting through the canine of a dog 100 days after operation. The tooth chamber is clogged with newly-formed dentinelike tissue.

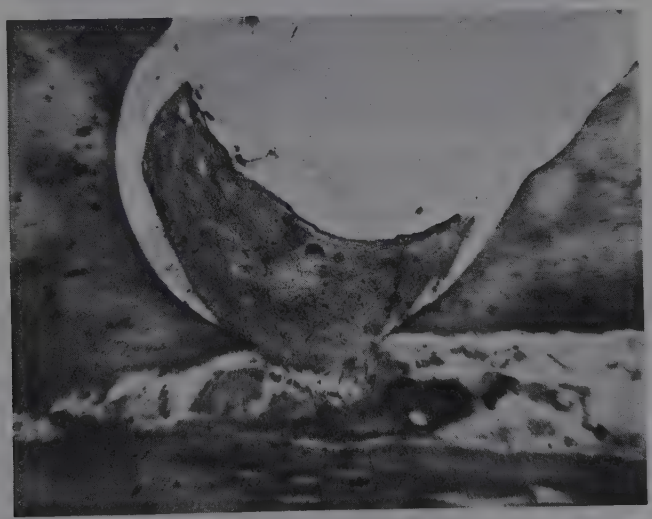


FIGURE 37. Region of operation in a tooth section. Dentinelike implant; newly-formed osteoid tissue is seen in the tooth canal.

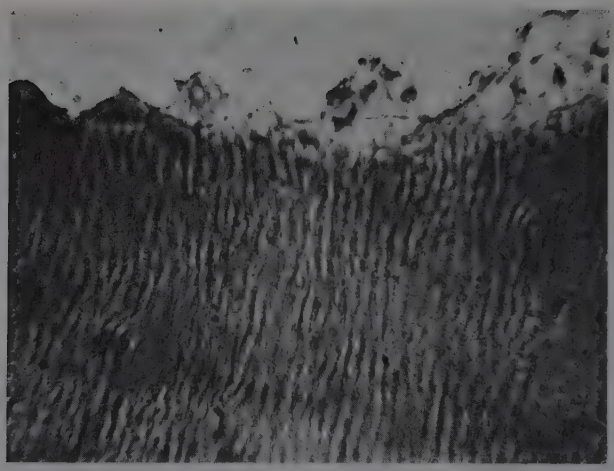


FIGURE 38. Dentine canaliculi in the wall of a tooth and odontoblasts. 10 × 60

FIGURE 39. Newly-formed dentine peeled off from tooth wall. 10 × 60

The fate and structure of the bonelike plug formed from the implant inside the tooth chamber is of great interest. It consists of tiny particles of dentine, densely and organically fused by an amorphous, calcified substance, and it becomes denser with time. There are no cells or cell appendages in this tissue; 45—60 days after operation, the disorderly mixed and fused dentine shavings present in the plug maintain their initial structure, and dentine canaliculi can be clearly seen in it; the individual particles are relatively loosely fused (Figure 40a). Later, 100 days after the operation, the structure of the shavings changes slightly, the dentine canaliculi in many of them are less distinct, the amount of basic cementing substance in them

becomes larger, and the entire structure of the plug becomes more amorphous and dense (Figure 40b). These facts indicate that the tissue of the implant lives and develops, that processes of metabolism occur in it as part of the general metabolism of the tooth and the whole body. The bonelike plug formed from the implant reliably protects the tooth from infection. Even a year after the operation, despite the fact that the phosphate-cement filling fell out, the plug so well covered the chamber and the pulp that no caries or pulpitis were observed in the tooth. The implant adheres tightly to the edges of the dentine chamber, which do not regenerate themselves and only fuse tightly with the implant.

The data obtained show that despite the fact that tooth tissue is not capable of regeneration under ordinary conditions of damage, it proved to be capable of regeneration and metaplasia under our experimental conditions (strong destruction).

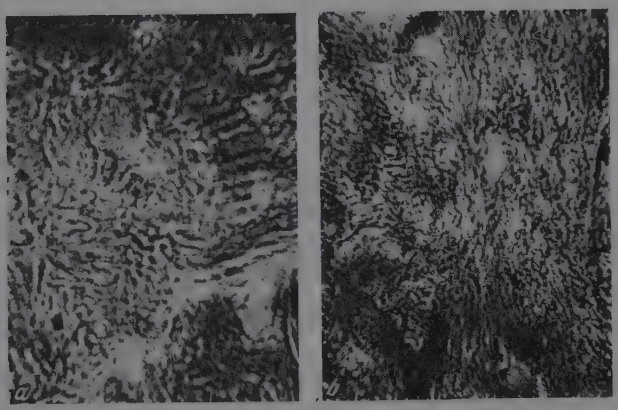


FIGURE 40. Structure of dentinelike tissue in tooth chamber:

a – less dense; b – denser. 10 × 60.

After termination of our experiments, we found published reports of something in this direction which had already been done in stomatological practice, albeit on the basis of different assumptions and with the use of a somewhat different technique. The well-known stomatologist, Prof. G. L. Feldman (1932), proposed a new approach to tooth therapy with inflamed pulp. He emphasized the fact that all medication which preserves the pulp or disinfects the root canal in vivo is harmful and causes inflammation in the surrounding periapical tissue, that the medication used necrotizes the pulp stump and causes scarification. He reached the conclusion that "the antiseptic method, based on the use of various antiseptics in the treatment of pulpite teeth, discredited itself" (p. 18). In the past investigators were of the opinion that any damaged pulp should die and that "there is no true regeneration of pulp tissue." However, the author (Feldman) reached the conclusion, on the basis of his experimental and clinical data, that if antiseptics were avoided and certain biological conditions created, regeneration of pulp might be induced. In experiments with a dog he bored a chamber in

a healthy tooth, amputated the pulp, and covered its stump with dentine filings. In all the cases the pulp of the root canal did not die, but underwent metaplasia and was transformed into a dense osteoid tissue with bundles of calcified dentine filings embedded in it, which filled the bottom of the chamber in the form of a dense mass. Irregular dentine formed around it. The wound healed. When the chamber was treated with phenol or cobalt, however, (in the absence of dentine filings) the pulp degenerated and died, and no metaplasia occurred.

The investigator practiced his biological-aseptic principle of tooth therapy successfully for 2 years without antiseptics in cases of inflammation and purulent decomposition of pulp. He removed the inflamed pulp and covered it with a biological implant.

Our data basically agree with those of Feldman. The main difference is that he was interested primarily in the pulp, while we were interested in the dentine. He showed that it was possible to preserve damaged pulp under conditions of its metaplasia into an osteoid tissue under the influence of dentine filings, and our data fully confirmed this finding, as well as the phenomenon of de novo formation of irregular dentine. Our data showed, in addition, the possibility of atypical regeneration of dentine from dentine filings, and the possibility of biological filling of the tooth chamber. The study of regeneration has shown that regeneration of one organ stimulates the regeneration and development of other organs. This means that induction of regeneration of tissues in the tooth, e. g., in caries, may lead not only to healing of caries, but also to strengthening and healing of other teeth. The regeneration of dentine by the destruction method is a new method of biological filling of teeth. Its advantages are:

1. It permits keeping the dental tissues alive.
2. It stimulates de novo formation and development of pulp and dentine tissues.
3. It may result in strengthening and healing adjacent teeth.

It must be pointed out, however, that much has still to be done to elaborate this method: experiments of homoplastic transplantation of dentine filings must be designed, the possibility of using lyophilized dentine filings elucidated, a better technique of biological filling of teeth elaborated, possibilities of causing regeneration of tooth enamel studied, etc.

Thus we reached the conclusion that the lack of regeneration in dental tissues (pulp, dentine, enamel), observed in stomatological practice, is due primarily to the harmful effect of medication, which kills live cells and tissues, and to the lack of conditions necessary for regeneration. If such conditions are created, the tooth tissue may be stimulated to regenerate. Dentine filings may be the building material for regeneration, but they may also heal affected pulp and change the physiological state and histological structure of the tooth tissues.

Regeneration of dental tissues brought about by the induction method

In recent years the possibility of regeneration of dentine and cement of the tooth root in dogs has been demonstrated (Gavrilov, 1957). The tooth root was exposed by alveolotomy, an orifice was drilled, and the region of the

defect covered with a mucous-periosteal scab. After 10 days, the region of the defect was filled with fibroblasts; after 15 days, a homogeneous predentine was formed; after 30 days, there was dentine with canaliculi and cement, and after 90 days, hard dentine and cement were seen. These data show the possibility of true regeneration of dental tissues by transformation of young, undifferentiated, connective tissue filling the region of the defect. Such an experiment, however, can be performed on the root but not on the crown of teeth which are located above the amphodont. In stomatology, however, the crown of the tooth affected by caries is usually treated and filled.

The question arose as to whether regeneration of dental tissues can be caused if a piece of differentiated connective tissue is placed into the crown where fibroblasts cannot penetrate. In this case the connective tissue could transform into dentine or bone. To answer this question in the affirmative, it could be said that, in principle, a new method for the treatment of teeth was found by biological filling based on metaplasia and induction.

The experiment was carried out on dogs. As in the previous experiment, we used a No. 3 bore and drilled a chamber to the pulp of the tooth canal in the crown of the canine in the upper or lower jaw, under aseptic conditions. The chamber was approximately $5-8 \times 3$ mm; 2 – 3 pieces of amphodont or aponeurosis (galea aponeurotica) were autotransplanted into the chamber, almost to the tooth surface. Part of the implant entered the pulp of the tooth canal. Above the implant we placed a phosphate-cement filling which protected it against falling out and infection. The tissue of the amphodont was taken from the gum which was first thoroughly washed with 70% alcohol, after which the outer epidermis was cut off. A piece of aponeurosis was taken from the head of a dog on which cranial surgery was performed. We implanted a small amount of dentine filings, together with the pieces of amphodont or aponeurosis, obtained during the boring of the crown of the tooth. The filings could enhance induction and, therefore, metaplasia of the implanted tissue. The implant was wetted slightly with a penicillin solution prepared in a physiological solution. The animals were killed at different times, up to 100 days after operation. The phosphate-cement plug remained in place in all except one case. The histological treatment of the teeth was the same as in the previously described experiment. All the animals remained in good health after the operation and did not suffer from toothache. The results of the experiment were clear-cut and distinct in all cases: the biological implant was transformed into a dense, hard, bonelike tissue which solidly plugged the tooth chamber (Figure 41). The amphodont was transformed to true bone or dentine, while the aponeurosis was transformed to only a mineral-dense tissue without any structure of true bone. Under the influence of the implant, the pulp in the tooth canal was transformed into a dense osteoid tissue; on the tooth wall there was de novo formation of dentine.

The transformation of the implanted tissues occurs in 2 stages: in the 1st stage, within the first 15 days after the operation, the implant has not yet been fully transformed into bone or osteoid tissue; in the 2nd stage, lasting from 30 – 100 days after the operation, a complete and thorough transformation of the implant into osteoid or dentinelike tissue takes place.

FIGURE 41. Chamber of tooth of a dog covered with an induced bonelike tissue

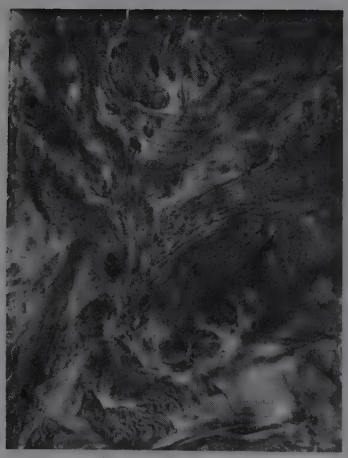


FIGURE 42. Implanted amphodont 7 days after operation; fibroblasts and thick, dense, collagen fiber, 10×60

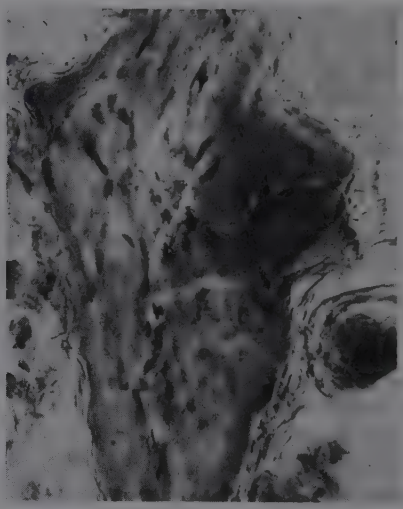


FIGURE 43. Transformation of amphodont into bone in the tooth chamber 7 days after operation, 10 × 60

The transformation of the implanted amphodont begins 7 days after the operation and is irregular in different sectors. A piece of amphodont located in the tooth canal maintains its original histological structure, and is not transformed into bone (Figure 42). It consists of considerably large and rare fibroblasts with distinctly delineated nuclei, with nucleoli and spindle-shaped, elongated cytoplasm, among which one sees a multitude of dense,

thickly convoluted and interwoven collagen fibers which do not show any signs of mineralization or transformation into bone. An entirely different picture is observed in that piece of amphodont which is located in the tooth chamber. Under low-power magnification of the microscope, transformation of amphodont into bone can be seen. In the amphodont tissue are numerous dentine filings, and the tissue, particularly at the surface of the chamber closer to the phosphate-cement plug, consists of layers of compact bone which stain differently from the nondifferentiated part of the amphodont. Under high-power magnification the transformation of the amphodont into bone is seen quite clearly (Figure 43). The fibroblasts change drastically in shape, becoming long, narrow, and embedded in the basic bone substance. The latter appears by direct transformation of collagen fibers, which widen, thicken, and are transformed into a fibrous bone. This, however, does not yet terminate their development; the fibrous bone is transformed into compact bone. At later stages, e. g., 43 days after the operation, a very dense, light bone appears in the amphodont, which is located in the tooth chamber, and few osteocytes are embedded in the basic bone substance (Figure 44). Pieces of amphodont which happened to enter the pulp of the tooth canal are also transformed into bone, but much more slowly than those in the chamber, and the bone developing from them also differs in structure from that in the amphodont located in the chamber. This is a dense, mineralized, compact bone with an irregular structure and consists of fused, altered amphodonts, pulp, and dentine filings. Acting as a plug it obstructs the lumen of the tooth canal (see Figure 41).

FIGURE 44. Further transformation of amphodont implanted in the tooth chamber into dentinelike tissue with embedded osteocytes, 43 days after operation, 10 × 60

FIGURE 45. Aponeurosis, implanted into the the tooth chamber, mineralizes but does not not transform into bone, 30 days after operation, 10 × 40

A piece of aponeurosis, transplanted into the chamber of the tooth crown and into the tooth canal, develops entirely differently from this. Under normal

conditions, aponeurosis consists of loose, gelatinous, connective tissue containing few and rare fibroblasts, and a great amount of interstitial substance. After transplantation into the chamber, this tissue, into which many dentine filings are embedded, becomes mineralized and dense, acquires an osteoid appearance, but does not differentiate and does not transform into bone (Figure 45).

Under the influence of dentine filings present in the tooth canal pulp, the odontoblasts become activated and produce new layers of canalized dentine on the inner wall of the tooth, which are deposited on the old, dark, also canalized dentine, in the form of a light zone (Figure 46). With time the layers of the newly formed dentine become thicker, and sometimes peel off from the old dentine.

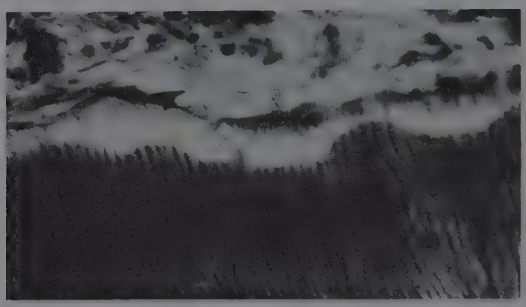


FIGURE 46. De novo formation of a canalized plast of dentine (light edge) under the influence of dentine filings, implanted into the tooth chamber, 7 days after operation, 10 × 60

Considerable change occurs in the pulp. After implantation of tissues, aseptic inflammation appears in it and it is strongly, and sometimes for a long time (10—15 days after operation), infiltrated mainly by polynuclears and partially by lymphocytes. In it are also formed small cavities filled with fluid, the cavities sometimes so large that the tooth canal looks empty in sections. The connective tissue of the pulp matures in the process, and fibroblasts appear in dense rows on the canal walls.

Thus, in autotransplantation into the tooth chamber and tooth canal of a dog, amphodont is transformed at first into a fibrous and later into a compact bone, while aponeurosis becomes mineralized, dense, and hardens but does not transform into true bone. Consequently, even when maintained under the same conditions, connective tissues of different origin react differently, exhibiting different potentialities. Transformation of transplanted amphodont into bone without prior multiplication of its cells or formation of young connective tissue, is an example and proof of the possibility of direct metaplasia. A similar example of direct metaplasia of dense connective tissue into bone was given by P. V. Sipovskii (1961). In our experiment, the transformation of transplanted connective tissue into bone depended on the effect of implanted dentine filings, or maybe on the effect of the phosphate-cement filling. This osteogenic effect is an induction, and the dentine filings and the tooth chamber dentine are inducers. The amphodont tissue is the reacting system, and its origin is the internal condition for the process of induction.

All the foregoing, the phenomenon of direct metaplasia of a piece of amphodont implanted into the tooth chamber, is induction similar to embryonic induction, e. g., transformation of ectoderm in the early gastrula into nerve tissue under the inducing effect of the invaginating presumptive chordomesoderm, or similar to the transformation of embryonic mesenchyme into the cartilage capsule of the acoustic vesicle (Spemann, 1936; Filatov, 1939). In these examples, tissues which are very plastic and pluripotential are dealt with, while in our method a differentiated tissue of an adult vertebrate, and of a high vertebrate at that — a mammal — is being transformed. These data are similar to those of Levander (1964) on the induction of tissues in adult rabbits: when a piece of bone is implanted under the skin of a rabbit, bone is formed from the local connective tissue, and when a piece of muscle is implanted, muscle is formed.

There is a basic similarity between our data and those of Levander; both cases deal with induction of tissues in the body of an adult mammal. However, there is also a basic difference: in Levander's experiment there is induction of tissues at an uncharacteristic site, while our experiments dealt with regeneration by induction of a tissue of a segment of an organ which does not regenerate under ordinary conditions of damage. The induced tissues in Levander's experiments are rapidly resorbed, while in our experiment they are lastingly and stably preserved.

Our data on regeneration of dental tissue by induction coincide basically with data on the regeneration of cranial bones by induction, which we described in the previous chapter. In both cases organs are dealt with which are not capable of regenerating under ordinary conditions of damage; in both cases mammals are dealt with, and the same means of regeneration are used. The main difference between the two is that in the experiments on cranial bones, the newly formed segment of the organ appears from immature connective tissue, while in the experiments on dental tissue, mature differentiated connective tissue is the source of new formation. Therefore, in the former case, an anatomically and histologically typical cranial bone appeared; in the latter case, the tissue formed is not at all typical; dentine canaliculi are missing in it. In the former case, organogenesis and histogenesis take place, while in the latter only histogenesis occurs. If the starting material was young fibroblasts, dentine canaliculi would also appear. Thus:

1. In autotransplantation of a piece of amphodont into the chamber of a tooth of an adult dog, the amphodont is transformed into bone or dentine by direct metaplasia.
2. Under the same conditions aponeurotic tissue becomes mineralized and hardens, but does not transform into bone or dentine.
3. The process of transformation of the connective tissue of the amphodont into bone, under the conditions just indicated, occurs by induction. The tooth tissue regenerates by induction.

Concerning the positive aspects and the necessity for improving this method of causing regeneration of dental tissue by induction, the same statement can be made in principle as was already made concerning the method of regeneration of dental tissue by destruction. The common characteristic of the two methods is a new biological principle for healing teeth in stomatology: avoidance of using medication which destroys living tissue, and the suggestion of creating biological conditions which will

lead to regeneration of dental tissues which do not regenerate under ordinary conditions. We assume that the data given are of both theoretical and practical value. At present attempts are being made in stomatological practice to use new biological principles. First, a number of attempts have been made to replace a lost tooth with a live, transplanted tooth. The most promising efforts have been the transplantation of tooth anlagen to adult dogs and rats (Lapchinskii and Malinovskii, 1940, 1940a; Lapchinskii, 1941, and others). It has been shown that teeth anlagen transplanted into soft tissue cannot develop, and they die; those transplanted into bone (femur or maxillary) can grow and differentiate nicely. Second, attempts are being made to replace a missing tooth alloplastically, inserting into the gum pierced metallic or plastic prostheses which are overgrown by connective tissue and thus become fastened in place (Vares, 1957, and others). The third biological principle may be the one described earlier in which the bases are the phenomena and laws of regeneration, the method consisting of an attempt to act on tooth tissue, change its conditions and cause in it inductive and metaplastic transformations, i. e., restore the lost capacity for regeneration. This method, in contrast to the 2 other biological methods, may be used not only for replacement of missing teeth, but also for treatment of diseased teeth. After proper development the method may give positive, promising results.

Chapter IV

RESTORATION OF LOST REGENERATIVE CAPACITY OF THE MYOCARDIUM

Definition of the problem and selection of objectives

As already pointed out, our work on the problem of loss and restoration of regenerative capacity of extremities of tailless Amphibia and other vertebrates was of an experimental-theoretical nature. We made a step forward in the direction of practicality when we became interested in the problem of loss and restoration of the regenerative capacity of dental tissues and bones of the cranial vault. Although these organs are of great importance for human life, they are not vital organs and thus, in theoretical and practical respects, the most interesting and important work would be that on the problem of restoration of vital organs which do not regenerate under ordinary conditions of damage. Among these belong the heart and the brain. These considerations prompted us to start work on regeneration of these organs. It is well known that cardiovascular diseases are the foremost and major cause for mortality in the population in the USSR, as well as in a number of other industrially developed countries. Death due to these diseases comprises 40% of the overall mortality rate of the population of the USSR. In these diseases death is due to cardiac insufficiency, which, in many cases, is caused by damage to the cardiac muscle. Myocardial infarctions belong in this category, and are frequently called by physicians the coronary disease of our century. Also in this category are various kinds of myocarditis, chiefly of an infectious nature (diphtheria, scarlet fever, influenza, rheumatism, lues, etc.), myocardiodystrophy, wounding, echinococci, etc. Emphasis should be given in particular to the importance of thoracic surgery, which is successfully used in surgical treatment of various heart diseases, and in which it is of great importance to stimulate regeneration of the myocardium. It is not without good reason that the academician A. N. Bakulev (1964) emphasized the fact that stimulation of regeneration of the myocardium is one of the acute problems of modern medicine.

At present physicians treat diseases of the myocardium by creating conditions of prolonged rest for the patient, and using a battery of cardiovascular means which improve the work of the heart. Under these conditions the foci of necrosis are resorbed and replaced by connective-tissue scar. The damaged segments of the myocardium are not restored. Thus, the efforts of physicians are directed toward bringing about scarification of the myocardium. As a result of scarification, hypertrophy of the remaining part of the cardiac muscle occurs, and there is some compensation of the disturbed cardiac activity. Although this fact is of great importance for

human life, as it allows the patient to live and work, the scar tissue is not capable of contraction and cannot replace the sometimes very large sectors of the damaged cardiac muscle. Under the pressure of the blood the scarified wall distends more and more, becomes thin, and forms an aneurism which may burst suddenly, leading to the patient's death. Thus it is not at all a small matter for the patient that regeneration or compensatory hypertrophy of cardiac muscle occurs after damage to the myocardium. It would be extremely important to learn to restore true cardiac muscle, instead of scar tissue, in the foci of damage in the myocardium. Modern medicine, however, is not yet capable of doing this, as it does not know the rules of loss and restoration of regenerative capacity of organs and tissues in animals, and does not have methods for the restoration of organs which do not regenerate under ordinary conditions of damage.

Having been interested in this problem for a long time, and having established, as we think, at least the basic rule of loss and restoration of regenerative capacity of organs and tissues in animals, we considered it possible to pose the problem of achieving restoration in mammals of damaged cardiac muscle which does not regenerate under ordinary conditions of damage. Having posed this problem, in approaching the search for a scientific basis for its solution, definition, and elaboration, we foresaw great difficulties, but, nevertheless, we thought that we could and should begin work in this direction. The main point in this work was, on the one hand, to find a new approach, and on the other hand, to overcome the scepticism of the representatives and defenders of the old approach who attempted to deny, not only our new theory but even the new facts we established. Thus, A. G. Knorre (1962) came out with his paper in which he condemned our approach, and cast doubt on our data which show the possibility of experimentally causing regeneration of myocardium in mammals. Vis-a-vis our active experimental approach to the controlled change of the nature of animal organisms toward restoration of regenerative capacity lost in phylogenesis and ontogenesis of animals, he presents his own descriptive-comparative approach. He suggests studying embryogenesis and histogenesis of myocardium in a comparative series of vertebrates, mammals in particular, to compare the myocardium's reactions to damage in animals of various taxonomic groups, and only after the culmination of such study, to think of an approach to the problem which occupies us at present. The importance of data which could be obtained by descriptive-comparative study of phenomena of regeneration cannot be denied. It is clear, however, that this approach alone, without taking into account and utilizing principles and data obtained in experimental studies, is unsuitable for the solution of the great biological problem of active controlled change of the nature of animal organisms, in particular the capacity of animal organs for reparative regeneration. A basically new approach is required to elaborate and solve this problem, and we make the first attempt to provide such an approach. It is understandable that this will be organically foreign to the representatives of the old contemplative approach to life phenomena, and they will not show a favorable attitude to the new direction in the study of regeneration.

Data on the incapacity of myocardium to regenerate

Even after cutting or partial removal, the skeletal muscles in young chicks, mice, rats, and rabbits are able to regenerate (Zhinkin, 1945, 1948; Clark LeGras, 1946; Studitskii, 1959, and others). In adult dogs and human beings, however, wounds heal by scarification (Marchand, 1901; Goldzieher and Makai, 1913; Studitskii and Ignat'eva, 1961). The myocardium of mammals and man belongs among organs with a very weak capacity for reparative regeneration. Experiments on regeneration of myocardium had already been conducted over 75 years ago (Martinotti, 1888; Mircoli, 1889, and others), and they gave a quite clear-cut result, which is in full agreement with data from human pathological anatomy, namely, cardiac muscle in all the investigated species of adult animals did not regenerate after injury of various kinds. Data on this problem are available in a number of reviews, monographs, and dissertations (Marchand, 1901; Anichkov, 1912; Goldzieher and Makai, 1913; Klose, 1923; Gesse and Gesse, 1924; Rumyantsev, 1953; Kashaev, 1953; Kolosova, 1961; Kochetov, 1961; Polezhaev, Akhabadze, Muzlaeva, and Yavich, 1965).

In experiments on rats, rabbits, cats, and dogs, it has been shown that after the heart was cut and stabbed (e. g., when the heart was stabbed through the thoracic wall) or when opened, phenomena of inflammation arose in the focus of damage in the myocardium; granulation tissue developed and was later transformed into a dense connective-tissue scar. Stumps of muscle fibers do not form myoblasts or muscle bundles (Martinotti, 1888; Mircoli, 1889; Bonome, 1889; Berent, 1892; Elsberg, 1899; Traina, 1899; Tikhov, 1899; Göbel, 1901; Klose, 1920; Lavrov, 1948, 1949; Khlopkov, 1948; Thomas and Ziffrin, 1952; Rumyantsev, 1953; Kashaev, 1955; Tähkä, Turunen, and Hjelt, 1957; Antipov, 1962). Stumps of muscle fibers do not dedifferentiate. Signs of regeneration in the myocardium are reduced to amitotic division of nuclei and formation of an excrescence on the stumps of the muscles. Kolosova (1961) carried out an extensive comparative study of the myocardial reaction to damage in a loach, frog, lizard, chicken, and cat, and she found that wounds in the hearts of all these healed by scarification. Only in the frog did a certain amount of smooth and striated muscle fibers form in the scar.

A similar result was obtained with rats, rabbits, and dogs in experiments in which the myocardium was damaged by burns (Martinotti, 1888; Moritz and Atkins, 1938; Walls, 1949; Polezhaev, 1959). Only T. V. Tarapina (1958, 1962) says that she observed the formation of myoblasts in the myocardium of rats. We could not confirm these data, however. This researcher probably diagnosed as myoblasts, fibroblasts or cells in the endothelium of capillaries.

The absence of regeneration of muscle fibers in the heart, and scarification of foci of damage upon the introduction of foreign bodies, steel, and gelatin needles into the myocardium of rabbits (Oppel, 1901; Anichkov, 1912) were also described. In these thoroughly executed studies, it was found that nuclei enter the myocardium from the stumps of the muscle fibers and participate in the formation of the scar. The scar not only includes connective tissue, but is also of myogenic origin. These elements, however, do not form muscle fibers or myoblasts. They have a special morphological characteristic and were called "myocytes." Along the

longitudinal axis of their elongated nuclei, there is a crenated zone of chromatin, the cell cytoplasm is weakly manifested, the cells divide amitotically; in contrast to fibroblasts, they do not form collagen fibers. Later a considerable number of reports were devoted to the myocytes, and some investigators reached the conclusion that these are not muscle cells, but are of connective tissue origin (Rumyantsev, 1960). According to our data, the myocytes arise from these and other elements, and also from cells of pericardium and the adventitia of the cardiac vessels. Their appearance indicates lack of regeneration in the heart muscle fibers.

Numerous studies have been devoted to the experimental investigation of infarctions in the myocardium of dogs, rabbits, cats, rats, and even mice, guinea pigs, and hamsters. Infarctions were usually obtained by ligating the left descending branch of the coronary artery (Kolster, 1893; Karsner and Dwyer, 1916; Ikhteiman, 1934; Ring, 1950; Barboni and Ganassi, 1954; Johns and Olson, 1954; Vinogradov, 1955, 1957; Fux and Hughes, 1955; Polezhaev, 1959; Kolosova, 1961; Lushnikov, 1961, and others). In the infarcted zone, the dimensions of which depend on the heart dimensions and the localization of the ligature, ischemia develops followed by coagulation necrosis, lysis of the necrotized tissues, and their replacement by a connective-tissue scar. The muscle fibers do not regenerate. In only a few studies was any mention made of the fact that in granulations, independent of the muscle stumps, few undifferentiated muscle fibers appear which degenerate later (Ring, 1950; Barboni and Ganassi, 1954a).

Scarification of heart wounds also occurs upon resection of considerable segments of the myocardium and simple sewing together of the wound edges (Göbel, 1910; Lampertico, Lesca and Perrone, 1958; Temesvari, 1954, 1955); Murray, 1947; Carter and MacMillan, 1950; Sergievskii, 1958; Dillon, Postelwait, and Durham, 1961).

Focal necroses in the myocardium arise in different kinds of myocarditis, caused by the administration into the animal body (rabbit, dog, cat, rat) of cardiac glycosides (adrenalin, sparteine, arsenic, enzymes, cholesterol, protein, etc.) and also diphtheria bacilli, streptococci, and staphylococci (Levitskii, 1904; Fleisher and Loeb, 1910; Anichkov, 1912; Selye, 1961, and others). The foci of damage are scarified; the muscle fibers of the heart do not regenerate.

A similar result is obtained from the action of food, physical factors, and stress. Necrosis of cardiac muscle appears in rabbits running in a treadmill, in orthostatic collapse, when tied to a stand during exposure to loud sound, fright, when they were kept on a special diet, etc. (Meessen, 1939; Fanfani, 1954; Groover, Selgeskog, Haglin, and Hitcheock, 1963; Wilgram, 1958; Selye, 1961, and others). The foci of damage in these cases become scarified.

Partial data on man are available and are presented below.

Fisher (1868) collected 452 cases of various wounds in the myocardium, of which healing took place in 72. Since then a great amount of such data has accumulated, and at present, operations on the heart have become routine for thoracic surgeons. At first, the histological picture was described in the different cases of wounding of the cardiac muscle. Subsequently, data were accumulated showing the dynamics of the process of healing in the myocardium (Hesse and Hesse, 1923; Ageichenko, 1947; Porksheyan, 1955; Antipov, 1962, and others). Along the edges of the wound there is necrosis of muscles, edema appears, infiltration of tissues with polynuclears,

lymphocytes, and polyblasts takes place, the necrotized tissues are later resorbed, numerous macrophages appear, and granulation tissue develops and is transformed into scar tissue. Plasma and giant cells are formed; eosinophils and numerous myocytes appear. On the muscle stumps the nuclei divide amitotically, but muscle fibers do not regenerate.

It is somewhat strange that despite the wide occurrence of myocardial infarctions in man, relatively few studies present a detailed description of the consecutive changes in the histological picture of the healing process of an infarcted cardiac muscle (Levine, 1929; Mallory, White, and Salcedo-Salgar, 1939; Zolotova-Kostomorova, 1950; Kaliteevskaya, 1957; Barrie and Urback, 1957; Zaikin, 1959, and others). Necrosis starts initially in the muscles, then in the connective tissue.. The necrotic parts are subjected to fibrocytosis and lysis within 4—6 weeks, sometimes 4 months. Processes of inflammation and infiltration of the coagulated tissues with elements of the white blood cells and polyblasts are replaced by lysis, development of granulation, and then scar tissue. After 6—8 weeks the infarct heals with a scar. The remaining part of the myocardium hypertrophies. In many cases aneurisms appear which often (in 30—35% of cases) burst. No regeneration of muscle fibers ever occurs in the foci of damage.

In the appearance of various myocardites, mainly of infectious origin, a general phenomenon may be observed: death of the cardiac parenchyme, and its replacement by proliferated connective tissue (Mönckeberg, 1924; Abrikosov, 1947, and others).

Many cases of myocarditis and myocardial infarction are accompanied by hypertrophy of cardiac muscle. The volume of the different muscle fibers increases, proliferation of muscle nuclei are observed, and the stroma may proliferate. There is no increase in the number of muscle fibers. Hypertrophy is a compensation for lack of regeneration; therefore, the tendency of some investigators (Vorontsova and Liozner, 1955, 1957; Liozner, 1962; Sarkisov, 1962, 1963) to identify these phenomena with each other is highly artificial and very erroneous. The unfounded qualitative identification of different phenomena of regeneration and hypertropy leads to denial of the possibility of formulating a general rule of regeneration (Liozner, 1962).

Thus, our brief review of the published data shows that, after various injuries in adult animals and human beings, the myocardium heals by scarification and no regeneration of muscle fibers takes place in the heart.

Data on regeneration of the myocardium

Numerous data are available in published reports showing that the cardiac muscle may regenerate under certain conditions. These phenomena have been described mainly in children and newborn animals.

It has been reported that in some cases of diphtherial myocarditis of children, spindle-shaped cells and myoblasts are formed, and the muscle fibers split longitudinally at the edges of the foci of damage (Heller, 1914; Fraenzel, 1915; Kermenli, 1920; Schamshin, 1920; Warthin, 1924). *

* The second-fourth works are cited according to Mönckeberg (1924), since they were not available to us.

Following piercing of the cardiac muscle in newborn kittens, and burning of the myocardium of newborn rats with a redhot wire, true regeneration of muscle fibers of the heart occurs (Rumyantsev, 1953; Robledo, 1956). The nuclei divide mitotically and amitotically in the muscle stumps, myosymplasts or thin muscle appendages are formed from them, and they coalesce with each other or split longitudinally.

Explanation of myocardium

For a long time researchers could not obtain differentiation of a growing nondifferentiated tissue in tissue cultures outside the body from cells taken from the hearts of chick embryos or mammals (Rumyantsev, 1932; Khlopkin, 1940; Fisher, 1946). Recently, however, it has been shown that under special conditions (in a medium consisting of pure embryonic extract, etc.) secondary differentiation of muscle fibers can be obtained from hearts, from chick embryos, and even human fetuses (Grigor'ev, 1957, 1960; Kochetov, 1961; Agrell, 1965).

Transplantation of sections of the myocardium

Pieces of myocardium from embryos of different age (chicken, rabbit, mouse, cat, human fetus) and from adult animals were transplanted into the anterior chamber of the eye to replace the removed skeletal muscles, and into the peritoneal wall (Törö, 1942; Lavrov, 1948, 1949; Khlopkov, 1948; Chen' Di, 1957; Studitskii, 1954; Ermakova, 1957; Kirpichnikova, 1958). In some cases of transplantation of tissue from young embryos, a muscle syncytium appeared with signs of secondary differentiation. Studitskii (1954) described the formation of myoblasts in experiments on homotransplantation of myocardium in young chicks. In other cases, nondifferentiated muscle syncytium and cells of connective tissue grew out.

Attempts to stimulate regeneration of myocardium

Törö (1937, 1939), on the basis of M. P. Tushnov's (1930) ideas on histolysates, used extracts of embryo hearts to stimulate growth of explants of myocardium, and to stimulate regeneration of damaged cardiac muscle. The extracts stimulated the growth of the explants, lengthened their lives, and enhanced contraction and multiplication of nuclei. A similar phenomenon was observed by Kh. S. Yuldybaev (1946). In myocardial injuries, the extracts stimulated proliferation processes rather than muscle of connective tissue (Mohr, 1952; Nieth, 1949); the muscle fibers of the heart, however, did not regenerate.

Biological stimulation of cardiac activity

Although Törö (1939) could not stimulate reparative regeneration of cardiac muscle, a number of investigators have succeeded in showing that protein-free extracts from cardiac muscle of cattle (Recosen), similar to the cardiolysates of Tushnov (1930), stimulate the activity of isolated hearts of frogs and warm-blooded animals. They are used successfully in clinical practice, mainly in Western Europe (see Polezhaev, Akhabadze, Muzlaeva, and Yavich, 1965). These preparations improve the metabolism of the myocardium, increase the contractile capacity, excitability, and conductability of the cardiac muscle, regulate the rhythm, reduce extrasystole, improve the EKG, prevent the toxic effects of cardiac glycosides and other poisons, dilate the coronary arteries of the heart, and in some cases lower the increased blood pressure. They are used in myocardiopathy, degeneration of the myocardium, postinfectious myocarditis, stenocardia, and myocardial infarctions. Similar preparations were used in the USSR successfully by Rumyantsev (1951, 1953) and Abdulaev (1961).

Our approach to the problem of regeneration of myocardium

We have thus seen that when our studies began (1954 — 1955), published data accumulated, indicating that in various kinds of injuries the myocardium of adult humans and mammals does not regenerate. It can regenerate, to some extent, at early stages of postnatal ontogenesis in the same organism. Consequently, the myocardium's capacity for reparative regeneration is lost during ontogenesis, as is true for reparative regeneration of extremities of tailless Amphibia, the cranial bones in dogs and men, and for a number of other organs and tissues in many animal species. This means that there is reason to try to restore the lost regenerative capacity of cardiac muscle in adult mammals and man as has been done in the study of regeneration of extremities in vertebrates and cranial bones in mammals.

To be satisfied with the idea that after damage of the myocardium, its intact part hypertrophies and that there is some sort of regeneration, would mean to refuse to pose and elaborate the problem in practice. The problem does exist, however, in reality, dictated by the true needs of life, and requires a concrete approach.

We proposed that the loss of regenerative capacity of cardiac muscle, as well as that of the extremities and bones of the cranial vault, is due to a decreased capacity of the main component tissues of mesodermal origin for destruction and dedifferentiation; therefore, these phenomena must be greatly enhanced in order to restore regenerative capacity. This can be realized in various ways. As we found from our experiments on the restoration of lost regenerative capacity of extremities in tailless Amphibia or cranial bones of dogs, the most effective means is direct action on the given organ. Additional traumatization or chemical treatment of the tissues of the amputated wound surface of an extremity in a tadpole or adult frog, which have lost the capacity for regeneration, results in restoration mainly of this extremity, and not of the second one of the pair which was amputated at the same time with the former. However, if such surgical interference

were applied to damaged myocardium, for example, in the case of infarction exposing the chest of a gravely ill person, that would worsen his condition and maybe result in his death. The path of physiological action on a body with a damaged myocardium seems, therefore, to be more appropriate. In the search for a way to do this, the principles of phasicity and organo-specificity are used as guides. To cause regeneration of a damaged cardiac muscle, it is necessary to introduce into the animal body substances which act selectively on this muscle and cause destruction and dedifferentiation in it. Such substances are the decomposition products of myocardium. As pointed out earlier (Part I, Chapter 5), decomposition of tissues and regeneration is caused by products of tissue decomposition (Nasonov, 1941; Polezhaev and Ramenskaya, 1950). According to the principle of organo-specificity, biologically active substances which are the decomposition products of myocardium should act most strongly on the myocardium (Ebert, 1954; Mahler, Walter, Alman, and Bylbenko, 1956, and others). We prepared these substances by enzymatic hydrolysis, and we called this method of affecting the damaged myocardium "the method of chemical organospecific traumatization" (Polezhaev and co-workers, 1958, 1959). It is clear that this method may be used not only with myocardium, but also with any other organ, if the biopreparations are made accordingly from tissues of other organs.

Hydrolysates or lysates of tissues were used successfully by Tushnov (1930, 1938) to stimulate weakened functions. Products of the autolysis of tissues were also used to stimulate healing of wounds in the skin and other tissues (Sel'kov and Vasil'ev, 1935; Sozon-Yaroshevich and Goldberg, 1936, and others). The method of specific tissue therapy, i. e., of treating certain diseases with tissue preparations (transplants, extracts), was used successfully by Rumyantsev (1951, 1953). This method, as well as the tissue therapy method of Filatov (1945), is based in general on enhancement of regenerative capacity. Specific therapy with lyophilized tissues was successfully applied abroad (Niehans, 1948, 1955; Kuhn, 1956).

The organospecific effect of tissues and tissue extracts or homogenates is indicated by biochemical data (Abderhalden, 1922), by physiological and experimental embryological data (Spemann, 1936; Filatov, 1939), and data obtained in the study of regeneration (Korschelt, 1927; Weiss, 1939; Polezhaev, 1945; Tumanishivili, 1958, and others)and immunology (Vyazov, 1962). We also conducted a special study and found that lysates or extracts of tissues in large doses suppress, and in small doses stimulate, the growth of certain organs (heart, liver, brain, spleen, skeletal muscles) of mice and rabbits (Polezhaev, 1960). Biopreparations act in 3 different ways: 1) the same preparation acts on different organs; 2) a preparation acts selectively on an organ identical to that from which it was prepared; for example, a muscle preparation stimulates growth of muscle; 3) a preparation acts not on the organ from which it was prepared, but on another organ physiologically connected with the latter; for example, a liver preparation acts on the brain.

In hydrolysates or protein extracts from tissues, the active principles may be: proteins; macromolecular fragments of a protein molecule, the so-called proteoses, i. e., polypeptides and peptides which are strong stimulators of growth and cell division (Baker and Carrel, 1928); or amino acids which are a good nutrient medium for the culture, and the ratios of which greatly affect the capacity for growth of a given tissue culture (Lockart and Eagle, 1959). The active principles may also be nucleic acids, especially RNA,

the presence of which always characterizes growth and regeneration of tissue (Brachet, 1960; Kedrovskii, 1959), or their decomposition products. All these may have a direct effect on protein biosynthesis. This conclusion is in agreement with data of German investigators (Dyckerhoff and Gaus, 1965), which show that the process of chemical regeneration of different organ tissues in animals and man can be stimulated by organospecific RNA, prepared from the tissues of identical animal organs.

In this connection, we decided to test the effect on the damaged heart of hydrolysates of myocardium and some other preparations, acting on the biosynthesis of proteins and nucleic acids.

This experimental morphological study of the induction of regeneration of myocardium required the use of a complex of methods from the fields of histology, histochemistry, physiology, biochemistry, and surgery, in order to know what changes occur in the morphology, physiology, and chemistry of the heart muscle.

Materials and methods

During the 10 years of work we operated on hearts from more than 2,000 adult, white, mongrel, male rats weighing 300 – 400 g, on 180 rabbits of the "Chinchilla" strain, more than 40 dogs, and 50 cats. The operations were carried out under narcosis in aseptic conditions. The heart was approached medially in rabbits by opening the chest, and in rats, dogs, and cats through the 4th intercostal space, accompanied by tracheotomy and artificial respiration using a DP-1 apparatus. Subsequently, we dissected the pericardium and performed the heart operation, following which the wound was sewn up layer after layer. The myocardium was damaged in different ways: 1) by ligation at the base of the branch of the left descending coronary artery, leading to the formation of an infarct; 2) by burning with a platinum electrothermo cautery; 3) by a strong vacuum using the tip of a vacuum apparatus (residual pressure 5 mm mercury); 4) by electrodiathermal coagulation resulting in conic burn damage to the heart wall, the apex of the cone being directed toward the endocardium and having a diameter and depth of 5 × 4 mm in rats, 12 × 6 mm in rabbits, and 25 × 12 mm in dogs. In the latter 3 methods, we damaged the anterior wall of the left ventricle not far from the apex of the heart in a standard position. The wound was sewn up layer by layer. The animals were killed at different periods from several minutes to one year after the operation. The heart was fixed in Zenker's solution, according to Shabadash in 10% formalin. Paraffin serial cuts, 7 – 8μ thick, were stained with hematoxylin-eosin, iron hemotoxylin, according to Van Giesen, according to Mallory with azan, according to Heidenhain with azure II-eosin. Staining for RNA according to Brachet, for DNA according to Feulgen, and for glycogen according to Shabadash, was also done.

The electrocardiogram (EKG) was taken with an EKP-4 (EMA factory) electrocardiograph, or using the 059 model with photorecording by needle electrodes. In the biochemical investigation, the autolytic activity was determined by the increment of amine nitrogen according to Van Slyke; the intensity of protein synthesis was judged by the incorporation of labeled

amino acids into protein: of cysteine S^{35}, glycine C^{14}, and tyrosine C^{14}; the number of sulfhydryl groups was determined by amperometric titration; the RNA and DNA content of the tissues was determined by the Schmidt and Tannhauser method (1945), RNA was determined by the Meibaum method (1945), and DNA according to Webb and Levy (1955). In order to prepare hydrolysates, we isolated cathepsins from the spleen of cattle (Fruton and Bergman, 1939). The details of the methods used are given in our monograph (Polezhaev, Akhabadze, Muzlaeva, and Yavich, 1965).

Various ways of damaging the myocardium

First we must elaborate the method and elucidate the significance of the nature of damage for the healing of the cardiac muscle.

In an experiment in which the myocardium of an adult rat was burned, we found that the tissues in the focus of damage are completely destroyed while the adjacent tissues surrounding them change greatly, are inflamed, edematous, invaded by polynuclears, lymphocytes, and polyblasts, residues of necrotized tissue being destroyed by macrophages. Subsequently, young granulation tissue develops, rich in vessels, which is transformed into a dense scar. In the muscle stumps the nuclei divide amitotically. The cells of connective tissue divide mitotically and no regeneration of muscle fibers occurs in the heart. The healing process of the myocardium is rapid, terminating within 10—20 days after the operation.

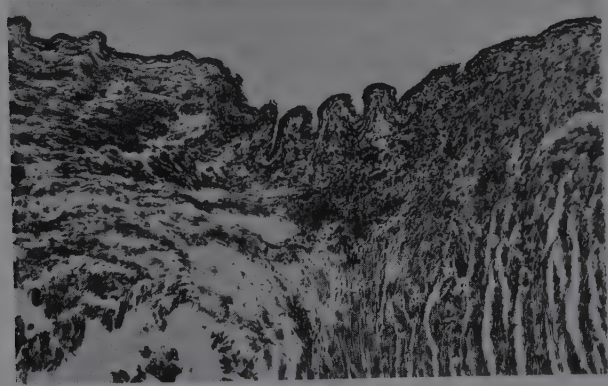


FIGURE 47. Wrinkled scar 6 days after cardiac muscle of a rat was subjected to burn, 7 × 8

As a result the region of the defect forms a depression, the bottom of which is covered by a wrinkled scar (Figure 47).

The experiment with ligation of the left descending coronary artery was carried out on adult rats and kittens aged 2—5 weeks. In both species of animals there were infarctions in the myocardium in 100% of cases. They healed more rapidly in the kittens than in the rats, and the area of the infarctions was strongly contracted; this could have given the impression

of regeneration of myocardium, which in reality did not occur. Although the site of application of the ligature was the same at the base of the left descending coronary artery, the dimensions of the infarcted zone varied. As a rule, it was very extensive and encompassed the anterior and posterior walls of the left ventricle. The infarcts were hemorrhagic (Figure 48), later paled, and the epicardium was covered with a whitish scar tissue. At the later stages the heart was flabby, and the walls of the left ventricle were thin. After operation phenomena of ischemia and hemorrhage appeared in the infarcted region. The muscle stained irregularly; some areas were very pale, while others were hyperchromic. The blood vessels were greatly dilated and overfilled with blood. Later the muscle fibers degenerated and dissolved; their nuclei distended, became pale and disappeared, and pyknosis of nuclei were also observed; the fibers were frayed, distended, and lost their striation; the myofibrils became fragmented and disappeared. The cells of connective tissue were more resistant and remained longer than the muscle fibers, and stained well; 36 — 72 hours after the operation, an intense infiltration of polynuclears appeared, they decomposed, and a mass of lymphocytes appeared, followed by polyblasts. Some of the muscle nuclei, as well as the connective tissue cells, remained intact and participated in the development of connective-tissue scar. A similar phenomenon was described during the healing of myocardial infarction in man (Smith, 1904 — 1905; Barrie and Urback, 1957). The connective tissue begins to grow as early as 3 days after the operation, and replaces the necrotized tissues from the edge and the surface. After 10 days these tissues are fully replaced by connective tissue and a dense network of blood vessels. Subsequently, the connective tissue is transformed into a solid scar which, at a later stage of the experiment (after 200 days), becomes hyalinized. The stumps of the muscle fibers are not destroyed or dedifferentiated, but are overgrown with connective tissue and do not regenerate.

FIGURE 48. Hemorrhagic myocardial infarction in a rat, 3 days after ligation at base of the left descending branch of the coronary artery

In experiments with damage by strong vacuum to cardiac muscle in adult rats, there was a very rapid destruction of muscle fibers and numerous, quite viable nuclei, surrounded with cytoplasm (Figure 49a), remained in the empty sarcolemmal cases. It is interesting that these destroyed muscle fibers stain with picrofuchsin, according to Van Giesen, as connective tissue. The extensive focus of damage is filled with a mass of spilled blood. Phenomena of inflammation, lysis, and phagocytosis of the necrotized muscles are similar to those just described. The muscle fiber stumps do not regenerate, and myoblasts are not formed. In the focus of damage a very thin scarified wall of the heart can be seen (Figure 49b).

Thus, the results of these experiments on damage of cardiac muscle in rats and kittens by burn, ligature of the branch of the coronary artery, and by the action of strong vacuum were the same and in full agreement with previously published data: the muscle fibers of the heart did not regenerate and the focus of damage scarified.

Different results were obtained in our experiments with diathermal coagulation of a sector of myocardium in adult rats. In this experiment,

the focus of damage was small, much smaller than the area of infarction in the above described experiments; tissues were not destroyed in the focus, as in the previous case with burning, but remained in the form of a conical plaque.

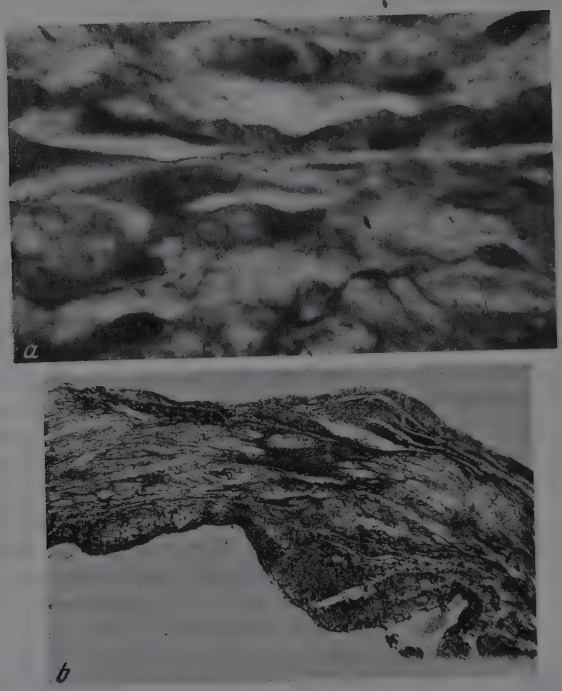


FIGURE 49. Rat cardiac muscle damaged by strong vacuum:

a – nuclei with cytoplasm which appeared in the muscle fibers 5 days after operation, 10 × 60; b – thin cardiac muscle 11 days after operation.

The necrotized tissues were infiltrated with erythrocytes (hemorrhagic inflammation), polynuclears and lymphocytes, and were filled with a serous exudate, and later polyblasts appeared. On polynuclear decomposition some of the cells died, but segments of nuclei of the other cells were transformed into dense chromatin balls and grains which gradually increased in volume, became less dense and surrounded by cytoplasm, and transformed into cells of the lymphocyte and polyblast type (Figure 50). A similar change has been described in the literature (Makarov, 1957, 1961, 1962, 1963; Esslova, 1953; Ermolova, 1956; Nowak, 1962, and others). We have observed this phenomenon in various experiments on cardiac muscle, extremities of various vertebrates, and other cases. We assume, therefore, that Makarov (1963) was right when he reached the conclusion that the time is now ripe for a revision of the problem of the potencies of segmented leukocytes, and possibly the idea of Mechnikov (1883, 1892) may be right, that these cells in the focus of damage may be transformed into connective-tissue cells.

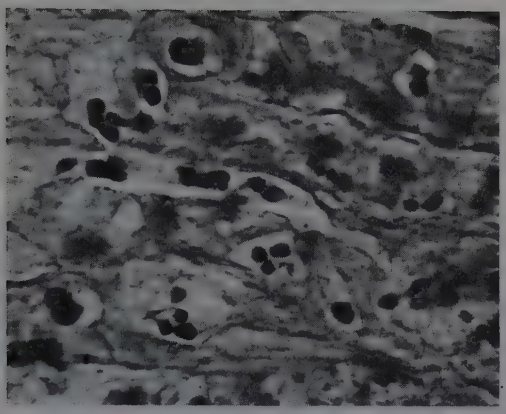


FIGURE 50. Decomposition of polynuclears into segments and formation from the segments of round chromatin grains in the focus of damage in the myocardium of a rat, 4 days after damage, 10 × 90

In 3 — 5 days after the operation, growth of connective tissue begins from the edges toward the center of the focus of damage, replacing the necrotized muscles. Thirteen days after the operation the lysis of the muscles is completed, and the focus is filled with granulation tissue in which, from the 10th day on, collagen fibers begin to develop, and gradually a connective-tissue scar matures and becomes particularly dense at the edges of the old muscles.

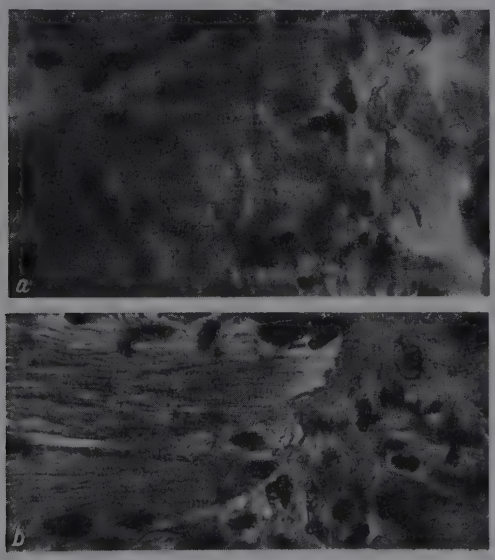


FIGURE 51. Stumps of muscles in the heart of a rat after diathermal coagulation of a segment of the heart:

a — 5 days after operation; b — 10 days after operation. 8×100.

The stumps of the muscles in the border zone are slightly impaired, not dedifferentiated, do not form myoblasts or muscle bundles, and are overgrown with connective tissue and do not regenerate (Figure 51a, b). In this respect our data agree completely with those of Harrison (1947) who did not observe regeneration of muscle fibers, following diathermal coagulation damage of myocardium in adult rabbits.

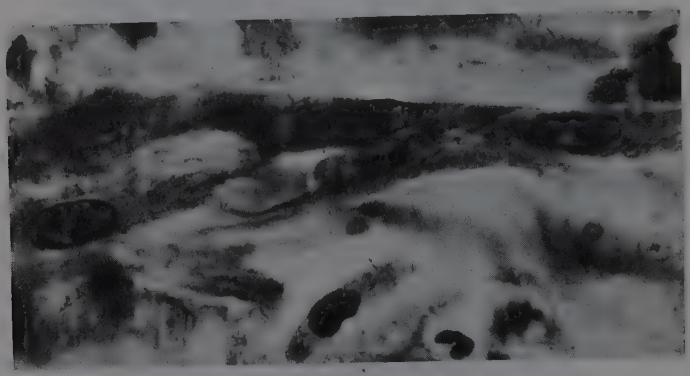
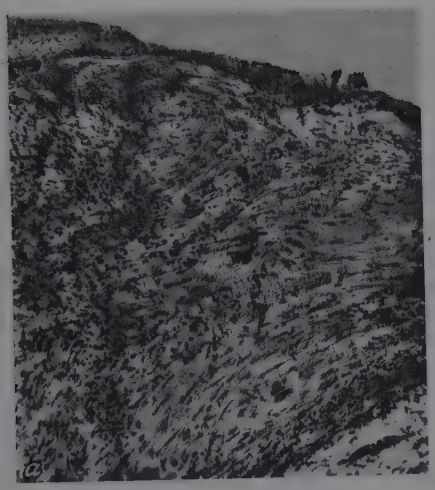


FIGURE 52. Chains of myoblasts in the center of the focus of damage in the cardiac muscle of a rat, 7 days after operation, 10 × 90

In addition, however, to the lack of regeneration of muscle fibers on the stumps, we observed another phenomenon which has never before been described, namely, de novo formation of small islands of muscle in the center of the focus of damage, independent of the muscle stumps in the border zone. As early as 7 days after the operation, together with the destroyed coagulated muscle fibers, myoblasts and chains of myoblasts appeared in the center of the focus in places where there were very few cells (Figure 52). Myoblasts are cells with large, light, bladderlike nuclei with a distinctly defined nuclear membrane, 1–2–3 large nucleoli, and a very basophilic cytoplasm forming a thick appendage which usually grows on one side of the nucleus. The myoblasts form a syncytium. Together with myoblasts numerous polyblasts are found, some of which become transitional forms to myoblasts. It may be assumed, therefore, that these myoblasts arise from polyblasts under the influence of tissue-specific decomposition products formed from decomposing coagulated muscles, similar to what was observed by Levander (1956) in his experiments on the implantation of dead skeletal muscles under the lateral skin of a rabbit. On the other hand, the possibility cannot be excluded that the myoblasts are myogenic cells released from the muscle stumps and transformed into muscle fibers under the influence of tissue-specific decomposition products of muscles; in the absence of the latter, they could have transformed only into myocytes.

Twenty days after the operation, the myoblasts are transformed into islets of muscle fibers located in a thicket of connective-tissue scar (Figure 53a). These fibers have elongated, amitotically dividing nuclei and a well-developed cytoplasm; they form syncytia, but are not striated (Figure 53b). In 45 days after the operation, these newly formed islets of cardiac muscle decompose, disappear, and are finally replaced by a scar.



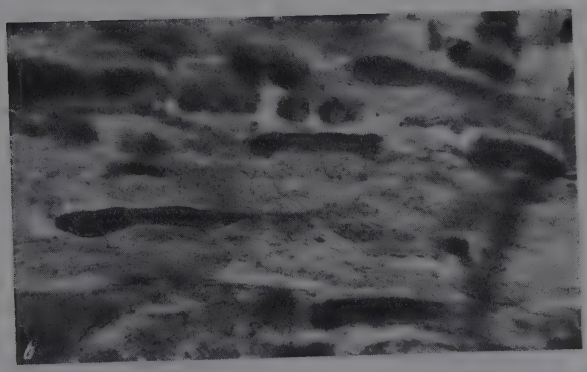


FIGURE 53. De novo formation of undifferentiated muscle fibers 20 days after coagulation of the myocardium of a rat:

a – × 35; b – 10 × 8.

In diathermal coagulation of the wall of the heart in rabbits and dogs, similarly as in rats, the stumps of the muscles did not regenerate, nor was there de novo formation of muscles in the center of the focus of damage, independent of the muscle stumps in the border zone.

The most convenient method of inflicting damage to the myocardium, in order to study its regeneration, was electrodiathermal coagulation. This allowed the researcher to obtain a focus of damage of a standard small dimension in the same place each time (and this is very important, as the directions of the muscle fibers are very different in the different sectors of the cardiac muscle), and to observe de novo formation of small islets of muscles even in the control. Subsequently, therefore, almost all the studies were carried out using this method in experiments on old male rats, weighing 300 – 400 g, and on adult "Chinchilla" rabbits weighing 2.5 – 3.0 kg.

Thus we could draw the following conclusions. Burning, strong vacuum, and ligation of the branches of the coronary arteries cause damage in the

myocardium which leads to scar formation in the foci of damage. Diathermal coagulation causes de novo formation of muscles in the center of the focus, independent of the muscle stumps in the border zone. Consequently, the resultant regeneration or scar formation depends on the nature of the damage to the myocardium, which means that there is a basis for the enhancement of the regeneration process of muscle fibers in the heart. The stumps of muscles in the focus of damage are not dedifferentiated, but are overgrown with a connective-tissue scar and do not regenerate.

Stimulation of regeneration of the cardiac muscle

In order to enhance destruction and dedifferentiation, and to cause regeneration of the damaged tissues of the cardiac muscle, we used decomposition products of myocardium and enzymatic hydrolysate. The latter contained traces of protein, a small concentration of peptides and polypeptides, and a great amount of amino acids, the composition of which was determined by paper chromatography (Mant'eva, 1959). We also determined nitrogen: total, protein, nonprotein, and amino nitrogen, and we performed protein analysis by electrophoresis. For comparative purposes, we tested the effect of protein extracts from cardiac and skeletal muscles which contained a great amount of native protein, polypeptides, peptides, and a smaller quantity of amino acids than did the hydrolysate. Further, we used Vitamin B_{12}; thyroidin; the antiseptic stimulant of Dorogov (ASD)—its third fraction; freshly prepared, highly polymerized ribonucleic acid (RNA), and dibasol. Vitamin B_{12} actively participates in metabolism and restorative processes, and stimulates regeneration of skeletal muscles in mammals (Litver, 1955), the peripheral nerve of rats (Znachkova, 1956), and the extremities of tailless Amphibia (Petrov and Taranets, 1957). Thyroidin enhances basic metabolism and oxidative processes, increases the rate of differentiation of tissues, and stimulates regeneration of skeletal muscles in mammals (Voitkevich, 1947; Belkin, 1947). RNA plays an important role in protein synthesis, participates in processes of regeneration, leads to restoration of the regenerative capacity of extremities in axolotl, following their suppression by X-ray irradiation (Polezhaev and co-workers, 1961—1963). ASD stimulates the regeneration of tissues in mammals (Braun, 1952; Tezekbaev, 1959; Khvorostukhin, 1961, 1962). Dibasol stimulates regeneration of myocardium in dogs (Sinitsyn, 1962).

These preparations in certain doses, mainly in the form of subcutaneous injections, were used immediately after the myocardium was damaged. The methods for the use and preparation of some of them have been described by us in detail in special papers and monographs (Polezhaev and co-workers, 1965).

In the experiment with hydrolysate, the result obtained was positive and quite clear, although not that which we expected. The hydrolysate strongly stimulated destruction and dedifferentiation of tissues in the focus of damage, and also the de novo formation of muscles inside the focus of damage, independent of the stumps of muscles in the border zone. A similar but weaker effect was also obtained with other stimulants, except for RNA and dibasol which act differently.

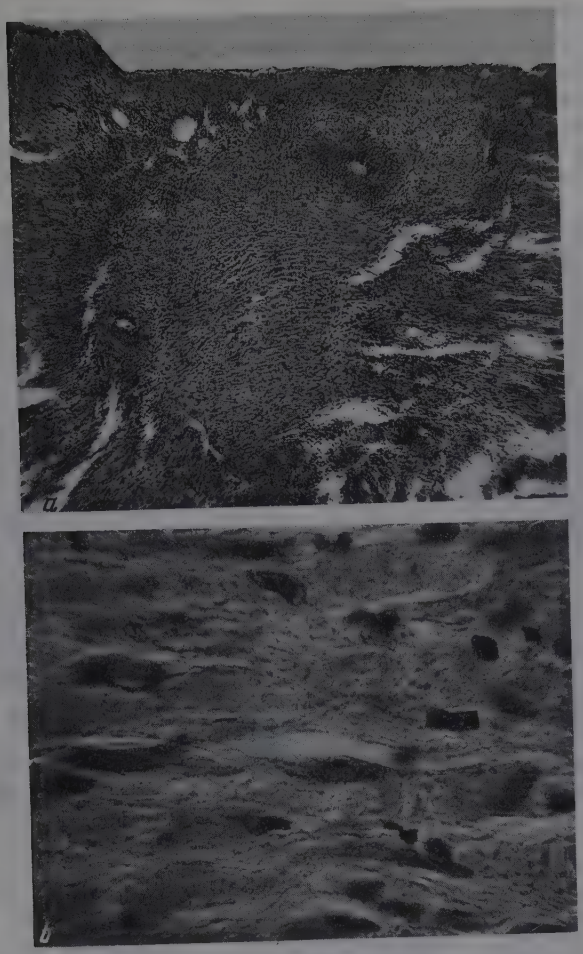


FIGURE 54. De novo formation of muscles in the focus of damage in the myocardium 13 days after coagulation; experiment with hydrolysate:

a – general appearance of the focus of damage, × 40; b – newly formed muscle fibers, 8 × 100.

The hydrolysate causes an intense reddening and increase in volume of the cardiac muscle. Large sectors of hemorrhage appear in the focus, the tissues become infiltrated with erythrocytes and leukocytes. The vessels distend. Lysis of the necrotized tissues is enhanced 2.5-fold; the focus of damage was clean of necrotized tissues and filled with granulation after 5 days, as compared with 13 days in the control. The process of de novo formation of myoblasts is greatly enhanced, and after 13 days almost the entire focus is filled with newly formed, histologically nondifferentiated, muscle fibers (Figure 54a, b). The latter, as in the control, are long, thick, succulent and well-developed, have a syncytial structure and amitotically dividing nuclei, but cross sections are either lacking or are poorly developed.

They are separated from the muscle stumps in the border zone by a dense connective-tissue scar; they cannot function, and they decompose after 2 months and are replaced by a dense collagenous scar. The reason for their decomposition is the pressure of the scar, as we determined in special experiments designed for this purpose.

The stumps of the muscles in the border zone dedifferentiate; they lose their cross striation and the myofibrils, their sarcoplasm becomes light with fine granules, they form club-shaped dilations, and basophilic muscle bundles with nuclei split off from them. In addition numerous myoblasts are formed (Figure 55). However, no proliferation of muscle fibers from the dedifferentiated stumps occurs, as this is prevented by the scar, the maturation and solidification of which is enhanced under the influence of the hydrolysate.

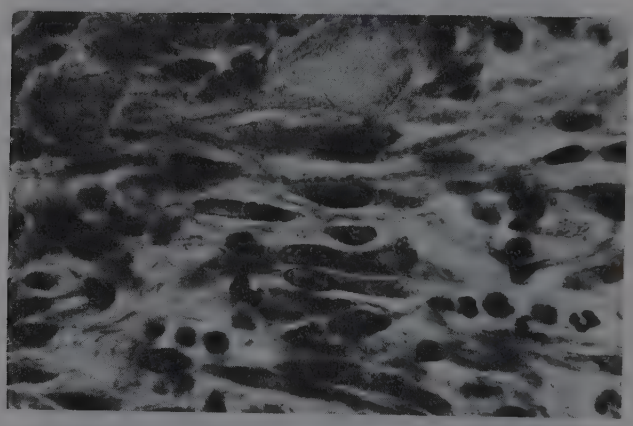


FIGURE 55. Dedifferentiation of a stump of muscle and formation of a muscle bundle from the stump, and numerous myoblasts in the granulation tissue; experiment with hydrolysate, 5 days after operation. 8 × 100

A similar, but less distinct, picture was observed in experiments with extracts of cardiac and skeletal muscles and vitamin B_{12}. ASD did not give positive results in our experiments. Thyroidin enhanced lysis of the necrotized muscles and de novo formation of muscles inside the focus of damage, and even caused some growth and differentiation of muscle fibers from strongly dedifferentiated stumps of muscles (Figure 56), but the latter phenomenon was very limited since there was simultaneously a rapid maturation and increase in density of the connective-tissue scar.

RNA caused a stimulation of mitotic division of the cells of connective tissue and of amitotic division of the nuclei in the stumps of muscles, dedifferentiation of the latter, and intensive formation of myoblasts and outgrowths of muscle from the stumps extending into the focus. A similar picture was observed of the action of dibasol. Both preparations suppressed the process of de novo formation of muscles in the center of the focus of damage, independent of the stumps of muscles, but stimulated regeneration of muscle fibers on the stumps of muscles in the border zone (Figure 57). This process is not very intense, however, and occurs only in those places where the connective-tissue scar is less dense. Both RNA and dibasol facilitate

leukocyte infiltration of the focus. Dibasol, in contrast to RNA, leads to some rarefication of the collagen fibers, and thus facilitates the growth of regenerating muscle fibers into the granulation and scar tissues. These fibers may normally differentiate, and can be preserved and function for a long time.

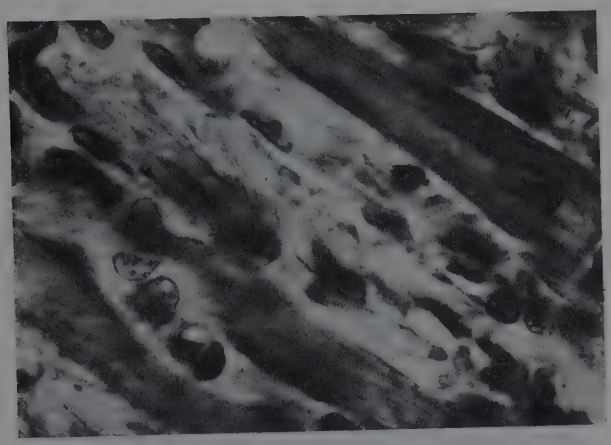


FIGURE 56. Regeneration of dedifferentiated stumps of muscle fibers of of the heart 11 days after operation; experiment with thyroidin, 10 × 90

FIGURE 57. Stimulation of regeneration of stumps of muscles in the heart of a rat, 17 days after operation; experiment with dibasol, 10 × 60

In experiments with rabbits, the hydrolysate also stimulated de novo formation of muscles in the center of the focus of damage in the heart, although less so than in the case of rats. In experiments on dogs we succeeded in causing regeneration of cardiac muscle, using the method of N. P. Sinitsyn (1962) which will be discussed further on.

In our studies of the effect of different preparations on the regeneration of myocardium, we also tested the effect of mixtures, e. g., hydrolysate + thyroidin, RNA + hydrolysate, RNA + hydrolysate + thyroidin, hydrolysate + vitamin B_{12} + dibasol, etc. Some of these complexes were more, while others

were less effective. The last complex was quite effective in experiments with rats. In this case there was a very strong dedifferentiation of muscle fibers in the border zone (Figure 58a), formation of myoblasts in the stumps of muscles (Figure 58b, c), and simultaneous de novo formation of islets of differentiated muscles in the center of the focus (Figure 59a, b) and their union with muscle fibers regenerating in the border zone. The major part, however, of the focus of damage was still filled with a connective-tissue scar.

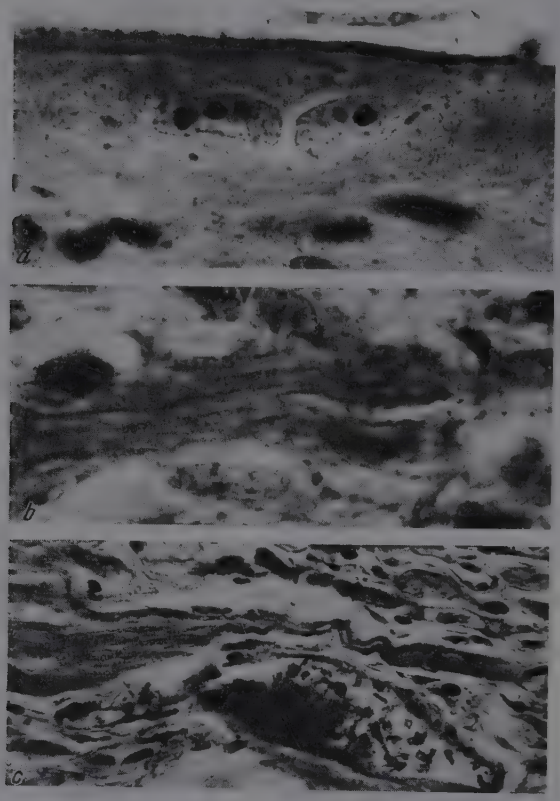


FIGURE 58. Stimulation of regeneration of cardiac muscle in a rat in an experiment in which a complex of myocardial hydrolysate + vitamin B_{12} + dibasol was used:

a – dedifferentiation of muscle fibers in the border zone, 7 days after operation, 10 × 100; b – formation of myoblasts on the stumps of muscles, 5 days after operation, 10 × 60; c – regeneration of muscle fibers from their stumps, 30 days after operation. 10 × 60.

Thus, the preparations we studied have a 2-fold effect: some of them (hydrolysate, vitamin B_{12}, and others) stimulate only de novo formation of muscles inside the focus of damage, independent of the stumps of muscles

in the border zone, while others (RNA, dibasol) stimulate only the regeneration of muscles on their stumps in the border zone. In either event regeneration is interfered with by the development of a dense, connective-tissue scar. Thus, it turned out that it is not enough to enhance the destruction and dedifferentiation of cardiac muscle if its regeneration is wanted, but the growth and development of the connective tissue must also be changed.

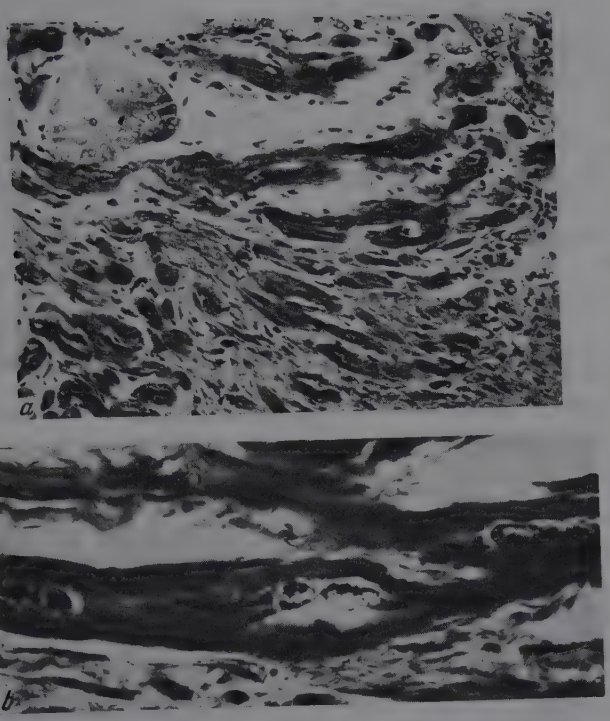


FIGURE 59. Stimulation of regeneration of cardiac muscle in rats, using the same complex of preparations as in Figure 58; de novo formation of muscle masses in the center of the focus of damage, 21 days after operation:

a – 7 × 20; b – 10 × 60.

Inhibition of scarification

In order to change the development of connective tissue, and to secure the growth of the regenerating muscle fibers through the softened scar in the focus of damage of the myocardium, we used inhibitors of scarification. Hyaluronidase is an active inhibitor. Umanskii and Samarova (1953) prepared this enzyme from bovine testes and used it to inhibit the development of scar which usually appears on the amputation wound surface of a nonregenerating extremity of a lizard. B. S. Kasavina and co-authors (1959) improved the techniques for the preparation of hyaluronidase and prepared

ronidase and lidase, which are capable of resorbing scars in skin wounds or at sites of transection of the spinal cord in rats (Matinyan and Andreasyan, 1961). Hyaluronidase acts only locally, however, and when introduced into the bloodstream by injection, it decomposes rapidly and loses its activity. We decided, therefore, to use other preparations: pyrogenal, a preparation of spleen, trypsin, leukocyte serum, and a preparation of vitreous body. Pyrogenal is a bacterial lypopolysacharide analagous to the American preparation piromen, which was successfully used by American investigators, in order to change the development of a glial scar appearing upon cutting the spinal column in rats and dogs (Windle, 1959). Pyrogenal acts similarly to piromen and facilitates regeneration of nerve fibers in the severed spinal cord (Nesmeyanova, Brazovskaya, and Iordanskaya, 1960, 1961, 1962). A similar effect was shown by trypsin in similar experiments (Turbes and Freeman, 1953; Freeman, Dougall, Turbes, and Bowman, 1960). Transplantation of pieces of spleen, or injection of a preparation made of this organ, act on phenomena of inflammation and scarification, and in a number of cases, successfully heal blindness, deafness, and sterility, resulting from an inflammatory injury to the optic or auditory nerves and obstruction of the fallopian tubes (Rumyantsev, 1951, 1953). It is possible that splenin, a protein-free extract of the spleen, also has a similar effect (Komissarenko, 1960). The leukocyte serum of G. K. Khrushchov (1945) is a horse serum enriched with metabolic products of leukocytes which facilitate cell division and growth of tissue cultures (Fischer, 1946). This preparation acts on connective tissue and stimulates healing of wounds. The vitreous body preparation removes ordinary and keloid scars acting on connective tissue (Mikhel'son, 1947).

We used all these preparations in the form of injections by a method which we have already described in detail (Polezhaev and co-workers, 1965). The preparations of vitreous body and leukocyte serum were not effective in our experiments. Pyrogenal and a spleen preparation produced positive results in experiments on rats, while trypsin was active in experiments on rabbits. There is something common and something different in the action of the different inhibitors of scarification.

All the aforementioned preparations change the development of connective tissue and granulation in such a manner that the regenerating muscle fibers grow from the stumps of muscles in the border zone in certain places, mainly in the periphery of the focus of damage and along the large vessels. These fibers grow through the connective tissue barrier, differentiate, remain for a long time (during the entire course of the experiment, i. e., up to one year) in the region of damage in the form of bridges retaining their function. The volume of the regenerating muscles is not large.

Pyrogenal has some specific effects on tissues in the focus of damage. First, it greatly reduces the infiltration of the tissues by leukocytic cellular elements; there are very few polynuclears and lymphocytes in the focus. Evidently, it reduces the permeability of the walls of the blood vessels. There is a serous fluid and a mass of erythrocytes in the focus. Second, the development of connective tissue is altered.

There are fewer polyblasts, histocytes, and fibroblasts in the focus of damage than in the control. Lysis of coagulated tissues is carried out mainly by fibrocytosis. Collagen fibers are formed early in the focus; they are thick and not as dense and closely interwoven as usual. In some

places, especially under the epicardium and in the center of the focus, they are are loose with spaces between them along which, as through canals, regenerating muscle fibers may grow (Figure 60). The scar is not as dense and does not press the stumps of the muscles in the border zone as strongly as in the control, and is not peeled off from the muscles of the border zone.

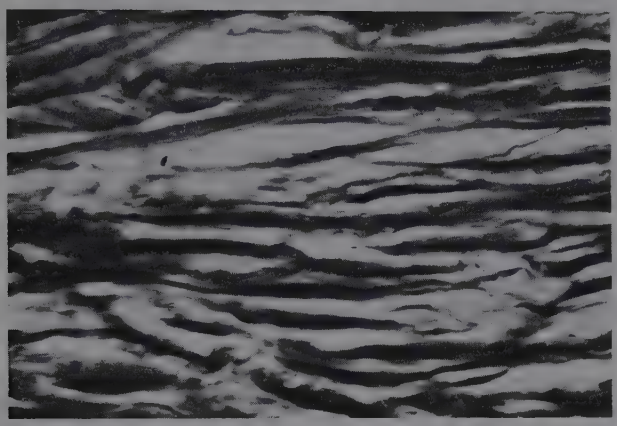


FIGURE 60. Collagen fibers and lumina between them in the focus of damage of the cardiac muscle of a rat; experiment with pyrogenal, 90 days after operation. 10 × 60.

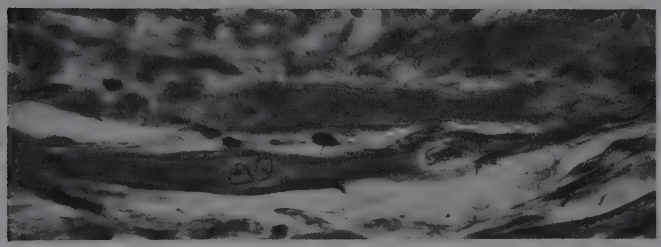


FIGURE 61. Muscle regenerates on muscle stumps proliferating through the scar. Amitotically divided nuclei are seen in the regenerate; experiment with pyrogenal, 10 days after operation. 10 × 60.

Stumps of muscles are dedifferentiated 3—5 days after the operation, and on the 10th—13th day, long, narrow appendages grow out from them; in some places they grow into the slits between the collagen fibers and the granulation tissue (Figure 61). In these outgrowths the nuclei divide amitotically. Later, some outgrowths grow further and differentiate (Figure 62). The regeneration of muscle fibers occurs relatively late (30—60 days after the operation) at the periphery of the focus of damage where narrow strips and bridges of regenerating muscle fibers may appear. The latter are fully differentiated: they have nuclei, myofibrils, cross striations, intercalated discs,

and waves of contraction, a fact which indicates that they are functional. At later stages of the experiment, the regenerated muscle fibers frequently died under the pressure of the dense scars but were partially preserved.

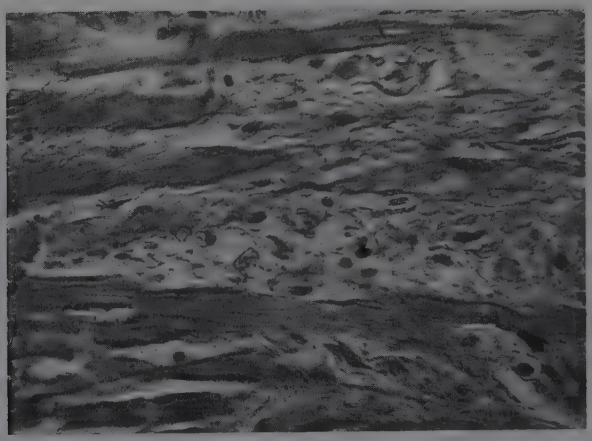


FIGURE 62. Muscle fibers proliferating through the scar in the focus of damage; experiment with pyrogenal, 13 days after operation. 10 × 60.

In experiments with a spleen preparation, the development of connective tissue, healing, and regeneration of the myocardium were similar to those in the experiments with pyrogenal, but there were also some differences. The invasion of the focus by polynuclears and lymphocytes was stronger, collagen fibers formed a denser interweaving pattern than in the experiments with pyrogenal, and de novo formation of muscle fibers in the center of the focus was not as strongly suppressed as in the experiment with pyrogenal.

A similar picture is observed in experiments with rats. In rabbits the action of these preparations is weaker and the result poorer. On the other hand, the effect of trypsin was considerably stronger in experiments with rabbits than in those with rats. In rabbits we observed regeneration of narrow muscle fibers in the scar tissue on the bottom of the wound. In the experiments with dogs we tested only spleen preparations and the result was negative; only a dense scar developed in the large focus of damage (diameter of 25—30 mm). The data obtained forces us to take into account the importance of species specificity in the reaction of animals to the different biopreparations.

Combination of stimulators of myocardial regeneration and inhibitors of scar formation

We have seen that stimulators of the regeneration of myocardium considerably enhance the volume of de novo formation of nondifferentiated muscles in the center of the focus of damage, or cause some regeneration of muscle fibers from the stumps of muscles in the border zone, but they

do not prevent the development of a dense scar under the pressure of which these regenerated muscle fibers decompose. Inhibitors of scar formation change its nature and allow stable regeneration of differentiated muscle fibers from some of the stumps of the muscles in the border zone, but the volume of the latter is very small and, as a rule, de novo formation of muscles in the center of the focus of damage is suppressed. In this connection, we attempted to use combinations of stimulators and inhibitors to increase the volume of regeneration and to obtain differentiation and prolong the maintenance of the regenerated muscle fibers. We tested the effect of a number of combinations in experiments on rats and rabbits: hydrolysate of myocardium + pyrogenal; hydrolysate + spleen preparation; hydrolysate + trypsin: vitamin B_{12} + pyrogenal; RNA + pyrogenal; RNA + spleen preparation; hydrolysate + dibasol + vitamin B_{12} + spleen preparation, and a number of other mixtures. The different combinations proved to have different activities, and affected rats and rabbits differently. In experiments with rats the combination hydrolysate + pyrogenal was active, while in experiments on rabbits it was weakly active, but these animals were greatly affected by hydrolysate + trypsin, although the effect differed only slightly from that of trypsin alone. In a number of cases stimulators and inhibitors, e. g., RNA and pyrogenal, were quite active when tested separately, but when used in combined form were mutually neutralizing. RNA facilitates migration of leukocytes into the focus of damage and cell division in the connective tissue, while pyrogenal considerably reduces the migration of leukocytes, depleting connective tissue of cellular elements and arresting its development. RNA stimulates the formation of myoblasts from the stumps of muscle fibers, while pyrogenal stimulates the formation of appendages from the stumps. It would seem that together the 2 preparations should enhance regeneration of muscle fibers, but in our experiments this did not occur.

In other cases, however, combinations of stimulators and inhibitors were more active than their separate ingredients, and caused regeneration of muscle fibers of the heart to be better, to be of a greater volume, and to occur in a more stable manner than did the stimulators or inhibitors alone.

In experiments using a combination of hydrolysate + pyrogenal with rats, there was rapid filling of the focus of damage in the myocardium with newly formed nondifferentiated muscle fibers, 10—13 days after the operation, but there also appeared a quite dense scar at the edge of the focus of damage. Later, on the other hand, 30 days after the operation, regeneration of muscle fibers from their stumps in the border zone occurred (Figure 63). Strips, and sometimes bridges, of differentiated regenerating muscle fibers appeared in the periphery of the focus (Figure 64a, b), and individual muscle fibers could penetrate deeply into the focus (Figure 65a, b). The scar, however, interfered with the desirable coalescing of the newly formed fibers in the center of the focus and those regenerating from the stumps of muscle fibers. As a result the newly formed muscles were preserved longer than in the control, or in the experiment in which hydrolysate alone was used (instead of 45—60 days, 90—120). Nevertheless, they decomposed and were replaced by connective-tissue scar. Only differentiated muscle fibers were preserved, these regenerating at the periphery of the focus from stumps of muscles in the border zone.

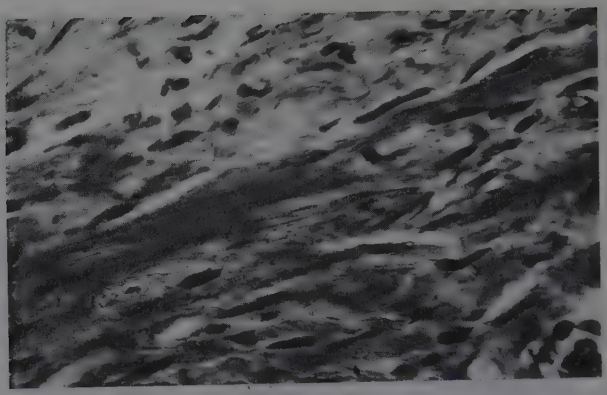


FIGURE 63. Muscle fibers proliferating through the focus of damage in the myocardium of a rat. Experiment with myocardial hydrolysate + pyrogenal, 21 days after operation. 40 × 60.

FIGURE 64. A muscle bridge in a focus of damage in a rat heart. Experiment: myocardial hydrolysate + pyrogenal, 158 days after operation:

a – general appearance of the focus of damage, 7 × 8;
b – individual regenerated muscle fibers. 10 × 60.

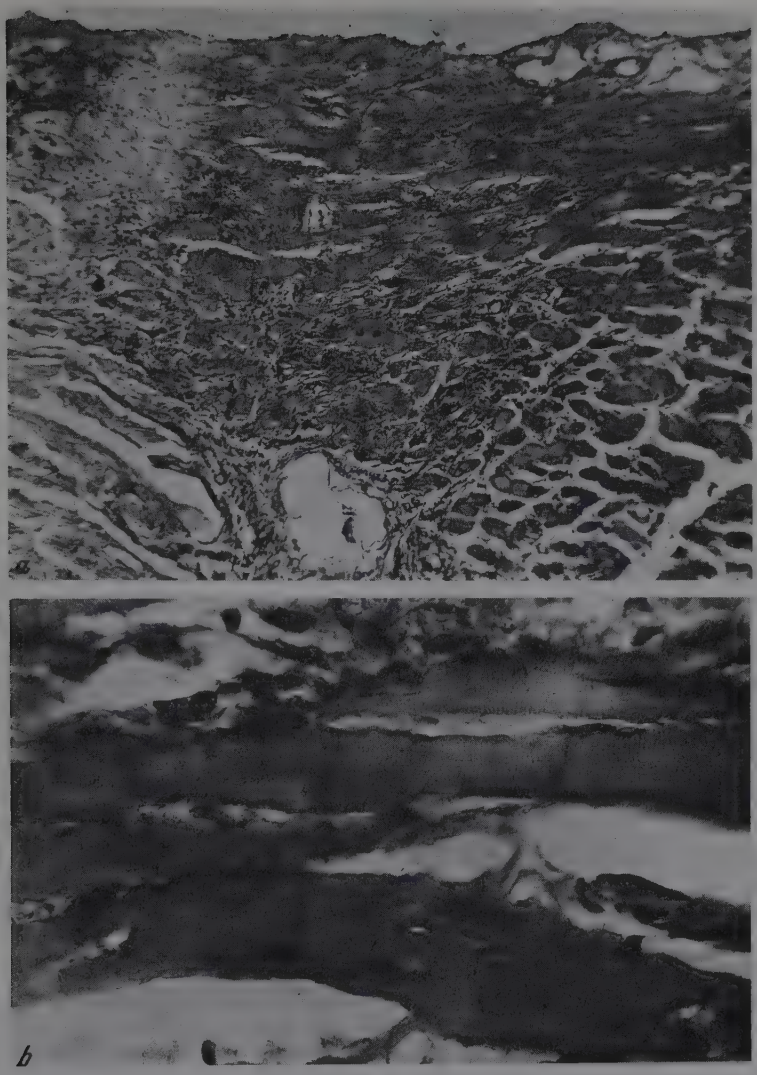


FIGURE 65. Bridge of regenerated muscles in the heart of a rat, 324 days after operation; myocardial hydrolysate + spleen preparation:

a – general appearance of the focus of damage, 10 × 8; b – regenerated muscle fibers. 10 × 60

In experiments with rats, using a combination of vitamin B_{12} + pyrogenal, the process of regeneration of the myocardium was similar to the one just described, but some of the newly formed muscles in the center of the focus could combine with muscle fibers in the border zone and differentiate.

In experiments in which a combination of hydrolysate + spleen preparation was used, there was also de novo formation of an islet of nondifferentiated

muscles in the center of the region of the defect, and partial coalescence of these fibers with the stumps of the muscles in the border zone.

We are now conducting experiments designed to discover the best stimulants of regeneration of myocardium, inhibitors of scarification, and complexes of the 2. There is still much to do, as the results so far obtained have not solved the problem. It is very important to find the strongest stimularots of regeneration of muscle fibers from their stumps in the border zone, which will also simultaneously inhibit scarification. At present only one such preparation is known, dibasol, but its effect is weak and entirely insufficient. Muscle fibers regenerating from stumps of muscles in the border zone are differentiated; they function and are preserved better and longer than nondifferentiated muscles arising in the center of the focus of damage independently of the stumps of muscles in the border zone.

Regeneration of heart muscle by induction

We have established the 2 main routes of regeneration of cardiac muscle: 1) outgrowth from the stumps of muscles in the border zone, and 2) de novo formation in the center of the focus of damage among the granulation tissue, independent of the muscle stumps. In the former method of regeneration it is it is quite clear that myoblasts or outgrowths of muscle fibers are formed from the stumps. The second way is not clear; both the source and way in which the myoblasts are formed are not known. We put forward the assumption that in de novo formation of muscles the myoblasts are formed by induction, similar to embryonic induction or regeneration by induction of the bones of the cranial vault, or dental tissues in our earlier experiments. Coagulated muscles releasing some tissue and/or organospecific substance may be the inducers, while the reacting material may be cells in granulations (polyblasts or myogenic elements released from the stumps of the muscles) which are incapable of transformation into myoblasts and muscle fibers without the inductive effect.

In order to check this assumption we designed a special experiment on rabbits. We have already pointed out that the introduction of foreign bodies (steel or gelatin needles) into the myocardium of an adult rabbit, did not lead to regeneration of the damaged muscle fibers, but that scar is formed (Oppel, 1901; Anichkov, 1912). As an inducer, we (Polezhaev, 1962) implanted into the cardiac muscle of the rabbit a piece of skeletal muscle kept for 48 hours in a 1% aqueous solution of trypan blue according to Levander (1956). The implant was killed without coagulation of the tissue protein. The animals were killed at different periods (from 1 — 90 days after the operation) and histological examination of the cardiac muscle was performed.

It was found that the implant was dead; it did not contain intact nuclei, cross striation, or myofibrils. It released trypan blue and greatly damaged the surrounding cardiac muscle which turned into granulation tissue.

Numerous polynuclears, lymphocytes, and polyblasts, and a connective tissue capsule accumulated around the implant. Subsequently, the polyblasts invaded the implant and turning into macrophages, destroyed it. In 3 — 5 days after the operation the polyblasts, which were located around the implant, began

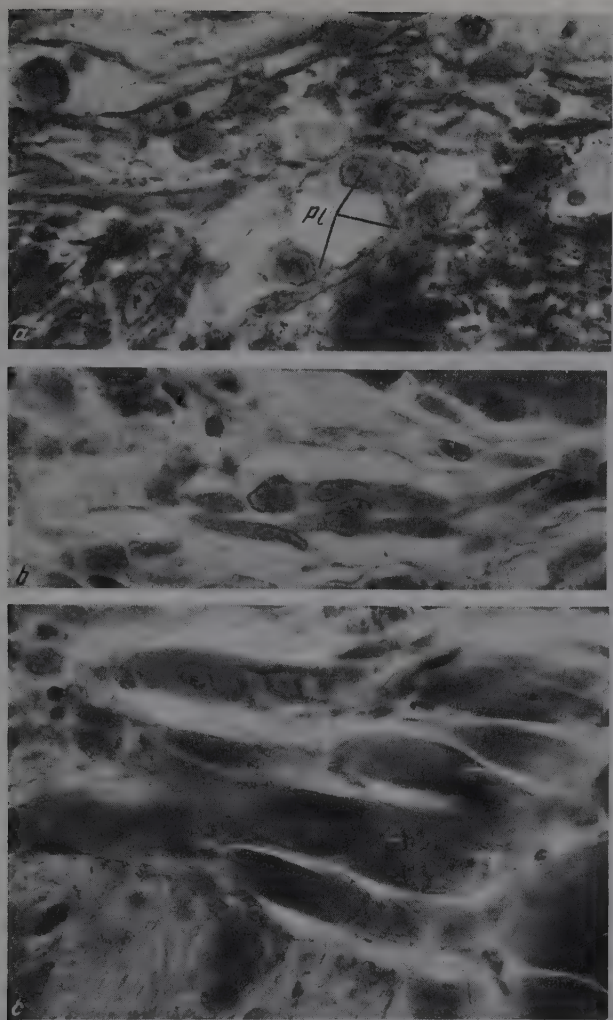


FIGURE 66. Induction of muscle fibers in the heart of a rabbit by implantation of a piece of skeletal muscle treated according to Levander, 10 × 60:

a — polyblasts with basophil appendages (pl), 3 days after operation; b — chains of myoblasts, 5 days after operation; c — muscle syncytium, cytoplasm basophilic, nuclei divide amitotically, 24 days after operation.

to change: the nucleus became lighter, a nucleolus appeared in it, the cytoplasm became basophilic, and an appendage was formed in one direction from the nucleus (Figure 66a). After 7—10 days they transformed into typical myoblasts. The nuclei, which were round, became elongated; the membrane was distinctly outlined; the cytoplasm formed long basophilic appendages. The cells united into chains with a syncytial structure (Figure 66b). Small islets of newly formed muscles appeared in the

vicinity of the decomposing implant after 13—24 days. They consisted of thick, very strongly basophilic muscle fibers interwoven into a syncytium, and contained a great number of embryonic-type, light, bladderlike nuclei which divided very intensely, amitotically, and formed accumulations and chains (Figure 66c). Later, myofibrils and weak cross striations appeared in some of these muscle fibers. Islets of newly formed muscles were surrounded with connective-tissue scar of increasing density; they compressed the latter, atrophied after 25—30 days, and died. Their development and fate were very similar to those observed in de novo formation of islets of muscles in the center of the focus, independent of the stumps of muscles. The cells from which the myoblasts arose, the nondifferentiated or weakly differentiated muscle fibers which were formed from them, and their fate (i. e., decomposition under the pressure of the surrounding dense scar) were also similar.

In this experiment the cardiac muscle regenerated by induction. This makes very probable the assumption that the neoformation of muscles in the center of a focus of damage, independent of the stumps of muscles in the border zone, also occurred by induction. In this experiment the inducers were coagulated muscles. The hydrolysate of the myocardium and some other stimulators of regeneration enhanced the decomposition which, in its turn, increased the intensity of their release of inducing substances, and thus enhanced the volume of induction. Obviously, polyblasts were the reacting material in both cases. We cannot exclude, however, the possible participation of myogenic elements released from the stumps of muscles in the granulation, and incapable of transforming into myoblasts and muscle fibers without an inducer.

Innervation in the regeneration of cardiac muscle

An extensive amount of published data is available on the innervation of the myocardium under normal conditions and in the process of scarification in various injuries: infarctions, myocardites, etc. (Khabarova, 1961; Kolosov, 1961, and others). We point out only the fact that as a result of technical difficulties, the nerve endings in the left ventricle, the damage of which was dealt with in our investigation, have not been thoroughly studied so far. In addition, not a single study has appeared concerning innervation during regeneration of the myocardium.

Being interested in the latter problem, we felt it was important to elucidate whether the development of a dense connective-tissue scar at the edges of the focus of damage did not interfere with the proliferation of nerve trunks and endings into the tissues of the focus. The absence of differentiation of muscle fibers arising in the center of the focus of damage, independent of the stumps of muscles in the border zone, and the subsequent decomposition of these muscles could be explained by disturbance of their normal innervation. From the published data we know very well that in denervation of extremities in Amphibia, their muscles atrophy (Hamburger, 1928, and others). On denervation the normal differentiation of regenerating skeletal muscles does not occur (Ignat'eva, 1954, 1956).

A study was conducted on rats in which the cardiac muscle was damaged by diathermal coagulation, and other experiments were performed in which the

myocardium was damaged and stimulation of regeneration of myocardium was accomplished by the action of a combination of RNA+ hydrolysate of myocardium; the nerve fibers and their endings were detected by impregnation with silver nitrate, according to Kampos and Rasskazova (Gusareva, 1964, 1965). It was found that during the first 5 days after coagulation reactive changes take place in the nerve trunks around the focus of damage , while already on the 7th day thin nerve fibers with growth cones begin to proliferate in the focus (Figure 67a). By 10–13 days after the operation, the entire focus of damage is penetrated by a dense network of nerve fibers and nerve endings (Figure 67b). The latter are preserved during all the subsequent experimental period, i.e., for 90 days. The data obtained are in agreement with those from the published reports and show that the absence of regeneration of muscle fibers from their stumps in the border zone is not due to the absence of nerve fibers or their endings in the focus of damage.

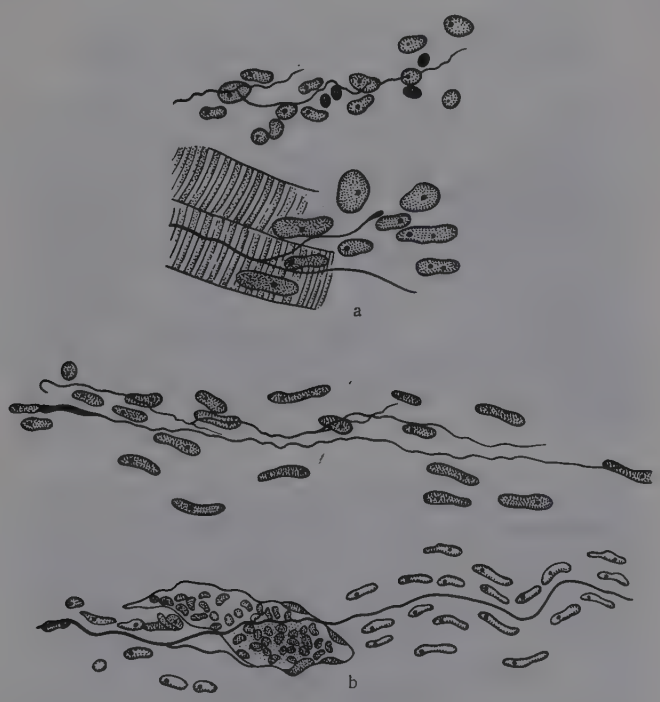


FIGURE 67. Regeneration of nerve fibers in the focus of damage in the heart of a rat:

a – 7 days after operation; b – 60 days after operation (according to Gusareva, 1964).

In the experiment in which regeneration of damaged myocardium was stimulated by a combination of preparations, two important factors have been established: 1) innervation of differentiated muscle fibers regenerating

from stumps of muscles in the border zone (Figure 68a); 2) innervation of the nondifferentiated newly formed muscle fibers located in the center of the focus of damage, these having arisen independently of the stumps of muscles in the border zone (Figure 68b). The latter fact shows that the absence of differentiation of the newly formed muscles is not associated with the absence of innervation in them, but is due to some other factor. Evidently, the responsible factors are the lack of connection between these muscle fibers and the old muscles of the border zone. Probably, for normal differentiation of the regenerating muscle fibers and their functioning, a connection with the old muscle fibers is essential. It is possible that this is due to the peculiarity of structure of the cardiac muscle, i. e., the presence in it of insertion plates which probably play the role of sites of attachment of the different segments of the muscle fibers. When the continuity of the connection of these segments is disturbed, their normal function is also disturbed. Both of these problems concerning the role of innervation in the regeneration of muscle fibers, require further investigation.

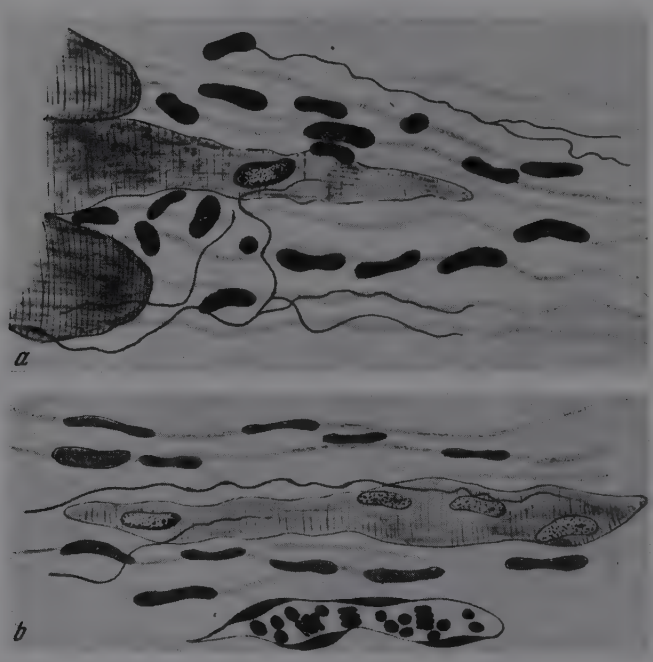


FIGURE 68. Regeneration of nerve fibers in the cardiac muscle of a rat following stimulation with hydrolysate of myocardium + RNA:

a – innervation of regenerating muscle fiber in the border zone, 17 days after operation; b – innervation of nondifferentiated, newly formed muscle fibers in the center of the focus of damage, 60 days after operation (according to Gusareva, 1965).

Data from electrocardiographic investigation

Electrocardiographic investigation carried out by N. A. Zakharova-Muzlaeva (Muzlaeva, 1964) shows that after diathermal coagulation damage of cardiac muscle in rats and rabbits, the electrocardiogram (EKG) constantly depicts 3 stages: an acute, subacute, and scarification stage (Figure 69 a—d). In rats in 28—30% of cases, the EKG, in the scarification stage may become normal for a brief interval and then again record a scarification stage. Normalization corresponds morphologically to de novo formation of islets of muscles in the center of the focus of damage, while the EKG transition into the scar stage corresponds to the decomposition of the newly formed muscle. In rabbits with diathermal coagulation damage of the myocardium, there is no de novo formation of muscles in the center of the focus of damage, and the EKG does not normalize.

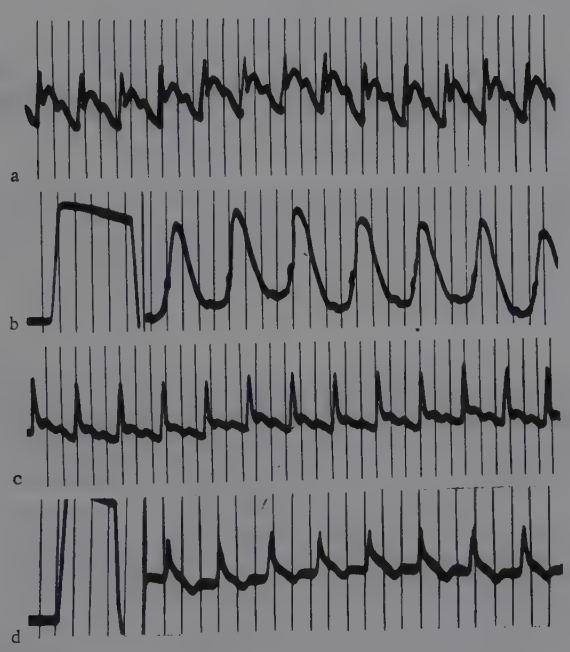


FIGURE 69. Electrocardiogram of a rat at different times after infliction of damage to the myocardium:

a — normal; b — acute stage; c — subacute stage; 4 — scar stage.

Under experimental conditions, the EKG of the rats and rabbits becomes normal at the time of regeneration of muscles in the focus of damage. Upon the action of a myocardial hydrolysate and a number of other stimulators, the de novo formation of muscles in the center of the focus of damage occurs early (10—13 days after operation), and the EKG also becomes normal at this time (Figure 70 a—c). Under the action of pyrogenal and other inhibitors of scarification, regeneration of muscle from the stump occurs later (30 days

after operation), and the EKG also becomes normal at this time. If, however, the regenerating muscles decompose in the experiment, the EKG again returns from normal to the scar stage.

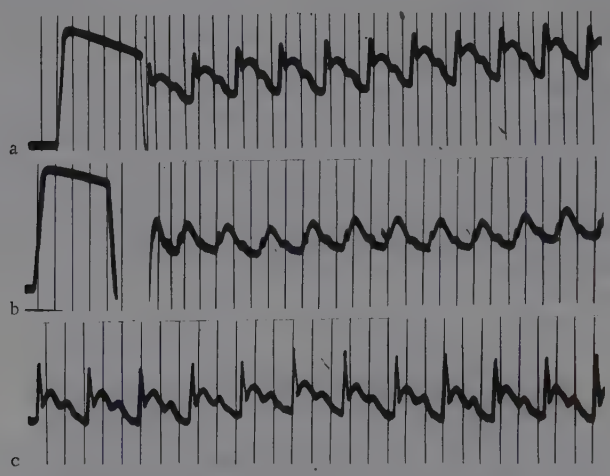


FIGURE 70. Electrocardiogram of a rat with experimentally damaged myocardium, upon stimulation of its regeneration:

a – thoracic lead in a normal animal; b – acute period; c – normalization 13 days after operation in an experiment with myocardial hydrolysate.

Thus regeneration of cardiac muscle is accompanied by normalization of EKG. In some cases, however, normalization of EKG is observed even in the absence of regeneration of muscle fibers, and quite frequently in the case of partial regeneration of cardiac muscle. Therefore, the phenomenon of normalization of EKG may be explained by the fact that in this experiment several metabolic conditions are created in the damaged cardiac muscle which, on the one hand, stimulate the process of regeneration of muscle fibers, and on the other, restore the impaired bioelectric activity of the myocardium. It is possible that among these conditions one is vascularization, which prevents the formation of a bioelectrically inactive zone in the focus of damage and mainly around it (in the perinecrotic region). It is possible that among these conditions there is restoration of the disturbed ionic equilibrium in this zone.

As a rule, normalization of the EKG in our experiments indicated a favorable course of the healing process of the damaged cardiac muscle, or its partial normalization. Thus, normalization of the EKG after myocardial damage may have a certain diagnostic and prognostic significance.

Electrocardiographic studies carried out in 8 experimental series of rats and rabbits, using various stimulators of the regeneration of myocardium, allowed us to establish the finding that stimulators do not, as a rule, alter the shape or the height of the main peaks of the EKG, the duration of the PQ and QT intervals, or displacement of the ST segment. In the majority of cases they cause a shortening of the electrocardiographic

stages: in the subacute and scar stages 1.5- to 2-fold, and in these cases a relatively early normalization of EKG takes place. Of the stimulators tested by the EKG data, the best was the hydrolysate of myocardium and vitamin B_{12}. These preparations themselves do not produce any pathological shifts in the EKG; they enhance the electrocardiographic stages and cause early normalization of EKG in 50—60% of cases.

Inhibitors of scarification affect the shape and dynamics of the changes in the T peak, which in the early part of the scar stage acquire a biphasicity, and later a negative shape, or become immediately positive. All the inhibitors caused normalization of EKG at later times (30—45, and even 60 days after the operation) and maintain it until 90—120 days in 50—60% of cases. Pyrogenal and leukocyte serum reduce the height of the R peak during the period of treatment of the animals, on the average, by 30—50%. The effect of inhibitors of scarification on the EKG is evidently associated with their specific effect on the development of connective tissue and inhibition of the process of scarification. In experiments on rabbits a high percentage (71) of normalization was obtained in an experiment with trypsin.

In combined application of stimulators and inhibitors, the EKG becomes normal at later times during the experiment in 50—100% of cases. Changes in EKG depend on the specific properties of each individual preparation. Thus pyrogenal reduced the height of the R peak on the average by 30%, no matter what the other stimulator in the complex was. Vitamin B_{12}, or a hydrolysate in a complex with trypsin, increased the voltage of the R peak 2-fold in rat experiments (Figure 71a, b). When taken separately, these preparations did not have such an effect on EKG.

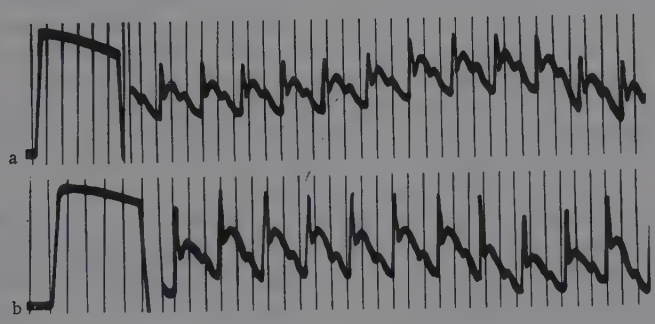


FIGURE 71. Electrocardiogram of a rat:

a — normal; b — 12 days after infliction of damage to the myocardium and administration of vitamin B_{12} and trypsin. Amplitude of peak R increased 1.5-fold as compared with normal.

The best combinations in the rat experiments were vitamin B_{12} + trypsin; hydrolysate of myocardium + pyrogenal; hydrolysate of myocardium + spleen preparation. In the rabbit experiments they were: hydrolysate of myocardium + trypsin. In the former and latter cases the EKG became normal in 100% of cases.

Data from histochemical investigation

L. V. Akhabadze (1964, 1965) carried out a histochemical investigation of the distribution of RNA (Brachet reaction), of DNA (Feulgen reaction), and glycogen (the Shabadash method) in the myocardium, upon damage alone, damage and use of stimulators of myocardial regeneration, inhibitors of scarification, and a combination of inhibitors and stimulators. It has been found found that RNA is distributed mainly in young cellular elements with high capacity for regeneration of the specific tissue of the heart: in myoblasts, newly formed muscle fibers, dedifferentiating stumps of muscles, sprouts growing out from them, strips and bridges of the muscle. The intensity of the reaction to RNA is higher in the muscle fibers of the heart than in connective tissue. In the experiment with pyrogenal, for example, the intensity of the reaction to RNA was higher in the less mature than in mature connective tissue where it was weak. The nature of distribution of RNA changes, depending on the structure of the regenerating elements. In a normal fiber of the heart muscle, RNA is not very abundant, and is localized in the sarcoplasm along the entire fiber and in the nucleoli of the nucleus. In myoblasts and nondifferentiated muscle fibers, RNA is distributed throughout the cytoplasm and in the nucleoli.

DNA is localized in the nuclei of muscle fibers and in cells of connective tissue and white blood elements. In addition it is present in the focus of damage in the myocardium, in the fragments of nuclei, and in the intercellular substance during the period of the 17th—45th day after the operation. In the latter event, it may be due to the presence of nuclear substance released from decomposing nuclei.

Glycogen in the normal muscle fiber of the heart is distributed in a diffuse manner in the myofibrils and Q discs. In the experimental animals its distribution is very variable. It is distributed diffusely in the form of granules; it is abundant in the stumps of the muscle fibers in the experiment with pyrogenal. In myoblasts and in regenerated undifferentiated and differentiated muscle fibers, it is distributed diffusely, and in differentiated muscle fibers it is sometimes located mainly on one side of the fiber, a phenomenon also observed in normal fibers.

Results of histochemical investigation complementing the data of histological investigation, allow us to conclude that the regenerated muscle fibers are characterized by signs which indicate the possibility of obtaining normal structure and function.

Data from biochemical investigation

Biochemical investigation was carried out starting from the principle of regeneration in stages. In order to restore the lost regenerative capacity of an organ, it is necessary to enhance phenomena of destruction and dedifferentiation of the main component tissues of mesodermal origin and, in this case, of the cardiac muscle. Special attention should be devoted to protein and nucleic acid metabolism, which is the basis of self-reproduction of living beings. On the one hand, it is necessary to know the composition of the substances which act (preparations used) on the regeneration process

in the myocardium, and on the other hand, the biochemical changes in the myocardium due to its damage and to the means used. We knew the chemical composition of a number of preparations, stimulators of regeneration in myocardium, and inhibitors of scarification (vitamin B_{12}, thyroidin, RNA, trypsin, dibasol, pyrogenal, etc.). We examined the composition of some other preparations (myocardial hydrolysate, extracts from cardiac and skeletal muscles) (Mant'eva, 1959; Yavich, 1963).

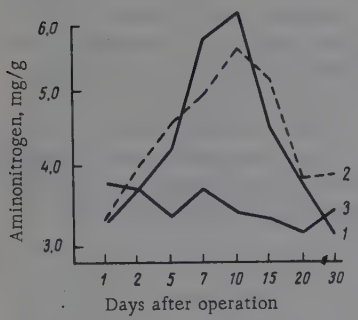


FIGURE 72. Autolytic activity in tissues of the focus of damage in the myocardium:

1– without administration; 2 – following administration of hydrolysate of cardiac muscle to the animal; 3 – control.

Biochemical investigation carried out by M. P. Yavich (1963) in experiments on rats showed that diathermal coagulation damage leads to regular changes in the protein and nucleic acid metabolism in the area of the defect, without greatly affecting the metabolism of the myocardial tissues distant from the latter. Thus, the autolytic activity of tissues is considerably enhanced on the 5th–10th day after the operation, i. e., during the period of lysis of the necrotized tissues, and approaches the initial level after 20 days, during the period of development of scar tissue which contains islets of newly formed muscles. The effect of the hydrolysate changes the autolytic activity very little (Figure 72).

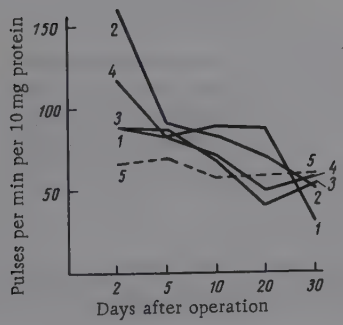


FIGURE 73. Changes in the total incorporation of cysteine S^{35} and tyrosine C^{14} into proteins of the cardiac muscle, upon damage of the muscle, and administration of myocardial hydrolysate to the animals:

1 – damage of tissue; 2 – the same, administration of hydrolysate; 3 – adjacent tissues; 4 – the same, administration of hydrolysate; 5 – control.

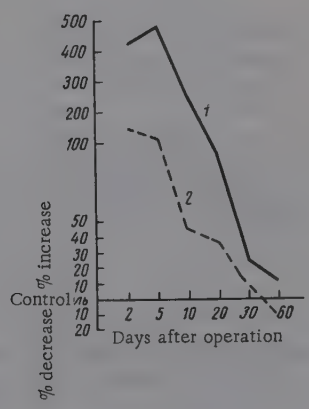


FIGURE 74. Changes in the amount of incorporation of cystein S^{35} and glycine C^{14} into the proteins of the focus of damage in the myocardium:

1 – cysteine S^{35}; 2 – glycine C^{14}. Data are expressed in percent; control – 100%.

In the course of healing of the damaged myocardium in the first days after the operation, the intensity of protein synthesis in the tissues of the focus of damage increases greatly, as indicated by data on the incorporation of labeled amino acids into the proteins of these tissues. After 30–60 days, the intensity of protein synthesis returns to the control level (Figures 73,74).

The number of sulfhydryl groups in the region of the defect decreases on the 10th day, returns to normal on the 20th, and rarely decreases 30 days after the operation (Figure 75).

The amount of RNA in the tissues in the region of the defect in the myocardium decreases by 30% at first, one day after the operation. It reaches maximum after 5 days, exceeding by 55 — 82% the normal level, and on the 30th day it decreases. After 60 days, it approaches the control level (Figure 76).

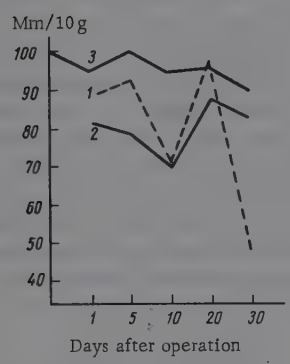


FIGURE 75. Amount of SH-groups in the tissues of the focus of damage in the myocardium:

1 — without administration; 2 — following administration of myocardial hydrolysate to the animals; 3 — control.

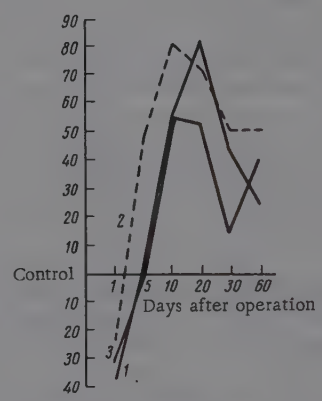


FIGURE 76. Changes in the RNA content of the tissues in the focus of damage in the myocardium:

1 — without administration of biopreparations to the animals; 2, 3 — following administration of vitamin B_{12} and pyrogenal, respectively. Data are expressed in %; control — 100%.

The concentration of DNA in the region of the defect decreases by the 10th day, returns to normal by the 20th, and drops sharply by the 30th day after the operation.

Lysis of coagulated tissue, development of granulation tissue, and appearance of myoblasts in the region of damage are characterized by enhancement of the processes of protein decomposition and synthesis; by a decrease in the amount of proteinaceous sulfhydryl groups; by a decrease followed by increase in the concentration of RNA and DNA.

The de novo formation of muscles in the center of the focus of damage is associated with a reduction in the decrease in autolytic activity, with an increase in protein synthesis, and with an increase in the number of sulfhydryl groups.

The stimulators of regeneration of myocardium (myocardial hydrolysate, vitamin B_{12}) and the inhibitor of scarification, pyrogenal, tested in the biochemical studies, affect protein and nucleic acid metabolism in the tissues of the region of the defect.

The hydrolysate of myocardium activates protein metabolism, mainly the synthesis of protein, and does not affect the nucleic acid content. Vitamin B_{12} increases the amount of RNA and DNA in the tissues of the defect area, and also stimulates the incorporation of glycine C^{14} into protein 14 days after operation. Treatment of the animals with pyrogenal enhances the intensity of protein synthesis in the tissues of the focus of damage at later stages of the experiment, 30 days after operation.

Thus the biochemical investigation, on the one hand, characterizes protein and nucleic acid metabolism in the damaged myocardium, and on the other hand, shows that different preparations may affect differently the synthesis of proteins and of nucleic acids during the healing processes and regeneration of cardiac muscle.

In other words, the results of the biochemical study support our general concept of regeneration: external effects (in this case the preparations we used) lead to a change in metabolism which is the basis of the processes of regeneration. The latter, i. e., the change in metabolism, results in a change in physiology, in this case leading to electrocardiographic and structural changes, i. e., to changes in the course of the processes of development of connective tissue and muscle fibers. The final result, scarification or regeneration of muscle fibers in the focus of damage in the myocardium, depends on the course of the metabolic processes which may be regulated in a controlled manner if the basic links and conditions which alter them are known.

Data in published reports on the regeneration of myocardium

Our first data on the possibility of obtaining regeneration of cardiac muscle in adult mammals (Akhabadze, 1957; Zakharova, 1957; Mant'eva, 1957; Polezhaev and co-workers, 1958) met with skepticism from some investigators. In this connection we have already pointed out the paper by Knorre (1960). After 2 years, however, our data were confirmed in other laboratories (Sinitsyn and Kochetov), and still later in a third laboratory (Andreev). At present they are also being confirmed by the data of G. I. Nepomnyashchikh (1966), A. S. Vitkus, and I. A. Danelyus (1966), O. N. Survillo and E. G. Naumets (1966), and E. G. Naumets (1966).

N. P. Sinitsyn (1948) worked successfully for many years on heart transplantation, at first in frogs, and later in mammals. From this work he went on to experiments on resection of large parts of the heart ventricles in the dog, opening the cavities of the ventricles. He showed the possibility of resection of extensive areas of the ventricles up to 26 cm^2 (Sinitsyn, 1959). If he sewed up the edges of the wound tightly after the resection, it healed with a scar. However, if he sewed the wound and put on it a large cover of live tissues (muscle fascia) or of nonliving materials (suede, capron, textile), the area of the wound healed with a scar in the midst of

which, on the side of the endocardium and the thrombus, true cardiac muscle fibers regenerated among the connective tissue bundles (Sinitsyn, 1959, 1960). These muscles did not die but remained viable, i. e., for the entire period of observation (up to one year), the muscles regenerated independently of their stumps in the border zone and were separated from them by a connective-tissue scar, as was the case in a number of our experiments.

In other experiments performed by Sinitsyn (1962), he restored the resected regions of the cardiac ventricles of a dog by a double-layered caprone bag, inside of which he placed ground muscle tissue, according to the data of Studitskii (1959) on the regeneration of ground skeletal muscle. However, only connective-tissue scar developed inside the capron bag. Underneath the bag, as in his previous experiments, the muscle bundles regenerated. The application of dibasol and vitamin B_{12} allowed him to stimulate the regeneration of cardiac muscle from the edges of the area of the defect. Pentoxyl and vitamin D_1 had no effect. Cortisone, methylthiouracil, and removal of the thyroid gland suppressed regeneration of the myocardium.

Sinitsyn emphasizes the importance of the wall thrombus and the distance between the edges of the wound for the regeneration of cardiac muscle. We think that the transplants are powerful stimuli or inducers greatly and lastingly enhancing the destruction and dedifferentiation of the myocardium, thus causing its regeneration. Pharmacological preparations enhance these phenomena. Recently, Sinitsyn (1965, 1966) obtained very interesting data on the regeneration of papillary cardiac muscles. He assumes that development of fatty tissue at the site of neoformation, which may change the enzymatic reaction in local tissues, is a condition for regeneration.

We repeated Sinitsyn's experiments on adult dogs, and we were able to confirm his main results (Polezhaev and co-workers, 1964, 1965). After diathermal coagulation of a sector of the left ventricle with a diameter of 25—30 mm, reaching in depth to the endocardium, there was always scarification of the focus of damage. The result did not change when the experimental animals were treated with a spleen preparation. When, however, we resected large sectors of the left ventricle or the apex of the heart, opened the ventricular cavities, and sewed up the wound with a piece cut out from the right ventricle of the heart of another dog, then regeneration of muscle fibers occurred. By 315 days after the operation the region of the defect was well marked. From the side of the epicardium, the pericardium adhered to it tightly (Figure 77a), and from the side of the endocardium, a dense light sector of scar tissue of smaller dimensions could be seen (Figure 77b). Also seen in sections in the thicket of the dense connective-tissue scar were hyaline cartilage and bundles of regenerated striated cardiac muscles which were located closer to the endocardium (Figure 78).

N. N. Kochetov (1959, 1961) pressed strongly the soft tissues of the thigh in rabbits and dogs. As a result, numerous tiny foci of granular and lumpy decomposition of muscle fibers appeared in the myocardium of these animals. If the foci were small, encompassing small sectors of 1–2–3 muscle fibers, the latter regenerated. If the foci were larger, regeneration of muscle fibers did not occur, and the wound was covered with connective-tissue scars which prevented their regeneration.

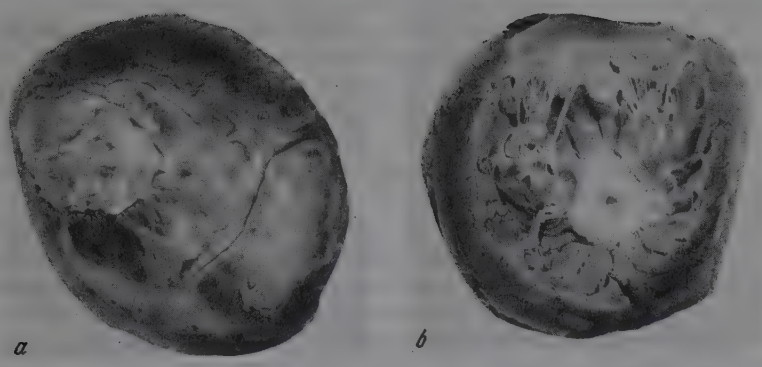


FIGURE 77. Heart of a dog; resection and plastic surgery of the myocardium according to N. P. Sinitsyn, 315 days after operation:

a – external appearance; b – internal appearance.

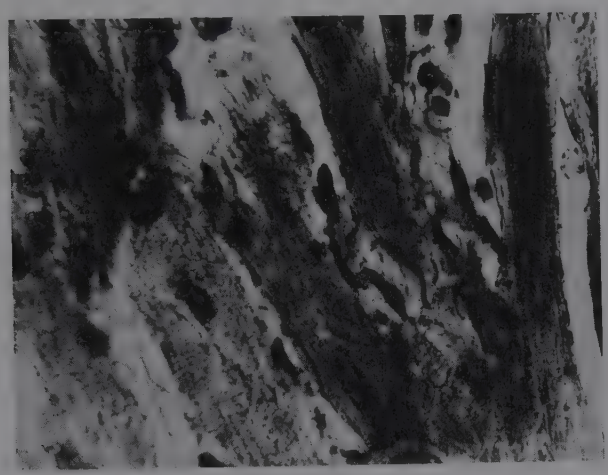


FIGURE 78. Heart of a dog; resection and plastic surgery of the myocardium. Bundles of newly formed muscles in the scar, 315 days after operation. 10 × 60.

S. S. Saidrasulov (1963), in S. V. Andreev's laboratory, afflicted, pierced, and cut wounds in the cardiac muscles of rats which were covered with scars. When he administered to the animals a therapeutic complex consisting of a mixture of substances facilitating protein biosynthesis (vitamin B_1, B_6, B_{12}, ATP, methionine, RNA, and DNA) stimulation of the regeneration of muscle fibers was observed. The DNA and RNA were low polymers which, according to our data, are inactive. In this connection, and also because the effect of the individual components of the mixture had not been previously studied, the nature of the active principle remains unclear.

In the experiments of Sinitsyn (1959), Kochetov (1959, 1961), and Saidrasulov (1963), as well as in others, normalization of EKG was recorded during myocardial regeneration.

N. N. Rumyantsev (1961) traumatized intensively the hearts of adult dogs, pressing and crushing the apex of the heart with pincers, a procedure analogous to our experiment on additional traumatization of nonregenerating extremities in tadpoles and adult frogs (Polezhaev, 1933, 1948). As a result, he established that regeneration of muscle fibers in the heart occurred in the form of bridges along the edges of the focus of damage. Simultaneously, the impaired function of the myocardium was restored. Kolosova (1961) observed limited regeneration of muscle fibers in the scar of the myocardium of a frog.

The data from a histochemical investigation, carried out by Kochetov (1961), Rumyantsev (1953, 1960), and Saidrasulov (1963) on the distribution of RNA, DNA, and glycogen in the regenerating muscle fibers in the hearts of rats, rabbits, dogs, and frogs, agree with our data.

L. M. Grigor'ev (1957, 1960) and Kochetov (1961) obtained secondary differentiation of muscle fibers in the heart of a chicken and in human fetuses in tissue cultures outside the body.

G. I. Nepomnyashchikh (1966) autotransplanted a piece of cardiac or skeletal muscle into the myocardium of a dog. As a result myoblasts and muscle tubules formed in the region of implantation, but further differentiation was prevented by development of a dense scar.

A. S. Vitkus and I. A. Danelyus (1966) brought about formation of myocardial infarctions in rabbits by ligating the branches of the coronary arteries of the heart. In order to stimulate regeneration of myocardium, they used vitamins B_6 and B_{12}, an extract prepared from hearts of rabbit embryos, and a protein hydrolysate prepared from blood fibrin of cattle. The latter was the most active preparation. It enhanced lysis of necrotized tissues and phenomena of regeneration of cardiac muscle in the zone of necrosis.

E. G. Naumets (1966) and O. N. Survillo and Naumets (1966) transplanted homoplastically pieces of lyophilized cardiac muscle into the myocardium of dogs. The transplants were resorbed and replaced by connective tissue and overgrown with blood vessels and muscle fibers.

The foregoing data confirm the findings of our investigations: the possibility of obtaining true reparative regeneration of muscle fibers in the hearts of adult mammals and Amphibia; data on the importance of destruction and differentiation in these processes; data on the role of connective tissue in the inhibition of regeneration of myocardium and, finally, our general concept that controlled change of the metabolism of the body or organ can change its capacity for reparative regeneration.

Practical application

The data on the stimulation of cardiac muscle may find practical application after appropriate elaboration of methods.

There are 2 ways to approach the application to practice, one surgical and other physiological. The method of resection and restoration of sectors of cardiac wall leading to regeneration of bundles of muscle fibers, suggested by Sinitsyn (1959, 1962), may be used in thoracic surgery in certain operations on the human heart in which there are infarctions, aneurysms, echinococcal injury, etc. Experiments on animals showed that it was

possible to perform resection of the infarcted region in the myocardium or of its aneurysm (Murray, 1946; Sergievskii, 1959). Consequently, in principle, the method of resection and restoration may also be used on man, at least in those cases where the body is young and strong enough to withstand a serious cardiac operation. To use it, however, on persons with infarction, arteriosclerosis, stenocardia, and hypertonia would be risky at present, albeit possible.

In this connection it seems to us that the physiological way, according to our studies, has better prospects. Here, stimulation of cardiac muscle regeneration, without a serious heart operation, is done using a syringe and preparations stimulating regeneration of myocardium and inhibiting scarification. The physiological method could be used in diverse diseases of the cardiac muscle: various types of myocarditis, myocardial dystrophy, microinfrarctions, wounds, etc. This method is very simple and can be used on people and animals of varying age; in certain cases it could lead to improvement of the general body metabolism, the metabolism of the heart, and its rejuvenation. Subsequently, after appropriate elaboration, it could probably be used also in infarctions. Inasmuch as the cardiovascular diseases are presently the primary cause of mortality in our country's population, as in other industrially developed countries, the importance of work in this direction is obvious.

We have now started work on testing the efficacy of our physiological methods for the treatment of diphtherial myocarditis in adult rabbits (Polezhaev, Kolchin, and Solntzeva, 1965; Polezhaev, Kolchin, and Sadokova, 1966). It is well known that the administration of certain doses of diphtheria toxin to rabbits produces severe diphtherial myocarditis in animals which, in principle, is the same as in man (Anichkov, 1912). Having worked out the methodology, we obtained, with single intravenous administration of certain sublethal doses of diphtheria anatoxin, development of diphtheria myocarditis in 100% of the cases (control). The mortality was 20—25%. Histological examination carried out at different times of the experiment showed the following picture. At first the blood vessels in the cardiac muscle dilate, become filled with blood, and stasis occurs. The walls of the vessels burst and foci of hemorrhage appear. Edema arises. The muscle fibers undergo granular degeneration; the cross striation disappears, the myofibrils decompose into tiny granules, the nuclei divide frequently amitotically, and the fibers swell. Later the muscle fibers undergo waxlike and fatty degeneration, myolysis, and lumpy decomposition (Figure 79, a—c). They lose their structure, become dull homogeneous, or show numerous tiny droplets of fat; their nuclei necrotize or swell and die. The fibers decompose into lumps (Figure 79d) and dissolve. In very many places in the myocardium, foci of destruction filled with blood appear (Figure 79c). These are filled with cells of connective tissue which is transformed into granulation tissue and subsequently into solid scar tissue (Figure 79d).

In 7—10 days after administration of diphtheria anatoxin and development of phenomena of myocarditis in the experimental rabbits, we injected the animals with a myocardial hydrolysate. Not a single rabbit of the experimental group died in the course of the experiment. Only 5 days after administration of the preparations, the animals showed signs of recovery. The lumps of decomposed muscle fibers coalesced and uneven muscle fibers appeared, which were of varying thickness not yet regularly

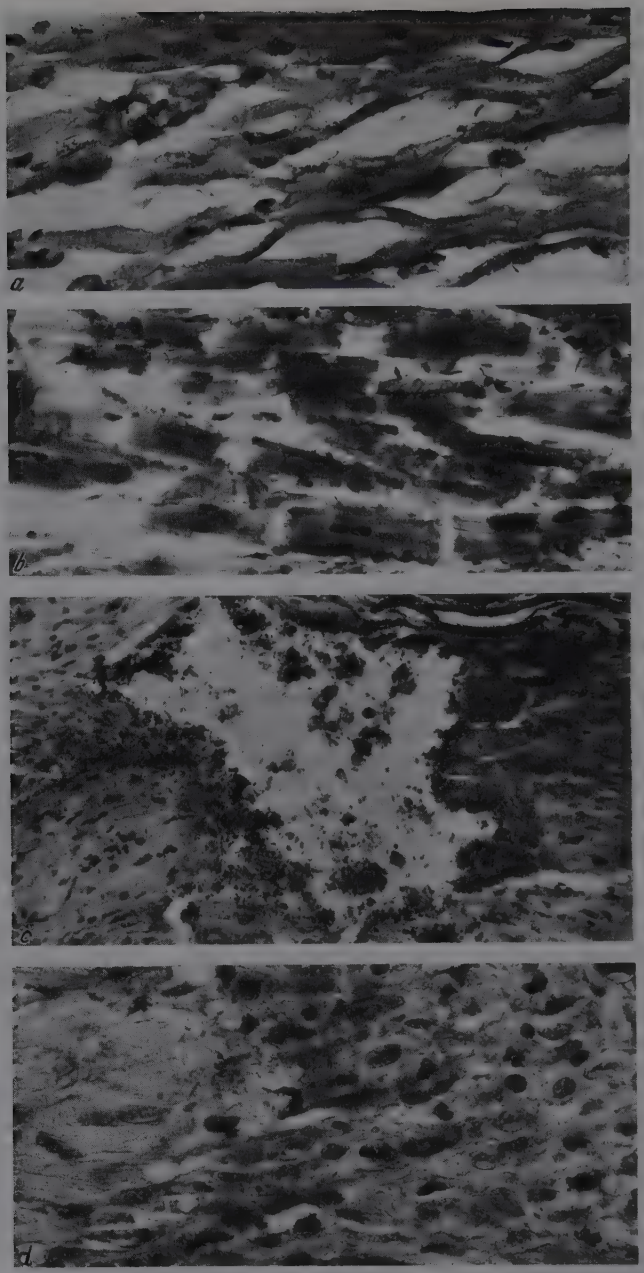


FIGURE 79. Change in cardiac muscle of a rabbit under the influence of diphtherial anatoxin, 7 × 60:

a – hyaline degeneration of muscle fibers; b – lumpy decomposition of muscle fibers; c – foci of decomposition of muscles and hemorrhage; d – scar tissue formed in foci of muscle decomposition.

differentiated (Figure 80a–c). From the stumps of the muscle fibers, thin, fine appendages grew out and coalesced with each other, or with the thick stumps of muscles which retained traces of waxy degeneration (Figure 80 a, b). As a result, the foci of myocardial decomposition were filled with regenerating muscle fibers which impregnated the connective-tissue scar (Figure 80d); the general structure of the cardiac muscle became normal in great contrast to the structure of the untreated myocardium.

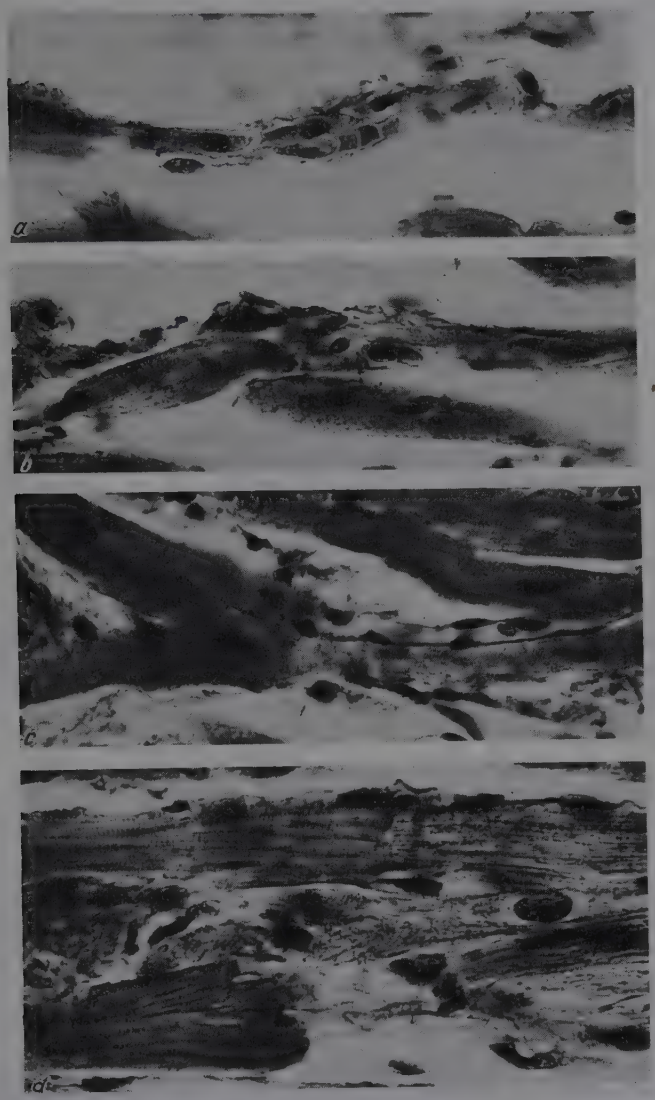


FIGURE 80. Stimulation of regeneration of cardiac muscle in diphtherial myocarditis in a rabbit. Administration of myocardial hydrolysate, 7 × 60:

a – d – consecutive stages of regeneration of muscle fibers after degeneration and lumpy decomposition.

As shown by our physiological investigation, the EKG of untreated rabbits passes from normal into a characteristic scar stage during the development of diphtherial myocarditis, while it becomes normal in treated animals. The blood pressure decreases regularly during the development of diphtherial myocarditis in the untreated animals, while in the treated animals it rapidly becomes normal (Figure 81a, b). The Bassette-Warner constant returns to normal (Figure 81c, d). Thus, data from morphological and physiological studies are in agreement, and show normalization of the damaged structure and function of the cardiac muscle, as a result of treatment by the stimulation of the regeneration method.

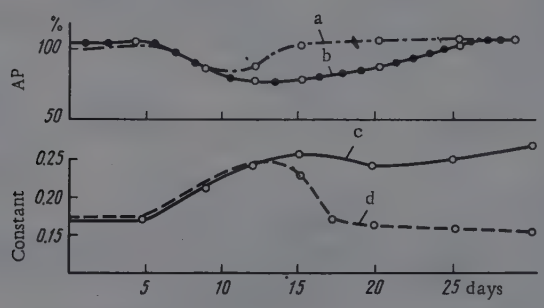


FIGURE 81. Changes in arterial pressure (AP) and Bassette-Waler constant in diphtherial myocarditis and stimulation of regeneration of myocardium in rabbits:

a — AP of treated rabbits; b — AP of untreated rabbits;
c — Bassette-Waler constant of untreated animals;
d — the constant in treated rabbits.

These data show that the foregoing conclusions concerning the practical aspect of studies on stimulation of regeneration of damaged cardiac muscle in myocarditis are quite valid.

Conclusion. Goals and prospects of this investigation

Until recently, medicine has been treating diseases of cardiac muscle (various myocardites, infarctions, wounds, etc.) by trying to accelerate scar formation in the foci of damage. No methods were available for the regeneration of cardiac muscle. This was due to the fact that physicians obeyed the old rules of the theory of regeneration on the basis of which the problem of restoration of lost regenerative capacity of myocardium could not be posed.

Now, on the basis of the new approach to phenomena of development and regeneration, and on the basis of new principles of regeneration, it becomes possible to pose the above problem and to suggest a new approach and concrete methods, which allow, experimentally, true regeneration of cardiac muscle in adult mammals (rats, rabbits, dogs). Thus a new way is opened for the treatment of cardiovascular diseases.

The problem of obtaining regeneration of cardiac muscle is not yet solved, however. Much has still to be done. It is necessary to find new, more effective preparations (stimulators of myocardial regeneration, inhibitors of scarification, and combinations of both) and new methods which allow for obtaining prolonged and stable regeneration of differentiated and functioning cardiac muscle fibers in large volume. A number of goals are clear: to elucidate how the work and working capacity of the regenerating cardiac muscle change; to determine how the preparations used in our experiments affect these changes; to study the role of the nervous system in the regeneration of myocardium; to determine the changes in protein, nucleic acid, and carbohydrate metablolsim in the processes of respiration, and in the enzymatic properties of the regenerating myocardium, using methods of biochemistry, histochemistry, autoradiography, cytospectrophotometry, etc.

The elaboration of these and other problems will be of great importance for biology and medicine. *

* Four reviews were written on our book: L.V. Polezhaev, L.V. Akhabadze, N.A. Muzlaeva, and M.P. Yavich "Stimulation of the Regeneration of Cardiac Muscle" (1965) by N.N. Kochetov (Uspekhi Sovremennoi Biologii, 62, 446–449, 1966), A.A. Voitkevich (Izvestiya AN SSSR, Seriya Biologicheskaya, No.4, 646, 1967), A.G. Lapchinskii (Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya, No.5, 91–93, 1967) and P.P.Rumyantsev and L.L.Zhinkin (Zhurnal Obshchei Biologii, 28, No.1, 122–125, 1967). The latter is a critical review. Our response to the critical remarks made by these reviewers will be published in a paper by L.V. Polezhaev "Ways of Studying Regeneration in Cardiac Muscle" (Zhurnal Obshchei Biologii, 29, No.4, 1968).

Chapter V

STIMULATION OF PHYSIOLOGICAL REGENERATION OF NERVOUS TISSUE IN THE CORTEX OF THE CEREBRAL HEMISPHERES

The brain, as well as the heart, is a vital organ in man and mammals; however, neither of which is capable of regeneration. Therefore, it is very important to determine whether it is possible to cause regeneration of damaged nerve tissue in the brain, and in particular in one of its most important components, the cortex of the cerebral hemispheres. This question is of great theoretical and practical significance. According to Pavlov's principle of nervism, the function of any of the organs of the human and animal body is regulated by the nervous system. From data from experimental embryology, we know that morphogenesis of organs which achieved a certain stage of development in normal ontogenesis of animals is also regulated by the nervous system (Spemann, 1936; Ivanova, 1948). Regeneration of organs depends to a great extent on the nervous system (see Weiss, 1925, Schotté, 1926, Singer, 1952). On the other hand, from pathological data we know very well that after various mechanical, infectious, toxic, and other injuries, the nerve tissue of the brain or spinal cord of the central nervous system (CNS) is not restored and, as a result, severe disorders, nervous and psychic diseases in particular, appear. Therefore, it is quite clear that it would be very important to prove the possibility of stimulating growth, development, and regeneration of the central, vegetative, and peripheral nervous systems in animals and man.

Data on the incapacity of nerve tissue for regeneration

There is a widespread belief in biology and medicine that the nervous tissue of the cortex of the large hemispheres is not capable of regeneration because of the inability of the neurons to divide mitotically. This belief is based on several groups of facts.

The previously mentioned data from clinical practice and pathological anatomy indicated that the changes in damaged nervous tissue in the brain and spinal cord are irreversible.

Data obtained in experiments on animals showed that the capacity for reparative regeneration of nervous tissue of the spinal cord and brain is absent under ordinary conditions of damage in birds, mammals, and man, and that the defective areas are filled with a scar or with a cyst containing fluid (Marchand, 1901; Goldzieher and Makai, 1913; Windle, 1959, and others).

For a long time no one had succeeded in obtaining growth and multiplication of nerve cells in tissue cultures outside the body (see Rumyantsev, 1932; Khlopin, 1940; Fischer, 1946, and others).

Data which were included in all Soviet and foreign textbooks on the embryology and histology of man and mammals said that the nervous tissue of the brain and spinal cord is basically formed during the period of embryogenesis (Zavarzin and Rumyantsev, 1946; Zavarzin and Shchelkunov, 1954; Knorre, 1959, and others). The neuroblasts form neurons which are capable of mitotic division but in the postnatal period lose this capacity as well as the capacity for amitotic division. The spongioblasts form glia cells which preserve the capacity for division even in the postnatal period.

All these data led the majority of histologists and embryologists to the conclusion that nervous tissue is stable, incapable of either physiological or reparative regeneration, that the same number of neurons that appeared in the brain during embryogenesis remains until the end of life of the animal or man, or that their number diminishes due to the death of nerve cells. Under known conditions, only peripheral nerves, i. e., dendrites of nerve cells, but not the neurons themselves, may regenerate. Nerve cells are capable of multiplying only in lower animals.

New data on the regeneration of nervous tissue

Together with the above mentioned data, particularly in the last 2 decades, other data began to accumulate indicating that nervous tissue of the central nervous system of higher vertebrates and men is capable of regeneration under certain conditions.

First, we should mention studies indicating the possibility of obtaining regeneration of large sectors of peripheral nerves in mammals and man by transplantation of nerves, fixed in formalin (Anokhin, 1940; Anokhina, 1954). Biological methods were elaborated which allowed restoration of an almost completely removed sciatic nerve in rabbits (Efimov, 1959; Aimanbetov, 1959).

Extensive work was carried out which showed that in rats and dogs it was possible to obtain regeneration of the spinal cord after it has been severed; however, much has still to be done to solve the problem completely and to use these results in clinical practice. Regeneration of severed nerve fibers of the spinal cord is prevented by the development of a dense hyaline scar in the region of the injury (Windle, and co-workers, 1959). If the development of the hyaline scar in rats, cats, and dogs with a cut spinal cord is arrested and it is softened by injection of piromen, pyrogenal, ACTH, cortisone, trypsin, or hyaluronidase, the nerve fibers proliferate into the scar, the cut ends of the spinal cord join, and the sensory and motor functions of the paralysed endings, and of other organs of the posterior part of the body are restored (Windle and co-workers, 1959; Turbes and Freeman, 1953; Freeman, Dougall, Turbes, and Bowman, 1960; Neseyanova, Brazovskaya, and Iordanskaya, 1960, 1961, 1962; Matinyan and Andreasyan, 1961, 1966). The use of these inhibitors of scarification facilitates curing of a number of surgical and infectious diseases, accompanied by the formation of scarified synapses in organs, and by damage of the brain or spinal cord.

Very interesting work was done by Muralt and co-workers on stimulating regeneration of peripheral nerves (Muralt, 1946; Lüscher and Muralt, 1947; Koechlin, 1955; Koechlin and Muralt, 1947; Jent, Koechlin, Muralt and Wagner-Jauregg, 1945). These investigators obtained from the brain and spinal cord, and also from peripheral nerves, a substance which accelerates the growth of nerve dendrites, called by them NR. From several tests proposed by these investigators, the most suitable one was the cornea test: a circular cut is made in the cornea of a rabbit and the regeneration rate of the cut radial nerves of the stems is followed in vivo. It was shown that injection of NR accelerates regeneration of these nerves 4–5 times. The investigators showed that this substance is of a non-proteinaeous nature, present in the dialyzable fraction of the extract, thermolabile, and most active at low concentrations ($1:10^{-5}$).

Studies on growing tissues outside the body showed that the most potent growth accelerating factor is embryonic extract (see Rumyantsev, 1932; Khlopin, 1940; Fischer, 1946; Bauer, 1954, and others). Very close in activity is an extract from Roux sarcoma. In the last 15 years, a series of interesting studies was carried out by Levi-Montalcini and co-workers (Levi-Montalcini, 1952, 1955, 1959, 1965; Levi-Montalcini and Hamburger, 1953; Levi-Montalcini, Meyer, and Hamburger, 1954; Levi-Montalcini and Angeletti, 1961, 1963; Levi-Montalcini and Cohen, 1960; Cohen, 1960, and others). These investigators showed that when nerve tissues from spinal or sympathetic ganglia of chick embryos are grown in a hanging drop outside the body, the growth of dendrites of nerve cells can be considerably stimulated by addition to the culture of a nucleoprotein extract from mouse sarcoma, snake venom, the protein extract from the submaxillary glands of mice, or protein extract from sensory or sympathetic nerve ganglia of rats, mice, cats, cows, or man. The latter extracts specifically stimulate only the growth of ganglia of the sympathetic nervous system of chick embryos outside the body, or of mice inside the body. Following injection of newborn mice with these extracts, the volume of the chain of their sympathetic ganglia increased 5-fold, and after injection of adult mice, it increased 2-fold. Hyperinnervation of the internal organs and hypertrophy of the neurons of the sympathetic ganglia themselves occurred. According to the investigators, the nerve growth factor is of a proteinaceous nature.

Let us point out that the studies of Muralt and Levi-Montalcini have demonstrated stimulation of the growth of nerve dendrites or hypertrophy of nerve cells, but not multiplication of neurons.

Very important data have been obtained in experiments on the growth of nervous tissue outside the body. Under ordinary conditions of growth, this tissue dies, but it grows well and ganglia are formed from the neuroblasts when embryonic mesenchyme is added to it (Grigor'ev, 1931, 1932). It has been shown that when appropriate life conditions for the explanted neurons are created, they can be maintained for several weeks, months, and even years (Costero and Pomerat, 1951; Hogue, 1953; Geiger and Becher, 1953; Mastrogiovani, 1958). The neurons in explants from the cortex of the cerebral hemispheres or sympathetic ganglia of man, rabbit, dog, and ape are capable of migration (Levi and Meyer, 1941; Hogue, 1953; Murray and Stout, 1947; Geiger, 1957, 1958) and of mitotic division (Murray and Stout, 1947; Geiger, 1957, 1958; Mastrogiovani, 1953). The cultivation of nervous tissue is facilitated by trypsinization (Mastrogiovani, 1958); the addition of brain extracts leads to an increase in the viability of the explants (Geiger, 1957), while addition of

cortisone increases the number of mitoses in the neurons 8-fold (Geiger, 1957). The effect on growth and on the condition of explanted nerve cells of a number of medical preparations, chemicals, and other preparations has been demonstrated (Geiger, 1963).

A considerable number of old reports (see Marchand, 1901) and new reports concerning the question of division of nerve cells in the body accumulated. Discussions have taken place on many points (Zhabotinskii, 1958, 1960; Koblov, 1960). Reviews sometimes appeared in which some generalizations were attempted (Braude, 1955; Shestopalova, 1956, 1960, Ivanova, 1960; Rampan, 1956, 1961; Kirsche, 1960, 1965; Milenkow, 1962; Vasile, Hurduc, and Gheorghium, 1961; Krivitskaya, 1963, and others). Division of neurons by mitosis, amitosis, and budding has been described. The major part of these studies was done on sympathetic spinal ganglia and ganglia of various internal organs in rabbits, dogs, cats, horses, and man (Al'tshul', 1940; Troitskii and Rudenskaya, 1950; Kolosov, 1951; Rampan, 1951—1961; Levinson and Leikina, 1952; Rzhevutskaya, 1952; Koblov, 1953, 1961; Radostina, 1953, 1957; Nikiforov, 1954; Tkachenko, 1955; Strukov and Lapin, 1956; Shetopalova, 1956, 1957, 1958; Torskaya, 1959, and others). Amitotic division of neurons in the cortex of the cerebral hemispheres, the retina of the eye, the cerebellum, and other parts of the brain under normal and pathological conditions has been demonstrated in a number of studies on young and adult humans, mammals (apes, dogs, rabbits, sheep, goats, wolves, and rats), and birds (Popoff, 1875; Saltykow, 1905; Schreber and Wengler, 1910; Levinson and Leikina, 1952; Pas'ko, 1953, 1956; Nikiforov, 1954; Rampan, 1956—1961; Tereza, 1955; Afanas'ev and Kotovskii, 1956, 1959, 1961; Gladkii, 1958; Oleneva, 1959; Kosmarskaya, 1961; Vasile, Hurduc, and Gheorghiu, 1961; Milenkow, 1962; Krivitskaya, 1963; Torskaya, 1963, 1964; Polezhaev and Karnaukhova, 1961, 1963, and others). The occurrence of binucleate neurons was described in the published data long ago. However, they were considered in different ways: as incomplete cell division, as a pathological condition, as an anomaly, as an abnormality, as a result of cell fusion. A demand was made to demonstrate all the consecutive stages of amitosis* of neurons in order to prove that binucleate cells are one of the stages of amitotic division (Rand and Courville, 1947). This requirement has now been fulfilled. I. I. Rampan (1956—1961) and later, also other investigators described the consecutive stages of amitosis in nerve cells: binucleolated, binucleate, and bicellular**. The neuron may divide into cells of equal or unequal size. Division of the neuron begins from the nucleolus and terminates by division of the body of the cell and its dendrites, some of which are formed again. According to I. V. Torskaya

* In connection with the use of new methods in cytology, mainly autoradiography and cytospectrophotometry, some morphologists in recent years revised the phenomenon of amitotic division of cells, and some of them even doubt the actual existence of amitosis (see Dobrokhotov and Liozner, 1962). Some investigators reached the conclusion that cases of direct division of polyploid nuclei and nuclear division in general accompanied by equal division of the nuclear DNA, and cases in which the division of the nucleus is preceded by synthesis and replication of DNA should not be considered as amitosis. We cannot touch upon here the complicated problem of mitosis and amitosis which is described in special monographs. We shall further define mitosis as that division of the cell during which morphological patterns are observed of known phases of mitosis, while by amitosis we shall refer to cell division during which these phases are not observed and the nucleus and the cytoplasm divide by splitting.

** However, the binucleolated neuron does not always become a binucleate neuron, and the latter divides cytotomically.

(1963, 1964), amitotic division of nerve cells begins with the appearance of a cleft in the nucleus. This investigator studied the cortex of the cerebral hemispheres and the nuclei of the mesencephalon, midbrain, and spinal cord in adult dogs revived after clinical death. She reached the conclusion that after the death of some of the neurons, they are replaced by amitotic division of differentiated neurons and possibly of neuroblasts stored in the cortex of adult animals.

FIGURE 82. Regeneration of the anterior brain with an additional ventricle: right, 150 days after an operation on axolotl; left – a non-operated anterior brain, ×160 (according to Kirsche, 1964).

After mechanical trauma or hemorrhage in the brain, mitotically dividing neurons were also found in the cortex of the cerebral hemispheres of adult rats and rabbits, near the focus of damage (Popoff, 1975; Mondino, 1885; Coen, 1888; Sanarelli, 1890; Tedeschi, 1897; Saltykow, 1905; Syui Tzin, 1959; Afanas'ev and Kotovskii, 1956, 1961; Polezhaev and Karnaukhova, 1962, 1963). There are indications, however, that neurons divide only till the metaphase and later perish (Soskin and Grigor'eva, 1964; Soskin, 1965). It is possible that the fate of mitotically dividing neurons depends on the degree of damage and localization, i. e., the nearness to the edge of the brain wound.

The foregoing data show that not only death but also multiplication of neurons takes place in the brain of mammals and man, i. e., a process of physiological regeneration. In this connection it is of special interest to know whether reparative regeneration of parts of the brain takes place in vertebrates at any stage of ontogenesis. There are a number of reviews on this problem (Spatz, 1930; Boeke, 1935; Windle, 1955; Kirsche, 1960, 1965; Windle, 1959).

Among teleostean fishes, the possibility of regeneration of the tectum opticum has been demonstrated in *Carassius carassius* L. (Kirsche, 1960, 1961) and of the anterior brain in *Gastrostens aculatus* L. (Segaar, 1962) and *Lebistes reticulatus* (Maron, 1963). The regeneration of the tectum opticum has been particularly well studied. After the infliction of a small defect, complete regeneration of the brain takes place in 300 days. If a large defect is inflicted, regeneration also occurs, but it is frequently atypical. The source of regeneration is cells of the matrix or ependyma. Multiplication of these cells occurs mitotically (Kirsche, 1960, 1961; Maron, 1963; Pflugfelder, 1964, 1965).

Much has been reported and is available on the regeneration of the brain in caudate and tailless Amphibia. The subjects may be divided into 3 groups: 1) regeneration in embryonic stages, 2) regeneration in larvae, and 3) regeneration in adult individuals. The data in the first group show the possibility of quite extensive restoration of the brain after removal of

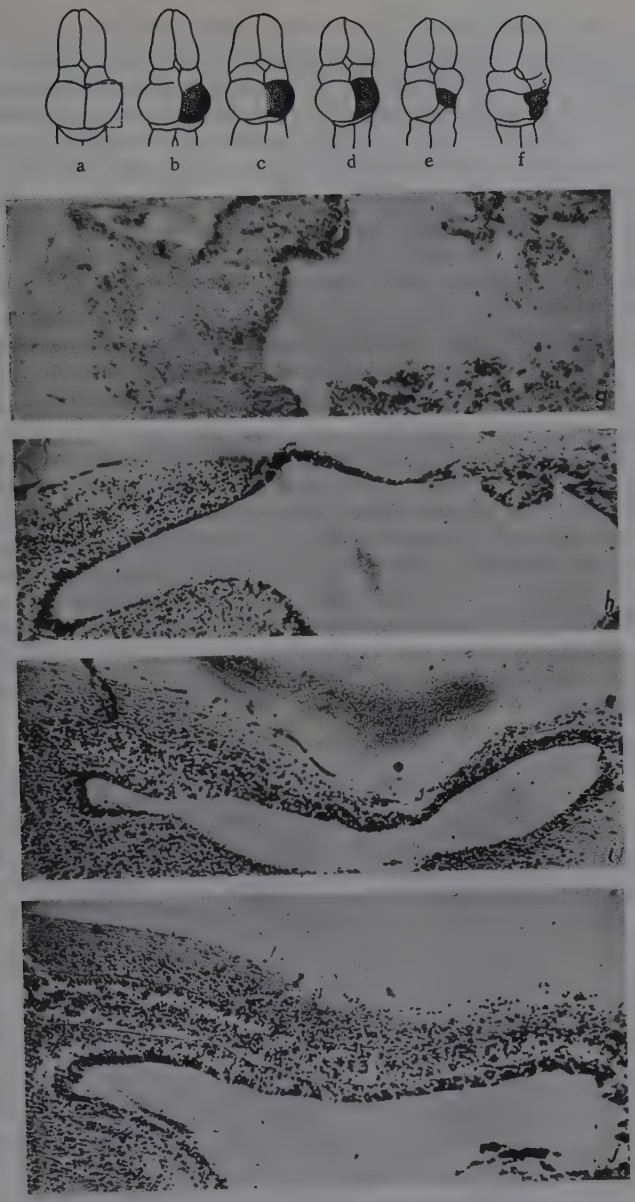


FIGURE 83. Regeneration of the midbrain (tectum opticum) in tadpoles of Rana temporaria after its removal on the right side at Stage Ic; on the left, nonoperated side of the brain:

a – the shaded areas represent the areas of brain extirpation; b – c – different cases of regeneration of the midbrain (stained) 30 days after its removal; g – j – consecutive stages of regeneration of midbrain, perpendicular sections; on the right, experimental; on the left, control. Stained according to Nissl.

sectors of the nerve plate or the nerve tube (Bell, 1907; Fischer, 1914; Burr, 1916; Spirito, 1930; Detwiler, 1945; Harrison, 1947; Wiener, 1954—1955; Romanovsky, 1958, 1959). Of considerable interest to us are the data of the second and third groups obtained in experiments on tadpoles, frogs, tritons, and axolotls of various ages after they are pierced or cut, or after removal of various parts of the anterior, midbrain, medulla oblongata, and cerebellum (Danilewski, 1891; Weissfeiler, 1924, 1925; Sibbing, 1954; Srebro, 1957; Jordan, 1958; Kosciuszko, 1958; Kwiatkowski, 1961; Kirsche, 1964, 1965). In larvae and young animals, regeneration of the brain occurs much faster and is more complete than in adult animals. After removal of large parts of the brain atypical regeneration often occurs: additional ventricles are formed in the brain, and the white and gray matter is distributed irregularly, brain walls of irregular shape appear, etc. (Figure 82). The regenerating brain is sometimes better developed than the brain on the unoperated side, sometimes it is poorly developed, or there is no regeneration at all. In the latter cases, as a rule, there is a compensatory development of the adjacent parts of the brain. Upon removal of very large sectors of the brain, regeneration is very limited or nonexistent (Kirsche, 1964). Probably for this reason, V. N. Petrov and L. M. Belova (1963) did not observe regeneration after removal of the entire anterior brain in axolotls. The source of brain regeneration is the ependyma or matrix cells (Serebro, 1957; Kirsche, 1964). The latter, as well as neurons, multiply mitotically (Srebro, 1957; Jordan, 1957; Kwiatkowskii, 1961; Kirsche, 1964, 1960, 1961, 1965). However, some investigators have not observed mitosis (Sibbing, 1954).

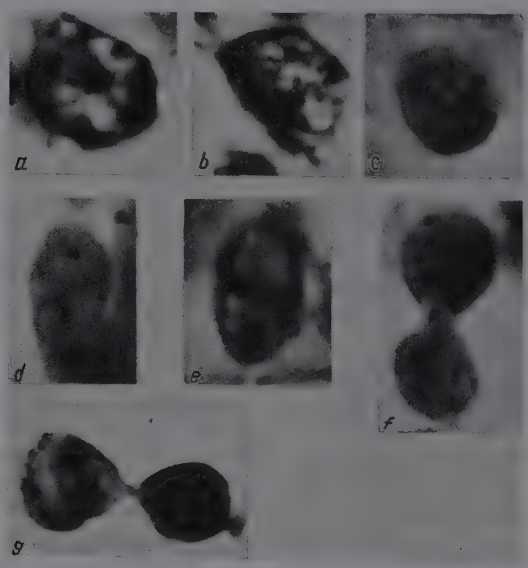


FIGURE 84. Consecutive stages (a – g) of amitotic division of ependyma and neuron cells during regeneration of the midbrain in tadpoles of Rana temporaria

We have also observed regeneration of the midbrain, anterior brain, and forebrain after unilateral removal in tadpoles of Rana temporaria at stage Ic, according to L. Ya. Blyakher (Polezhaev and Malyovanova, 1965; Malyovanova and Polezhaeva, 1966). Regeneration in these cases occurs rapidly, and the regenerate differs very little in dimensions from the brain on the unoperated side one month after the operation (Figure 83). The sources of regeneration are both ependyma cells in the ventricles, and neurons. In the ependymal cells, mitosis can be observed, but in addition, these cells and the neurons also multiply amitotically (Figure 84).

In adult mammals and in man, as shown in numerous experiments of physiologists and by clinical data, there is no reparative regeneration after extirpation of different parts of the brain. In the last 10 years, studies have been reported in which there have been findings indicating that after partial decortication in 2—4 week old kittens, puppies, and chicks, the area of the defect is filled with nervous tissue (Kogan and Ivannikova, 1955; Ivannikova, 1956, 1960; Dzidzishvili, 1956; Menisashvili, 1960, 1966; Polezhaev, Matveeva, and Zakharova, 1957). Anatomically the brain is restored. However, later experimental and histological investigations showed that what occurred was not regeneration of nervous tissue, but displacement of the latter into the defect area from the adjacent sectors (Klossovskii and Vasil'ev, 1961; Ivannikova, 1963; Belova, 1964; Fanardzhyan, Chilingaryan, Papoyan, and Paravyan, 1962; Polezhaev and Reznikov, 1966, 1967). The number of amitotically dividing neurons in the nervous tissue increases in the process, and mitoses appear in the marginal area. It is of interest that, despite the presence of quite extensive defects in the cortex of the kittens and puppies, its conditioned reflex activity, disturbed during the operation, is fully restored, while this does not happen in adult animals (Kogan and Ivannikova, 1955; Ivannikova, 1957, 1960). Similar morphological and physiological data were obtained in experiments on chicks and hens (Ponomareva, 1959).

After the action of certain doses of ionizing radiation on the cortex of the cerebral hemispheres, the cerebellum, and other parts of the brain in embryos, and during the postnatal period of ontogenesis in a number of mammalian species (mice, rats, rabbits, guinea pigs, and apes), the neuroblasts and nerve cells are replaced by new ones arising by mitotic division of the surviving cells (Manina, 1964). On this basis, the investigator concludes that the nervous tissue of the brain of mammals, at least during embryogenesis and in the early stages of postnatal ontogenesis, does not lose its capacity for (physiological) regeneration.

Summarizing the foregoing facts, we point out that at present the following possibilities have been proven:

1) not only of restoration, but also of stimulation of regeneration, of peripheral nerves in mammals;

2) of obtaining partial regeneration of a severed spinal cord in mammals by changing the development of the glial scar;

3) of prolonged (up to one year) preservation of nerve cells in the cortex of the cerebral hemispheres in man, ape, dog, rabbit, and other mammals in the presence of certain conditions of explantation, and their mitotic division;

4) of the presence of amitotic division of neurons in the cortex of the cerebral hemispheres and in other parts of the brain in adult man, mammals, and birds under normal and pathological conditions (taking into account the continuous death of nerve cells in the brains of these organisms, it may be

said that a process of physiological regeneration of nerve tissue of the brain occurs in these species); and

5) of reparative regeneration of different parts of the brain at all stages of ontogenesis in fish and Amphibia, in the absence of reparative regeneration in young and adult mammals.

From points 3 and 4, it follows that from the absence of a capacity for reparative regeneration of nerve tissue of the brain of mammals and man, the conclusion cannot be made that the neurons lost the capacity for division. However, this problem has not yet been solved.

Our approach to the problem

On the basis of analysis of the data from published reports, and partly of our own experimental data, we reached the conclusion that the nervous tissue of the brain is in a state of physiological regeneration. We shall discuss this problem after we review the results of our new investigations. We shall only point out here that this process certainly can be changed, enhanced, or weakened, and that this fact may be of great theoretical and practical significance. We assume that changing, enhancing the process of physiological regeneration of nerve tissue in the brain, may improve the condition of the entire central nervous system, of the higher nervous activity (HNA), and of the psychic condition of man, and thus present a new way to treat a number of nervous and psychic diseases (Polezhaev, 1961; Polezhaev and Karnaukhova, 1962). Stimulation of physiological regeneration of nerve tissue in the brain of man and animals should necessarily lead to a change of the animal's entire physiology, metabolism, molecular, structural, and consequently functional, renewal of the brain. In pathological cases, in the presence of certain nervous-psychic diseases, it may lead to normalization of the disturbed psychic activity of man.

On the basis of Pavlov's study, the scheme is as follows: external or internal living conditions cause an overtension of the nervous system; a shift in the physiological processes of stimulation and inhibition of the nervous system and in the metabolism of the nervous substance of the brain, primarily in the cortex of the cerebral hemispheres; an accumulation of excessive amounts of excretion products of the nervous tissue of the brain, and therefore autointoxication, weakening of the force of the nervous processes in the brain cells; and create inability for spontaneous restoration of nerve cells and finally psychic disease. Persons with a weak type of nervous system, a fragile system already disturbed under the influence of ordinary living conditions, are most prone to succumb to psychic diseases.

Pavlov (1951) rightly emphasized the fact that the main property of the central nervous system, and of the cortex of the cerebral hemispheres on which is based the subdivision into types of nervous systems, is the force of the nerve cells, determining the working ability and resistance of the nerve cells in the cortex and subcortex of the brain and their possibility to be restored after damage and psychic disease. Consequently, the main biological goal in the struggle with primary functional psychic diseases is to increase the force and resistance of the nerve cells of the brain. This problem is difficult, but real and soluble.

In the history of the development of psychiatry, three main stages or directions may be pointed but:

1. The psychological stage when psychic diseases were considered mainly in the framework of psychology; the psyche was considered as an autonomous phenomenon, not directly dependent on the physiology and biology of the organism; in this period the theory of physical and psychic constitution was greatly developed (Kretschmer, and others).

2. The physiological stage, and this may be considered since the times of I. M. Sechenov and I. P. Pavlov, who began to look on psychic activity as reflexes of the brain depending on environmental conditions, as a function of the organism depending on its physiology. The theory of inhibitory centers, conditioned reflexes, a second signal system, processes of stimulation and inhibition enabled scientific investigation of the physiology of higher nervous activity and deeper penetration into the basis of psychic life of healthy and sick persons; a possibility opened up of widely experimenting on animals and causing and removing experimental neuroses.

3. The biological stage, which began spontaneously within the physiological stage on an empirical basis, has only begun to develop. It is characterized by the idea that the psyche, as are all the other functions of the organism, is based on biology and changes according to the same laws of individual and historical development as does the organism as a whole, with all its organs and organ systems. Within the biological, and still formative stage, a new stage of chemical and physical studies has already been born. This is understandable since the biology, metabolism, and development of an organism are closely associated with chemical and physical processes and rules. These rules can be understood correctly and controlled consciously, however, only after a suitable basis and development of investigations at the biological stage are available. Therefore, at present the main stage in the development of psychiatry is the biological one.

The psychological direction indicated psychotherapy for treatment of psychic diseases, the physiological direction indicated sleep therapy and other methods of restoring the process of inhibition and metabolism in the nerve cells. These methods undoubtedly are of great importance for the therapy of psychic diseases, but they cannot affect in a radical manner the biological basis of the nerve cells because they are not based on knowledge of the biology of development and restoration of organs and tissues, in particular of nerve cells. Pavlov (1890) stated: "Contemporary physiology of animals is mainly the physiology of destruction of live tissues... it is still very far from that happy state of knowledge of the other side of the life process, the knowledge of restoration of organs".

The biological direction suggested some effective methods for the treatment of such diseases as schizophrenia: insulin shocks, electric shock, etc. However, the empirical nature of this direction limited greatly its possibilities. A scientifically well-founded biological direction in the therapy of psychic diseases should be based on the knowledge of the main principles of loss and restoration of regenerative capacity of organs and tissues during evolution, ontogenesis, pathology, and experimental conditions; on the knowledge of the biology of development of nerve cells, the possibility of enhancing growth and development of the brain; on increasing the resistance

and working capacity of nerve cells and the entire nervous system, up to changing its type or constitution. At present, biology and the study of regeneration have already accumulated such knowledge as allows not only destruction, but also restoration of organs, tissues, and, therefore, functions of the mammalian and human organism.

Goals and method of investigation

The main goal of our study was to stimulate strongly the process of physiological regeneration of nerve tissue in the cortex of the large hemispheres in order to cause its molecular and structural renewal, and to change (improve) the functioning of the higher nervous activity. Initially, the experiments had to be done with healthy animals and later, under pathological conditions: to cause experimental neuroses and other phenomena. There is a sufficient basis for the assumption that it is easier to achieve this task than to cause reparative regeneration of the nervous tissue of the central nervous system, e.g., after partial decortication.

In order to restore a disturbed psyche as well as to restore lost regenerative capacity, one must change its basis, namely metabolism, in a controlled manner, starting from the basic law of regeneration and the principles of phasicity and organospecificity.

A priori, it may seem that a desirable result could be obtained primarily in those cases in which emaciation or atrophy of nerve tissue occurs in the brain. Among these are phenomena of old age, involution of the psyche, schizophrenia, etc. In schizophrenia, emaciation of the nervous system is observed. According to pathological data, the main changes in advanced cases of schizophrenia are phenomena of atrophy in the cortex of the cerebral hemispheres and in a number of internal organs (liver, kidneys, thyroid gland, sex glands, adrenals, etc.). The neurons in the cortex are sclerotized or swollen and are subject to neuronophagia. It may be assumed that the atrophy of the internal organs is due to degenerative changes in the vegetative nervous system. In that case, the main changes in schizophrenia are atrophy in the cortex and in the vegetative sympathetic nervous system. In order to overcome the atrophy of these elements, their physiological regeneration, their growth, and their development must be stimulated. The aforementioned data show that this is an entirely feasible task.

To carry out the task just discussed, and starting from the data on restoration of lost regenerative capacity of extremities in vertebrates, the method of traumatization of nerve tissue of the brain could be used. However, this proposal seems inhumane. Therefore, this method must be replaced by another, sufficiently effective method, but one that is admissible and humane. The problem must be carefully examined. It is quite interesting that psychiatrists themselves already used the method of traumatization, albeit starting with entirely different goals and considerations: this is the method of prefrontal leukotomy (see Gurevich, 1949). This well known method consists of surgical dissection of the nervous tissue of the frontal parts of the cerebral hemispheres with a scalpel. The aim was to destroy in this way the formation of pathological interneuronal connections, which were thought to appear in the brain in schizophrenia and other psychic diseases. This method has been very widely used in the United States.

Clinically, it sometimes gave a high percentage (70—80) of recovery. However, at the same time, in many cases it resulted in deep, severe and irreversible changes: hemorrhage in the brain, formation of coarse scars, large cysts with fluid in the brain, atrophy of nerve tissue, etc. (Shevchenko, 1954). The method of prefrontal leukotomy, therefore, was justifiably discontinued, and forbidden in psychiatry. However, its essence must be more deeply understood and what is useful extracted from it. The basic presumption of this method, namely the appearance of pathological interneuronal connections in the brain in schizophrenia, has not at all been proven. Nobody proved it: it is highly improbable that such pathological connections, if they have arisen, could be removed by surgery following which scars must appear in the focus of damage, which would again disturb the restored connections. Finally, prefrontal leukotomy was used and gave good results only in the case of so-called organic but not functional diseases: in asthenia, reactive states, various psychopathies, etc. These cases could hardly be due to organic changes in the interneuronal connections.

We assume that the essence of leukotomy is traumatization: dissection with a scalpel leads to the formation in the nervous tissue of decomposition products, biologically active substances, which result in the restoration of disturbed metabolic processes, physiology, working capacity, and resistance of the nerve cells in the cortex and in other parts of the brain; in enhancement of its physiological regeneration and, therefore, in restoration of the psyche. This probably explains the clinical success of prefrontal leukotomy. It is interesting that this method enables researchers to remove conditioned reflex fear in rats (Streb and Smith, 1955). The fact, however, that it leads to the formation of scars and cysts in the brain, and atrophy of nervous tissue, makes it unacceptable in psychiatry.

In the previous chapters of this book, we have seen that the method of traumatization can be replaced by methods of chemical treatment of animals with certain preparations, or by transplantation of certain tissues. These methods could also be used in psychiatry, an approach which would be quite admissible, humane, and painless. An attempt could be made to use decomposition products of brain tissue prepared in a certain manner. It would be necessary to take into account the presence of the hematoencephalic barrier (Shtern, 1935; Kassil', 1963), the dose, the site, and the means of introduction of the preparation, etc.

An extract of degenerating nerve trunk stimulates growth of nerve tissue in the spinal cord of a chick embryo outside the body (May and Trillard, 1954). Additional destruction of the peripheral nerve stimulates its regeneration (Bobrovskii, 1957). Brain extracts from chick embryos stimulate the growth of tissue cultures outside the body (Ditmar, Lipp, and Augustein, 1957), and when acting on the explanted skin from the back of chick embryos, they facilitate the growth of the feather anlagen (Sengel, 1964). An acetone brain extract (neuroketone), when administered subcutaneously, increases the conditioned reflexes and reduces the neurotic condition of rats (Chiesa and Martini, 1958). A similar administration to adult rabbits of a suspension of lyophilized hypothalamus from embryos of rabbits or calves increases the electrogenesis in the cortex of the cerebral hemispheres to 300%, as compared with the previous level, and exerts a lasting pacifying effect on the animals (Velley, 1959).

In mice, rats, rabbits, and dogs convulsive attacks of epilepsy can be caused by injection of some chemicals or suspensions prepared from brains of animals which are in a state of epileptic seizure (Kroll, 1933; Propper, 1938, and others), or by the action of strong sound (Krushinskii, 1960). Parenteral administration of preparations made of nervous tissue from the brain can prevent or stop the onset of such seizures (Cerletti, 1952; Venturi and Pinelli, 1959). A similar result can be obtained in experiments with "acoustic" epilepsy, using bromine (Krushinskii, 1960) or adenosinetriphosphoric acid [ATP] (Mosketi, 1959). Parenteral administration of preparations made of nervous tissue from the brain also gave some positive results in the treatment of certain neuroses and psychoses in human patients (see Khoroshko, 1912; Rumyantsev, 1951).

Our analysis forces us to touch on the problem of attempts at biogenic therapy of neuropsychic diseases and its basis. Attempts were made to use ACS for this purpose (Protopopov, 1942; Frumkin, 1942; Sluchevskii, 1942), nonspecific "biogenic stimulants" of Filatov (1945), and species nonspecific serum of N. G. Belen'kii (1950). These treatments did not give a clearcut positive result, however, probably because they could not have a specific effect on the brain and the entire nervous system. The use of tissue- and organospecific preparations acting mainly on the brain and the central nervous system and enhancing the regenerative capacity of the organism were more successful. M. K. Petrova (1946) using sympathomimetin, a hydrolysate of blood fibrin (Chukichev, 1958) which acts mainly on the sympathetic nervous system, successfully treated experimental neuroses in dogs. A. A. Malinovskii (1960), in experiments with "acoustic" epilepsy in rats, showed that implantation of skin preserved according to Filatov (1945) is much more effective than injections of an aloe extract. In contrast to the extract, the implantation enhances considerably the processes of inhibition in the central nervous system of rats and increases the resistance of the nerve cells to strong stimulation. At present, data are available which show that tissue preparations made from various preserved materials (skin, liver, placenta, spleen, aloe leaves, etc.) differ in their action. Filatov showed that similar substances appear in all these preparations upon preservation: "biogenic stimulants, "probably certain dicarboxylic (malic, succinic, etc.) and amino acids. However, this does not exhaust their action, and each of them has its own specificity, as rightly pointed out by Rumyantsev (1951, 1953). He very successfully treated a number of diseases, in particular neuropsychic diseases, on the basis of the organospecificity principle, using specially composed "bouquets" of tissue preparations. A similar effect is obtained upon using an organospecific method of treatment of a number of diseases with live cells (Niehans, 1948), and with preparations made of lyophilized tissues (Kuhn, 1956; Derozhi, 1959). Lyophilization, preserving the biological activity of tissues, greatly reduces their antigenicity and the danger of allergic reactions. Initially, in taking the blood or urine of a patient, which organs are damaged and to what degree is determined by the Abderhalden reaction for proteinases (Abderhalden, 1922). Later, according to the results obtained, a "bouquet" of tissue preparations are composed which are then used in suspensions for injection. A good effect is obtained. A number of diseases and anomalies, not amenable to other forms of treatment, can be dealt with successfully.

In speaking of treatment of neuropsychic diseases, the following are some forms which have been treated: oligophrenia, mental deficiencies of various origin, epileptic seizures, encephalopathies (various cases of child paralysis of the brain, after-effects of encephalitis, congenital injuries, Little's disease, infantilism, hypophyseal deficiency), etc. (Haubold, 1956; Goldstein, 1956; Destinus, 1957). After treatment of the children, their intellect, memory, attention, speech, mobility, tonus, and general development improved, epileptic seizures disappeared, and the children did much better in their studies. The brain developed and the occiput, which had been flat, became convex and round.

Biogenic therapy of psychic diseases by amino acids should also be mentioned (Andreev, 1960, 1961, 1962) as should therapy by tissue preparations made of endocrine organs. We pointed out earlier (Part I, Chapter 5) that amino acids are a very good nutrient medium for the growth of tissue cultures and are capable of detoxifying the action of poisons. In this respect, glutamic acid is of special interest. A good effect is also obtained with tissue preparations from the sexual and thyroid glands (Ol'shevskaya and Sorokina, 1955), or by thyroid therapy alone (Danziger, 1958). It is known that thyroidine enhances the oxidative processes and stimulates regeneration of muscles and soft tissues (Belkin, 1947, 1947a; Voitkevich, 1947; Studitskii, 1959). Thyroidine stimulates mitotic cell division in the ependyma of the brain in tadpoles (May and Mugard, 1955; Baffoni, 1959). Injections of an extract of the hypophysis considerably increases the number of cells in the brain of tadpoles and rats (Zamenhof, 1941, 1942; Zamenhof, Mosley, and Schuller, 1966). Intraperitoneal injections of extracts from brains of guinea pig embryos considerably stimulate the growth of a nonoperated, and the regeneration of an operated, anterior brain in axolotls, and increase the number of mitoses and total number of nerve cells in the brain (Kirsche, 1965).

Thus, a number of biological preparations stimulate processes of development, regeneration, growth, and multiplication of nerve cells on the one hand, and have a therapeutic effect in the treatment of some neuropsychic diseases, on the other.

It is known that starvation, accompanied by subsequent feeding, stimulates processes of physiological regeneration of various organs (see Vorontsova and Liozner, 1955). Probably, this fact lies at the basis of starvation treatment of certain diseases. And probably successful treatment of schizophrenia with dosed starvation (Nikolaev, 1963) is due to the stimulation of biological regeneration of nerve tissue in the brain and entire nervous system of the patient, and not to the protective effect of the system of interoreceptive analysors, as assumed by the researcher.

Various biological methods of stimulation of physiological or reparative regeneration of the nervous tissue of the central nervous system in lower and higher vertebrates are available, and some of these are used or may be used for the treatment of a number of neuropsychic diseases. Consequently, work in the direction of discovering and elaborating the best and the most effective of these methods is well-founded and has good prospects. Theoretically, the most effective methods should be those of direct action on the nervous tissue of the brain, thus stimulating its physiological regeneration.

Stimulation of physiological regeneration of nerve tissue in the cortex of the cerebral hemispheres

In our experiments on the stimulation of physiological regeneration of the nerve tissue in the cortex of the cerebral hemispheres, we started from the principles of phasicity and organospecificity, and we attempted to act directly on the cortex in order to cause a maximal effect (Polezhaev and Karnaukhova, 1962, 1963). In order to obtain traumatization in the parietal region of the cortex of the cerebral hemispheres, we performed a deep linear cut to the ventricles; into the brain wound, we implanted an agar plate 3X2X0.5 mm in dimension and which had been kept for 48 hours in a 1% aqueous solution of trypan blue. To enhance trauma, destruction, and dedifferentiation of nerve tissue in the cortex, we implanted a piece of cerebral hemisphere cortex in a state of decomposition due to treatment with a 1% aqueous solution of trypan blue for several hours. In order to elucidate the effect on the brain of the decomposition products of nerve tissue rather than of trauma, 1—2 drops of a brain homogenate treated with a 1% solution of trypan blue were introduced into the brain through a tuberculin syringe needle. The experiment was performed under aseptic conditions on adult white rats weighing 250—300 g. We approached the brain, cutting through the dura mater, and cut out a 6X3 mm rectangular piece of the parietal bone. The homogenate injections were made through a round, 2 mm aperture bored in the parietal bone by a boring machine. The animals were killed at different times, from 1 to 30 days after the operation. The brain was fixed in 10% neutral formalin. Vertical paraffin sections were stained with thionine or toluidine blue, according to Nissl and with hematoxylin-eosin.

Altogether four series of experiments were performed:

1. Control: nonoperated brain
2. Implantation of agar into the brain
3. Implantation of a piece of brain into the brain
4. Injection of a brain homogenate into the brain.

The nervous tissue of the cortex of the cerebral hemispheres in the nonoperated animals had a normal 7-layered structure. In the molecular layer there were rare cells of micro- and macroglia, and few normal or dystrophic and degenerating neurons. Therefore, the latter appear and die in the molecular layer, evidently because of lack of conditions for normal development and functioning. From the walls of the vessels of the pia mater, light, round or oval-shaped cells separate and migrate into the molecular layer. In each of the 2nd—7th layers of the gray matter of the cortex, two properties are observed: 1) amitotically dividing neurons; there are no mitoses in the neurons, and 2) neurons of different size and also at different stages of development or maturity (Figure 85). There are large pyramidal cells with well-developed cytoplasm containing tigroid and numerous dendrites; and there are smaller cells difficult to distinguish from macroglia cells, with a large nucleolus and weakly developed cytoplasm, with a small amount of tigroid and poorly developed dendrites. Evidently, neurons continue to grow and develop in the cortex of the cerebral hemispheres even in adult animals. This is also indicated by the fact that few degenerating and dying neurons and amitotically dividing neurons are found in the cortex.

From the ependyma, which covers the cavities of the lateral ventricles, oval-shaped or round cells with a weakly developed cytoplasm (Figure 86)

emerge and move into the subependymal layer, and subsequently into the white matter, forming rows. They migrate into the gray matter of the cortex. The process of appearance and migration of glia cells in the mouse brain was studied by autoradiography (Smart and LeBlond, 1961; Smart, 1961). These investigators showed that the glia cells, migrating and dividing mitotically, are transformed and consecutively form oligodendrocytes and astrocytes. We have also seen mitoses in glial cells, in the subependyma, and in the white matter. According to our observations, however, some of of the migrating cells are transformed in the white substance into cells of neuroblast and neuron type. Evidently, from the ependyma and subependyma, cambial type cells, capable of forming neurons as well as glia cells, emerge (Figure 87).

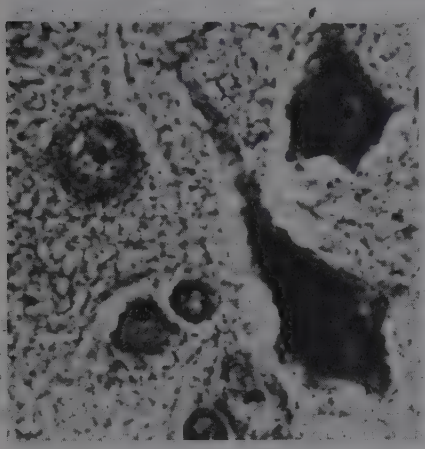


FIGURE 85. Neurons of various size and stages of development in the cortex of the large hemispheres of nonoperated rats

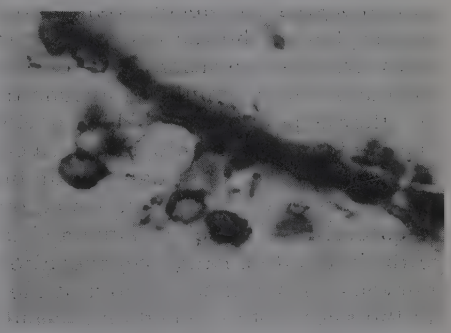


FIGURE 86. Emergence of cells from the ependyma into the sub-ependymal layer in adult nonoperated rats

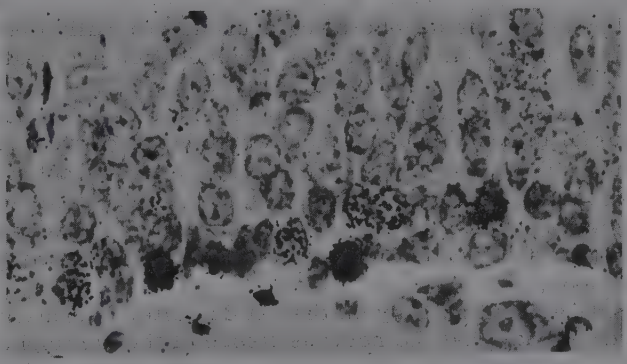


FIGURE 87. Autoradiogram from the region of gyrus dentata in the hipocampus of young rats, labeled with H^3-thymidine at the age of 10 days, and killed 2 months after injection. Labeled cells are located mainly in the basal layer, ×640 (according to Altman and Das, 1965).

All the foregoing facts: the presence of normal and dying neurons in the molecular layer of the cortex; death and amitotic division of neurons in the gray matter; formation of cambial type cells from the ependyma and subependyma, and their migration to the white matter and formation of neuroblasts and neurons there; and finally, the presence of poorly differentiated neurons in the gray matter, all those indicate that physiological regeneration of nervous tissue occurs in the normal cortex of the cerebral hemispheres of adult rats, and probably of other mammals as well.

In all 3 experimental series, some common phenomena were observed. In the first 3–5 days after the operation, hemorrhage and intense aseptic inflammation were seen. The nervous tissue in the operated area, and particularly at the site of implantation of the agar or the piece of brain, or the administered homogenate, underwent strong destruction and infiltration with polynuclears, which decomposed after 2–3 days and were replaced by lymphocytes, polyblasts, monocytes, and glia cells. Polyblasts transformed into macrophages which avidly phagocytized the substance of the implant together with drops of trypan blue, as well as decomposing erythrocytes and decomposition products of the local tissue. The macrophages showed energetic mitotic division during the period from 3–10 days after the operation. In the experiment with agar, a great amount of serous fluid accumulated around the latter, while this was not observed in other experiments. Also observed, 10–13 days after the operation was the beginning of development of a connective-tissue glial scar with collogen fibers in the focus of damage or brain wound. Finally, the region of the defect, an extensive brain wound or a wound canal from the inserted syringe needle, fills with scar tissue and in the experiment with agar, also with a cyst with fluid. Reparative regeneration of nervous tissue does not take place. The nervous tissue of the cortex of the cerebral hemispheres is greatly damaged in the area of the wound as a result of wounding by the scalpel or syringe needle, of action of the implant, and of nematogenic and histogenic cells infiltrating the tissue. In this area, numerous nerve cells undergo dystrophy and degeneration (Figure 88) and die. The nuclei of the degenerating neurons become dark, their contours become irregular, and they become cracked. The bodies of the neurons are compressed, wrinkled, and numerous; small and large vacuoles appear in the cytoplasm. After becoming wrinkled and pyknotic, the degenerating neurons gradually dissolve or undergo phagocytosis. This process of degeneration and death of neurons diminishes gradually with distance from the edges of the wound.

The most interesting changes occur outside the focus of damage in the nervous tissue of the operated and unoperated hemispheres. Phenomena of strong destruction and dedifferentiation of nerve tissue, intense stimulation of amitotic and, in some cases, mitotic divisions of neurons, and physiological regeneration of nerve tissue occur. These phenomena occur in both hemispheres, but are more intense in the hemisphere which was operated upon.

It is strongly evident already on the 1st day after operation, and even more so, on the 3rd–5th day that the nervous tissue of the hemispheres, in particular that of the operated hemisphere, stains a pale color. This decrease in staining intensity is preserved for a long time, 13–20 days, and does not completely disappear even 30 days after operation. It is due mainly to changes in the neurons. The blood vessels on the surface of the hemispheres in the pia mater,

and those penetrating the cortex, are dilated and filled with blood. In neurons with an intermediate degree of differentiation in the cortex of unoperated animals, there are round, quite light, but nevertheless thionine-stained nuclei, with a blurred middle-sized nucleolus, and cytoplasm with tigroid and dendrites (Figure 89, a). In the experimental animals, the neurons are strongly dedifferentiated and pale (Figure 89 b). The body and the nucleus of the cell become very light and round, and increase in dimensions, as if blown up. In the cytoplasm, the tigroid disappears and its remnants are finely dispersed. The nucleus increases in size and becomes round, the nuclear membrane is thin with sharp contours, and chromation granules stick closely to its periphery. Such activated or dedifferentiated neurons begin to divide intensively, amitotically. Amitosis begins with the division of the nucleoli. The nucleolus increases greatly in dimensions, and a particle splits off from it and migrates to the opposite pole of the nucleus, where it increases gradually (Figure 89 c) and becomes equal in dimension to the original nucleolus. This is the binucleolated phase of amitosis. Subsequently, around the nucleoli, nuclear substance accumulates, a septum is formed, and the nucleus divides in two (Figure 89 d). Sometimes a bridge is seen connecting the incompletely divided nucleus of the neuron (Figure 89 e). Later, the nucleus finally divides but remains included in one cytoplasmic body (Figure 89 f, g). This is the binucleate phase of amitosis. Following this, the body of the neuron divides (Figure 89, h). The main dendrite can divide longitudinally, or may migrate to one of the daughter cells, while it is evidently formed de novo in the other daughter cell. In general, in amitotic division of neurons, some of the dendrites, and probably the axon, are formed anew. It is possible that the nerve connections in the cortex of the cerebral hemispheres, and in the entire brain, change constantly and are remade, preserving at the same time their functional and morphological integrity in the central nervous system. This problem, as well as the fate of the axons of dividing nerve cells, should be specially studied using silver impregnation methods.

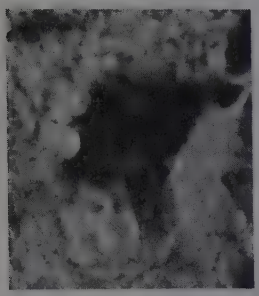


FIGURE 88. Dystrophy and degeneration of nerve cells in the cortex of the large hemispheres in the vicinity of the zone of damage in adult rats, 7 × 90

Neurons with 3, or even 4, nucleoli often appear, and after dividing, they continue to divide further, forming nests consisting of 3 or 4 neurons and corresponding satellite cells (Figure 89 i, j).

Stimulation of amitotic division of neurons, and their massive amitoses, are already observed after one day and reach a maximum 3—5 days after the operation. This process goes on both in the operated and in the nonoperated hemisphere.

The process of death of neurons which takes place in the nerve tissue of the cortex of the hemispheres is also enhanced, and at a distance from the focus of damage occurs in a manner different from that at the edges of the damaged area. Under the influence of implants many neurons become swollen, pale and burst, after which their bodies are compressed and decompose into fragments and grains, and these gradually dissolve and disappear (Figure 90). Neuronophagia does not take place. Instead of the lost neurons

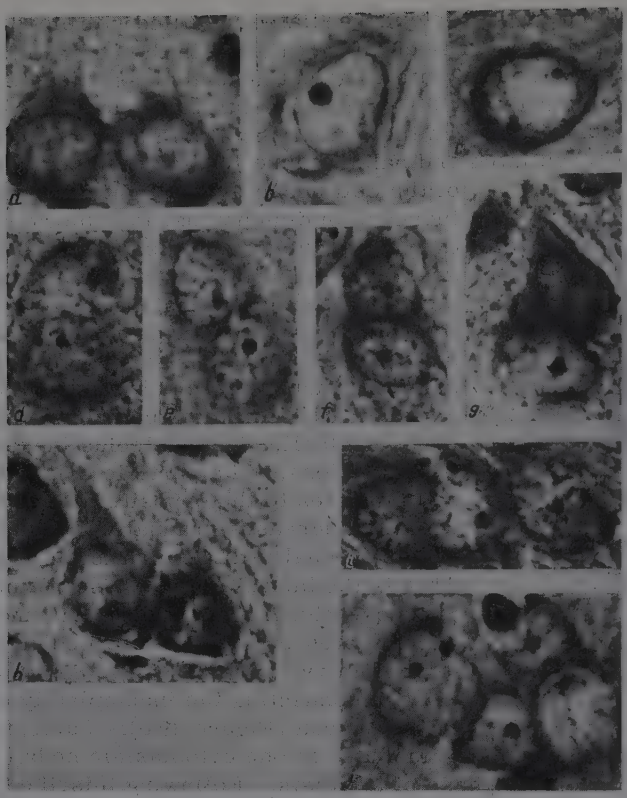


FIGURE 89. Consecutive stages of amitotic division of neurons in the cortex of the large hemispheres upon stimulation of physiological regeneration of the nerve tissue of the brain in rats, 10X90:

a – neurons in a normal animal; b – i – neurons one day after implantation of agar into the brain; d, c – binucleolated stage of amitosis of neurons; d, e, f, g – binucleate stage of amitosis in neurons; h – division of body of the neuron; i, j – division of neurons into 3 and 4 cells.

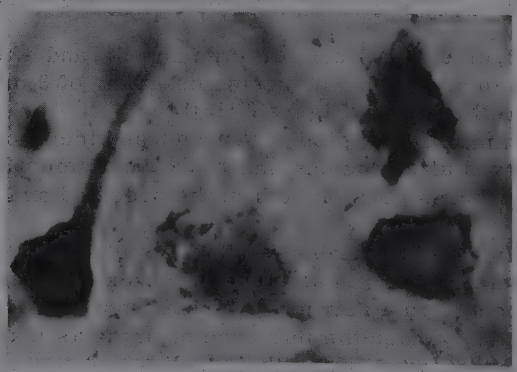


FIGURE 90. Degeneration and death of neurons 13 days after operation on the brain in rats, 7×90

empty spaces appear in the neuropil, which are rapidly filled with new young neurons (Figure 91). The latter have a round, light nucleus with a large nucleolus and a weakly developed cytoplasm with tigroid and characteristic dendrites. By 20—30 days after operation, a similar process of replacement of defunct neurons by new ones is also observed at the edges of the wound. A similar picture, described by E. V. Torskaya (1963, 1964), was seen in the cortex of the cerebral hemispheres of dogs revived after clinical death. The new neurons, according to our observations, arise in 2 ways: by amitotic division of differentiated neurons, and by dedifferentiation of cambial type cells which migrate into the gray matter from the white matter, subependyma, and ependyma. A number of continuous transitions from such cells to poorly differentiated and highly differentiated neurons takes place. This process of transformation of cambial cells into nerve cells, as we have already pointed out, also occurs in the brain of non-operated animals; it is greatly activated on stimulation of physiological regeneration. The cells of the ependyma become high, light and large, and it can be clearly seen that light cells emerge from them and migrate into the subependymal layer, and later into the white matter (Figure 92). When in the white matter, a nucleolus appears in the nucleus of the cambial type cell, and the cytoplasm forms unipolar or bipolar dendrites containing tigroid (Figure 93). Under the influence of stimulation, the glial cells in the gray matter (Figure 94a) and in its molecular layer (Figure 94b) also become similar to nerve cells. In the latter case, however, this phenomenon is temporary, and subsequently, the altered cells return back to their previous condition. It was not possible to follow the further fate of the altered glial cells in layers 2—7 of the gray matter of the cortex. Haranghi (1963) described transformation of glial cells into nerve cells after wounding the brain of adult mice.

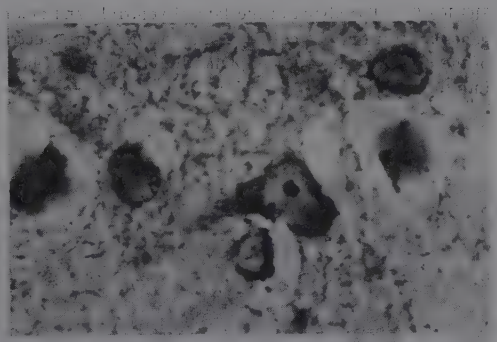


FIGURE 91. Appearance of young neurons in the place of those that died, at a distance from the focus of damage in the large hemispheres of rats 13 days after operation, 7x90

Mitotic division of neurons was observed only in the experiment with implantation into the brain of a piece of brain treated with trypan blue, and to a very limited extent in the experiment with injection of brain homogenate. Numerous prophases and metaphases, and few anaphases and telophases appeared around the implant in the cortex of the cerebral hemispheres between

3 and 10 days after operation (Figure 95 a, b, c, d). Ordinarily, during mitosis the neuron was surrounded by a vacuole with fluid, and alongside it was a smaller, glial, satellite cell. There were many mitoses in addition to amitoses among the glial cells (Figure 96 a, b); the mitoses were much more numerous in nerve cells than in glial cells. Dividing nerve cells retained signs which distinguish them from dividing glial cells and macrophages. Various phases of mitosis in neurons of the cortex of the cerebral hemispheres of a rabbit, following removal and autotransplantation of a conical piece of nerve tissue, have already been described by Saltykow (1905). In his experiments, as well as in ours, there is no doubt that organospecific and tissue-specific stimulation took place. It is characteristic that, in general, mitoses in neurons in the brain of adult mammals and man have been described only in cases of injury.

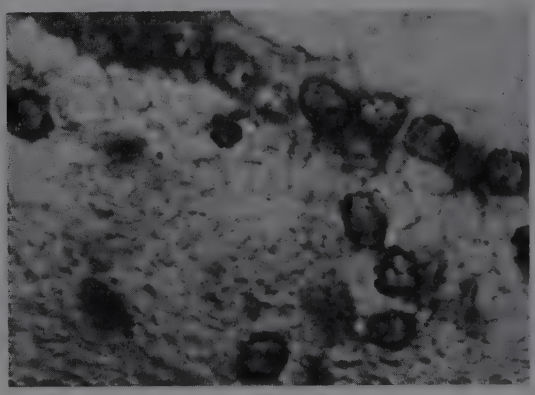


FIGURE 92. Ependyma of the lateral ventricle and emergence from it of light cells entering into the white substance of the cortex 5 days after implantation of a piece of brain into the brain, 7 × 90

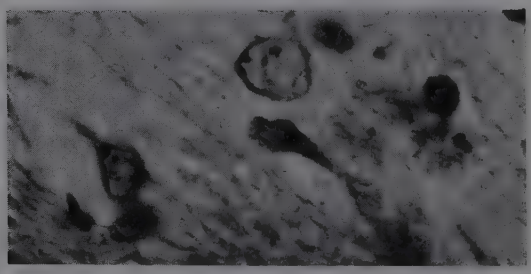


FIGURE 93. Glial cells in young neurons in the white matter of the cortex of the large hemispheres of rats, 7 × 90

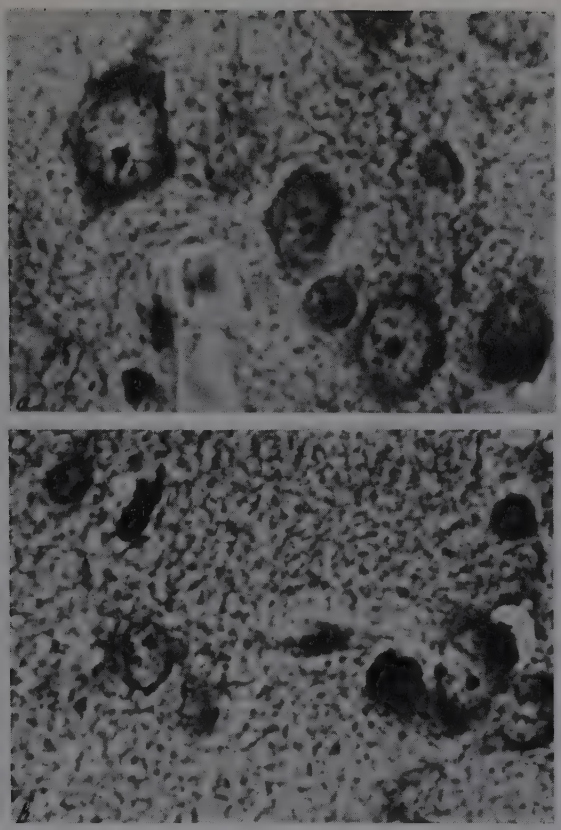


FIGURE 94. Activation and temporary appearance of glial cells as nerve cells in rats 5 days after implantation of a piece of brain into the brain, 7×90:

a – the 5th layer of the cortex of the large hemisphere; b – molecular layer.

Thus, we can see that the treatment we used in all the cases considerably stimulated physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres, but to different degrees: most strongly in the experiment with implantation of a piece of brain into the brain, less so in the experiment with the homogenate, and the least in the experiment with agar. In the first case, the decomposition products of nervous tissue and the factor of trauma played a role in the process; in the second case, basically the same products acted as in the first; and in the third case, trauma was the major factor. On the whole, the effect of these factors was the same. Various processes were stimulated: amitotic and mitotic division of neurons, and production and transformation of cells of the cambial type. The latter is of great importance for the understanding of processes of normal development and physiological regeneration of nerve cells, since a new, powerful source for the appearance of nerve cells in the brain is thus found. Suppression of its function may lead to pathological conditions: atrophy and withering of nerve cells and depletion of the nervous system. Its stimulation

may lead to normalization of pathological phenomena or, under conditions of a healthy organism, it may lead to enhanced higher nervous activity.

The last point we mention here is the migration of neurons from the second layer into the molecular layer of the gray matter of the cortex of of the cerebral hemispheres, which we observed in some experiments on implantation into the brain of brain or agar (Figure 97). In these cases, inflammation of the brain membranes usually took place; they were greatly emaciated, and neurons moved to such areas, resulting in a decrease in their numbers in the second layer. Consequently, despite their dendrites and the complex interwoven mesh between them, neurons from the cortex of adult animals have the capacity for translocation under certain conditions. In the normal state, such phenomena are considerably weaker: in the molecular layer, narrow chains or paths of nerve cells coming from the second layer are observed.

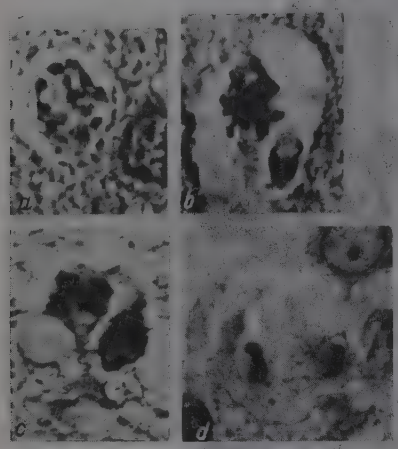


FIGURE 95. Mitoses in neurons 3 days after implantation of pieces of brain into the cortex of the large hemispheres of rats, 10x90:

a – prophase; b – metaphase; c – anaphase; d – telophase.

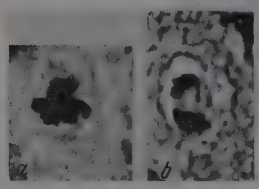


FIGURE 96. Mitoses in glial cells in the same experiment as in Figure 95, 10x90:

a – metaphase; b – telophase.

Continuation of experiments on the stimulation of physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres

The data we obtained indicated a number of goals: to provide quantitative proof for the stimulation of division of neurons in the cortex of the cerebral hemispheres; to establish which stimulators are the most effective; to determine the active principle in the decomposition products of nervous tissue, used by us as a stimulant for proliferation of nerve cells; to elucidate whether the stimulatory effect of preparations can be prolonged; to investigate the best means by which to introduce the preparation in order to effect stimulation; to establish whether the processes of stimulation and inhibition which basically characterize the higher nervous activity of animals are changed on administration of stimulants. These problems have been studied by K. Yu. Reznikov (1965), Reznikov and S. P. Kolchin (1964, 1965), Kolchin and Reznikov (1964, 1965), and Reznikov and V. M. Kondik (1966).

Experiments were performed on adult, white, male rats weighing approximately 250 g each. For qualitative estimation of the effect of stimulation on sections of certain parts of the cortex of the cerebral hemispheres,

the total number of neurons was counted (usually 5,000—6,000 cells), as well as the number of nondividing, mononucleate neurons, and the number of neurons at various stages of amitotic division: binucleolate, binucleate, and bicellular. Subsequently, we calculated the index of division of neurons, i. e., the ratio between the number of dividing neurons to the total number of neurons expressed as per mil (‰).

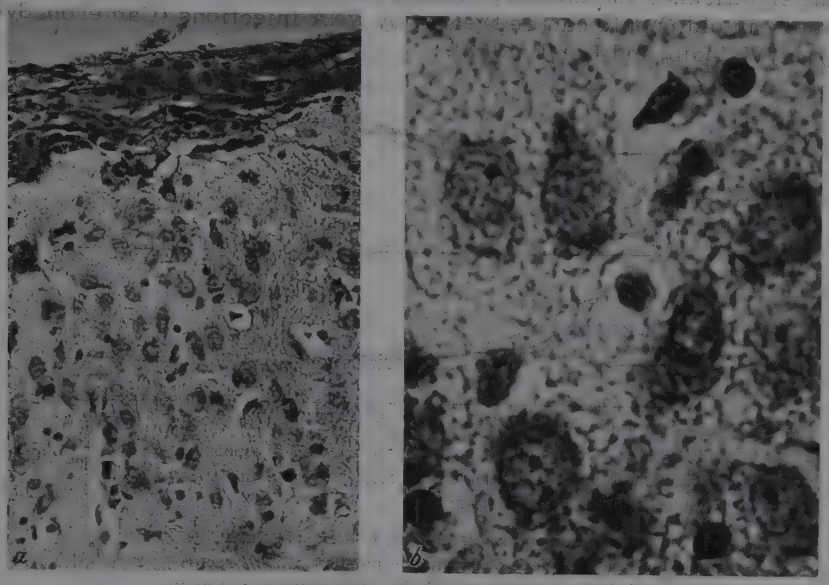


FIGURE 97. A stream of neurons migrating from the second layer of the cortex into the molecular layer, toward the sector of inflamed brain membranes in the zone of damage to the cortex, 5 days after operation;

a — 10×20; b — 7×90.

The study showed that in the normal cortex of the cerebral hemispheres, the number of dividing nerve cells remains at a certain constant level, while it changes regularly after a single administration into the brain of a preparation of a stimulant (Figures 98,99): it increases drastically and reaches a maximum after 3—5 days, subsequently decreasing and returning practically to the initial level after 10—17 days. At these times, the number of nondividing mononucleolate neurons regularly decreases, while the number of dividing binucleolate, binucleate, and bicellular neurons regularly increases. This means that upon stimulation, division of nerve cells indeed occurs, and at the expense of the decrease in the number of nondividing cells, the number of dividing neurons increases correspondingly. The intensity of stimulation is the same in the operated and nonoperated hemispheres.

In order to test the activity of stimulants in the brain, we introduced various preparations: 0.85% physiological saline, homogenate from the cortex of the cerebral hemispheres from adult rat brains, highly polymerized

freshly prepared RNA, which was very effective in our experiments on the restoration of lost regenerative capacity of the cardiac muscle in adult rats (Part II, Chapter 4) and of axolotl extremities after X-ray irradiation and suppression of regenerative capacity (see following chapter) When these methods were used for the administration of preparations, even an injection of physiological solution proved effective, but RNA was 5 times as effective. It must be stressed that, according to modern theories, RNA is of great significance for memory in man and animals and that weakening of memory in old people can be treated by RNA injections (Cameron, Sved, Solyom, Wainrib, and Barik, 1963).

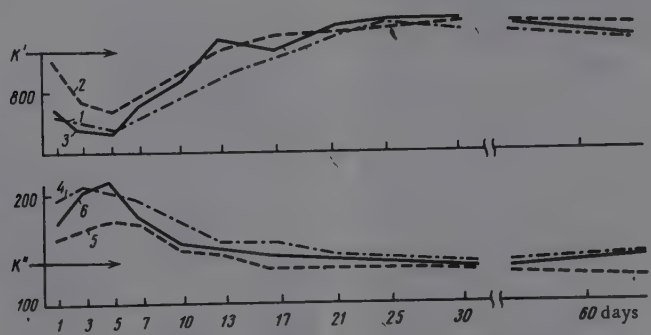


FIGURE 98. Dynamics of changes in the numbers of mononucleolated and binucleolated nerve cells following the administration of biostimulants into the brain:

1 – numbers of mononucleolated cells in the experiment with implantation of a piece of brain; 2 – numbers of mononucleolated cells in the experiment with the administration of a brain homogenate; 3 – numbers of mononucleolated cells in the experiment with RNA: K' – average arithmetic number of mononucleolated cells in nonoperated animals; 4 – numbers of binucleolated cells in the experiment with implantation of brain; 5 – numbers of binucleolated cells in the experiment in which a brain homogenate was used; 6 – numbers of binucleolated cells in an experiment with RNA: K" – arithmetic mean of the number of binucleolated cells for nonoperated animals (according to Reznikov, 1965).

On material obtained from autopsies in the Moscow Hospital of First Aid imeni Sklifosovskii, it has been shown that stimulation of division of nerve cells was not observed in the cerebral hemispheres of human brain from persons who had died of various diseases of the heart, kidneys, etc., which have no direct effect on the brain. However, in the cerebral hemispheres of persons who died at various times from trauma to the brain, a stimulation of the division of neurons took place, as it did in the case of injection of the aforementioned preparations into the brains of animals.

Trying to determine the active principle causing division of neurons, we designed an experiment with dialysis of a brain homogenate, the dialysate (dialysable fraction) being taken at high and low concentrations. It has been found that the nondialyzable fraction was inactive, a high concentration of the

dialysate was weakly active, while a low concentration of the dialysate (dilution $1:10^{-6}$) was very effective. Since proteins do not pass across the membrane upon dialysis, the active principle evidently must be of a non-protein nature and low molecular weight. A study should be conducted to determine the physical and chemical properties of the active principle. At present, we can say that the results of our investigations were similar to those of Muralt and co-workers (1946). The main difference was that in Muralt's experiments, the NR factor stimulated regeneration of peripheral nerves, i. e., of dendrites of nerve cells, while in our experiments, there was stimulation of division of neurons. In the experiments of Levi-Montalcini (1965) the active principle which stimulated the growth of dendrites of nerve cells was evidently of a proteinaceous nature, while in our experiments, the factor stimulating division of nerve cells was evidently of a nonprotein nature.

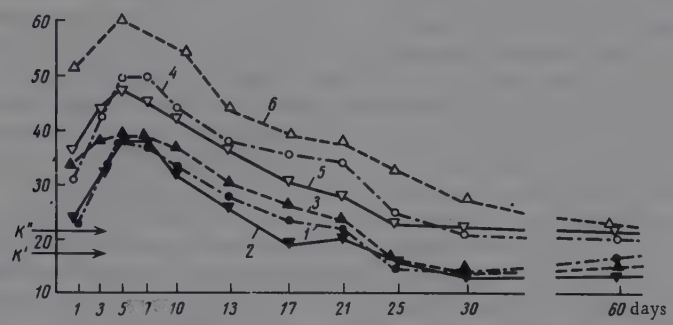


FIGURE 99. Dynamics of changes in the numbers of binucleate and bicellular groups of nerve cells upon the administration of biostimulants to the brain:

1 – numbers of binucleate cells in the experiment with implantation of brain; 2 – numbers of binucleate cells in the experiment with administration of brain homogenate; 3 – numbers of binucleate cells in the experiment with administration of RNA: K' – Average arithmetic number of binucleate cells for non-operated animals; 4 – numbers of binucleate groups of cells in the experiment with implantation of brain; 5 – numbers of bicellular groups of cells in the experiment on the administration of homogenate; 6 – numbers of binucleate groups of cells in the experiments with RNA: K" – average arithmetic number of bicellular groups of cells for the nonoperated animals (according to Reznikov, 1965).

Experiments with 4-times-repeated administration of stimulators into the brain at intervals of 3–5 days, showed that the effect of enhancement of nerve cell division in the cortex can thus be prolonged 4-fold. These data demonstrate the possibility of obtaining lasting division of neurons, a fact which is of great practical importance since brief stimulation of physiological regeneration of nerve tissue is, by far, not always sufficient.

It is very important to know by what route a preparation could be introduced in order to reach the desirable effect of stimulation of nerve cell division in the brain. The study showed that intramuscular or subcutaneous injection of RNA, or a dialysate of decomposition products of nervous tissue of the brain at low concentration, did not stimulate the

physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres. Evidently, the action of these stimulants was interfered with by the hematoencephalic barrier. Administration of these preparations directly into the brain allows overcoming this barrier and causes stimulation of neuron division. This intervention causes almost the same (somewhat weaker) effect as implantation into the brain of a piece of agar or a piece of brain, but it is physiologically more favorable. Implantation results in considerable destruction of nervous tissue and formation of coarse scars or cysts; this, of course, is no less harmful than leukotomy. Injection of a preparation into the brain with a syringe needle avoids the appearance of these phenomena; nevertheless, it is dangerous as a needle may wound the vessels in the brain and cause hemorrhage with all its complications. Further experiments showed that this danger, too, can be avoided and a stimulatory effect obtained if the preparation (RNA or brain dialysate) is administered through the spinal cord into the ventricles of the brain.

The question of whether processes of stimulation and inhibition in the central nervous system can be changed by injecting stimulators of neuron division is of great importance. In order to study this problem, we used the physiological method of Shon (1926), which allows the researcher to determine the degree of expression of certain phenomena by sleep duration signs, and by the time required for causing a sleeping animal to react to a stimulus (Kolchin and Reznikov, 1964, 1966). The control rats were given a certain dose of chloral hydrate intraperitoneally and were put to sleep. In the experimental group, the animals were, in addition, injected in the brain or cerebral ventricles with stimulators of neuron division (brain dialysate). The results were clear-cut and highly interesting. An injection of stimulators regularly and strongly changed the intensity of the processes of stimulation and inhibition and their relationships, in time, these changes being directly associated with morphological changes, particularly with enhancement of division of neurons in the cortex of the cerebral hemispheres. These data confirm our starting thesis that by changing the biology of the nervous tissue, the researcher can change in a controlled manner higher nervous activity, animal behavior, and, therefore, man's psychic conditions. Work in this direction may lead to highly important results, namely elaboration of new biological methods for the treatment of a number of neuropsychic diseases.

Sources of physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres, and neuronogenesis

As already pointed out, from experiments on rats, we concluded that physiological regeneration of the nervous tissue in the cortex of the cerebral hemispheres occurs in 2 ways: by amitotic division of nerve cells, and by differentiation of cambial-type cells formed from the ependyma and subependyma and migrating into the white and gray matter. The first source for the formation of neurons is quite clear, and its potency may be determined by calculating the amitotic index. The presence of the second source requires additional proof. If our conclusion is correct, that the brain of adult mammals and man has a source which continuously produces

cambial cells and thus replaces the neurons which die in the process of ontogenesis, then the concepts on the development of brain and neuronogenesis would change. According to the prevailing ideas, entered into textbooks on histology and embryology (Zavarzin, 1933, 1939; Zavarzin and Rumyantsev, 1946; Zavarzin and Shchelkunov, 1954; Knorre, 1959; Eliseev, 1964; Petain, 1959, and others), even in the time of Wilhelm His (1889), the development of the brain of mammals and man is as follows. During embryogenesis, a nerve tube appears with a widening in its anterior part. From the cells of the internal layer of the nerve tube, there is formed a multilayered matrix from which cells undergoing mitosis, migrate to the outside into the mantle layer and form neuroblasts, which are transformed into neurons and spongioblasts, which are transformed into glial cells. Gradually, the cells migrate further to the surface, and a mixture of layers is formed in the cortex. At a certain moment in embryogenesis in mice during the first days after birth, according to A. A. Manina (1964), the neurons stop dividing mitotically. The matrix transforms into a monolayer ependyma which does not produce neuroblasts and spongioblasts anymore. This basically terminates the development of the brain, and the mass of formed neurons survives throughout the life of the animal and man, or it diminishes progressively as a result of the death of some of them. According to these ideas, the lifespan of nerve cells is measured not in hours, days, or weeks, as is true for all the other cells of the animal and human body, but in many years and decades. However, nobody provided any direct evidence for this conclusion. Such profound stability of nerve cells contradicts the data of biochemists indicating an exceptionally intense metabolism in the brain neurons. It has been established by the use of labeled atoms that the rate of turnover of amino acids labeled with S^{35} methionine in the heterogeneous proteins of the brain, is extremely high. The period of the half-life of the alkali-soluble lipoprotein fraction of the microsomes, which is similar in composition to the Nissl substance, is 94 minutes (Gaiton and Richter, 1953). The slowest is the metabolism of the proteins in the nucleus, mitochondria, and the supernatant fraction, lasting 3—6 hours after the injection of S^{35} methionine (Clouet and Richter, 1959). Incorporation of labeled amino acids (leucine C^{14}) into the proteins of the brain is more rapid than it is into the proteins of muscles, but slower than into the proteins of the liver. The half-life of the proteins in the brain of a mouse, determined by the incorporation of leucine C^{14}, is less than one hour (Lajtha, 1959). The highest protein metabolism in rats and rabbits occurs in the ganglial cells. In these cells, the average lifespan of proteins, determined chemically, is 12.8 days, while that determined autoradiographically is 9 hours, and for glial cells it is 25 days. The average duration for the existence of proteins in the nerve cells of the brain is 50—70 times less than in skeletal muscle or connective tissue. More rapid turnover occurs in rats, mainly in the gray matter of the cortex of the large hemispheres, while in the white matter and the myelin membranes, the proteins are metabolically inert (Davison, 1961).

The concept of the stability of nervous tissue in the cortex of the cerebral hemispheres, of its incapacity for structural, cellular turnover, was based on data demonstrating the absence of mitoses in neurons of the cortex in adult animals, the absence of matrix in these cells and of neuroblasts, and the incapacity of cortical nervous tissue for reparative regeneration. However, all these data must be revised at present. Histologists have for a long time

exaggerated the significance of mitosis. Now it has been quite well established that amitosis is not an abortive, but a normal cell division for tissues in a number of organs. Rampan (1956—1961) and other investigators (see preceding in this chapter) showed all the consecutive phases of amitotic division in neurons of mammals, birds, and man, and we were able to confirm this finding from our material (brains of rats, cats, and frogs). The incapacity of the cortex of the cerebral hemispheres for reparative regeneration can probably be explained not by the incapacity of neurons for division, but by the lack of conditions which are essential for the development of nervous tissue: presence of a fibrous structure, tension, etc. which is essential for the migration and differentiation of nerve cells. Instead of these conditions, which are essential for the organogenesis and histogenesis of the brain, scars appear after mechanical damage to the cortex, and these interfere with its reparative regeneration. Probably, incapacity for reparative regeneration is also due to the loss of the nervous tissue's capacity for destruction and dedifferentiation, characteristic of the brain of frog tadpoles, but absent in the brain of mammals and man. Data on this, as well as those showing the presence of cambial-type cells in the nervous tissue of the brain, were obtained in our experiments on partial decortication in kittens (Polezhaev and Reznikov, 1966).

In our experiment, we used kittens aged 2—5 weeks. Under aseptic conditions and urethane narcosis, we removed the nervous tissue in the parietal or occipital region of the right hemisphere from a 20×15 mm area, 5 mm deep. We removed sectors 1, 2, and 3 of the arched convolutions above the Sylvan fissure and the entire occipital region encompassing the g. suprasylvius medius, g. suprasylvius posterior, g. postlateralis, or g. ectosylvius medius, g. suprasylvius medius, and g. postlateralis.

We stitched the cut in the dura mater with silk or covered it with a piece of a fibrin film, stretched on a cellophane membrane. From above, the area of the defect was covered with a previously cut out rectangular piece of bone, and the skin was sewn up. Histological examination of the nervous tissue in the region of the brain was conducted at frequent intervals from one day to 5 months after the operation.

As pointed out earlier, there was no reparative regeneration of nervous tissue in the cortex of the cerebral hemispheres. In the regions of the defects, cysts with fluid, or hyaline-connective-tissue scars appeared, or the area was filled with displaced surrounding nervous tissue. Destruction of nervous tissue was very intense at the edges of the defect, and numerous neurons died. Additionally, we observed a fair number of mitoses which later died at the stages of prophase and metaphase, a finding which is in agreement with observations of other investigators (Soskin and Grigor'eva, 1964; Soskin, 1965). There were numerous amitoses in neurons and, on the whole, their description coincides with that found in experiments with rats (see preceding).

The changes in the ependyma, subependyma, and white matter in the operated and nonoperated cerebral hemispheres of kittens were, on the whole, similar to those in rats. The ependyma was thickened, and light and dark cells from the ependyma entered the subependymal layer and migrated into the white and gray matter of the cortex. Evidently, under the influence of trauma, these cells transformed into neuroblast-type cells, not only in the white matter, but also in the subependymal layer, in the vicinity of the ependyma (Figure 100). These are cells with a large, round nucleus, large nucleolus, and uni- or bipolar dendrites of the cytoplasm with tigroid, and

they are quite numerous in the white matter. There are a number of transitions from little-differentiated small cells to more highly differentiated cells of the neuroblast or neuron type (Figure 101). These cells are numerous in the cortex of the cerebral hemispheres in 2–3 week-old kittens, but they are absent in 5-month-old animals.

The foregoing results justify the assumption that differentiation of cambial type cells into nerve cells occurs not only in the brains of rats, but also in cats, and probably also in other mammalian species, including man. This problem must be further studied. The assumption may be made that differentiation of nerve cells from cambial cells is stimulated by the factor of trauma or by the action of tissue-specific and organospecific substances released from the damaged nervous tissue. Adguhr (1920) described the presence of nerve cells in the vicinity of the ependyma of the spinal cord of a bull. Altman and Altman (1962) observed neuronlike cells in the chiasma of the optic nerves in pigeons after unilateral removal of an eye.

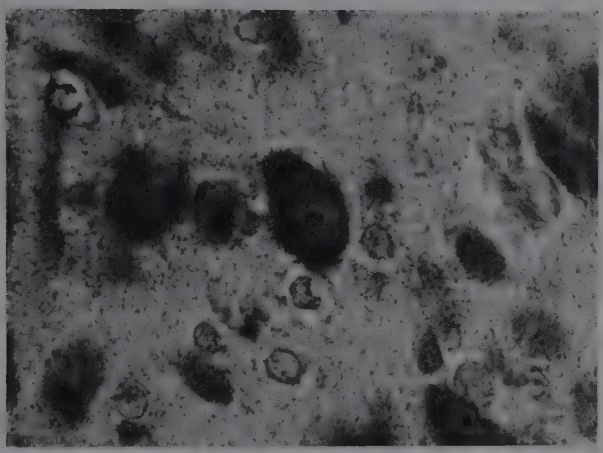


FIGURE 100. Formation of nerve cells in white matter in the vicinity of the ependyma in a kitten 3 days after operation, 7×90

Rampan (1956—1961) voiced the opinion that the ontogenetic and phylogenetic development of the nervous system, and in particular of the brain, in vertebrates and man proceeds by quantitative addition of dividing (amitotically) nerve cells. Altman's (1962), data, based on experiments in which he damaged the corpus geniculatum and studied autoradiographically the brains of adult mice, using thymidine-H^3 as a precursor of DNA synthesis, are in agreement with the very important conclusions just reported. Silver grains were found not only in the glial cells which divided mitotically, but also in numerous neurons surrounding the focus of damage. This indicates DNA synthesis or division of neurons. Similar data were obtained by other investigators (Angevine and Sidman, 1961). American investigators (Altman and Das, 1965, 1965a, 1966, 1966a) showed by autoradiography that in rats, in the postnatal period of ontogenesis, microneurons appear in the brain, and that in the apendyma and subependymal layer of the third and lateral

ventricles, there are indifferent cells labeled with thymidine-H^3, which migrate into the hippocampus and there differentiate into neurons. According to recent data, incorporation of thymidine-H^3 may take place in cells which divide amitotically as well as in mitotically dividing cells (Khrushchov and Zaborskaya, 1964; Brodskii, 1965). In addition to this pathway of nerve cell formation there may also be another, their appearance from cambial cells as indicated by the results of our investigations.

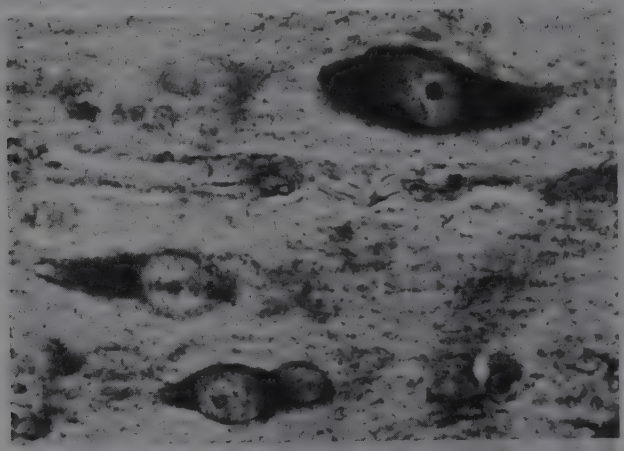


FIGURE 101. Formation of nerve cells in the white matter at a distance from the ependyma in a kitten 3 days after operation, 7×90

It is now important, using new and modern methods, to review the entire problem of neuronogenesis during the development of the brain in mammals and man, this not having been extensively studied since the time of His (1889).

Induction of neuronlike cells

The extensive material (160 cases) in our experiments on partial decortication in 2–5 week-old kittens allowed us to make an unexpected and, at least to us, very interesting observation. In those cases in which the brain wound remained open, or when the dissected dura mater above it was sewn up, there was proliferation of a glial-connective-tissue scar and formation of cysts with fluid. In some of the other cases, when the brain wound was covered with a fibrin and cellophane membrane, special neuronlike cells appeared in the glial-connective-tissue capsule formed around the membranes. They appeared in the capsule, below it, in the region of the wound, but at a distance from the residual nervous tissue from which they were separated by a large blood clot, and they even appeared outside the membranes. In a number of cases, these were separate cells (Figure 102 a–c), but in other cases, there were large accumulations or nests of cells (Figure 103). Neuronlike cells appeared between 3 and 30 days after the operation, and then disappeared, losing their specific features and acquiring the appearance and shape of connective-

tissue cells. Neuronlike cells are bubblelike, square, triangular, or elongated with one long main dendrite, a cytoplasmic appendage, and they are uni- or bipolar. The nucleus is large, round, light, with a large nucleolus. The cytoplasm forms appendages resembling the appendages of nerve cells or neuroblasts, or an uneven, quite narrow ridge of cytoplasm resembling the cytoplasm of nerve cells. The cytoplasm of the neuronlike cells stains intensely with thionine according to Nissl, similarly as in nerve cells. The neuronlike cells are mostly large, considerably bigger than connective-tissue cells; however, there are also cells of smaller dimensions among them. In some cases, in their external appearance, they are very similar to large nerve cells with a large tapering main dendrite.

Forming accumulations, the neuronlike cells sometimes consist of several dozens of cells. They are located in vacuoles or cavities filled with fluid. Sometimes the accumulations resemble one giant nerve cell in shape. In contrast to giant multinucleate cells, they do not have a syncytial structure, they do not have one common cytoplasm in which a great number of nuclei are concentrated. In the accumulations, the cytoplasmic borders of the individual cells can be clearly seen.

We concluded that the appearance of neuronlike cells can be explained by induction, as being a result of an organospecific or tissue-specific effect of decomposition products of nervous tissue (inducer), which are abundant in the brain wound on the plastic reacting material (evidently, cells of the polyblast type, or cells accompanying the cerebral vessels, or fibroblasts, or some other cells), under such conditions that a capsule forms from this material around the implant (condition of tension). Formation of true nerve cells does not occur, as the induced cells differ in shape from neurons, and in addition, they do not live very long. Nevertheless, the phenomenon of induced formation of neuronlike cells is well established.

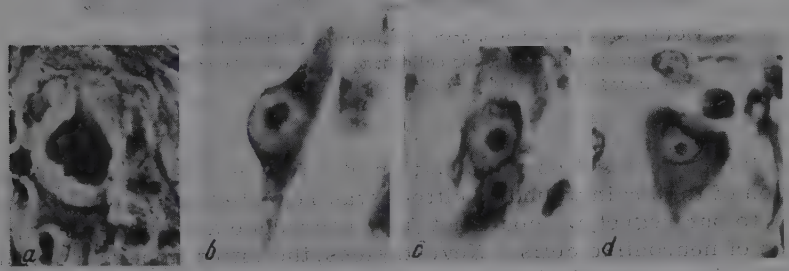


FIGURE 102. Induction of neuronlike cells (a–d) in the connective tissue capsule which has arisen around the cellophane membrane transplanted into the dissected area of the dura mater above the brain wound of a kitten 3 and 7 days after operation, 10×90

Having made this observation, we designed some additional experiments to check it (Polezhaev and Cherkasova, 1966). First, we repeated the following experiment: we removed a piece of nervous tissue from the right cerebral hemisphere in kittens aged 2—4 weeks, and covered the brain wound with a piece of fibrin and cellophane membrane. In addition, we homoplastically implanted into the connective tissue under the skin of the head of these kittens an inducer, a piece of nervous tissue from the cortex of the cerebral hemispheres, which had been kept for 5—6 hours in a 1% aqueous solution of

trypan blue according to Levander (1956). A similar experiment of homoplastic implantation of an inducer under the skin of the flank was performed on adult rats. In these experiments of implanting a piece of nervous tissue (inducer) under the skin, we could elucidate the question of whether cells of subcutaneous connective tissue can react in the inducer and transform into neuronlike cells; whether there is any regional difference in the reactive capacity of connective tissue cells in the area of the head and in the lateral area, and whether induction could be obtained in animals of various species (rats and kittens) of different ages. Levander worked only with adult rabbits. In our experiments on induction of the bone of the cranial vault, we used dogs, rats, and mice; in experiments on the induction of dental tissue, we used dogs; and we used rabbits in experiments on induction of cardiac muscle.

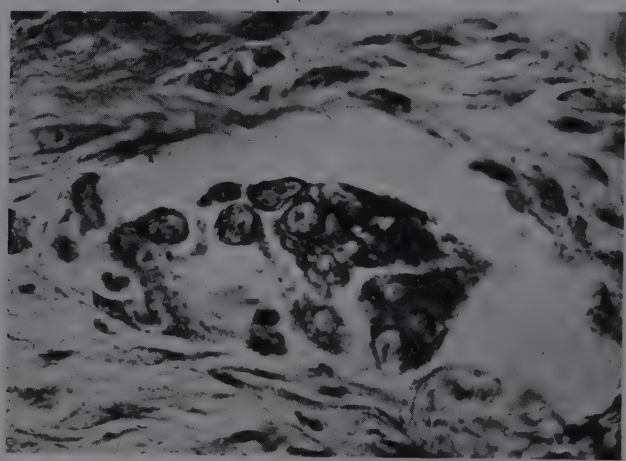


FIGURE 103. Induction of nests of neuronlike cells formed in the connective tissue capsule around the cellophane membrane placed above the brain wound of a kitten 7 days after operation, 7×90

When the experiment on implantation of a fibrin-cellophane membrane in the region of the brain wound in kittens was repeated on a limited number of animals, to our regret, we could not fully reproduce a distinct effect of induction of neuronlike cells. Nevertheless, the latter appeared in the connective tissue capsule formed around the implant. We could not establish precisely the origin of these cells. Evidently, they arose from polyblasts, fibroblasts, or cells which accompanied the vessels in the pia mater.

In our experiments on implantation of a piece of brain under the skin of kittens and rats, we observed reactive changes of fibroblasts during a period of 1—3—5 days after the operation. In the experiment on kittens these changes were more strongly manifested when the inducer was implanted under the skin of the head. The fibroblasts became bubble-shaped, triangular, or square. The nucleus became round and light, and the nucleolus enlarged. The cytoplasm formed either long appendages or a margin around the nucleus, and stained intensely with thionine (Figure 104, a, b, c). Similar, but less intense changes were observed in experiments on adult rats in which

inducers were implanted under the skin of the flank (Polezhaev and Karnaukhova, 1962).

Our data on induction of neuronlike cells should be compared with the scanty published data on the transformation of nerve cells into cells of a different type of tissue, and of the tissue into nerve cells. In the aforementioned work of Haranghe (1963) it was stated that after infliction of a pierced-cut wound in the brain of a mouse, some time later glial cells not far from the area of the wound began to change and become more like nerve cells. The structure of the nucleus changed, a nucleolus appeared, and appendages grew out from the cytoplasm, and the cell became similar in shape to a nerve cell. Benitez, Murray, and Chargaff (1959) added to a fibroblast culture grown outside the body of an adult rat, a certain amount of RNA or a microsomal fraction obtained from liver, kidney, or yeast. As a result, the fibroblasts changed heteromorphously and became very like nerve cells morphologically. The change was temporary and depended on the concentration of the added preparations and on other conditions. These data are very similar to ours. In our experiments on the stimulation of regeneration of cardiac muscle by injection of rats with RNA, however, no such changes in fibroblasts were seen. Evidently, the result of the experiment depends greatly on the experimental conditions.

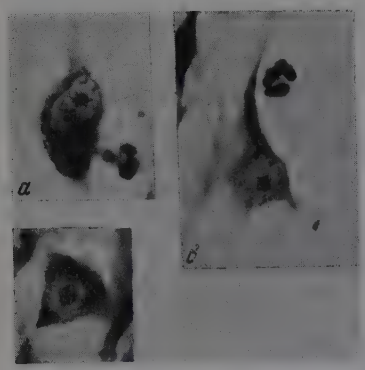


FIGURE 104. Induction of neuronlike cells (a, b, c) from fibroblasts under the skin of the head upon implantation of a piece of brain and a cellophane membrane 5 days after operation, 10×90

The experimental data obtained by K. A. Lavrov (1960) are of great interest*. This investigator implanted into the brain of an adult rabbit pieces of skeletal muscles from a rabbit embryo. Induction of malignant tumors in the brain and a simultaneous deep change in the glial and nerve cells occurred. The nuclei of these cells and the body surrounding them retained their specificity, but their appendages and the neuropil were transformed into striated muscle fibers. Peculiar "neuromuscular hybrids" appeared. This phenomenon was massive and diffuse. Evidently, it was due to malignant changes of the nervous tissue, under the influence of the implants which greatly enhanced its plasticity.

Similar results were obtained in Wilde's (1961) experiments on organotypical differentiation of dissociated cells in a tissue culture outside the body. Cross striation appeared in the bodies of the developed neuronlike cells; they were characteristic of muscle fibers, or melanin pigment.

This data from published reports as well as our own, show that under certain conditions, nerve and glial cells, even in adult mammals, are capable of transformation which proceeds like embryonic induction. Consequently, by creating such conditions, the direction of development can be changed in a controlled manner, as can the quality of cells and tissues; metaplasia can be caused even in the highly differentiated nervous tissue, or its cells made to

* These data were reported by the investigator in 1959 in Leningrad at a conference of histologists on the subject of "Determination and Plasticity of Tissues," dedicated to the memory of academician A. A. Zavarzin.

appear similar to connective tissue cells. This highly important problem, both theoretically and practically, must be further studied.

Conclusion

As a result of an analysis carried out on the basis of data from the published reports and our own experiments, we reached the conclusion that it was essential to revise the ideas concerning the capacity for regeneration of nervous tissue in the brain, and of the entire central nervous system of vertebrates, and in mammals and man in particular. The capacity for reparative regeneration of the brain was demonstrated in embryos, larvae, and adult lower vertebrates: fishes, caudate and tailless Amphibia. It was not found in mammals even at the early stages of postnatal ontogenesis. The brain of mammals, and probably of man, however, is capable of physiological regeneration and, at that not only during embryogenesis, but also in adults. This conclusion is based on the following groups of facts.

1. On one hand, death of nerve and glial cells may be constantly observed in the nervous tissue of the brain at various stages of normal ontogenesis of mammals, while on the other hand, their multiplication may be observed. The glial cells multiply amitotically and mitotically, while the nerve cells multiply mitotically only until a given stage of embryonic and, in some cases (e.g., in mice), of early postnatal development; after this stage they divide only amitotically.

2. In addition to multiplication, nerve and glial cells can be formed in another way, by originating from the matrix or cambium. It had already been known that in embryogenesis, neuroblasts producing neurons, and spongioblasts producing glial cells, originate from the matrix; both the neurons and the glial cells migrate from within, from the matrix to the surface of the cortex of the cerebral hemispheres. In adult mammals, as has already been shown by autoradiography, cells constantly arise from the subependymal layer of the ventricles, multiply, and migrate into the white and gray matter of the cortex of the cerebral hemispheres, later being transformed into glial cells: oligodendrocytes and astrocytes. According to our data, in adult rats and kittens aged 10 days to 5 months, ependymal cells continuously emerge from the monolayer, enter into the subependymal layer, and migrate from there into the white and gray substance to the surface of the cerebral hemispheres. These are cambial cells. They form not only glial, but also nerve cells. The latter are present in the white substance and sometimes also in the subependymal layer. Evidently, they are also present in the gray matter, as there are cells there which cannot readily be determined as nerve cells or glial cells. Under the influence of brain trauma, or following the administration into the brain of decomposition products of nervous tissue, these cells and some of the cells which are present in the molecular layer of the cortex of the cerebral hemispheres temporarily acquire the shape of neurons.

3. Experiments with cultures of nervous tissue from the cortex of the cerebral hemispheres outside the body, taken from a number of mammalian species and man, show that nerve cells may live and multiply for a long time, up to one year.

4. Replacement of dead neurons by new ones occurs following the action of ionizing radiation, some extreme conditions (revival of animals after clinical death, etc.) and other pathological phenomena.

Thus, we reached the conclusion that the nervous tissue of the cortex of the cerebral hemispheres, of other parts of the brain, and of the entire central nervous system is not stable, but is in a state of constant physiological regeneration, with a continuous turnover, not only molecular but also structural and cellular, during all periods of mammalian and human life.

Published data show that a number of chemical factors (extracts from muscle sarcoma, snake venom, protein extracts from submaxillary glands or sympathetic ganglia, nonprotein extracts from nervous tissue of the brain and spinal cord — NR) may stimulate the growth of nerve dendrites and hypertrophy of neurons.

Our experiments on adult rats and kittens show that physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres can be considerably stimulated if it is acted upon by decomposition products of nervous tissue from the brain, or by a highly polymeric, freshly prepared RNA. The introduction of these substances into the brain, as well as the factor of brain trauma, cause intense destruction and dedifferentiation of nervous tissue. Some of the cells die; the remaining glial and nerve cells become activated and as if rejuvenated. The nuclei of the neurons increase in size, become round, light, and large nucleoli appear in them. The cytoplasm becomes light, the tigroid in it disperses and disappears, and the appendages increase in size. Such activated neurons divide intensely amitotically, passing through 3 stages: binucleolate, binucleate, and bicellular. In the case of a very strong effect, mitotic division of neurons appears; being abortive in many cases, however, it reaches the stage of metaphase, and then the cells die. Glial cells multiply intensely mitotically and amitotically.

On the other hand, stimulating effects strongly activate the processes of production of cambial-type cells from the ependyma and the subependymal layer, their migration into the white and gray matter, and the transformation of some of them into nerve cells. The later are seen particularly clearly in the white substance and in the molecular layer of the cortex of the cerebral hemispheres.

Under certain conditions, under the influence of decomposition products of nervous tissue from the brain, some connective-tissue cells and other cells (evidently cambial and glial and cells accompanying the cerebral vessels) may transform into neuronlike cells. A process of induction of neuronlike cells occurs. Similar phenomena were also obtained in a culture of fibroblasts in vitro under certain other experimental conditions.

Upon stimulation of physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres, a regular change occurs in the functioning of the higher nervous activity, the processes of stimulation and inhibition. Thus, making controlled changes in the physiological regeneration of the nervous tissue of the brain permits regulation of higher nervous activity, behaviour of animals and, in principle, the psychic activity of man. This opens up a new way for combating neuropsychic diseases on the basis of regulation of the biology of the nerve cells and of the physiological regeneration of nervous tissue.

The new data radically change the ideas on development, regeneration, and pathology of the nervous system that have prevailed. The incapacity of

the nervous tissue of the brain and spinal cord for regeneration is due not to the loss of the capacity of nerve cells for division, as had been assumed earlier, but to the lack of conditions essential for organogenesis and histogenesis: the lack of a certain fibrous structure necessary for the migration of nerve and glial cells, conditions for their interaction with the brain membranes, etc. These conditions are present in the normal nervous tissue of the cortex of the cerebral hemispheres of the brain, and they are absent when the brain is damaged and becomes scarified. The data on the presence of cambial cells in the brain suggest the need to reconsider the problem of neuronogenesis. A number of phenomena in the pathology of the nervous system, and in particular of the nervous tissue of the brain associated with its emaciation and atrophy, may be considered as resulting from suppression of its physiological regeneration. Therefore, stimulation of the latter may serve as a new, powerful means for treating such diseases and some other pathological phenomena in the central nervous system, and in the brain in particular. Thus, the direction in the study of regeneration we developed opens up new prospects for the study of development, regeneration, and physiology of the nervous tissue of the brain, this being of great importance for biology and medicine.

Chapter VI

RESTORATION OF REGENERATIVE CAPACITY SUPPRESSED BY X-RAY IRRADIATION

Introduction and statement of the problem

New problems have arisen in biology and medicine in connection with the intensive elaboration of the problem of nuclear irradiation. It is well known that at certain dosages ionizing radiation, and X-rays in particular, causes radiation sickness when the total body of man and animals has been irradiated, and that local irradiation causes local injuries to tissues: erythema, atrophy, formation of radiation ulcers which do not heal for a long time, and which are transformed into malignant tumors in many cases. Modern biology and medicine do not yet have effective means to combat the general and local radiation changes. It is understandable, therefore, that the struggle against radiation changes is one of the most concrete modern biological and medical problems, in the elucidation of which large teams of biologists, physicians, physicists, and chemists participate. We attempted to approach this problem from the positions of experimental morphology and the theory of regeneration, turning our main attention to local radiation changes.

It is known that at a given dose, local X-ray irradiation causes local radiation changes on the one hand, and inhibition or suppression of the capacity for reparative regeneration of organs and tissues on the other hand. In this connection, a hypothesis evolved that local radiation damage is due to suppression and distortion of the regenerative capacity of the irradiated tissues (Furth, Upton, 1954; Mole, 1958; Gorizontov, 1958; Kraevskii, 1960; Strelin, 1960; Polezhaev, 1959; Polezhaev and Ermakova, 1960; Studitskii, 1961, and others). From this hypothesis, it follows that if regenerative capacity suppressed by irradiation could be restored, local radiation injuries could be cured, and subsequent malignancy of irradiated tissues prevented. However, formulating this hypothesis, none of our predecessors tried to restore the regenerative capacity suppressed by irradiation, since they did not have any approach to this problem, and since biology and medicine accepted until very recently the concept that irradiated cells are irreversibly damaged. We made the attempt because, in the course of many years, we had been specially interested in elaborating the problem of loss and restoration of regenerative capacity of organs in the processes of ontogenesis and phylogenesis of animals. This work allowed us to establish the rule of loss of regenerative capacity during ontogenesis and phylogenesis of animals, and to propose a number of biological methods for its restoration.

On this basis, we decided to check the efficacy of some of these methods for the restoration of regenerative capacity suppressed by X-ray irradiation. This attempt met with success, and we could show that restoration of regenerative capacity suppressed by irradiation prevents or cures local radiation injuries.

Data from the literature

In the history of the problem of change in the capacity for reparative regeneration of organs and tissues in animals, several consecutive stages may be distinguished.

First, it has been established that a given dose of X-ray irradiation inhibits and suppresses the capacity for growth and development of organs and tissues. At that, it has been found that various tissues have different degrees of radioresistance; the most sensitive to X-rays are embryonic and little differentiated cells and tissues characterized by high mitotic activity. This statement was called the law of Bergonier and Tribaundo (Bergonier et Tribaundo, 1906). As is well known this law serves as the basis for radiation therapy of malignant tumors: at a certain dose, X-rays suppress development (destroy) of little differentiated cells of carcinoma or sarcoma, preserving the viability of the adjacent healthy differentiated tissues. Tissues of adult mammals can be arranged in a certain order in regard to increase in radio-resistance to X-rays; lymphoid tissues, bone marrow, thymus, ovaries, testes, mucous membranes, salivary glands, hair follicles, sweat and sebasceous glands, epidermis, pericardium, lungs, digestive glands, thyroid gland, connective tissue and vessels, muscle tissue, cartilage and bone tissue, and nervous tissue (Graevskii and Shapiro, 1957). Similar data are given by B. Raevskii, (1959) and Fritz Niggli (1961). At a given dose, X-ray irradiation also inhibits and suppresses the capacity for reparative regeneration of organs and tissues in lower and higher animals. This has been shown in Hydra (Zavarzin and Strelin, 1929), Planaria (Curtis and Hickman, 1926; Curtis, 1928; Shevchenko, 1938; Wiegand, 1930), roundworms (Stone, 1932, 1933; Zhinkin, 1932, 1934, and others), caudate and tailless Amphibia (see Brunst, 1937; Brunst, 1960), and also in mammals in which the regeneration of muscles, nerves, bone, skin, and other tissues was studied (see Mikhailov, 1959, 1965).

It must be pointed out that the dose of irradiation which suppresses the capacity for reparative regeneration of various organs and tissues in different animal species, and in animals of the same species at different ages, will always differ and will always be higher in higher animals than the dose which causes radiation sickness from total body irradiation. Therefore, we agree with V. I. Mikhailov (1965), that the regeneration of the different tissues changes little upon total body irradiation of animals (rabbits, rats, mice, dogs, etc.) with a dose of 500–800 r, which causes radiation sickness. This is quite understandable as the dose which suppresses the regeneration of muscles is 2,000—3,000 r, and that which suppresses the regeneration of epithelium is 5,000—10,000 r, etc.

The next stage in the study is a cycle of works showing that upon transplantation of nonirradiated tissues into irradiated organs, regeneration takes

place, the source of which is the cells of the transplants. These studies were initiated by Umanskii (1937), who irradiated the hind extremities of axolotls up to the body wall with a dose of 7,000 r, protecting the remaining part of their bodies with lead, and thus suppressed the capacity for regeneration of organs. When he homoplastically transplanted cuffs of skin or muscle or tubular bones of nonirradiated extremities into the irradiated extremities, and amputated the extremities in the zone of the transplants, regeneration of organs corresponding to the origin of the transplants took place. Thus, upon transplantation of skin or muscles from extremities into the irradiated extremities, regeneration of extremities occurred, while transplantation of skin or muscles from the tail, resulted in the regeneration of tail-like organs. Consequently, transplanted nonirradiated tissues were the source of regeneration. The irradiated tissues, as Umanskii assumed, changed irreversibly and completely lost the capacity for regeneration. The facts established by Umanskii (1937) were confirmed by a number of investigators (Umanskii and co-workers, 1938—1953; Thornton, 1942; Trampusch, 1951, 1957, 1958, 1959; Stinson, 1963, 1964; Lazard, 1964, and others). Experiments were made in different modifications. In axolotls, they irradiated the forelegs and the hind extremities or tails, and transplanted various nonirradiated tissues taken from other organs into the irradiated organs. The result was always the same: the transplanted nonirradiated tissues were the source of regeneration.

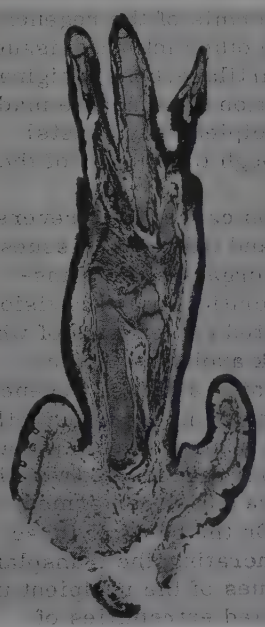


FIGURE 105. Longitudinal section of an axolotl extremity, regenerating after implantation of destroyed muscles from extremities of newborn mice into the skin sleeve of an axolotl extremity; 104 days after operation. The regenerating tissues (skeleton, muscles, connective tissues, skin) are seen on a basis consisting of skin, connective tissue, and the remnants of the transplanted tissues.

Recently Trampusch (1965) announced that if a previously prepared nonirradiated nerve implanted under the lateral skin is brought into the stump of a denervated and irradiated extremity of axolotl, regeneration of the extremity occurs. However, if pieces of nonirradiated spinal cord are implanted into the stump of irradiated extremities, finlike organs regenerate. Regretfully, in this very interesting preliminary communication, much still remains unexplained: the dose of irradiation is unknown; it is not clear what the source of regeneration was: nonirradiated connective tissue cells, carried along with the nerve or the implanted spinal cord, or local irradiated tissues of the recipient. Therefore, the final opinion on these data should be deferred until the investigator's next publication.

Similar experiments were also performed by us without irradiation (Polezhaev, 1960). We amputated the hind extremities of axolotls close to the body with exarticulation at the iliofemoral joint. Only the skin cuff, the sciatic nerve, and the femoral artery were left in place. Subsequently we implanted into this sleeve, ground muscles from the extremities, pancreas, or spleen of newborn mice or rat embryos. The nerve and the artery were

pulled through the implant, and the skin sewn up. Several days after operating, we cut off the skin at the distal end of the organ. As a result, the extremities of the axolotls regenerated, nevertheless, after a long lag, 90—100 days after the operation. The implanted tissues survived, remained in place, and in the form of a thick cushion, separated the tissues of the regenerate from the tissues of the recipient (Figure 105). The skin was the source of the regenerate of the extremity. The epidermis of the regenerate originated from the epidermis of the skin, and all the other internal tissues of mesodermal origin (muscles, connective tissue, cartilage, bone) originated, therefore, from the dermis of the skin. The assumption also may be made that the connective-tissue and muscle cells of the recipient (myoblasts) penetrated the wound surface of the organ, going through the thicket of the implant tissues, an action which is less likely.

However, Umanskii's opinion (1937) that irradiation causes an irreversible suppression of the regenerative capacity of tissues, and that these tissues, as a result, cannot participate in the regeneration of organs after transplantation of nonirradiated tissues, was subjected to criticism and revision. E. Ch. Pukhal'skaya (1940) transplanted to nonirradiated extremities of white axolotls the skin from irradiated extremities of black axolotls. After amputation, a white extremity with some black irradiated skin was regenerated. Liozner (1941, 1946) transplanted into irradiated extremities of axolotls pieces of muscles and skeleton taken from the tail of nonirradiated animals. After amputation through the transplant, tail-like organs regenerated with the participation of the skeleton of the recipient. Sidorova (1949) performed similar experiments and obtained similar results. On the basis of these data, all these investigators concluded that when regenerating, the transplanted nonirradiated tissues may include the irradiated tissues of the recipient in the process of regeneration. Sidorova (1951) irradiated extremities of axolotls with a dose of 3,500 r, and transplanted into them nonirradiated pieces of heart, liver, and lungs taken from other axolotls. After amputation of the organs, some cases showed regeneration of typical or atypical extremities on the transplant. In such cases, the transplant was not the source of regeneration, but was a factor causing restoration of the regenerative capacity suppressed by irradiation. The latter conclusion, however, cannot be considered final, as the dose of irradiation, 3,500 r, could be insufficient to suppress the regenerative capacity of the extremities in axolotl. It is known (Brunst, 1937) that suppression of the latter to the extent of 95% occurs only with a dose of 10,000 r, while at a dose of 4,000 r, suppression occurs only in 53% of cases. Therefore, Umanskii (1937) customarily used a dose not less than 7,000 r in his experiments.

In recent years, Lazard (Lazard, 1965, 1965a) has shown that if, after irradiation of extremities in axolotls with a dose of 4,000—5,000 r, tissues of testes are implanted into the tissues of these organs and the extremities then amputated in the zone of the transplant, a typical or atypical regeneration of extremities takes place. In the control, extremities, irradiated with the same dose and with no implantation of tissues, could not regenerate after amputation. A similar result was obtained by this author upon implantation of embryonic tissues into the tissues of the extremities.

The present stage of these studies, at which the work in our laboratory was mainly accomplished (Polezhaev, 1959, 1965, 1966, 1966a; Polezhaev and coworkers, 1960—1965; Teplits, 1962, 1964, 1965; Tuchkova, 1963, 1964, 1965,

1966), is characterized by attempts to restore regenerative capacity, suppressed by X-ray irradiation, by using new biological and chemical methods, and to study the mechanism of suppression of this capacity. The results of this stage, must be discussed separately and in greater detail than the previous ones. It is very important to establish what is common and what is different in the phenomena of loss of regenerative capacity of organs in the processes of ontogenesis and phylogenesis on the one hand, and the action of ionizing radiation on the other. It is no less important to investigate the question of whether the new biological and chemical methods for restoring regenerative capacity, suppressed by X-ray irradiation, can be applied in the fight against local radiation injuries. This is a problem of direct practical importance.

Results of our own experimental study

We started our investigation not with an attempt to make a further step in the direction of previous studies by other investigators, but with an attempt to use directly for restoring regenerative capacity suppressed by X-ray irradiation, some of those methods which we used for restoring regenerative capacity lost during ontogenesis and phylogenesis of animals. The objects of our study were extremities of axolotls possessing a high capacity for reparative regeneration. The regeneration was studied from different aspects by numerous investigators. Our investigation was a complex one in which we used methods from experimental morphology, histology, histochemistry, and biochemistry.

Materials and methods

Altogether, we used in this experiment 1,346 axolotls of white and black strains (*Amblystoma mexicanum*), aged 8—12 months, with a body length of 12—15 cm. Of these, we used 456 animals for biochemical investigation. In each separate series of experiments, we used 10—20 animals. In the biochemical investigation, we used 6 extremities at each time of fixation. We irradiated to the base, both hind extremities, and covered the remaining part of the body with a lead plate 4 mm thick. Irradiation was performed in RUP-1, RUM-1M, and RUP-200 X-ray instruments without a filter, at a distance of 24 cm from the object to the anticathode. The instrument carried 210 volts with a current intensity of 15 ma, and the irradiation dose was 7,000, 8,000, or 10,000 r. In total body irradiation of axolotls, we used a 0.1 mm Al filter, and the irradiation dose was 1,000 r. After local irradiation, we allowed a latent period of 15—30 days, and then amputated the feet of both irradiated extremities. The next amputation was made at a 5 mm distance from the previous wound surface, which healed smoothly, or from the regenerating extremity, the plane of amputation always passing through the zone of irradiation. We eliminated from the experiment those cases in which the irradiated extremities became necrotized and fell off, leaving only

nonirradiated tissues in their place. We considered as typical regeneration cases in which 5- or 4-toed extremities regenerated. We considered atypical regeneration those cases in which 3- or 2-toed extremities regenerated. The absence of regeneration meant smooth healing of wounds or formation of conical appendages or elevations. Transplantation of tissues was carried out under ether narcosis without observing sterile conditions; the incision was closed with braided silk sutures. For histological examinations, the extremities were fixed in 10% formalin in Helley's fluid to which acetic acid was added, and according to Shabadash. Serial paraffin sections, 5—7μ thick, were stained with hematoxylin-eosin, with iron hematoxylin according to Van Giesen, with Mallory and with azure II-eosin. The extremities were fixed after 1, 3, 5, 7, 10, 15, 20, 25, 30, 60, 90, 120, and 180 days following amputation. From the homogenates, we prepared smears which were examined directly in the fresh state, and after fixation in methyl alcohol; the smears were stained with Nile blue sulfate and toluidine blue. In the histochemical examination, we determined the distribution of nucleic acids in the sections and smears, and we performed Brachet's reaction test for RNA, and Feulgen's test for DNA. In the biochemical investigations we determined the concentration of RNA and DNA in the tissues of the extremities according to Schmidt and Tannhauser. In the cytological examination, we studied the structure of the cells and the processes of amitotic and mitotic division; the number of mitoses was counted in 3,000—5,000 cells, separately for the epithelium of the skin, blastemal cells, myoblasts, cartilage and connective tissue.

For the purpose of restoring regenerative capacity suppressed by X-ray irradiation, the animals were injected with various biopreparations into the stumps of the amputated extremities from the base to the wound surface or into the muscle of the back. The preparations used were: homogenates, prepared from fresh tissues, lyophilized, frozen at -18°, or killed by boiling for 3 minutes (muscles from the femur, skin, liver, testes, spleen, red bone marrow), of axolotls, white and black rats, and rabbits. Homogenates of muscles, liver, and testes were prepared by cutting the tissues with scissors and grinding in a porcelain mortar; the ground tissues were diluted in 0.65% physiological saline, in the case of cold-blooded animals at a ratio of 1 g/3 ml of solution for muscles and testes, and 1:2 for liver, and filtered through a double layer of gauze; the skin was cut and ground in a mortar with sand, diluted 1:4 in a physiological solution, centrifuged for 10 minutes at a velocity of 2,000 rpm, and the supernatant was used. The fresh homogenates of rabbit liver and spleen were ground in a mortar, diluted, and immediately used in amounts of 1.0 ml; 1.0 ml of the solution contained 0.1 g of wet weight of liver, 0.03 g of spleen, and 0.03 g red bone marrow from the epiphysis of the humerus and femur. For lyophilization, we placed the tissue suspensions in a test tube: liver 2.5 g, spleen 1.0 g, muscle from the femur 2.5 g, skin of rat (shaven and washed with alcohol) 2.5 g, red bone marrow of a rabbit 0.3 g. The test tubes were quickly frozen in a mixture of dry ice and 96% alcohol at a temperature of -78° and dried under vacuum with a residual pressure not exceeding 100 μ of mercury, the first day at a temperature of -78 to -10°, and the second day at room temperature. The test tubes with the dried tissue were sealed under vacuum. Before use, the suspension of dry material was ground to powder which was diluted in 10.0 ml of 0.65% physiological saline for cold-blooded animals; we used 1.0 ml of solution for one injection.

From the rat liver, we isolated cell nuclei according to the modified method of Shovo (Georgiev, Ermolaeva, and Zbarskii, 1960), RNA and DNA by the phenol method (Georgiev, 1959), and protein by the method of Sevag (Sevag, Zackmann, and Smolenz, 1938). We also used acidic protein prepared from milk by the method of S. S. Perov (1947) and A. P. Sadokova (1960). The acidic protein was dissolved in the following solvent: KCl, 1 g; NaCl, 1 g; K_2HPO_4, 5 g; H_2O, 1,000 ml. The proteins were free of nucleic acids (Dische reaction and with orcinal), and the RNA and DNA were free of protein. The preparations were administered once daily every day or every other day, altogether 4—7 times, with a single dose in the case of nuclei consisting of 13 mg, DNA 85 μg, RNA prepared in the laboratory 125 μg, commercial preparation 250 μg, acidic protein 10 mg, liquid heptamyl in a single dose of 0.1 ml with an RNA content of 3.88 mg. The heptamyl, a preparation made by the French firm, Delaland, is a metabolite of nerve cells and of cardiac muscle; it contains RNA 33.8%, pure terpenes 11.0%, cineol, borneol, organic solvents, and acidrax (neuroleptic and uretic). The muscle hydrolysates were obtained by us enzymatically according to a method we described earlier (Polezhaev, Akhabadze, Zakharova, and Mant'eva, 1958, 1959); 0.5 ml of the hydrolysate diluted in physiological saline and containing 4.3 μg of dry substance were introduced into the muscle of the back immediately after amputation of the extremities, and 3 more times every second day. The regeneration results were established on the basis of the number of amputated extremities, and not on the basis of the number of animals. Thus, if we irradiated and amputated 40 extremities in 20 axolotls, and if in 10 animals regeneration was obtained in which one extremity regenerated in each, we considered this to be a positive result in 25% of cases, not in 50%.

Regeneration of nonirradiated extremities

After amputation of nonirradiated extremities, complete typical regeneration occurred in 100% of cases within 45—60 days. The regenerated extremities had normal structure consisting of a cartilaginous and bony skeleton, muscles, connective tissue, skin, nerves, and blood vessels.

Irradiation and amputation of extremities

After irradiation of the hind extremities with a dose of 7,000—10,000 r and their subsequent amputation, after 3 months smooth healing of the wound took place (Figure 106a) in the majority of cases. Sometimes small conical appendages regenerated. In a few cases there was regeneration of atypical or typical extremities. Regeneration of extremities was absent on the average in 93% of cases. We did not succeed in establishing a statistically significant difference of the results of regeneration as a function of the irradiation doses used. However, necrosis of tissues in the extremities clearly depended on the irradiation dose. A dose of 7,000 r caused necrosis of the toes; a dose of 8,000 r caused necrosis of the toes and feet; a dose of 10,000 r caused necrosis of the feet in 75—100% of cases.

FIGURE 106. Lack of regeneration of extremities after X-ray irradiation and amputation of extremities in axolotls (3 months after operation):

a – general view of the extremities; b – longitudinal histological section of the extremity.

In the case of smooth healing, the amputation wound surface of the extremity was covered with skin and loose connective tissue. The skeletal elements in the muscles were ragged. At the distal end of the skeletal elements, cartilage formed (Figure 106, b).

Irradiation, transplantation of muscles, and amputation of extremities

In order to check the efficacy of Umanskii's method (1937), we performed experiments on irradiation of extremities with a dose of 7,000 r and subsequent (5—7 days later) transplantation into the shin muscles of pieces of muscles taken from nonirradiated extremities of other axolotls. After the transplant had taken, the extremities were amputated through the zone of transplantation. In all the cases, small appendages appeared on the amputation wound surface, consisting of skin, connective tissue and small cartilages, clearly separated from the old skeletal elements and muscles (Figure 107). Thus, the transplants are the sources of regeneration. The volume of regeneration was small and proportional to the volume or mass of transplanted tissues.

Irradiation, amputation of extremities and injections of hydrolysate from muscles of extremities

The decomposition products of tissues stimulate processes of regeneration (II, Chapter 1). When hydrolysis products of cartilage are introduced into the skin of an extremity of axolotl, an additional extremity forms (Silaev, 1941; Fedotov, 1943; Zelinskii, 1946). If the cartilage hydrolysate is placed under the epithelium of the wound surface of an extremity of an adult Bombina bombina, the regeneration of an atypical extremity takes place

instead of smooth wound healing (Polezhaev and Ramenskaya, 1950). Subcutaneous injection of hydrolysate of myocardium stimulates regeneration of damaged cardiac muscle (Polezhaeg, Akhabadze, Zakharova, and Mant'eva, 1958, 1959). In connection with these data, we attempted to use injections of an enzymatic hydrolysate of muscles from rat extremities to restore the regenerative capacity of axolotl extremities, suppressed by X-ray irradiation with a dose of 7,000 r. The result was negative; the irradiated extremities did not regenerate after amputation and 3 hydrolysate injections.

FIGURE 107. Formation of small appendages after irradiation and amputation of extremities into which pieces of muscles taken from other axolotl extremities were transplanted; 3 months after operation

Irradiation, amputation of extremities, and injection of a homogenate of axolotl muscles

It has been established that following destruction of main tissues in axolotl extremities, these organs completely regenerate (Polezhaev, 1934, 1936, 1937, 1937a, 1945, 1960). The cells of the destroyed tissues are the source of the regeneration material.

On the other hand, we know that protein extracts from cardiac and skeletal muscles may stimulate regeneration of damaged myocardium (Polezhaev and co-workers, 1958, 1965). Consequently destroyed tissues may be both the regeneration source and stimulant.

Based on these data, we performed an experiment in which we injected homogenates prepared from muscles of extremities of black axolotls into the stumps of extremities of white axolotls irradiated with a dose of 7,000 r, immediately after their amputation (Polezhaev, 1959). The injections were given once daily for 5 days, always into the right hind extremity. The homogenates contained dissociated cells and nuclei. As a result of the injection, the homogenate reached the wound surfaces of both the left and right irradiated hind extremities and concentrated there, forming dark elevations. In a number of cases one of the 2 irradiated extremities, right or left, completely regenerated. It was gray: its internal tissues of mesodermal origin formed from the homogenate and were pigmented, while the skin originated from the skin of the recipient and was white. Histological examination showed that the regenerated extremities had a normal structure, and the newly formed tissues differed clearly from the more highly differentiated old tissues, especially the skeletal tissues.

Similar experiments, in which homogenates of nonirradiated tissues from axolotl extremities or tails were injected into irradiated and amputated extremities of axolotls, were made by Polish investigators (Skowron and Roguski, 1958; Roguski, 1961; Lagan, 1961). The results were similar: after injection of homogenates of extremities, atypical or typical extremities regenerated; while after injection of homogenates of tail tissues, taillike organs regenerated.

Irradiation, amputation of extremities, and injection of a homogenate of rat muscles

As we have already pointed out, homogenates of muscles from nonirradiated extremities of axolotls could be not only the source of regeneration but also the factor leading to the restoration of regenerative capacity of the tissues of the extremities, suppressed by irradiation. In order to elucidate the importance of this factor, we performed an experiment in which the hind extremities were irradiated with X-rays at a dose of 7,000 r, and the animals were given injections of homogenates prepared from nonirradiated muscles of rat extremities (thigh). After a latent period following irradiation, the axolotls' hind extremities were amputated, and we introduced into the stump of the right extremity a homogenate of rat muscles. The result obtained was very interesting. After the injection, the irradiated extremities regenerated even more rapidly and better than in the experiment in which a homogenate of axolotl muscles was used. One irradiated leg, right or left one, always regenerated (Figure 108 a). In other words, regeneration occurred independent of the injection site. Subsequently, we became convinced that the same result is obtained whether homogenates or other preparations are injected into the back muscles. Regeneration occurred in 30—33 % of cases, i. e., in 10 axolotls, 6 of 20 irradiated extremities regenerated. The regenerating extremities had a typical anatomical and histological structure (Figure 108b). In addition, atypical extremities and various appendages also regenerated.

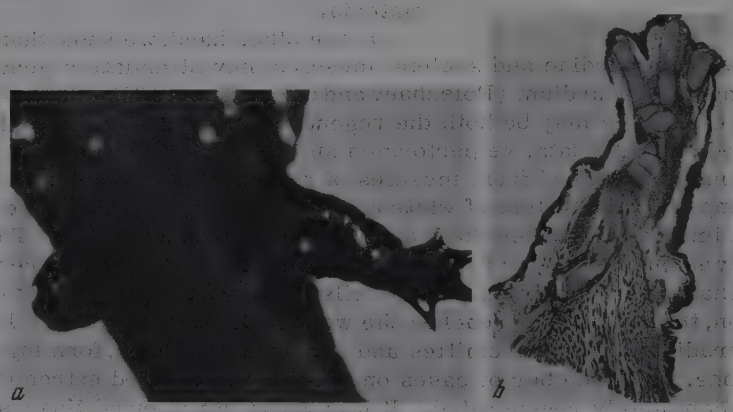


FIGURE 108. Regeneration of one of two irradiated and amputated extremities after an injection of a homogenate of rat muscle (3 months after amputation):

a — general appearance of the extremities; b — longitudinal histological section through the regenerated extremity.

The data obtained show that homogenates from muscles of extremities are endowed with the property of restoring the regenerative capacity of axolotl extremities suppressed by X-ray irradiation, since they cannot serve as a source of cellular regenerating material, as do homogenates of axolotl muscles.

We carried out a biochemical analysis of our preparations. Muscles from a rat hip were ground, and a 30 % extract was prepared containing large quantities of protein and nucleic acids. The extract dry weight was 1.65 %,

its pH 5.7, the total nitrogen 1.28, nonprotein nitrogen 0.359, protein nitrogen 0.921, and ammonia nitrogen 0.03 mg/ml. The total nonprotein and protein nitrogen was determined by microkjeldahl, and amino nitrogen by the copper method; pH was measured colorimetrically. Paper electrophoresis allowed us to establish the fact that the extract contained 2 components: one which moved toward the anode, and by its mobility was similar to serum albumin from rat blood; the other remained at the point of application. Chromatography analysis of free amino acids on paper showed on development of the chromatograph, 11 spots, 9 of which, by their position, coincided with lysine, histidine, aspartic acid, alanine, tyrosine, serine, valine, phenylalanine, and leucine (isoleucine); 2 were not identified. It is possible that the extract also contained peptides which coincided in their positions with the markers. The active principles of the extract could be proteins and nucleic acids. The decomposition products of protein (peptides and amino acid) are also present in hydrolysate of muscles, but they do not restore the regenerative capacity suppressed by irradiation.

Irradiation, amputation of extremities, and injection of homogenates from various tissues

Are the tissues from which we prepared the homogenates, and which we used to restore the regenerative capacity of axolotl extremities suppressed by X-ray irradiation, specific? To elucidate this question we performed experiments with homogenates prepared from fresh tissues of a rat: muscles, liver, testes, and skin. These homogenates were injected in axolotls with irradiated extremities by a method similar to the one in the experiment just discussed. The dose of irradiation was 7,000 r.

This experiment revealed that homogenates, prepared from fresh muscles from rats' hip, liver, and testes, were active, i. e., restored to approximately the same extent the regenerative capacity of axolotl extremities suppressed by irradiation. Always only one, right or left, irradiated extremity regenerated. The homogenates from skin were inactive.

Injections of suspensions of lyophilized, frozen and boiled tissues

Does the activity of tissues which restore the regenerative capacity suppressed by X-ray irradiation change in relation to its physiological condition? We tested the activity of suspensions prepared from boiled, frozen at -18°, and lyophilized muscles, liver and spleen of the rat, and from lyophilized red bone marrow of a rabbit. The irradiation dose was 7,000 r, the experimental design the same as reported .

Freezing the muscles destroyed the activity of the homogenates prepared from them. Boiling the muscles considerably reduced the activity of prepared homogenates. Homogenates from lyophilized muscles and spleen also possessed lower activity. Homogenates of lyophilized liver were highly toxic to axolotls. Almost all died during the first 7 days after injection. The entire skin

of the body was highly hyperemic and edematous; the tissues at the injection region were necrotized. The liver was very greatly enlarged, swollen, reddish-claylike externally, and yellow upon sectioning, and severely cirrhosed. Its cells were enlarged in volume, round, with numerous granules and lipid droplets in the cytoplasm, with pyknotic nuclei, and even with nuclei missing entirely. Fresh homogenates of liver and homogenates of lyophilized other organ tissue did not show the above effect. Lyophilization reduced the antigenicity of the tissues. Homogenates of lyophilized liver taken in excess affected the liver organospecifically, and caused toxicosis and death of the animals. Probably, the recipients did not offer sufficient resistance. One axolotl, which survived this experiment, had no regeneration of extremities.

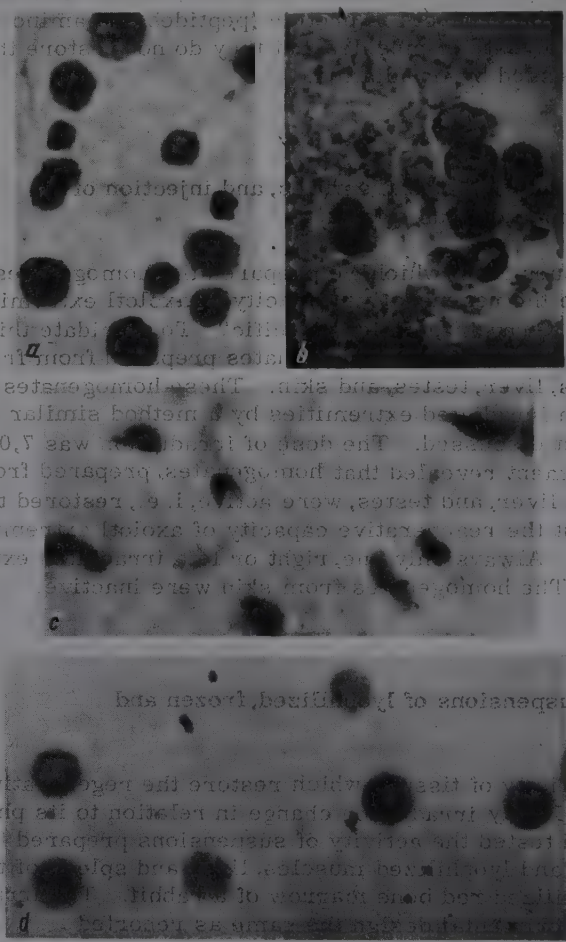


FIGURE 109. Cellular elements and nuclei in homogenates of rat tissues (after lyophilization):
a — liver cells; b — spleen cells; c — nuclei of muscles; d — nuclei of fresh liver cells.

Examination of smears prepared from fresh and lyophilized tissues showed that cellular structures changed greatly after lyophilization. The liver cell nuclei are destroyed (Figure 109 a) and do not give a good Feulgen reaction; the cytoplasm is also destroyed, but gives a clear Brachet reaction. The cellular elements of the spleen (Figure 109 b), bone marrow, and muscles (Figure 109 c) are also destroyed, but they also give distinct Brachet and Feulgen reactions.

Injection of cell nuclei, protein and nucleic acids

What is the active morphological or chemical component of the cell which restores regenerative capacity of axolotl extremities suppressed by X-ray irradiation? We tested the effect of nuclei, DNA, and RNA (ribosomal fraction) and also protein isolated from rat liver cells or protein isolated from milk (so-called acidic protein, Perov, 1946). The results of these experiments were unexpected.

It is known when the nuclear apparatus is irradiated, the cell chromosomes and DNA are strongly damaged. Therefore, it was expected that the best effect would be obtained in experiments by injecting nuclei and DNA from liver cells into axolotls with irradiated extremities. The results obtained differed from the expected. Both insufficiently purified nuclei of liver cells, and those which had been thoroughly purified proved to be inactive, and failed to restore the regenerative capacity of extremities suppressed by irradiation. DNA was also inactive. We repeated this experiment for several years in different variations. In all cases, when DNA was injected after a latent period, 20 days after irradiation, and the irradiated extremities were amputated at the same time or 2–2½ months later, there was no regeneration of organs. A positive result (regeneration of extremities in approximately 25% of cases) was obtained in those experiments in which DNA was injected immediately after irradiation, and the extremities amputated after 20 days or 3 months.

The results of experiments with protein, and particularly those with RNA, were much better. Liver protein was active and acidic protein was slightly more active. They restored the regenerative capacity of limbs in 33% of cases. A positive result was obtained both in those cases in which the extremities were irradiated, the protein administered after 20 days, and amputation carried out also after 20 days, and in cases when the extremities were amputated 2½ months after irradiation. Results were better in the latter cases. For these, even a single injection of protein proved effective.

A very good result was obtained in experiments with RNA. When highly polymeric freshly prepared RNA was injected once daily for 5 days, starting 20 days after irradiation, and the extremities amputated, typical and atypical regeneration occurred in 30–33% of cases. If RNA was administered several times for prophylactic purposes and irradiation was given 2 months later, followed in 20 days by amputation of the extremity, regeneration did not occur. Consequently, RNA is effective only as therapy, but not as a prophylaxis.

It is highly significant that only highly polymeric freshly prepared RNA is active. RNA stored in the laboratory refrigerator for 1½–2 months before

use; commercial RNA, dry, sealed in ampules; low polymer RNA; and RNA from heptamyl comprising 33.8% of its bulk, turned out to be inactive.

Thus, having established that protein and RNA are active, we made an attempt to improve the experimental result by the simultaneous use of RNA and protein following irradiation and amputation of extremities after the latent period. It turned out however, that no positive result was obtained in this case. An effect of mutual neutralization of the effects of the two separately active factors was observed.

Restoration of regenerative capacity of extremities in axolotls under the influence of homogenates of certain tissues, protein, or RNA may be obtained with various doses of irradiation: 7,000, 8,000, and 10,000 r.

Repeated amputation, cumulative effect

Having done the experiments with irradiation, suppression, and restoration of regenerative capacity of extremities in axolotls, we became interested in the question of what would be the result of regeneration after repeated amputation of organs. To elucidate this, we carried out repeated amputation in various experimental series of all the irradiated nonregenerated and regenerated extremities 3 months after the first amputation. The second amputation was always carried out in the zone of irradiation, i. e., approximately 5 mm proximal to the previous amputation wound surface, i. e., slightly distal from the knee joint.

We found that the second amputation considerably increased the percentage of regeneration of irradiated extremities. Thus, if regeneration occurred on the average in 7% of cases in axolotls after irradiation of extremities with a dose of 7,000—10,000 r, then it occurred in 17% of cases after the second amputation, and it was even higher in some experimental series. Similar results were also obtained in the experiments of Stinson (1964) on tritons (*Triturus viridiscens*): regeneration took place in 4% of cases after the first amputation of irradiated extremities, and in 12% after the second amputation.

An even more striking result was obtained in various experiments in which animals with irradiated extremities were injected with various homogenates of rat tissues, protein, and RNA. If atypical and typical regeneration of extremities occurred in 30—33% of cases after the first amputation, it occurred in 70—100% of cases after the second amputation. In these cases, both irradiated extremities regenerated (Figure 110). It is remarkable that in certain cases, for example, when skin homogenates or homogenates of frozen rat muscles were injected, there was no regeneration of extremities after the first amputation, while regeneration occurred in 100% of cases after the second amputation, which was performed 3 months after the first one. Consequently, parenterally administered preparations act on the animal body and on the irradiated organ tissues not immediately, but gradually, within 2—3 months, during which time such reorganization takes place as leads to continuous enhancement of regenerative capacity. This enhancement is due to the action of the administered preparations, and not only to the second amputation, as the percentage of regeneration in experiments

with irradiation and administration of preparations is considerably higher than in experiments with irradiation without the administration of preparations. We called this phenomenon of enhancement of the effect of restoration of regenerative capacity of irradiated organs, which occurred 2—3 months after injecting preparations, the cumulative effect. Knowledge of this effect is very important, not only theoretically but also practically: in order to obtain an optimum restoration of regenerative capacity suppressed by ionizing radiation, the restoring preparation, for example RNA, should be administered not at the time of the tissue resection, but 2 months before. The reality of the cumulative effect can be proven in a direct experiment. We irradiated axolotl extremities with a dose of 10,000 r, and after 20 days, we injected RNA once daily for 6 days. In the first series, the irradiated extremities were amputated at the time of the first injection, and in the second series 2 months after the injection, allowing time for the preparation to act on the irradiated tissues and cause the cumulative effect. As a result, extremities regenerated in 33% of cases in series 1, and in 100% of cases in series 2. After the second amputations of the extremities, regeneration occurred in 92% of cases in series 1, and in 100% of cases in series 2. Consequently, RNA indeed has a cumulative effect. It is interesting that DNA did not show any such effect.

Importance of the time factor and the trauma factor in the restoration of regenerative capacity, suppressed by X-ray irradiation

The increase in the percentage of regeneration of irradiated extremities after repeated amputation may depend on 2 factors: 1) the length of time from irradiation to the second amputation (in our experiments, 6—7 months), during which the regenerative capacity, which was suppressed by irradiation, may be gradually restored, and 2) the action of the trauma of the 2nd amputation. To answer this question, we performed the following experiments. To elucidate the role of the time factor, axolotl extremities irradiated with a dose of 8,000 r, were amputated 20 days, 3 months, and 6 months after irradiation. Atypical and typical regeneration of extremities occurred in 13% of cases in the first and second instances, while it occurred in 23% of cases in the third instance. In other words, with time, there was a certain spontaneous restoration of regenerative capacity suppressed by irradiation.

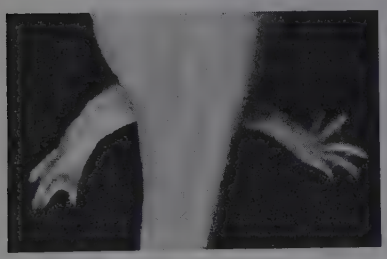


FIGURE 110. Regeneration of both extremities of axolotl after irradiation, amputation, injection of RNA, and amputation 3 months after the first

To elucidate the importance of the amputation trauma factor, the extremities were amputated 20 days after irradiation with a dose of 8,000 r, and then again 3 months after the first amputation. Our findings showed that 6 months after irradiation and upon the action of the secondary trauma, the

extremities regenerated in 53% of cases. Consequently, the trauma factor (repeated amputation) increased more than 2-fold the percentage of extremity regeneration within the same 6 months, as compared with the experiment in which only a single amputation was performed.

Thus, the time factor had only a slight effect, while the trauma factor considerably facilitated the restoration of regenerative capacity of the organ tissues suppressed by X-ray irradiation.

Traumatization and destruction of tissues of irradiated extremities

We have seen that amputation trauma is of great importance for restoring regenerative capacity of axolotl extremities suppressed by X-ray irradiation. We also know that this capacity can be restored if axolotls with irradiated extremities are injected with homogenates of certain tissues, protein, or RNA. On the other hand, we showed earlier that the regenerative capacity of extremities in tadpoles and frogs lost during ontogenesis can be restored by using methods of additional traumatization or chemical treatment (Polezhaev, 1933, 1948). In this connection, the question arises, is it possible to restore the regenerative capacity of extremities in axolotl suppressed by irradiation, by the method of strong additional traumatization or destruction of tissues? Such experiments were carried out in our laboratory by S. Ya. Tuchkova (1966).

FIGURE 111. Experiment with traumatization: irradiation and amputation of both extremities in axolotls. Right, an additionally traumatized extremity regenerates; left, a nontraumatized extremity does not regenerate.

We irradiated both hind extremities of axolotls to the base with a dose of 8,000 r. After 20 days, we amputated both stumps of the irradiated extremities. The stump of the organ of the left extremity served as control. The residue of the right experimental extremity was strongly traumatized mechanically. For this purpose, we pierced the tissues in the wound area to a depth of 5—10 mm with thin-eye forceps, crushing the tissues by closing the forceps. This manipulation was repeated 20—40 times during each session once daily, altogether for the first 5 days after amputation. In 3 experimental series (43 axolotls), we did not achieve any

positive result. In one experimental series with 20 axolotls, we did achieve a positive result: the left control extremities did not regenerate and the wounds healed smoothly, while the right experimental extremities regenerated in 50% of cases (Figure 111). A similar result was obtained in an additional experimental series with 17 young axolotls in which the extremities were irradiated with a dose of 6,000 r. In this experiment, a positive result was obtained in 9 cases (47%).

In another experiment by Tuchkova, the destruction method (Polezhaev, 1934) was used. We irradiated the 2 hind extremities of axolotls to the base with a dose of 8,000 r, and after 20 days, amputated in the distal or middle part of the shin. The left extremity served as control. The right extremity was used in the experiment, and we left a skin cuff and a prepared nerve above the wound surface during amputation. Into this cuff, we implanted ground muscles from irradiated axolotl extremities, and we sewed the skin together. Several days after operation, we cut off the skin above the implant. Some of the transplanted tissues fell off. However, they managed to affect the irradiated tissues of the residue of the organ. As a result, 3 months after operation, the experimental extremities regenerated in 14 of 33 cases, while the control showed smooth healing in 31 cases. In the subsequent 3 months, despite the lag, the control extremities also regenerated in 6 cases.

In traumatization and destruction, there are released from the tissues decomposition products, probably proteins and nucleic acids primarily, these acting on the irradiated tissues of the extremities of the recipient and restoring their suppressed regenerative capacity.

The results of experiments with destruction and restoration of regenerative capacity of irradiated extremities in axolotls are in accordance with the results of our previous experiments on the regeneration of extremities in axolotls after destruction of their tissues of mesodermal origin (Polezhaev, 1934, 1936, 1937, 1937a), and with results of similar experiments on regeneration of destroyed skeletal muscles in rats and chickens (Studitskii, 1959). The main difference between the results of these experiments is that in those on the regeneration of irradiated extremities in axolotls, the destroyed tissues served as an agent restoring regenerative capacity, while in the experiments on regeneration of nonirradiated extremities in axolotls, they were the source of the regeneration material. In Studitskii's experiments (1959) the destroyed tissues served as a stimulator of regeneration and a source of regeneration material. The results obtained are similar to the results of experiments on the stimulation of regeneration of skeletal muscles after irradiation and destruction in rats (Studitskii and Popova, 1962; Popova, 1964).

Thus, traumatization and destruction of tissues of irradiated organs may result in restoration of their regenerative capacity suppressed by irradiation.

Data from histological examination

What are the changes taking place in tissues after irradiation of extremities in axolotls and suppression of their regenerative capacity? And what

are the changes which occur in these organs after irradiation and experimental restoration of regenerative capacity?

In the process of normal regeneration of nonirradiated extremities in axolotls and tritons, development of the regenerate proceeds by formation of regeneration blastemata. This fact was established more than 50 years ago (Fritsch, 1911, and others; see Polezhaev, 1945; Vorontsova, 1949; Vorontsova and Liozner, 1957). For a long time, the mechanism of formation and the origin of the regeneration blastemal cells were not known. According to the old theory of regrowth, each tissue in the organ residue produces one like itself (Fraisse, 1885; Barfurth, 1891; Wendelstadt, 1901, 1904; Kazantsev, 1934, and others). However, subsequent studies showed that only the epidermis, nerve fibers, and blood vessels in the regenerative blastema are formed from tissues like themselves in the organ residue. The remaining main mass of morphologically homogeneous embryonic type blastema cells are of unknown origin. These cells may arise from the cellular elements of muscles, skeleton (cartilage, perichondrium, periosteum), connective tissue, and, partially from lymphocytes. A number of experimental, histological, and autoradiographic studies have shown that blastemal cells are formed by dedifferentiation of the aforementioned destroyed tissues of mesodermal origin (Butler, 1933; Polezhaev, 1935, 1945; Barakina, 1951, 1952; Anton, 1951; O'Steen and Walker, 1961; Hay and Fischman, 1961; Bodemer, 1962, and others).

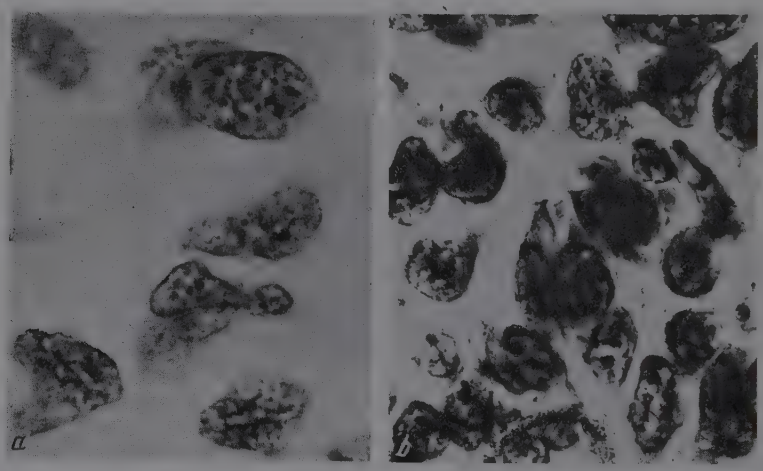


FIGURE 112. Cells appearing in the wound region after amputation of extremities in axolotls, 7×90:

a – cells of normal regeneration blastema; b – "coarse" cells appearing after irradiation of an extremity.

Histological changes occurring after irradiation and amputation of extremities, and those taking place during smooth wound healing have been described in published reports, but their histogenesis has not been studied in detail (Butler, 1933; Lichko, 1934; Brunst, 1937, and others). It has been shown that the amputation wound surface of the extremities

epithelializes after irradiation, and is covered with a connective-tissue scar. The skeleton and the muscles do not regenerate. E. Ya. Lichko (1934) assumed that the reason for the suppression of regenerative capacity of organs after irradiation is the proliferation of connective tissue which interferes with regeneration. However, this assumption has not been confirmed. V. V. Brunst (1937) and other investigators reached the conclusion that the reason for suppression of regeneration after irradiation is the inhibition of mitotic activity of cells. Butler (1933) and other authors concluded that irradiation suppresses the differentation of tissues in the regenerating organs. These last 2 assumptions served as the basis for modern ideas on the causes of suppression of regenerative capacity of organs and tissues after X-ray irradiation.

Studies performed in our laboratory led us to entirely different conclusions.

Tuchkova (1963, 1964, 1965, 1966, 1967) carried out a histological and histochemical investigation of the histogenesis of regeneration of extremities in axolotls: 1) normal nonirradiated; 2) after X-ray irradiation with a dose of 8,000 r and suppression of regenerative capacity; 3) after irradiation with 8,000 r, suppression of regenerative capacity, and restoration of regenerative capacity by administration of highly polymeric freshly prepared RNA. She found that in normal regeneration of extremities, deep destruction and dedifferentiation of tissues in the organ stump, and the formation of regeneration blastema take place (Figure 112 a). The embryonic type cells of the blastema have a cytoplasm and nucleoli which give an intensive Brachet reaction to RNA, and a large, light nucleus with fine chromatin granules. In the irradiation experiments, the organ stump tissues are also strongly destroyed but they cannot dedifferentiate; therefore, no embryonic type cells of the blastema are formed. Instead of these, "coarse" cells appear with smaller nuclei, a coarsely delinated nuclear membrane, large chromatin granules, no nucleoli, and a cytoplasm which gives hardly any Brachet reaction to RNA (Figure 112, b). Recently, B. M. Carlson (1966) showed that actinomycin D, which suppresses RNA synthesis, strongly inhibits the regeneration process of extremities in tritons, whereas the destruction process of tissues does not change but the dedifferentiation process is suppressed. The effect of X-rays and of actinomycin D on cells is similar. Carlson's data are in agreement with those of Tuchkova. In the experiment with irradiation and restoration of regenerative capacity, there is deep destruction of tissues in the organ residue and a 10—15-day arrest of restoration of the capacity for dedifferentiation and formation of cells of regeneration blastema which give Brachet and Feulgen reactions of the same intensity as in the control, i. e., in regeneration of nonirradiated extremities.

In all the 3 experimental series, a cytological investigation was carried out and attention given to the intensity and nature of mitotic division. This had to be done because the biphasicity of the process of regeneration had been established earlier. We have shown, that blastemal cells at the beginning of the regeneration process, during the phase of primary accumulation, arise almost without mitoses, due to their release from the tissue associations during tissue destruction and dedifferentiation. Later, in the secondary accumulation phase they divide intensely, mitotically. The mitotic index changes regularly in the process of regeneration (Polezhaev,

Mitotic activity during regeneration following amputation of extremities of normal animals, after X-ray irradiation and after irradiation and restoration of suppressed regenerative capacity in axolotls

Type of cells examined	No. of days after amputation	Normal regeneration				Irradiation and amputation					Irradiation, amputation, and restoration of regenerative capacity				
		No. of cells	No. of mitoses	No. of mitoses, ‰	No. of typical mitoses	No. of cells	No. of mitoses	No. of mitoses, ‰	No. of typical mitoses	No. of atypical mitoses, ‰	No. of cells	No. of mitoses	No. of mitoses, ‰	No. of typical mitoses	No. of atypical mitoses, ‰
Regenerating epithelium	1	6,000	2	0.33	–	4,000	3	0.75	1	0.25	4,000	1	0.25	1	0.25
	3	8,000	43	5.3	–	3,000	13	4.3	–	–	5,000	40	8	1	0.2
	5	5,000	47	9.4	–	3,000	23	7.6	1	0.33	5,000	39	7.8	1	0.2
	10	5,000	83	16.6	–	4,000	50	12.5	1	0.25	3,000	38	12.6	1	0.33
	15	4,000	47	11.7	–	3,000	30	10	1	0.33	4,000	50	12.5	1	0.25
	20	5,000	68	13.6	–	5,000	41	8.2	1	0.2	3,000	52	12	1	0.25
	30	5,000	42	8.4	–	4,000	36	9	3	0.75	3,000	41	13.6	1	0.33
	45					3,000	31	3.3	5	1.6	3,000	23	7.6	1	0.33
Subepithelial cells of the blastema or connective tissue	10	5,000	63	12.6	–	4,000	11	2.7	1	0.25	4,000	17	4.2	1	0.25
	15	5,000	48	9.6	–	5,000	11	2.2	4	0.8	4,000	16	4	1	0.25
	20	4,000	54	13.5	–	3,000	8	2.6	4	1.3	4,000	63	15.7	1	0.25
	30				–	3,000	9	3.0	2	0.66	4,000	48	12	–	–
	45					3,000	7	2.3	1	0.33	3,000	5	1.6	–	–

1935, 1935a, 1939, 1945; Polezhaev and Gintsburg, 1944; Basina, 1940; Litwiller, 1939; Chalkey, 1954, and others). Tuchkova showed that the mitotic index, i. e., the ratio between the number of mitotically dividing cells and the total number of cells expressed per mill, changes very little in the epidermal epithelium after irradiation of axolotl extremities, being reduced mainly during the later stages of regeneration, and decreases greatly (to one fourth) in the cells of mesodermal origin which should have formed the blastema cells (see Table on p. 239). Irradiation causes the appearance of atypical mitoses: non-equipolar, tripolar, with chromatin bridges, chromosome aberration. Upon restoration of regenerative capacity suppressed by irradiation, the mitotic index in the epithelial and blastema cells becomes normal, and the number of atypical mitoses is reduced.

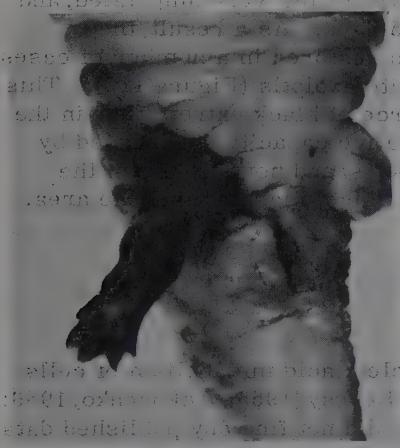


FIGURE 113. Regeneration of an extremity of a black axolotl transplanted to a white axolotl after its irradiation and administration of RNA to the animal

Thus, histological, histochemical, and cytochemical examinations showed that X-ray irradiation suppression of regenerative capacity of extremities in the axolotl occurs before the blastema cells begin to divide intensively mitotically and differentiate further. The reason for the suppression of organ regenerative capacity is the change in the capacity of their cells and tissues for dedifferentiation and morphogenesis, during which the morphological structure of the cells and their histochemical reactions and, therefore, also the capacity for mitotic division, change greatly. Upon restoration of regenerative capacity suppressed by irradiation, all these phenomena return to normal.

Sources of regeneration upon restoration of regenerative capacity suppressed by irradiation

We reached the conclusion that the changes in cells and tissues of irradiated organs are reversible, that impairment of the cell morphogenetic capacities caused by irradiation can be reversed, and that the morphological structure of the irradiated cells can be normalized; that under the influence of RNA and other preparations introduced into the animal body, the regenerative capacity of irradiated cells and tissues can be restored. However, this restoration may take place in 2 ways: 1) the irradiated cells themselves may be the source of the regenerative material, and 2) regeneration of the irradiated organ may occur due to migrating, nonirradiated (for example, hematogenic) cellular elements. To elucidate this question, Tuchkova (1967) designed a special experiment.

In white axolotls she amputated the right hind extremities close to the body and transplanted segments of thighs of black axolotls to the wound surfaces. Several days after the transplantation, the left hind extremities of the white axolotls were also amputated. After 3 months, the white axolotls showed regeneration of extremities of the same dimensions and age, those to the left, white and those on the right, black. Then, the 2 hind extremities were irradiated to the base with a dose of 8,000 r, while the remaining body was shielded with a lead plate. At 20 days after irradiation, the stumps of both irradiated extremities were amputated, and the animals were given RNA once daily for 5 days. As a result, the regenerative capacity of the extremities was restored in a number of cases, and black extremities regenerated in the white axolotls (Figure 113). This experiment shows that the regeneration source of black extremities, in the case of experimental restoration of regenerative capacity suppressed by irradiation, is local irradiated cells and tissues, and not cells from the extremities of the recipient (white axolotls) which migrated into the area.

Data from biochemical investigations

Upon total body X-ray irradiation, the nucleic acid metabolism of cells and tissues changes greatly (Howard, 1958; Hevesy, 1955; Dikovenko, 1956; Bilai, 1959; Kuzin, 1962, etc.). However, we did not find any published data concerning the changes in nucleic acid metabolism upon local X-ray irradiation of animal tissues. Nevertheless, we were interested primarily in nucleic acid metabolism because recent biochemical data make it well known that nucleic acids play an important role in the biosynthesis of protein, and because we had become convinced that injections of highly polymeric freshly prepared RNA lead to restoration of the regenerative capacity of axolotl extremities suppressed by X-ray irradiation. In this connection, our co-worker, N. A. Teplits (1962, 1964, 1965) examined the changes in nucleic acid metabolism in tissues of extremities of axolotls after amputation: 1) during normal regeneration; 2) after X-ray irradiation with a dose of 7,000, 8,000, or 10,000 r which suppresses their capacity for regeneration, and 3) after X-ray irradiation with the same dose and restoration of the suppressed regenerative capacity by the administration of RNA according to our method.

The biochemical investigation carried out 3 months after irradiation and amputation of axolotl extremities showed that the RNA content of the irradiated tissues decreased greatly, from 400 to 320 micrograms, and that the DNA content decreased from 240 to 190 micrograms per 100 mg of wet weight. During the restoration of the regenerative capacity, by injection of RNA or protein to animals with irradiated extremities, the RNA and DNA content in the tissues of the irradiated and regenerating extremities approached the normal level and even exceeded it, after both the first and after the second amputation.

Investigation of the changes in the nucleic acid content during regeneration or healing of wounds in the extremities in these three experimental series was continued for 100 days after amputation. It showed a quite definitive characteristic (see Figures 114, 115).

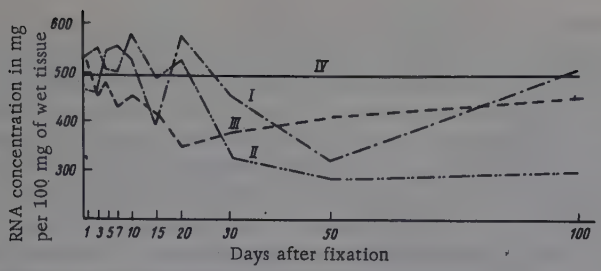


FIGURE 114. Curve of changes in the RNA content of the tissues of axolotl extremities:

I – during regeneration of nonirradiated extremities; II – upon healing of wounds after amputation of irradiated extremities; III – upon restoration of regenerative capacity suppressed by X-ray irradiation; IV – in normal animals without amputation.

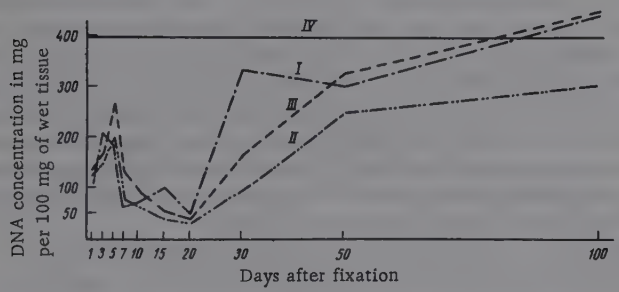


FIGURE 115. Curve of changes in the DNA content of the tissues of axolotl extremities.

Designations same as in Figure 114.

In all 3 series of experiments in the first 3—7 days after amputation, during the period of edema and inflammation, the RNA content of the stump tissues increased, exceeding the normal level (from 488 to 556 μg per 100 mg of wet weight). During the period of destruction and dedifferentiation, i. e., 15 days after the operation, the RNA content decreased (in series I, to 396; in series II, to 485; and in series III, to 416 μg). Twenty days after the operation the processes of destruction of the old tissues in the organ stump reached maximum and were at the same level in all 3 experimental series; in series I, growth and differentiation of the regeneration anlage took place and the RNA concentration reached maximum: 576 μg; in series II, regeneration did not occur and the RNA concentration reached 525μg; in series III, the RNA concentration was 353 μg. At 50 days after the operation in series I, the regenerate grew and the RNA content decreased to 325μg; in series II, there was no regeneration, the RNA content decreased to 285μg; in series III, the regenerate grew and differentiated, the RNA content reached 419μg. At 100 days after the operation, the extremities changed very little morphologically, the RNA content was 510 and 447μg, respectively: in series II, there was no regeneration and the RNA content was low, 299μg.

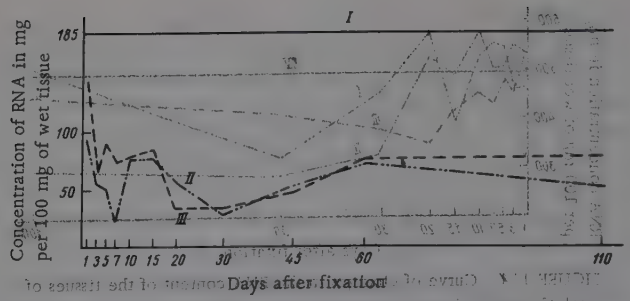


FIGURE 116. Curve of changes in RNA content of tissues of nonamputated extremities of axolotls:

I – normal; II – after irradiation; III – after irradiation and administration of RNA to restore the regenerative capacity suppressed by irradiation.

The DNA content which was 397 μg per 100 mg wet weight of tissue in the normal animal decreased sharply one day after amputation: in series I, to 125 μg; in series II, to 110; and in series III, to 136 μg. After some increase 7 days after the operation, during the period of edema and inflammation, toward the 20th day it decreased to 44, 34, and 40 μg, respectively. Later it increased again, and 100 days after the operation it was found to be 306, 442, and 451 μg, respectively. The period of accumulation of DNA in the tissues beginning 20 days after amputation is evidently associated with mitotic activity which was reduced in series II.

The dynamics of changes in RNA and DNA after irradiation and amputation of extremities in axolotls is due to the direct action of X-rays on the tissues, and to the amputation trauma factor. Teplits excluded the latter factor, irradiating but not amputating the extremities of axolotls, and in a second series of experiments, she did the same plus injecting highly polymeric freshly prepared RNA. In this series, the RNA and DNA content decreased regularly and strongly and did not reach normal level at any time up to 110 days. In the second series of experiments, however, this decrease was considerably less marked than in the first series (Figures 116 and 117). Consequently, X-ray irradiation strongly reduces RNA and DNA content of tissues of irradiated extremities, while the amputation trauma factor increases it, causing destruction and inflammation of tissues.

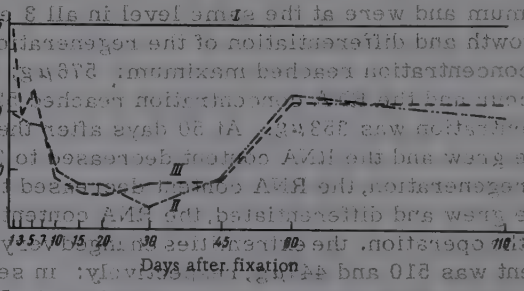


FIGURE 117. Curve of changes in DNA content of tissues of nonamputated extremities of axolotls.

Designations are the same as in Figure 116.

Teplits' biochemical data concerning the decrease in nucleic acid content during the stage of destruction and dedifferentiation of regenerating extremities, quoted earlier, contradict some histochemical data (Roskin and Kharlova, 1944; Yakovleva, 1943, 1953; Barakina, 1951, 1952; Studitskii, 1959, and others) according to which the RNA concentration increases during the dedifferentiation stage. Teplits explains this contradiction by the following biochemical data. She determined the nucleic acid content at consecutive stages of regeneration of nonirradiated axolotl extremities in the stump tissues and in the regenerative anlage separately. Her findings indicated that the stump tissues became strongly depleted of RNA and DNA during the entire 2 months of regeneration, while the nucleic acid content in the anlage decreased 1.5–2-fold, up to 15 days. At 20 days after the beginning of regeneration, the anlage RNA content was more than twice as high as the normal level. The increase in nucleic acid content in the regenerative anlage is directly related to its decrease in the old tissues of the organ residue. This fact is of great significance for understanding the mechanism and essence of regeneration of organs and tissues.

Thus, the biochemical investigation shows that nucleic acid metabolism is disturbed when there is local irradiation; the molecular and, later, the morphological structure of the cells, and the capacity for dedifferentiation and morphogenesis are altered. In experimental restoration of regenerative capacity of tissues suppressed by irradiation, nucleic acid metabolism returns to normal, followed by a normalization of all the aforementioned phenomena and properties of cells and tissues.

Are regenerating tissues radioresistant?

According to the law of Bergonier and Tribondot (1906), embryonic, young, poorly differentiated, and intensely proliferating tissues are radiolabile, and more strongly damaged by X-rays than the differentiated, less intensely proliferating tissues of the adult organism. This served as the basis for radiation therapy of malignant tumors, the poorly differentiated cells of which die under the influence of certain doses of ionizing radiation, while the cells of the adjacent healthy differentiated tissues remain alive.

Brunst (1937, 1960) showed that tissues of regenerating extremities of tritons and axolotls are more highly labile than tissues of normal extremities. He obtained regeneration of one of the hind extremities and then irradiated both paired hind extremities, the regenerating and the normal one, with an X-ray dose of 5,000 r. As a result, the regenerating extremities underwent regression and necrotized to the level of the old tissues of the organ, while the normal paired extremities survived and underwent no regression.

Studitskii (1961) and co-workers (Ivanova, 1961, 1963; Popova, 1964; Studitskii and Popova, 1962; Popova and Bulyakova, 1966) reached a diametrically opposed conclusion. According to them, regenerating, intensely proliferating, and RNA-rich tissues are radioresistant, and this property may be the basis for elaborating a new biological method of protection against ionizing radiation action. S. A. Ivanova (1961, 1963) irradiated rat extremities with an X-ray dose of 2,000 r, removing the

diaphysis of the femur subperiosteally. When the bone was removed immediately after irradiation, it did not regenerate, but if irradiation was carried out 8—14 days after its removal, when endochondrial ossification occurred in the regenerate, the bone could regenerate. In the experiments of Studitskii and Popova (1963) and Popova (1964), X-ray irradiation with a dose of 2,000 r inhibited regeneration of skeletal muscle in rats, when the muscle was irradiated before it was damaged. If, however, the muscle was removed, ground, and autotransplanted and then irradiated at various times between 3 and 14 days after operation, it regenerated and contained a high concentration of RNA. Finally, Popova and N. V. Bulyakova (1966) found that local irradiation with a dose of 2,000 r is less inhibiting to the mitotic activity of the epithelium of the cornea if the latter regenerates.

FIGURE 118. Regeneration anlage of an axolotl extremity at the cone stage; 20 days after amputation, × 24

Thus, 2 diametrically opposed views have appeared in the literature concerning the radiosensitivity or radioresistance of regenerating tissues. In view of this, we designed special experiments to elucidate the problem (Polezhaev, Teplits, Tuchkova, and Kilina, 1966).

In axolotls we irradiated both hind extremities to the base with 8,000 r, the right extremity being the regenerating one, the left kept intact. We performed 2 series of experiments. In series I, the regenerating extremity was at the cone stage (Figure 118), its tissues being characterized by a maximum RNA content (Teplits, 1964) and the highest mitotic index (Tuchkova, 1966). The tissues of the regeneration cone contained 405 μg RNA, while the tissues of the intact extremity contained 295 μg per 100 mg wet weight. The mitotic index in the blastema cells at the cone stage was 13.6%. In series II, the regenerating extremities were completely formed and of almost the same dimensions as the intact ones. In series I, the left intact extremities were amputated at the same level as the regenerating cone of the right extremities, and on the following day both extremities were irradiated. In series II the stumps of the regenerating and intact extremities were amputated and irradiated on the following day.

FIGURE 119. Extremities of axolotl after irradiation, amputation, and smooth healing of wounds:

a — an X-ray wound formed on the stump of the left extremity; b — longitudinal section of an extremity stump with formation of wound; c — tear of epithelial tissue in the region of the X-ray wound; d — mitosis in an epithelial cell at the edge of the X-ray wound; e — altered dermis in the region of the wound; f — connective tissue, poor in cells in the region of the wound; g — altered muscle fibers in the region of the wound.

The results were quite clear cut. In both series of experiments after irradiation, regeneration of the intact and regenerating extremities was inhibited to the same extent and did not depend on the regeneration stage, the RNA content, or the value of the mitotic index in the regenerate tissues. Already at 5 days after irradiation, there was not a single mitosis in the blastema cells of the regeneration cone, and irradiation killed them.

The data obtained showed that young embryonic-type cells of the blastema of regenerating axolotl extremities are considerably more radiosensitive than are cells of intact extremities. The conditions differed in the experiments of Studitskii and co-workers. Upon regeneration of tubular bone in rats, the cartilage, which has a higher radioresistance than the periosteum, was subjected to irradiation. The fact that the radioresistance increased somewhat after grinding the muscles in rats and damaging the cornea in mice, shows the importance of the trauma factor for restoring the regenerative capacity of irradiated tissues, and these data agree with ours. Finally, in these experiments of Studitskii and co-workers, the dose of irradiation was small: 2,000 and 1,000 r. These doses merely result in temporary inhibition of regeneration and not in complete suppression of tissue regenerative capacity.

On the whole, it can be said that regenerating tissues are radiosensitive and not radioresistant, and that the trauma factor is of great importance for restoring regenerative capacity suppressed by irradiation. Further studies are required to solve the problem conclusively.

X-ray ulcers and necrosis in irradiated tissues

In many cases, up to 60—100% in some experimental series, X-ray irradiation caused necrosis of the tissues of extremities. The degree of necrosis was proportional to the radiation dose. With a dose of 7,000 r, the fingers necrotized; with a dose of 8,000 r, the fingers and, in some cases, parts of the feet become necrotic; with a dose of 10,000 r, the fingers and parts of the feet necrotized in 75—100% of cases. In addition, 1—2 months after irradiation and amputation, necrosis and necrobiosis of a considerable part of the tissues of the stump were observed in many cases. The skin became dull white, and differed greatly from the healthy skin parts of the organ, and frequently Saprolegnia developed on it. However, in a number of cases regeneration of extremities still occurred after the second amputation along the zone of necrosis in the skin. The source of regeneration most probably was some other, not so extensive change in the tissue.

In a number of cases, up to 30—40% in some experimental series, one month after amputation of the irradiated extremities, X-ray ulcers appeared and they did not heal for a month. The wounds were located in the center or at the edge of the previous amputation wound surface (Figure 119 a). The wound edges were whitish; the bottom was open but not epithelialized. The wounds appeared both after irradiation and after injection of various preparations which did not prevent the formation of wounds. After the second amputation, the number of cases in which X-ray wounds were formed was distinctly reduced. This was observed both in the control and in the

experimental animals (irradiation plus injection of preparations). In the experimental animals after the second amputation, wounds appeared when DNA was used, and after a single injection of acidic protein or commercial RNA. After injection of highly polymeric freshly prepared RNA and a second amputation of organs, no wounds appeared. This merits serious attention from our point of view, as it must be taken into account in the elaboration of therapeutic methods for the treatment of X-ray ulcers in man, their having something in common with those in axolotls. The data we obtained in experiments with axolotls are in good agreement with the data of Tsanev and co-workers (1957), who treated successfully trophic and even X-ray ulcers in man by the application of RNA hydrolysate.

The fact that X-ray ulcers are never formed during the restoration of regenerative capacity suppressed by irradiation and during regeneration of the irradiated extremities, merits attention. The restoration of lost regenerative capacity prevents the formation of X-ray ulcers. This fact is a direct confirmation of the hypothesis that local radiation injury is directly associated with a change in the suppression of the capacity for reparative regeneration of animal organs and tissues.

Histological and histochemical studies show that destruction and degeneration of the irradiated tissues and complete absence of dedifferentiation and formation of regeneration blastema cells may be observed after irradiation and amputation of extremities of axolotls during the formation of X-ray ulcers (Figure 119b). The wound surface is open or covered with a thin layer of altered epithelial cells from which groups of dissociating cells mixed with erythrocytes tear off (Figure 119c). At the wound edge, the epithelium proliferates toward the center. Mitoses are observed in the epithelial cells (Figure 119d). There is no dermis in the wound region, and the basal membrane may also be absent. The dermis at the skin edges around the wound is highly altered, thin, breaks into fragments, and contains cavities with lymph fluid or blood (Figure 119e). In the wound region are much detritus, many fibrin clots, hemorrhagic foci, and blood vessels. The inflammatory reaction is poor. There are few polynuclears, lymphocytes and polyblasts, but there are macrophages which phagocytize the detritus. The connective tissue is loose, and contains few cells and numerous fibers (Figure 119f). The muscle fibers decompose into fragments and lose the cross striation and myofibrils; their nuclei acquire an irregular shape, often become pyknotic, and decompose into parts (Figure 119g). The cartilage is destroyed, stains weakly, and cavities appear in it. A strong reaction to RNA, according to Brachet, is seen in the cells of epithelium, bone, different sectors of muscle fibers, and cartilage.

Total body irradiation, radiation sickness, and the fight against it

If local radiation injury is directly associated with suppression and distortion of regenerative capacity, and if restoration of that capacity may result in either the prevention of X-ray wounds or in their healing, is it not possible to affect the cause of radiation sickness by those means which can restore regenerative capacity suppressed by irradiation?

To study this problem, we made a series of experiments in which axolotls were subjected to total body irradiation with X-ray at a dose of 1,000 r, causing development of radiation sickness in the animal. A. F. Ivanitskaya (1959) showed that this dose of radiation results in the death of 100% of the irradiated axolotls within one month, but that the duration of the disease depends on the season and the temperature of the external environment. Preparations were introduced into the muscles of the back before (prophylaxis) or after (treatment) irradiation of the animals. Each experimental series had its own control (irradiation). Irradiation was always carried out in February-March.

After irradiation, the animals always developed radiation sickness. They became thin, ate poorly, frequently threw up the food, developed Saprolegnia on the skin of their body, and all died within 30 – 45 days after irradiation. The liver and the spleen degenerated.

In order to affect the course of radiation sickness and to increase their survival time, the animals were injected with the following preparations: fresh homogenate of hematopoietic organs; liver, spleen, and red bone-marrow of rabbits; highly polymerized freshly prepared DNA; highly polymerized freshly prepared RNA; acidic protein. In many experimental series, for example, that in which homogenates and DNA were introduced for therapeutic purposes, there was no effect at all; the experimental animals died at the same time as the controls, and sometimes even earlier. The investigator succeeded in prolonging the survival time of the irradiated axolotls in experiments with prophylactic injections of protein and RNA 1 $^1/_2$ 2 months before irradiation, and also with therapeutic injections of RNA immediately after irradiation. In these cases, the animals' resistance to Saprolegnia infection increased, their general condition improved, and the average survival time increased almost 2-fold (61 days against 33 days in the control). In the case of a prophylactic injection of RNA 1 $^1/_2$ 2 months before irradiation, a cumulative effect was observed. One or two RNA injections were sufficient to obtain a positive result.

The data obtained do not confirm the data concerning the increase in the survival time of mice after injections of DNA (Libinzon, Konstantinova, Muksinova, Popova, and Rogacheva, 1963) and are in good agreement with data that show an increase in the survival time of mice after injections of RNA, prepared from yeast (Luchnik, 1960; Maisin, Dumont, and Dunjic, 1960).

Consequently, the same method — use of highly polymerized freshly prepared RNA — on local irradiation of extremities in axolotls may restore the regenerative capacity which was suppressed by irradiation, while its use with animals of the same species having had total body irradiation, can prolong the life of those animals in which radiation sickness develops.

Another interesting observation should be pointed out, also. In a number of experiments with local irradiation of extremities in axolotls, the animals died within 6—8 months after irradiation. However, axolotls, in which the extremities were irradiated with the same dose and which simultaneously were given RNA to restore the regenerative capacity of the extremities, did not die and appeared quite healthy. Evidently, local irradiation leads to the formation of radiotoxins which cause the animal's death, while injection of RNA somehow neutralizes the effect of these toxins.

Mechanism of suppression of regenerative capacity under the influence of X-ray irradiation

What happens during the suppression of regenerative capacity of organs under the influence of X-ray irradiation? Impairment of differentiation is observed, but this is of a secondary nature as the stage of formation of the regeneration anlage preceding its differentiation is suppressed. Suppression of mitotic activity is also of secondary significance. In the epithelium of the skin of irradiated extremities of axolotls, the mitotic activity hardly changes. In the cells of tissues of mesodermal origin, mitotic activity decreases to one fourth under the influence of irradiation, but these cells change drastically immediately after irradiation, 10 days before they begin to divide mitotically, after having accumulated in the regeneration blastema. In addition, however, they retain their mitotic activity but lose the capacity for morphogenesis.

The main change in the cells of tissues of irradiated organs is that of their capacity for morphogenesis. This change occurs while the tissues retain their capacity for destruction and lose their capacity for dedifferentiation. In the irradiated cells, the normal morphological structure disappears. The cells of differentiated tissues cannot transform into embryonic-type cells with the characteristic large, light, bubblelike nucleus, large nucleolus, and cytoplasm rich in RNA. They become "coarse", the nucleus shows a coarse membrane and large chromatin granules, there is no nucleolus, and the cytoplasm hardly gives any Brachet reaction to RNA. Biochemical examination shows that irradiation leads to a deep change in the RNA and DNA content of the irradiated tissues, i. e., to a great change in nucleic acid metabolism and, consequently, to a change in protein synthesis.

Upon the loss of regenerative capacity of organs and tissues during ontogenesis and phylogenesis on the one hand, and under the influence of ionizing radiation on the other, the common denominator is tissue loss of the capacity for dedifferentiation. What is different is that in the former case, the capacity for destruction decreases sharply, while in the latter case it is retained. The loss of the capacity of the tissues of organs for dedifferentiation and the loss of the regenerating capacity during ontogenesis and phylogenesis is a regular and adaptive phenomenon caused by the animal's transition from one set of living conditions to another, e. g., from aquatic conditions to terrestrial ones. The capacity for reparative regeneration of organs is replaced by progressive differentiation and progressive development of the nervous system, in the process of which, the metabolism and destructibility of tissues is altered. Upon the action of ionizing radiation, the capacity for reparative regeneration of organs is lost as a result of a drastic impairment of the animals' natural adaptation to their conditions of existence. In the former case, the capacity for dedifferentiation is due to the destructibility of tissues, while in the latter case, it does not depend on it. In both cases, a change in metabolism is at the base of the changes in the regenerative capacity, but the characteristic of the metabolic change differs greatly. However, as the change in metabolism of nucleic acids and proteins is common to both cases, the effects of external factors on this metabolism may be similar in the two cases.

Upon total body irradiation, as well as in the case of local irradiation of different organs or parts of the body, nucleic acid and protein metabolism, biosynthesis of protein, and other types of metabolism are disturbed. However, the picture is very complicated when the total body is irradiated. First, the hematopoietic organs are injured, radiotoxins are formed in the body, secondary radiation injury develops. Upon local irradiation, these phenomena do not occur, or appear to a limited degree. The primary damage is exerted in the locally irradiated tissues. The radiation dose in the case of radiation injury to these tissues is higher than in the case of irradiation of the hematopoietic organs. In connection with all this, the result of total body irradiation, radiation sickness, cannot be reduced merely to suppression and distortion of regenerative capacity of hematapoietic organs.

Mechanism of restoration of regenerative capacity of irradiated organs and tissues

What happens during the restoration of regenerative capacity suppressed by X-ray irradiation?

In experiments on transplantation of nonirradiated tissues to an irradiated organ, and regeneration of the latter after amputation along the transplant (Umanskii, 1937, and others), the main source of regeneration is the tissue of the transplant. In these cases, therefore, we cannot speak of restoration of the regenerative capacity suppressed by irradiation. The same may be said concerning the experiments in which homogenates containing dissociated cells from nonirradiated tissues were injected into irradiated and amputated extremities of axolotls (Skowron and Roguskii, 1958; Polezhaev, 1959, and others). The demonstration that irradiated tissues may participate somehow in the process of regeneration of nonirradiated tissues transplanted to irradiated extremities of axolotls (Pukhal'skaya 1940; Liozner, 1941, 1946; Sidorova, 1949), does not elucidate the mechanism of the process. Nor can we exclude the possibility of insufficient suppression of regenerative capacity of tissues after irradiation of organs, and its manifestation under conditions of regeneration of the transplant. The data on the regeneration of extremities of axolotls irradiated with a dose of 3,500 and 5,000 r after implantation into the irradiated organs of pieces of nonirradiated heart, lung, testes, and other tissues (Sidorova, 1951; Lazard, 1965) indicate restoration of the regenerative capacity of the irradiated organs. Here, two arguments can be raised: 1) the radiation dose was insufficient and the regenerative capacity was not completely suppressed; 2) these experiments have not shown what happens with the cells and tissues after irradiation and during the process of restoration of the suppressed regenerative capacity.

In our experiments described earlier, the dose of radiation was high: 7,000, 8,000, and 10,000 r. The regenerative capacity of the extremities was suppressed in 93% of cases. Experiments in which axolotls with irradiated extremities were injected with homogenates of rat tissue, and also pure RNA or protein, and the experiments with repeated amputation, traumatization, and destruction of the irradiated tissues, clearly proved that true restoration of regenerative capacity suppressed by irradiation was dealt with here.

Tuchkova's experiments on the regeneration of black irradiated extremities transplanted to white axolotls show the main source of regeneration to be the local irradiated tissues and not migrating cells from the nonirradiated tissues of the recipient.

Upon irradiation and restoration of regenerative capacity of organs suppressed by irradiation, the capacity for destruction or the destructibility of tissues, does not change noticeably. The capacity for dedifferentiation of the irradiated tissues is suppressed and then experimentally restored. Experiments on restoration of regenerative capacity suppressed by irradiation, by administering RNA or protein to the animals, show that changes at first occur in the chemical processes and probably in the molecular structure, and only later in the morphological structure of the cells. Administration of preparations (RNA, protein) or the action of an excess of decomposition products of cells and tissues, produced by strong traumatization or destruction, leads to restoration of nucleic acid metabolism and protein biosynthesis impaired by radiation, and to normalization of the histochemical reactions in the cells, normalization of mitotic activity of the cells, normalization of the capacity of the cells and tissues for dedifferentiation and morphogenesis.

The capacity for dedifferentiation and morphogenesis is based on a certain metabolism and molecular structure of cells. This metabolism and structure are labile and may be changed in a controlled manner, as a result of which the capacity for reparative regeneration of organs and tissues in animals and man also can be changed in a controlled manner.

Practical significance of the data obtained

These data provide, in principle, proof of the possibility of restoring the capacity for reparative regeneration of organs and tissues in animals, suppressed by X-ray irradiation. In addition, they also show that this can be achieved by using injections not only of homogenates of some fresh or lyophilized, and even boiled tissues, but even by using proteins, and particularly RNA, which is highly active and at the same time devoid of antigenic properties. The role of trauma and the significance of the cumulative effect in the restoration of regenerative capacity suppressed by irradiation has been demonstrated. Finally, it has been shown that the restoration of the regenerative capacity suppressed by irradiation may be used as a preventive treatment for local radiation injuries, necrosis, and X-ray ulcers which do not heal for a long time, and which greatly resist treatment, and are transformed into malignant tumors in some cases.

Radiation therapy of malignant tumors and some other diseases is now widespread in medicine. However, it is very limited by the fact that irradiation of tumors, in particular those of internal organs, greatly damages the adjacent healthy tissues, which may subsequently become malignant. This fact forces the leading physicians-roentgenologists to consider limiting the radiation therapy method (Kozlova, 1961). It is clear, therefore, that the search for a method for combating local radiation changes is of great importance.

The elaboration of methods for combating radiation sickness also may proceed in the direction of the search for means by which the restoration of regenerative capacity suppressed by irradiation can be enhanced and, thus, improve the condition of the patient. We have seen that injections of highly polymeric freshly prepared RNA may considerably prolong the life of animals after total body irradiation and development of radiation sickness.

Goals and prospects of this study

The problem of the possibility of cell restoration after intensive irradiation with ionizing radiation is now being widely discussed in radiobiological literature (see "Radiobiologiya", "Informatsionnyi Byulleten'", No. 7, 1965). Investigators (Korogodin, Luchnik, Paribok, and others) define the problem as follows: to restore irradiated cells means to create conditions under which the processes which take place in the cell after its irradiation cannot reach completion, as a result of which the cell does not die, but survives. However, in essence, this survival of the cell is not true restoration. We assume that our experiments have given proof of true restoration of cells after postradiation damage. The cells of tissues of irradiated extremities of axolotls underwent deep changes: their chemical (molecular) and morphological structure changed, the capacity for dedifferentiation, morphogenesis, and regeneration was lost. After administering RNA, protein, or homogenates of certain tissues to animals with irradiated extremities, all these changes ceased, the properties of the cells became normal, and their capacity for dedifferentiation, morphogenesis, and regeneration was restored.

However, the established phenomena require a number of further studies; much still remains unclear. The mechanism of changes in cells after irradiation, and the mechanism of restoration are not clear. It is not clear in what way such substances as protein and RNA can change irradiated cells in such a manner that their properties are altered similarly, i. e., the cells are restored. The essence of the changes during the period of development of the "cumulative effect" is not clear. It is very important to establish whether the regenerative capacity of organs and tissues suppressed by X-ray irradiation in mammals can be restored. If this possibility were proven, it would become possible to treat local radiation injuries, in particular X-ray ulcers, which do not heal for long periods of time, and to open up a direct way toward radical improvement of the now limited method of radiation therapy of malignant tumors and other diseases.

The foregoing brief enumeration of some of the present goals shows the wide prospects of this study in the elaboration of this problem and its great practical importance. This problem is a real and soluble one and common efforts of different biologists, first of all of experimental morphologists, histologists, histochemists, cytologists, biochemists, physiologists, physicians, physicists, and chemists are required for its elaboration.

Part Three

GENERAL CONCLUSION

We have just discussed the theoretical basis of the problem of loss and restoration of regenerative capacity of animal organs and tissues, and the results of its experimental elaboration. We shall now discuss the advantages of the rules of regeneration we formulated for the understanding of some other phenomena of morphogenesis and changes in some physiological properties of the organism during ontogenesis of animals.

Chapter I

RESTORATION OF DAMAGED INTERNAL ORGANS

We are convinced that the capacity for reparative regeneration of extremities, bones of the cranial vault, cardiac muscle, and nervous tissue of the brain weakens or is lost with the increase in the complexity of organization during ontogenesis and in a comparative series of vertebrates, and that it can be restored experimentally. The question arises, is it possible to restore this capacity in other cases of injury to other organs?

The data on skin regeneration are of interest in this regard. All the investigators unanimously point out that human skin possesses a low capacity for reparative regeneration. After wounding, burns, and certain diseases, disfiguring scars appear in human skin, and its normal structure is not restored (Marchand, 1901; Goldzicher and Makai, 1913; Korschelt, 1927; Needham, 1952; Vorontsova and Liozner, 1955; Liozner, 1960; Montagna and Billingham, 1964, and others). In this connection, in order to replace skin defects, especially on the face, physicians frequently use autoplastic surgery of the skin. However, in certain cases, with the use of certain means, not only is the process of healing or scarification of skin wounds stimulated, but regeneration of skin also may be caused, albeit not quite typical skin. We cannot discuss here in detail this extensive and interesting problem; we shall only point out some very few data. It is known that so-called trophic skin wounds in people occur frequently and that they heal with great difficulty; in a number of cases they do not heal for 10—20, and even 40 years, resulting in severe incapacitation of the patients (see Protopopov. 1950). Numerous methods for the treating of trophic wounds are available, but they are not sufficiently effective. Frequently, they do not allow wound healing, and recurrences are often observed after healing. A high vacuum method for the treatment of trophic wounds has been suggested (Safronov, 1957; Ivanov and Safronov, 1959). The wound surface and the skin edges are dissected, and the damaged tissues are subjected to treatment with a sharp metallic point connected to a vacuum pump. The scarified tissues are strongly traumatized and impregnated with blood, become sterile, and devoid of bacterial and fungal microflora. Intensive processes of regeneration occur on the wound surface, and 30—45 days after operation the wound heals completely. It is remarkable that in the region of the wound it is not a scar that forms, but skin, although the skin is somewhat atypical. Recurrences occur in 5—10% of cases (Ivanov and Safronov, 1959). The method of vacuum therapy of trophic wounds is in essence one of the variations of the traumatization method. It is not only highly effective, but allows for obtaining regeneration of human skin.

Consequently, skin regeneration instead of scar formation may be caused even in man. Using another method, Grigor'ev and V. I. Orlova (1967) succeeded in obtaining a traceless, scarless healing of extensive skin wounds in humans. Transplantation of a piece of cornea from a cadaver and implantation of preserved tissues stimulate the regeneration of damaged cornea and clarify human and animal cataracts (Filatov, 1945). Transplantation of pieces of skin preserved at 2 or 4° enhances the regenerative capacity and stimulates processes of skin regeneration in various ulcerous diseases of man. Probably, the active principle in the stimulation of skin regeneration in the experiments of Filatov (1945) is similar to that in A. A. Safronov's (1957) experiments.

A question of great interest is whether it is possible to stimulate regeneration of so-called internal glandular and other organs such as liver, spleen, lymph glands, thymus, lungs, and glands of internal secretion; thyroid, adrenals, pancreas, ovary, and testes. The question of the capacity for regeneration of these organs has been discussed in detail in the reviews by Vorontsova and Liozner (1955, 1957), Liozner and co-workers (1960), and in particular by A. A. Voitkevich (1964). Certain general properties are characteristic of these organs; proliferation of the remaining part after amputation of a part of the organ; hypertrophy, without neoformation of tissue growth on the wound surface; so-called regeneration hypertrophy (Vorontsova, 1953). Later, Liozner (1962, 1964, 1966) called this phenomenon of hypertrophy, regeneration. Vorontsova and Liozner assumed that the basis for the identification of hypertrophy and regeneration is first, the fact that both these phenomena are caused by amputation of a part of an organ, and second, in both cases there is growth, increase in the mass of the damaged organ. D. S. Sarkisov (1962, 1963, 1965) and Sarkisov and others (1966) accepted the opinion of these investigators and began to call hypertrophy "intracellular regeneration."

However, the identification of hypertrophy and regeneration triggered justifiable objections on behalf of many Soviet and foreign scientists (Polezhaev 1957, 1958, 1959, 1964, 1965; Voitkevich, 1964, 1965, 1965a; Grigor'ev, 1965; Needham, 1964; Rose, 1964, and other investigators). In all the pathological-anatomy textbooks (see Nikiforov and Abrikosov, 1931; Abrikosov, 1949; Abrikosov and Strukov, 1961; Davydovskii, 1952, 1958; Cameron, 1952; Wright, 1958; A. Needham, 1960, and others) phenomena of hypertrophy and regeneration are always considered separately in different chapters, since these phenomena are qualitatively different. Hypertrophy is a compensatory phenomenon, a reaction compensating for the impairment of the work, the function, of an organ. Regeneration is restoration of cells, tissues, and organs after their loss, injury, or death; it is not a reaction of the organ to a functional disturbance. The mechanisms of regeneration and hypertrophy also are entirely different. Hypertrophy is characterized by an increase in the size of cells or of other structural elements of the organ without multiplication or hyperplasia, i. e., cell multiplication, and frequently it is a combination of one and the other. In hypertrophy, dedifferentiation is not observed. In the regeneration of an organ, the cells do not undergo hypertrophy, the initial stage of the process is characterized by the appearance of dedifferentiation, migration of cells into the focus of damage, and only later, is it followed by cell multiplication. In hypertrophy, there is no morphogenesis on the amputation wound surface, the wound heals

smoothly and scarifies, and the shape of the organ is not restored. In regeneration, there is morphogenesis in the focus of damage, as a result of which the disturbed shape of the organ is restored.

Voitkevich (1965), in addition to the aforesaid, communicated new, highly important data on the differences between regeneration and hypertrophy. Working on the restoration of endocrine organs, he showed that their hypertrophy (thyroid gland, pancreas, testes, ovaries, adrenals) is always more strongly manifested, the smaller the organ residue, or the greater its functional damage. However, administration of the hormone of the gland, a part of which has been removed, compensates for the functional insufficiency and fully prevents hypertrophy. Phenomena of true regeneration are thus enhanced, for example, neoformation of tiny follicles in the region of the amputation wound on the surface of a resected thyroid gland. Hypertrophy of thyroid, pancreas, and adrenals does not occur even when the hypothalamic-hypophyseal system is disturbed: in destruction of the hypophysis, or of the nuclei of the hypothalamus, or resection of the neurosecretory-hypophysis tract, the trophic function of the hypophysis is excluded. Hypertrophy of endocrine organs is regulated by hormones and by the nervous system.

On the other hand, by the administration of certain substances or hormones, the hypertrophy of resected spleen, ovaries, and tests may be enhanced (Voitkevich, 1965; Poluektov, 1962, 1963, 1964; Raitsina, 1958, 1962; Kadilov and Mezhlumyan, 1966). In these cases, there is no regeneration, and a scar appears on the amputation wound surface. The mechanism of the process remains the same as in the case of resection of these organs without stimulation.

Voitkevich (1965, 1966) rightfully says that hypertrophy and regeneration are opposed, antagonistic phenomena. To illustrate this position, we give an example with cardiac muscle. After a myocardial infarction appears, there is no regeneration of muscle fibers, the focus of damage scarifies, and the remaining part of the cardiac muscle hypertrophies. This is well known from data of pathological anatomy. In contrast, in experimentally caused regeneration of damaged cardiac muscle, there is no hypertrophy (Polezhaev and co-workers, 1965). And it is by far not a matter of indifference to the body whether a damaged organ regenerates or hypertrophies. The criteria for the distinction between hypertrophy and regeneration, however, are the objective, aforementioned symptoms, and not a functional symptom which may be absent even when complete regeneration of an organ occurs.

The presence of the amputation wound surface is not an attribute of regeneration. Regeneration may occur without the amputation wound surface, for example, when a ligature is applied to an axolotl extremity (Nasonov, 1930). On the other hand, after amputation of certain organs, for example the extremity of a rat, or the liver in mice or tritons, there is no regeneration, and the wounds heal smoothly.

An increase in the mass or dimensions of the liver and of other internal organs after their resection is a phenomenon of growth but not of development, which is observed in regeneration of organs. The latter is a phenomenon of repeated development, and is characterized by a complex process of morphogenesis, analagous to embryonic development of organs, which is not observed in hypertrophy. At the same time, growth of organs during the normal process of ontogenesis of animals and during hypertrophy differs, proceeding in different ways and being determined by different

factors. In ontogenesis, the organs grow as they develop, they obey the laws of development; growth is regulated by the interaction of the parts of the organ, by the interaction of organs, by the influence of the internal humoral milieu, hormones, vitamins, conditions of external environment, influences of the nervous system. Morphogenesis of organs takes place in the process of growth. In hypertrophy, growth is accomplished according to entirely different laws, it is determined by a functional tension which arises in the organism as a result of the resection. There is no morphogenesis in this case.

Regeneration, growth, embryonic development, hypertrophy, hyperplasia, neoformation of malignant tumors, asexual and sexual multiplication, all these are qualitatively different phenomena of reproduction of living matter. They all have something in common, they all are based on biosynthesis of proteins and nucleoproteins, but they all differ basically from each other in the nature of this biosynthesis, the form, mechanism, and regularities of development. Therefore, there is no basis for their identification, and in particular for the identification of hypertrophy and regeneration, since it is not possible to identify with each other different forms of energy: thermal, electrical, mechanical, photic, chemical, and intra-atomic on the basis that they all are different types of energy. The transformation of one form of energy into another is of great scientific and practical importance. The same may be said of the possibility of transformation of one form of self-reproduction of a living being into another form, e. g., of the capacity for hypertrophy into the capacity for regeneration. In principle, this is possible. We became convinced that this is so in experiments on mammalian hearts: in creating certain conditions we may cause regeneration and not hypertrophy of cardiac muscle. Voitkevich (1965) showed that instead of hypertrophy of the thyroid gland, it is possible to obtain true regeneration, e. g., upon transplantation of fragments of the thyroid into the anterior chamber of the eye of rats and frogs (Bredikhin, 1962, 1963; Somova, 1964).

Liozner (1962) and co-workers tried to dissociate hypertrophy into 2 supposedly different phenomena: compensatory and regenerative hypertrophy, and relate the latter to regeneration. By compensatory hypertrophy they mean hypertrophy taking place in one of a pair of organs after removal of the other, e. g., hypertrophy of a kidney after removal of the other kidney; by "regenerative hypertrophy" they mean hypertrophy taking place after resection of an organ, e. g., a part of a liver lobe. However, according to data from pathological anatomy, and according to the investigators, the phenomena of hypertrophy in these 2 cases are similar, and only the means by which they were caused differ. However, it has already been shown that the presence of an amputation wound surface does not always lead to regeneration. Starting from the point of view of the aforementioned authors, reveals that it is unclear how to designate hypertrophy occurring in the liver after complete removal of 2—3 of its lobes without amputation of part of a lobe. All the remaining part of the organ hypertrophies. Is this compensatory or regenerative hypertrophy? Finally, the reason for both these phenomena is functional tension of an organ leading to its hypertrophy, no matter whether the organ was removed completely or only partially. It is quite clear that the division of hypertrophy into two supposedly qualitatively different phenomena, and attempts to designate hypertrophy which takes place

after traumatization (post-traumatic) as regeneration, is artificial to a very high degree. It clouds the issue, leads to denial of the existence of a general rule of regeneration (Liozner, 1962), and creates an illusion that the problem of regeneration of mammalian organs has been solved. Rose (1964) says that the identification of hypertrophy with regeneration confuses scientists. A. Needham (1964) voices a similar bewilderment.

The capacity for repair regeneration of organs in mammals is very limited. However, on the basis of the fact that a number of mammalian internal organs (liver, kidneys, etc.) are capable of hypertrophy, Liozner (1965) says that the present study of regeneration has passed to a new higher stage of its development, having proven that mammals are capable of regeneration of organs. This statement is utterly incorrect and misleading.

We cannot deny the importance of hypertrophy of internal organs for the human and animal body. We ought to work on hypertrophy, and the studies in this direction made by Liozner and his co-workers, Sarkisov, B. P. Solopaev, and others, are of no small importance. It does not follow from this, however, that hypertrophy can be identified with regeneration. To call a known phenomenon of hypertrophy by the name of regeneration is not a discovery, contributes nothing new to science and, finally, cannot raise the study of regeneration to a new level. Development of science is determined by discoveries and inventions, and not by new names or new interpretations of old, well-known facts.

Why, then, do they insist so much on the identification of two qualitatively different forms of self-reproduction, hypertrophy and repair regeneration? What do they gain by it? This identification allowed Vorontsova and Liozner (1957) to reach the paradoxical conclusion that all vertebrates, and mammals in particular, are capable of regeneration of organs. This statement made the impression that the problem of organ regeneration in mammals had been solved, that this conclusion is very progressive, and that it overthrows Weismannism in the study of regeneration, which supposedly consists of the admission that the capacity for reparative regeneration of organs in mammals is low. However, life showed the utterly erroneous nature of these statements. Weismannism in the study of regeneration does not at all consist of that statement, but of the idea that development and regeneration do not depend on environmental conditions. Finally, the accusation of scientists in adherence to Weismannism has recently lost its edge.

In this connection, the fact that dogs and rats are incapable of regeneration of extremities, tails, eyes, and even skin or testes, does not look odd to anyone anymore. It is better to try to understand what hypertrophy is, and what regeneration is, and how they can be changed.

Z. A. Ryabinina (1961) established a highly important fact: after removal of 70% of the mass of the liver in rats, there is hypertrophy of the residue at first, but later, 11—17 months after operation, the liver begins to atrophy: the RNA content of the cytoplasm and of the nucleoli of the liver cells, and the glycogen content decreases, the cell dimensions are reduced but the size of the nuclei does not change. Consequently, the hypertrophied organ is in a state of supertension which leads to its emaciation, and sometimes to its becoming malignant (Voitkevich, 1965).

As we have already pointed out, hypertrophy of an organ after resection of part of it proceeds by means of hypertrophy of its cells and by hyperplasia. Hypertrophy of cells is caused by their functional stress and leads to a very rapid restoration of the weight or mass of the organ, and to considerable compensation of its function, which has been greatly impaired as a result of its resection. This fact is highly important to the body, saving it from catastrophe. However, the body cannot exist for a long time merely on over-straining of organ functions. The importance of hyperplasia, the appearance of which normalizes the function of the organ, is therefore quite clear. The relationship between hypertrophy and hyperplasia differs greatly in different organs. For example, after resection of liver, hypertrophy of cells mainly occurs, while after resection of the thyroid gland and the ovary, hyperplasia predominates. The mechanisms of these processes differ. In hypertrophy of the cell, there is an increase in size as if by swelling of the cytoplasm, mitochondria, and other elements. In hyperplasia, there is cell multiplication without an increase in cell volume, and the different structural elements of the organ appear, e.g., new follicles in the thyroid gland. Hyperplasia occurs on the basis of physiological regeneration, the mechanisms of these processes being essentially identical. Hypertrophy of a cell cannot be called physiological regeneration. This is a phenomenon of a special kind, this is growth, caused by functional stress in the cell. We must point out in this connection that recently, with regard to the tendency of certain investigators to give an excessively broad meaning to the notion of regeneration, including in it hypertrophy, an attempt was also made to interpret the polyploid increase in cell size as physiological regeneration (Brodskii, 1964, 1965, 1966). Kravskii and Liozner (1966) go even further, stating: "In essence, the processes of recovery which take place after various pathological disturbances, basically represent some or other manifestiation of regeneration" (p. 3). Such a broad interpretation of regeneration is not justified, however, and leads to an ill-founded identification of different phenomena with each other, and does not, therefore, allow for establishing the rules governing these phenomena, in particular the rule of regeneration. In fact, if both the restoration of a lost organ and smooth healing of a wound are called regeneration, it is clear that it is impossible to establish any rule of regeneration whatsoever.

However, knowledge of the differences between phenomena of self-reproduction of living matter and their mechanisms is very important, both theoretically and practically. Hypertrophy of glands of internal secretion depends on the hypothalamic-hypophyseal regulation, it is conditioned by hormonal action (Voitkevich, 1965). For example, by enhancing the thyrotrophic function of the hypophysis, hypertrophy of the resected thyroid gland can be enhanced, while when its function is eliminated, hypertrophy of thyroid gland also can be prevented. The physiological regeneration of this organ does not noticeably change under these conditions. However, by the use of other means, for example, by administration of thyroid hormone, the physiological regeneration and hyperplasia of the thyroid gland and of some other organs can be stimulated. This fact is of great importance in the treatment of certain pathological conditions, and for the control of changes in phenomena of morphogenesis in animals and man.

It is possible to cause regeneration of such internal organs as liver, spleen, glands of internal secretion? And if the answer is yes, how is it to be done? Is it possible to use enhancement of phenomena of destruction and dedifferentiation for this purpose? In designing experiments on resection of part of a liver lobe in mice and rats, we did not succeed in obtaining regeneration of tissue from the amputated wound surface of the organ, even when using a hydrolysate of liver as a regeneration stimulant. On administering into brains of adult rats decomposition products of brain nerve tissue, we did not succeed in causing reparative regeneration in the focus of damage, but we obtained a strong stimulation of physiological regeneration of nerve tissue (Polezhaev and Karnaukhova, 1962, 1963; Reznikov, 1965, 1966). This fact once again indicates that reparative and physiological regeneration of organs are different phenomena, and that the latter cannot always serve as the basis for the former. On the other hand, these data show that enhancement of phenomena of destruction and dedifferentiation may, at least in some organs, stimulate their physiological regeneration in the absence of reparative regeneration. This fact is highly important. It may serve as a means for cellular renewal of an organ and improvement of its function (Kolchin and Reznikov, 1964, 1966; Reznikov and Kondik, 1966). These data justify the conclusion that enhancement of destruction and dedifferentiation of tissues, and methods based on this principle, may be powerful means for stimulating physiological regeneration and hyperplasia of organs and, consequently, may be a means of lasting and stable enhancement of existing functions or enhancement of disturbed functions of numerous internal organs, primarily endocrine, in which hyperplasia takes place. This conclusion requires experimental confirmation and further development, which may have very good prospects.

Thus, we reach the conclusion that even in those organs which do not have a capacity for reparative regeneration, and which so far nobody has succeeded in restoring (mammalian brain etc.), physiological regeneration can be stimulated and their physiological functions improved or restored, using methods based on enhancement of destruction and dedifferentiation of tissues composing these organs. However, these methods cannot enhance hypertrophy of cells and organs. The knowledge of these facts can hardly be overestimated.

Chapter II

RESTORATION OF LOST FUNCTIONS

Functions of the animal body may be lost in two cases: 1) upon the loss of an organ, and 2) in the presence of the organ but as a result of changes in the properties of its components (different tissues and cells, nervous system, internal humoral environment). Investigators who study processes of reparative regeneration of organs usually deal with the former case of change in functions: an organ is lost, e. g., an extremity, and correspondingly its function is lost, e. g., motor function. It is natural that when the organ is restored, its function is also restored. This situation is so obvious that investigators who study regeneration of lost organs are only secondarily interested in the problem of restoration of lost functions. The main attention is given to the process of regeneration of the organ as a result of which there is restoration of its lost functions. Therefore, for a long time studies of regeneration were primarily concerned with the morphological changes occurring after the loss of an organ.

With the development of the study of regeneration, however, investigation reached deeper levels, and morphogenesis was investigated in relation to physiological conditions. We long ago reached the conclusion that the decisive factor for the regeneration of an organ is the physiological state of its component tissues (Polezhaev, 1933, 1933a). If in an axolotl, which possesses a high capacity for regeneration, we artificially reduce the processes of decomposition of tissues in the region of the wound after amputation of an extremity, this organ will not regenerate, and the wound will heal smoothly. However, if we amputate an extremity in tadpoles or adult frogs which has lost the capacity for regeneration, and enhance destruction and dedifferentiation of tissues in the organ residue, regeneration of extremities can be reduced. Regeneration of organs also depends on the physiological influences of the nervous system, the internal humoral milieu, hormones, properties of the tissue components of the organ, effect of temperature, light, etc. Affecting the process of regeneration by changing any one of these factors, we can stimulate or inhibit the process and change it in a controlled manner. In so doing we shall also change the function of the regenerating organ.

This approach to the study of regeneration may be called morphofunctional, or the experimental-morphological approach.

In the study of processes of regeneration, special attention has been directed to the extent of restoration of functions changed by damage or amputation of organs. One of the first studies in this direction was Pavlov's work (1890) in which he determined biochemically the degree of restoration of artificially stimulated salivary glands, measuring nitrogen

in the excreted saliva by the Kjeldahl method. Later, numerous investigators began to study the degree of restoration of functions of damaged and regenerating organs and tissues. In our laboratory, this approach is widely used in experiments on stimulation of regeneration of cardiac muscle (Polezhaev and co-workers, 1965), and on stimulation of physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres of the brain (Kochin and Reznikov, 1964, 1966; Reznikov and Kondik, 1966, and others); in the laboratory of Studitskii (1959), in experiments on the regeneration of skeletal muscles; in the laboratory of Voitkevich (1963, 1964), in experiments on the regeneration of endocrine organs. The necessity for this type of study has been pointed out by Liozner (1962). Many more similar examples can be quoted.

In connection with this, we should point out that the degree of functional restoration of an organ does not yet indicate a similar degree of its morphological restoration. The function of a damaged organ may be restored as a result of compensation and not regeneration. Thus for example, in experiments with bilateral extirpation of the optic (occipital) regions of the cortex of the cerebral hemispheres of the brain in 2—5 week-old kittens, the impaired visual function is rapidly and fully restored (Kogan and Ivannikova, 1955), but, as shown by histological examination, the nervous tissue of the cortex is not restored in the process (Ivannikova, 1963). The function of the cortex was restored due to compensation and not to regeneration. On the other hand, the process of complete typical regeneration of an organ, e.g., an axolotl extremity, may take place without restoration of the organ's motor function: when the motor nerves are cut (Schotté, 1926 and others), or upon removal of the skeleton of the extremity (Brunst, 1932). Therefore, we can in no way agree with the opinion voiced by some investigators that the main index of regeneration of organs and tissues is the restoration of their function impaired by damage to the organs.

To review the analysis of cases in which an organ is not lost but its function weakens or is lost completely, numerous cases in human and animal pathology may be cited. Here are some examples: weakening of cardiac activity as a result of dystrophy of the myocardium; weakening of memory or of conditioned reflex activity with age in people; decrease in the protective forces of the body in fighting infections upon some kind of weakening of the body; male or female sterility in humans and other mammals as a result of impairment of the functioning of the sexual glands, and many other examples. A number of diseases of old age may be related to this category. Medicine and veterinary medicine possess a number of means to fight these pathological phenomena. A whole battery of pharmacological preparations is used by physicians for stimulation in cardiac insufficiency. To cure male impotence, male sexual hormones (methyltestosterone, methyltestosterone propienate, etc.) are used. However, it must be admitted that in the majority of cases, this kind of treatment is not sufficiently effective, and it gives only a short-lived effect but no healing. In the case of hormone insufficiency, the administration of hormone from without leads to further weakening of the reduced secretion of the given endocrine hormone. Weakening of functions with age, decrease of protective forces and of memory, and a number of other functions, unfortunately, cannot be treated successfully by the usual pharmacological means.

We assume that not pharmacological, but biological methods are necessary for the treatment of such phenomena. Impaired or weakened functions of organs and tissues can be restored, based on the regeneration theory, by methods which are used to restore weakened or lost capacity for reparative or physiological regeneration of organs and tissues. This fact is quite clear: physiological functions are always conditioned by the physiological state of the cells and tissues of a given organ and those organs correlated with it. Consequently, stimulation of physiological or reparative regeneration of organs may be expected to enhance physiological functions. We put forward and developed this idea in a number of our previous studies (Polezhaev, 1950, 1956, 1958, 1959, 1961, etc.). When an organ is acted on and its cellular composition renewed, its function, as a rule, should improve not briefly, but lastingly. It is possible to enhance the regenerative capacity of an organ and its function respectively, by enhancing destruction and dedifferentiation. Consequently, we have at hand a powerful means by which we can enhance normal functions or restore lost and weakened functions of various organs, not in an empirical manner but on a sound, scientific bases. Work on this plane constitutes a special direction of extensive and diverse studies and has great prospects. Several examples follow.

It has been pointed out earlier (Part II, Chapter 1) that additional traumatization of the organ residue, after amputation of an extremity in grass frog tadpoles, leads to tissue destruction and dedifferentiation, to rejuvenation, and to a lower degree of differentiation of the experimental extremity as compared with the control. The regenerative capacity in such experimental extremities is higher than in the control extremities. Consequently, the physiological properties of tissues of regenerating extremities were different from those of control extremities. We also pointed out that tissues of a regenerating tail in metamorphosizing tadpoles are more resistant to the thyroid hormone than are tissues of intact animals of the same age (Speidel, 1929).

We have seen (Part II, Chapter 4) that stimulation of regeneration of cardiac muscle in mammals leads to normalization of the electrocardiographic indices, i. e., to improvement of the physiology of the myocardium. Recently, Kolchin (1966, 1966a, 1966b) has shown that stimulation of regeneration of cardiac muscle in diphtherial myocarditis results in considerable improvement of the functioning and efficiency of the myocardium. This investigator (Kolchin, 1967) also showed that a myocardial hydrolysate and a number of other stimulants of cardiac muscle regeneration, can stimulate the work of the heart of a frog isolated according to Straub's method.

Studying the problem of stimulation of physiological regeneration of the nervous tissue of the cortex of the cerebral hemispheres, we became convinced that introduction of stimulants (RNA, brain dialysate) into the brain or cavity of the 4th ventricle of adult rats, leads, simultaneously with stimulation of amitotic division of neurons, to a strong physiological change in the functioning of the higher nervous activity, a change in the processes of stimulation and inhibition (Kolchin and Reznikov, 1964, 1966; Reznikov and Kondik, 1966).

Earlier, we described (Polezhaev, 1956) the finding that the functioning of the body's protective forces in white mice can be enhanced if the physiological regeneration of the reticulo-endothelial system is stimulated.

After receiving a lethal dose of Staphylococcus albus, the control animals died but the experimental animals survived. Similar results were obtained in experiments with mice infected with Salmonella typhosa (Tushnov, 1938).

These data confirm our concept that the enhancing of destruction and dedifferentiation of tissues can, in principle, enhance or restore the lost physiological functions of any organ. The significance of this conclusion can hardly be overestimated.

Our conclusions should be compared with some data from the literature.

Our principles and conclusions are in good agreement with those of Tushnov (1938), A. A. Bogomolets (1956), Filatov (1945), Rumyantsev, (1951, 1952) and Niehans (1948). There are some common and some different aspects in these principles and conclusions.

Tushnov (1938) developed a theory on lysates or interexcretions. According to his ideas, in the process of metabolism cells continuously excrete into the surrounding milieu metabolic products, intersecretions. These substances are organospecific and act primarily on the tissues of the same organ, in small doses, stimulating their development and functioning, and in large doses suppressing them. These statements relate to normal development and also to regeneration, as reparative regeneration is repeated development, and as physiological regeneration always occur in the process of normal ontogenesis.

Carrying out an enzymatic hydrolysis of tissues of a number of organs, and preparing from the organs hydrolysates or lysates analogous to the natural intersecretions, Tushnov demonstrated their high efficacy and specificity. In small doses, they greatly enhanced weakened functions of various organs and enabled treatment of the appropriate diseases in man and animals; enhanced the weakened function of the cardiac muscle; cured sterility in females and males, impotence in men, and reduced sexual function in women; normalized impaired function of the thyroid gland; enhanced weight increase in animals based on the enhancement of body muscle growth; enhanced the protective forces in fighting infections, etc. The principle of lysate therapy, similar to our principle of enhancement of regenerative capacity, directed the investigators' efforts not toward compensation of the missing substances by addition from without, but toward enhancement of their production by the body itself. Yuldybaev (1954, 1956), using lysate therapy according to Tushnov (testo- and ovariolysates), cured sterility in horses in 98% of cases. Tushnov also achieved the same effect by using testolysates. This example makes it clear that the essence of lysate therapy consists of enhancing the regenerative capacity of organs by a chemical approach. Tushnov's lysates were used by a number of investigators to stimulate the regeneration of tubular bones, and some other organs and tissues. There are data on stimulation of regeneration of tissues under the influence of various autolysates of the tissues of liver, skin, muscles, and other organs (Sel'kov and Vasil'ev, 1935). In our experiments, a hydrolysate of cartilage induced regeneration of extremities in adult Bombina, or proliferation of cartilage around the skeleton in the organ residue after amputation of extremities (Polezhaev and Ramenskaya, 1950). Hydrolysate of myocardium stimulated regeneration of damaged cardiac muscle in rats and rabbits (Polezhaev and co-workers, 1965), and caused its regeneration in diphtherial myocarditis in rabbits (Polezhaev, Kolchin and

Solntseva, 1965; Polezhaev, Kolchin and Sadokova, 1966). The hydrolysate enhanced destruction and dedifferentiation of tissues.

Bogomolets' (1956) theory on ACS includes data on stimulation of tissue regeneration. It has been shown that in small doses, ACS can stimulate regeneration of damaged tubular bones in rabbits (Bogomolets, 1944). In large doses, the myocytotoxic serum inhibits regeneration of extremities in axolotls (Striganova, 1954).

ACS acts on the principle of cytotoxic sera, i. e., in a different manner than do lysates, but like the latter, it also stimulates regeneration processes and enhances the protective, phagocytic, and other functions of the organism. It is possible that ACS also enhances destruction and dedifferentiation of tissues. This problem requires further investigation.

Filatov (1945) thinks that the basis for the therapeutic effect of tissue preparations is their property to stimulate regeneration processes. This conclusion was strengthened by experiments on the stimulation of healing of skin wounds in animals, and stimulation of regeneration of epithelium of the the cornea in frogs (Yanyk, 1940, 1950; C. Likhachev, 1953). We have shown that implantation of skin or muscles, preserved according to Filatov, induces the regeneration of extremities in adult Bombina (Polezhaev, 1948). This treatment enhances phenomena of destruction and dedifferentiation of tissues. Rumyantsev (1950, 1953) also points out that implantation of tissue preparations enhances the body's regenerative capacity.

The Niehans (Niehans, 1948; Kuhn, 1956, and others) principle of cell treatment gives results which are, to a high degree, similar to those obtained upon treatment by implantation of preserved tissues according to Filatov, and especially to those of Rumyantsev. The principle of this approach is that upon impairment of certain functions, e. g., sleep, growth of body or of some organs, which are primarily related to certain endocrine disturbances, it is possible to determine which organs are injured; subsequently, tissues of corresponding organs taken from cattle are lyophilized, and suspensions of the preparations are administered parenterally to the human patient. As a result, the impaired functions are restored, e. g., insomnia is cured, anomalies caused by endocrine disturbances, and in particular certain severe inborn psychic anomalies (as for example, certain forms of oligophrenia, etc.) are cured. At the basis of the normalization of the impaired functions is a change in the condition of the substrate, changed organ tissues. Regretfully, so far it is unknown what changes take place in tissues leading to normalization of functions in such cases. It is possible that the tissues change in a similar manner as that observed when destruction and dedifferentiation in these tissues are enhanced. Further studies are also required in this area.

In recent years, data have appeared showing that in order to restore a number of functions lost in human beings as a result of atrophy or degeneration of the tissues of certain organs, preparations of organospecific RNA (Dyckerhoff and Gaus, 1965) can be used successfully. These preparations do not have antigenic properties and are not toxic (Goossens and Gastrap, 1960). The administration of such preparations to deafened persons allows full restoration of hearing, while the administration of an RNA complex to old persons helps the hemoglobin content increase by 17%, and helps bring about rejuvenation.

Our brief review and comparisons made between our data and those from the literature lead to the following conclusions.

1. It is possible to restore impaired or lost functions of the animal and human body without loss of the respective organs, by changing the condition of cells and tissues in these organs or in other components and systems of the organism associated with them.

2. This restoration may be achieved by means through which one can restore lost regenerative capacity of organs and tissues, i. e., means which enhance destruction and dedifferentiation of tissues.

Thus, the formula for the rule of loss and restoration of regenerative capacity of organs in animals is applicable to a very wide group of cases of loss and restoration of functions without loss of the respective organs and tissues. This fact enables the investigator to greatly enlarge the scope of studies and to bridge the gap between the basic problems of biology and medicine. In this manner, a close association is being established between the short-term and long-term goals of theory and practice in these fields. It is possible to indicate such goals which quite recently seemed to be entirely unreal to physicians and biologists. For example, it becomes possible to pose and experimentally develop the goal of restoring [vision in] cortical blindness, i. e., blindness due to changes in the optic regions of the cortex of the cerebral hemispheres of the brain, and not to changes in the peripheral optic apparatus. It is possible to design ways to restore a number of lost or weakened important functions; this could not have been done until now on the basis of medicine's old approach. These ways also could not have been proposed on the basis of the banal idea of restoration of functions during regeneration of lost organs or on the basis of the very interesting ideas on the restoration of functions of a damaged organ by acting on the nervous system (Frankshtein, 1948).

Chapter III

CHANGES IN REGENERATIVE CAPACITY AND IMMUNITY

The question of the relationship between regenerative capacity and immunity is highly interesting and important. As far as we know, this question has not yet been touched upon by any investigators except in our studies (Polezhaev, 1957), although the phenomena of regeneration and immunity, have been extensively studied separately.

It is well-known that the basis of immunity or resistance to infections (bacterial, viral, and fungal) is a specific antigen-antibody reaction, the antigen being a protein. Mechnikov (1901, 1947) very early showed that immune reactions appear not only toward the proteins of the microflora but also to any foreign cells and tissues. This originated a new direction in immunology, study of the properties of the so-called cytotoxic sera or antisera, which was so successfully developed by Bogomolets (1942, 1956) in his theory on ACS. The high specificity and finesse of the immunological reactions made possible the use of immunological methods in the study of processes of ontogenetic morphogenesis. In this connection, a new direction was indicated, immunological embryology (see Vyazov, 1962). The phenomenon of immunity and the mechanism of immunological reactions change during animal ontogenesis. The nature of immunity during embryogenesis differs sharply from that in the postnatal period of animal development (Tokin, 1955). In studies of the regeneration processes, attempts were also made to use methods of immunology. Thus, it has been shown, that regeneration of extremities in axolotls can be inhibited by the use of myocytotoxic serum (Striganova, 1954). In this case, as well as in the study of embryogenesis by methods of immunology, the effect of immune reactions on processes of morphogenesis may elucidated and to some extent so also may the mechanism of morphogenesis. However, the question which interests us, concerning the relationship between regenerative capacity and immunity during ontogenesis of animals, still remains open.

We decided to approach an analysis of this problem, comparing the changes in regenerative capacity and in immunity during animal ontogenesis, and attempting to affect immunity by the same means used to change regenerative capacity (Polezhaev, 1957). The study was conducted on metamorphosizing tadpoles of the grass frog (Rana temporaria). As is well-known, in the process of metamorphosis the regenerative capacity of extremities, the tail, and other organs of tadpoles is lost. The analysis of this problem has been described earlier (Part II, Chapter 1 of this book). It has been shown that in the process of losing regenerative capacity, the capacity of tissues for destruction and dedifferentiation is greatly reduced, involving changes

both in epidermis and in tissues of mesodermal origin, as well as in the internal humoral milieu. During metamorphosis, the type of metabolism changes greatly in the animal: the intensity of aerobic respiration increases 2-fold, proteolysis is enhanced and protein synthesis in tissues is also increased, there is a reshuffling of proteins in all the organs and tissues, the water content of cells and tissues diminishes, the active reaction of the internal milieu changes in the acidic direction, etc. (Shaper, 1902; Groebbels, 1922; Adolph, 1926; Aleschin, 1926, and others). It may be assumed that these physiological, metabolic changes in the organism, taking place during metamorphosis, cause morphological and morphogenetic changes in the organs and tissues, and in particular changes in their capacity for reparative regeneration. All these ontogenetic changes can be looked upon, according to the biogenetic law, as a recapitulation of the phylogenetic changes, whereupon both the latter and the former are a result of the adaptation of animals to changing living conditions. Drawing this conclusion, we do not at all go into the mechanism or sequence of changes which take place in the process, a problem which is of great biological significance. What is important to emphasize is that the capacity for reparative regeneration of organs in tailless Amphibia, and in other animal species, changes regularly during their phylogenesis and ontogenesis, depending on the adaptive physiological changes in these organisms. This is understandable. It suffices to mention that at the basis of the physiological stages of regeneration, there is protein metabolism (proteolysis, protein synthesis), nucleic acid metabolism, the nature and level of oxidative processes, a certain active reaction of the internal milieu, etc. These physiological changes during regeneration will necessarily occur under the influence of similar physiological processes taking place during adaptive changes in organisms, for example, upon transition from aquatic to terrestrial conditions. It is also understandable that if the investigator were to change artificially in a controlled manner the general physiological conditions of the body, he would also regularly change its regenerative capacity. And indeed we could convince ourselves that this is so in a direct experiment, changing the capacity for regeneration of extremities in tadpoles by changing their living conditions (Polezhaev, 1955). The regenerative capacity of organs can be changed in a directed manner by changing the physiological stages of regeneration.

Does the immunity of these species of tailless Amphibia change during metamorphosis? At first, chance observations, made in the course of experiments on changing the regenerative capacity of extremities in tadpoles of the grass frog, and later in specially designed experiments, convinced us that immunity indeed changes during metamorphosis, and that these changes are regular but not identical in different infections. We dealt with two very widespread diseases of amphibians and fishes: red leg [Krasnutha in Russian] and Saprolegnia.

Red leg causes outbursts of epizootics in fishes, and sometimes leads to widespread death of commercial fishes, e.g., of carp and bream in the Azov Sea or of carp in ponds, resulting in great losses to our country's national economy. In the case of red leg of frogs and fishes, the skin reddens greatly, ulcers form on it, and as a result of ascites, the belly distends and the animals die. If an extremity of a frog is amputated and if the open wound is infected, the stump does not become epithelialized but

remains open, reddens intensively and swells, and the animal dies after some time, showing the signs just described. The causative agent of red leg or of hemorrhagic septicemia is considered to be B. septicaemiae ranarum or a virus (Goncharov, 1954). In ponds and in spawning farms, red leg of fish is combated by amelioration and sanitary hygienic methods. Attempts are being made to vaccinate fish against the disease agent. Other biological methods of combating red leg by increasing the resistance of the animals to infection have not been elaborated at all. Saprolegnia is manifested by injury to the skin integuments of the animals by the fungus Saprolegnia in the form of a mushy, grayish-white mould which eats away the skin; intoxication and death follows. Sometimes Saprolegnia massively affects the roe, which subsequently perishes. Healthy, well-fed animals in good physiological condition are usually much less prone to succumb to Saprolegnia and red leg than are diseased, weak animals or those with damaged integuments.

According to our observations, larvae and adult Bombina bombina are much more resistant to red leg and Saprolegnia than are frogs. Upon amputation of extremities and contact with sick animals, adult frogs succumb to red leg in 100% of cases and die, while adult Bombina bombina catch the disease and die in only a few cases.

The main experiment was performed on 1,800 tadpoles of Rana temporaria taken from the various stages of metamorphosis, according to Blyakher (1928) (see Part II, Chapter 1 of this book), and on 300 adult animals. We amputated the hind extremities of the tadpoles at the level of the knee or the foot, while we amputated the forelegs in the middle of the forearm or at the wrist in the adult animals. After amputation of the extremities, the animals were placed together with sick animals for 5—7 days and subjected to various treatments: varying temperatures, treatment with a 0.5% solution of NaCl, 5% solution of glucose, diluted in fresh water, and a solution of sulfadiazine at a concentration of 1:2,000. The treatment was carried out 10—15 hours daily, totaling up to 80 hours. Solutions of table salt were often used to combat Saprolegnia under laboratory conditions; on the other hand, it is known that these solutions, as well as glucose solutions, stimulate regenerative processes and allow the regeneration of extremities in tadpoles and in adult frogs (Polezhaev, 1948; Rose, 1944). Sulfadiazine was used to prevent infections in the operated frogs (Rose, 1944).

The experiments gave quite clear-cut and interesting results. The capacity for regeneration of extremities and immunity proved to be closely related to each other. After amputation of extremities at the level of the knee at stage I, regeneration of these organs takes place in 100% of cases, and the tadpoles do not succumb to red leg despite being infected. After amputation of extremities at stages at which they have already lost the capacity for regeneration, the tadpoles succumb to red leg and die: after amputation at Stage IIIa, in 10% of cases; at Stage IV, in 45% of cases. Consequently, with its development, the tadpole's resistance to red leg, as well as its capacity for reparative regeneration of organs decreases. A very interesting phenomenon is observed at Stage IIa—IIb, i. e., at the critical stage when the regenerative capacity of extremities is lost. The infected wounds at first heal smoothly or regeneration begins, forming regeneration buds. As soon as the animals reach Stage III—IV,

however, the wounds open up, the stumps of the extremities redden, and the animals die from red leg. During this critical period, there is a simultaneous and rapid change in regenerative capacity and immunity. These data are in good agreement with those of R. I. Belkin and K. A. Fride (1941) who showed that axolotls, immunized against B. septicaemiae ranarum, lose the acquired immunity after artificially caused metamorphosis. Our data on the reopening of infected wounds after the animals reach a certain age, or to be more precise, a certain stage of ontogenesis, may be compared with clinical data on human beings, showing that in a number of cases wounds which had healed long before may reopen without any obvious external reasons. It may be assumed that the reason for this is a change in the protective forces of the body (immunity), caused by physiological changes during ontogenesis.

From the data on red leg, we cannot conclude that during ontogenesis, immunity to various infectious diseases always decreases regularly in connection with the decrease of regenerative capacity. Immunity to Saprolegnia in tadpoles of the grass frog, on the contrary, regularly increases during metamorphosis. At Stage I, the tadpoles are easily infected and succumb to Saprolegnia, while they do not suffer from the disease at Stages IIIb—IV. Animals who develop Saprolegnia at Stages I or II are cured spontaneously during metamorphosis. Probably, a similar phenomenon may also be observed in other animals with regard to other phenomena.

Regenerative capacity and immunity can be changed: if the living conditions of animals, the course and rate of ontogenesis, gas exchange, oxidative and proteolytic processes, etc., are changed. At an increase in temperature, the morbidity rate of red leg and the percentage of mortality in tadpoles and frogs increase. On the other hand, a decrease in temperature to 4—6° increases the animals' resistance to this disease. A decrease in temperature reduces the percentage of regeneration of extremities in tadpoles at Stage I, and increases it at Stage II (Polezhaev, 1955). Good nutrition, especially vegetarian nutrition, increases the immunity and regenerative capacity of tadpoles and frogs. Oxygen insufficiency and excess of excretion products in the external environment sharply reduce the capacity of these animals for regeneration. Thyroidization considerably accelerates metamorphosis in tadpoles, and increases gas exchange, oxidative processes, and tissue differentiation (Helff, 1926). On the other hand, it accelerates their loss of regenerative capacity of extremities (Speidel, 1929; Liozner, 1931), and in human beings and other mammals, it accelerates healing of wounds (Belkin, 1947; Voitkevich, 1947, 1965). These data may serve as a basis for the asumption that thyroidization may enhance the resistance of tadpoles to Saprolegnia and reduce the resistance to red leg.

The simultaneous and different changes of immunity toward various diseases during ontogenesis is a general biological rule. It is observed not only in tailless Amphibia, but also in humans and plants. In this connection, we may refer to the well-known data from medicine that certain human diseases are encountered at a certain age: some infectious diseases (measles, chickenpox, mumps, scarlet fever, etc.) are met in childhood; arteriosclerosis, cataracts, hypertrophy of the prostate, cancer, etc. are found in advanced age (see Sakharov, 1935). M. S. Dunin (1946) showed that

immunity in plants, e.g., in potatoes, carrots, and mulberry, depends on a certain phase of their ontogenesis. Affecting these phases may enhance the resistance to plant diseases, a fact which is of great practical importance. These data on the ontogenetic rules of changes in immunity may be compared with data on the ontogenetic changes in regenerative capacity. Safronov (1967) showed that the appearance of tropic ulcers in human shins is accompanied in 100% of cases by a fungal infection and by a decrease in regenerative capacity of tissues. The use of vacuum therapy was successful in greatly enhancing regenerative capacity, in completely freeing the tissues from the fungal infection, and in achieving rapid and complete healing.

In conclusion, we point out that treatment of tadpoles and frogs with a table-salt solution increases the regenerative capacity of extremities, and simultaneously cures these animals from Saprolegnia, but this treatment does not affect red leg. Treatment of these animals with a solution of glucose enhances the regenerative capacity, but also increases the percentage of mortality from red leg. The mortality percentage can be reduced if the glucose solution is boiled. Treatment of animals with sulfadiazine does not noticeably affect the regenerative capacity, but prevents red leg. However, all chemicals could have acted directly on the causative agents of the animal diseases, e.g., on Saprolegnia, and not on the protective forces of the body. Therefore, these data cannot be considered a basis for the analysis of the problem concerning the relationship between regenerative capacity and immunity during the ontogenesis of animals.

The data quoted in this chapter allow the following conclusions to be made:

1. During ontogenesis of animals, for example, tailless Amphibia and other vertebrate species, the regenerative capacity and immunity change regularly and simultaneously. With the decrease in the regenerative capacity of organs, the resistance of the animals to some infectious diseases decreases, while that toward others increases.

2. A given pattern of metabolism, which changes regularly during ontogenesis, serves as the basis for regeneration and immunity. Changing this metabolism in a directed manner can affect both regenerative capacity and immunity.

On the basis of these conclusions, a certain new approach to the phenomena of regeneration and immunity may be outlined.

Chapter IV

REGENERATIVE CAPACITY AND TUMOR FORMATION

Our experimental study of the problem of loss and restoration of regenerative capacity of organs and tissues during ontogenesis and phylogenesis of animals, led us to the necessity of considering the problem of malignant growth. Even at the start of this study, we found unexpectedly that while, in some cases, destruction and dedifferentiation of tissues lead to regeneration of organs, in other cases they may lead to a drastic transformation of cells, to absence of regeneration, to toxicosis and death (Polezhaev, 1940, 1948; Polezhaev and Gintsburg, 1939). These latter phenomena are strongly reminiscent of phenomena of malignant neoplasia. We subjected these phenomena to a special experimental analysis, the results of which will be given in the following pages, after we discuss some other problems.

First, let us point out that we do not at all intend to give a review of contemporary data and theories on such an extensive and complex problem as the formation of malignant tumors. The elaboration of this problem led to the development of specialized large fields or disciplines: cancer morphology, cancer biochemistry, cancer virology, cancer genetics, etc. Our task is quite well defined, concrete, and narrow. It consists merely of a brief discussion of the characteristics of malignant neoplasms and comparison of them with phenomena of morphogenesis during the processes of normal ontogenesis and during regeneration, from the viewpoint of experimental morphology. This approach enables us to illuminate the connection between regenerative capacity and tumor formation and to point out some new ways to combat malignant neoplasms.

Pathological anatomy and experimental oncology accumulated vast experimental data allowing determination of certain characteristic features of malignant tumors (Abrikosov, 1949; Abrikosov and Strukov, 1961; Davydovskii, 1952, 1961; Shábad, 1947; Kaudri, 1958; Needham, 1942; Petrov, 1947—1962; Rumyantsev, 1932; Vasil'ev, 1961; Erenpreis, 1963, and others). First, cells of malignant tumors, formed from any tissues of any organ, differ qualitatively from normal tissue cells in their morphological, biochemical, and morphogenetic properties, and in the capacity for growth. Malignant cells are always less differentiated, younger than normal cells of tissues of the adult organism from which they arise. In becoming malignant, cells of malignant tumors appear by dedifferentiation of cells of the old tissues (anaplasia). Tumor cells multiply intensively but they do

not differentiate. Instead of differentiation, the cells die, necrosis of the tumor in its central part takes place. Malignant cells differ greatly in size: there are very small and very large cells. Cells of malignant tumors grow, infiltrating healthy tissues. Their growth is autonomous; it is not regulated by the ordinary physiological mechanism characteristic of human and animal organisms: morphogenetic influences, nervous and humoral factors. Tumors are characterized by the absence of specific functions characteristic of any normal organ. In a sense, the specific function of a tumor is only its toxic effect on the body. Metastases are characteristic of many tumors. Biochemically, tumors are characterized by glycolysis, proteolysis, altered nucleic acid metabolism, shift of pH in the acidic direction, and other peculiarities.

The briefly enumerated morphological and physiological peculiarities of malignant tumors indicate a similarity of their cells with embryonic cells, and with cells which appear during regeneration, a fact which numerous investigators had noticed long ago. Let us point out the features of similarity and difference between these cells.

Cells of an embryo and dedifferentiated cells of regenerating tissues in the first phase of regeneration, as well as cells of tumors, have a large, light nucleus with one or two large nucleoli and a small basophil cytoplasm, and they divide intensively. Glycolysis, proteolysis, a shift of the active reaction of the milieu in the acidic direction, and enhanced protein and nucleic acid synthesis are characteristic features of these cells. Cells of embryos, however, and of regenerating tissues respond to the environment's morphogenetic influences and differentiate, while cells of malignant tumors behave autonomously, do not differentiate, and kill the adjacent healthy tissues.

A number of theories have been proposed concerning the origin of malignant neoplasms.

1. The viral theory, according to which the normal cell becomes malignant as a result of the intrusion of a virus which may organically combine with the molecular basis of the chromosomes in the nucleus; this theory is true for some, but not for all, malignant tumors.

2. The genetic theory, according to which malignization is determined by a mutational change of the nuclear hereditary apparatus of the cell; this theory is in good agreement with contemporary data from biochemistry and molecular biology, but it has not been proved.

3. Konheim's theory, according to which the source for the formation of malignant cells is embryonic cells which survived in the adult organism and become malignant at a certain time due to the organism's changes connected with age, or due to other changes. Malignant tumors can be obtained experimentally by transplanting embryonic cells, or cells of regeneration blastema, into the peritoneal cavity or other parts of the body in Amphibia or mammals (Spemann, 1942; Waddington, 1947; Lavrov, 1960; Skowron and Walknowska, 1959).

4. Virchow's theory of stimulation or damage which emphasizes the fact that the initial factor of malignization is some damage to the cells (mechanical, chemical, or hormonal). This theory finds

confirmation in the fact that tumorous growth can be obtained experimentally by the use of a number of carcinogenic substances, and also by clinical data.

5. The theory of impairment of neuroregulation, in favor of which are the data obtained by Petrova (1946), who showed that after prolonged work with experimental neuroses in dogs, malignant tumors develop in many internal organs.

6. The theory of disturbance of the tissue relationships, put forward by Ribbert, according to which the reason for tissues becoming malignant is the weakening of the normal interaction of epithelial and connective tissues. In favor of this theory are recent data, which show that so-called cellophane cancers can arise from transplantation of chemically indifferent cellophane films into tissues (Oppenheimer, B. S., Oppenheimer, E. T., and Stout, A. P., 1948, 1952; Studitskii, 1962, 1965).

It can be seen that each of these theories has some factual basis, and probably correctly reflects certain aspects of the phenomena of malignant neoplasia but, regretfully, so far none of these theories gives a full, unitary explanation of the phenomena as a whole. Analysis of these phenomena allows us to come to the following general conclusions:

1. In malignization a deep qualitative, usually irreversible, change in the morphology, physiology, and biochemistry of the cell takes place.

2. An external factor is the reason for malignization.

3. The neoplasm appears in connection with some changes in the body due to age or to pathological conditions.

From these conclusions it follows that, if the action of external carcinogenic factors or the pathological effect is prevented, malignization may not occur. On the other hand, it also follows from these conclusions that malignization is due to some changes in the body occurring with age. The latter result in a decrease in the body's resistance. Consequently, to prevent the appearance of malignant formations, the resistance should be increased, the protective forces enhanced, the nerve trophism increased, and aging of the body inhibited. Malignization is a result of changes in the body as a whole which create a certain background or prerequisite, and of reactions of the cell or groups of cells to certain extermal effects, the sources of which may be different factors of the external environment or of the body itself.

At present, three main methods for treating malignant tumors prevail in medicine: 1) surgical removal of the tumor; 2) radiation therapy, and 3) chemotherapy. While the first method is nonspecific, the remaining two are based on the idea of a specific selective effect of physical and chemical factors on malignant cells which are more sensitive to these factors than are cells of healthy tissues.

Malignant tumors arise in diverse animal species and are formed from diverse tissues in practically all, or almost all organs, but in different percentages of cases.

Numerous investigators turned their attention to the connection between regenerative phenomena and formation of malignant tumors. However, the interpretation of this connection, given by different investigators, varied. B. P. Tokin (1959) concluded that the processes of regeneration and tumor formation are antagonistic. According to data obtained by him and his co-workers, the formation of malignant tumors cannot be readily obtained in tissues of regenerating organs in planaria, axolotls, and tritons which possess a high capacity for regeneration. On the other hand, carcinogenic substances, e. g., coal tar, inhibit the regeneration processes of extremities in tritons. A number of investigators succeeded, however, in obtaining cancer formation in axolotls and tritons by use of carcinogenic substances (methylcholanthrene, and others), and the malignant tumors appeared in the regenerating organs (tails of axolotls) (Umanskii and Ryzhkova, 1954; Ryzhkova and Us, 1954; Peredel'skii, 1941, 1942; Fedotov, 1941, and others). On the basis of these data Peredel'skii (1941) concluded that regeneration and tumor formation are not antagonistic processes. Umanskii and Ryzhkova (1954) point out that inhibition of regeneration by carcinogenic substances may be due to their toxic, and not to their carcinogenic, effect on tissues. Some investigators (Sarkisov, 1962, 1963, and others) point out that regeneration may be a prerequisite of, or the basis for, the development of malignant tumors. Clinical data, which show that tumors frequently appear in connection with injury, scarification, or regeneration of tissues, agree with this conclusion.

We assume that the aforementioned contradiction in the data available can be explained as follows. Damage may be one of the prerequisites for tumor formation, inasmuch as it reduces the resistance of the tissues and opens the gates for infections or for the action of carcinogenic substances. An incipient and developing process of regeneration is not able to arrest the process of malignant neoplasia. But on the other hand, the latter may be antagonistic not to regeneration itself, but to the regenerative capacity. Tokin (1959), discussing the relationship between the capacity for regeneration of organs and the capacity for malignant tumor formation, reached the conclusion that at present any defined rule in this respect cannot be established. As pointed out by some other investigators, however, such a rule does exist (Needham, 1936, 1942; Waddington, 1942, 1947; Gersch, 1951, 1957; Pflugfelder, 1950, 1954; Seilern-Aspang, 1960—1961). This rule consists of the fact that the incidence of cancer increases in connection with a decrease in the capacity for reparative regeneration of organs and tissues. Thus, in animals which possess a high capacity for regeneration (planaria, annelids, axolotls, tritons, and others), malignant tumors are very rare, or are not encountered at all, and it is difficult to induce them. On the other hand, malignant neoplasms often appear, and they are readily induced in animals with a low regenerative capacity, including mammals and man. This rule may be confirmed by data obtained in an ontogenetic series of animals: with the age of the animal, as a rule, the regenerative capacity decreases and, correspondingly, the tendency toward development of neoplasms increases. Malignant tumors most frequently affect middle-aged and old persons, rarely appear in young persons, and are very rare in embryos. Cancer is a disease of the later years. Tokin (1959)

announced that implanted sarcoma developed in 80% of cases in adult rats, while in only 6.4% of cases in rat embryos. Similar results were obtained following the use of carcinogens. Very interesting data were obtained in experiments on stick insects or caddis flies, Dixippus. These insects possess a high capacity for regeneration, and tumors do not occur spontaneously in them. After removal of the endocrine gland, corpora allata, the regenerative capacity is lost and, simultaneously, spontaneous tumors appear in these animals (Pflugfelder, 1938, 1939, 1950, 1954). In the turbellarian, Dendrocoeleum lacteum, the anterior end possesses a high capacity for regeneration, while the posterior end has low regenerative capacity. Administration of scarlet red into the posterior end of the body of these triclads causes an infiltrating tumor to develop, while if administered into the anterior end, it causes only hyperplasia of tissues (Seilern-Aspang, 1960). The administration of various carcinogens into muscles of the normally regenerating anterior extremities of tritons causes the formation of additional extremities in a high percentage of cases, and in a few cases it causes sarcoma (Breedis, 1952). In tritons (Triton cristatus) carcinogens readily induce malignant metastasizing tumors in the region of the trunk, where the regenerative capacity is low, but in the tail, which has a high regenerative capacity, these are spontaneously cured if part of the tail is amputated leaving a tumor at its base. In the former case the animals die, while in the latter they remain healthy (Seilern-Aspang and Kratochwill, 1962, 1963, 1965). It is of interest that in this case, the dedifferentiated epithelial cells of the malignant tumors differentiate into normal structures. If they were in the skin, they would transform into cornified, glandular, and pigment cells. If they metastasized into muscles, they would transform into cells of connective tissue and muscle fibers, according to the influence of the adjacent tissues.

These data allow us to state that the tendency to form malignant tumors is inversely related to the regenerative capacity of the animal and human body; or, the higher the regenerative capacity, the lower the capacity for tumor formation; and conversely, the lower the regenerative capacity, the more readily the animal or man succumbs to cancer. That statement is readily supported by radiobiological data. It is known that total body irradiation with ionizing radiation in a sublethal dose greatly enhances the incidence of cancer in man and animals. There is a decrease in the capacity for regeneration of the hematopoietic organs (bone marrow, liver, spleen, lymphatic glands). Local radiation injury, leading to malignancy, also decreases the regenerative capacity of organs and tissues. From this it follows that when the capacity for reparative regeneration of organs and tissues is increased experimentally, the resistance of animals and man to malignant tumors may be increased. We have already seen (Part II, Chapter 6) that the regenerative capacity of axolotl extremities, suppressed by X-ray irradiation, can be restored, thus curing the local radiation injury, which may prevent malignization of the tissues. Thus, the task of prophylaxis of malignant tumors in animals and man, by enhancing their regenerative capacity, is quite pertinent. This is one of the new ways of combating the appearance of malignant tumors. It is not difficult to see that this is directly connected with the problem of enhancing resistance to infections, or enhancing the protective forces of the body. It is of interest that during ontogenesis of animals and man, the following properties undergo regular

and simultaneous changes: regenerative capacity, resistance to diseases (immunity), and propensity to malignant tumors. The same factor is basic to these three phenomena: a regular change in the capacity of cells and of the entire living organism for self-reproduction, the latter, in the final account, depending first of all on the biosynthesis of proteins and nucleic acids. Therefore, if we know how to change this biosynthesis in a controlled manner, in a direction allowing the restoration of regenerative capacity lost or weakened in the processes of ontogenesis and phylogenesis, or by pathological or experimental conditions, we could find a way to prevent malignant tumors. As all the examples discussed in the second part of this book show, this is quite a real matter, and there are grounds for the assumption that this is a valid way toward the elaboration of some new methods of fighting (prevention of) malignant formations. We have in mind prophylaxis, and not treatment, since if a tumor has already appeared, increase of regenerative capacity alone may not be enough to fight the tumor.

We shall now consider the problem of whether some new elaborations of methods of treatment of malignant tumors can be derived on the basis of the theory of regeneration.

We have already pointed out that malignant tumor growth is characterized by the lack of differentiation. In those few cases in which spontaneous curing occurs, malignant cells differentiate and form normally functioning structures. The question logically arises, therefore, is it not possible to cause artificially the differentiation of malignant tumors, and thus cure the patient?

The reasons for the lack of differentiation of dedifferentiated (anaplastic) cells of malignant tumors may be their incapacity for morphogenesis, or the lack of morphogenetic effects of the adjacent healthy tissues, or both. Various investigators hold different opinions on this problem. Some (Waddington, 1935, 1942, 1947; Needham, 1936, 1942; Rose and Wallingford, 1948; Ruben, 1955, 1956; Kaudri, 1958; Tokin, 1959; Lazarev, 1963) think that the autonomous tumorous growth is due to the lack of controlling morphogenetic influences of the animal body (they call this "fields of individuation"). Other investigators (Törö, 1933; Huxley and DeBeer, 1936; Rondoni, 1957; Bernhard and Gross, 1959; Büngler, 1953; Burnet, 1957, 1959; Dürken, 1926; Fischer, 1927; Fischer-Wasels, 1934; Graffi, 1940, 1957; Reimann, 1938, and others) are of the opinion that tumor cells lose their plasticity or the capacity for morphogenetic reactions. We shall discuss some experimental data concerning this problem.

Upon the implantation of young rat embryos into tumors of adult rats, tubular structures appeared (Schotté, 1938). The investigator considered them to be the result of induction and change in the tumor tissue. They could have appeared, however, not from the tumor but from the implant. Waddington (1947) transplanted small pieces of chicken sarcoma into the cavity of the primary zone of the blastoderm of a chick embryo. After 24 hours, normal embryos developed, the tissues of which included tumor cells. In these cases we cannot speak of differentiation of the latter. Lustig and Matrey (1962) showed that the primary zone (organizer) of a chick embryo interferes with the infiltration of the blastoderm by cells of muscle tumors and that glandular structures sometimes appear in the latter, i. e., differentiation of tumor cells takes place. Brunst and

Sheremet'eva (quoted from Lazarev, 1963) amputated the tails of axolotls at the level of a spontaneous melanoma. The cells of the latter participated in regeneration, but the investigators did not describe any differentiation of these cells.

Rose and Wallingford (1948) implanted a tumor (Luké's carcinoma) of a frog kidney (Rana pipiens) into the anterior extremity of adult tritons (Triturus viridiscens) and amputated the organs along the transplant. In the regenerated extremities, cartilage, muscle, and connective-tissue cells appeared, which differentiated from the cells of the implant. The latter had nuclei of much smaller size than that of the nuclei of triton cells.

These interesting data, however, were subjected to criticism. Ruben (1955, 1956) transplanted the same adeno-carcinomas of frog kidney into extremities of Amblystoma larvae and adult tritons (Triturus viridiscens), amputated the extremities along the transplants, and did not observe any differentiation of the tumor cells during regeneration. He thinks that they could not differentiate under the morphogenetic influence of the organ residue.

Ya. G. Erenpreis (1963) implanted pieces of S-465 sarcoma into the region of the defects in tibiae of rats. The investigator observed transformation of the sarcoma cells into cartilage cells under the influence of the conditions of regeneration, and weakening of the destructive capacities of the entire tumor. However, he reached the conclusion that not the sarcoma cells themselves, but the stroma of the tumor differentiated.

Dulbecco (1960) communicated the findings of Ruben that upon cultivation outside the body in embryonic-calf serum, the round, Rous sarcoma cells are transformed into fibroblasts. This takes place only with the participation of normal fibroblasts added to the cells of the explanted sarcoma. If there is a shortage of normal fibroblasts, no such transformation takes place.

Seilern-Aspang (1961) chemically induced a very malignant chicken sarcoma and treated it with homogenates obtained from chick embryos at various stages. Sarcomas which had reached the dimensions of a pea soon disappeared in all these cases, or (in one case) became cornified. The sarcoma cells changed and were transformed into a dense connective tissue with closely packed cells. A similar result was obtained upon the use of homogenates prepared only from embryos at the early stages of development. In the control group the sarcoma reached the dimensions of a child's fist, and the animals died within 14 days.

Seilern-Aspang (1963) obtained even more interesting data in other experiments. In the first experiment, cells of Erlich's ascites tumor from a mouse, or spindle-shaped cells from a chicken sarcoma, were implanted into 3—4-day-old chick embryos. The cancerous cells formed monolayer epithelial films or vesicles. The sarcoma cells completely lost their malignancy and were transformed into muscle, connective tissue, and bone. In another experiment, axial organs (the nerve tube, cord, somites) from 2-, 3-, and 5-day-old chick embryos were implanted into malignant metastasizing tumors (spindle-shaped sarcoma) in chickens. In many cases, the tumor tissue adjacent to the inducer was transformed into fibrous cartilage (Figure 120a). In almost all the cases, the animals recovered, and the tumor was transformed into fibrous connective tissue (Figure 120d, c), while the control animals died from developing and metastasizing tumors. The results of this experiment, however, could be interpreted as normalization of the

tissue due to the death of the tumor cells. The third experiment solved this problem completely. Sarcomas were produced in the muscles of the shin in chickens by the use of dibenzanthracene. Pieces of such sarcoma and of human sarcoma, consisting of spindle-shaped cells of intermediate size, were cultivated outside the body. Cords of 4 to 5-day-old chick embryos were added to the explants of the tumors as inducers. Similar inducers were added to the control, which consisted of explanted fibroblasts from the hearts of 11-day-old chick embryos. The results were: the fibroblasts did not change in the control, while the tumor cells were transformed into a fibrous or hyaline cartilage in the experimental animals, and even into bone upon prolonged cultivation. However, if these pieces of tumors, together with the inducer (cord), were cultivated on a connective-tissue membrane (tunica vaginalis from rat testes), the chondrification of the tumors was especially successful (Figure 121 a, b, c). On the connective-tissue membrane under the influence of the cord, cartilage formed even from heart fibroblasts or from fibroblasts of the shin. Thus, these investigators confirmed experimentally the correctness of Waddington's assumption (1935, 1947) that it is possible to cause differentiation in tumors. They reached the conclusion that cells of malignant tumors possess morphogenetic plasticity to a greater extent than do embryonic cells.

FIGURE 120. Reverse development of a tumor (spindle-shaped sarcoma) induced by dibenzanthracene in a chicken in vivo (according to Seilern-Aspang and Kratochwil, 1963b):

a – tumor cells infiltrating the muscles, transformed into cartilage (2 months after the tumor became noticeable and 11 months after dibenzanthracene was administered); b – infiltration zone of the tumor 3 weeks after a single injection of a purified homogenate of axial organs of 4- to 5-day-old chick embryos. Tumor cells disappeared and between the muscles granulation tissue was found; c – central part of the tumor 14 days after injection of the homogenate mentioned in b. The tumor cells disappeared and instead connective tissue consisting of coarse fibers may be found.

It must be stressed that tumor cells may differentiate both upon explantation and, sometimes, also in the animal or human body (see Erenpreis, 1963). Therefore, it is quite understandable and to be expected that this phenomenon might be caused experimentally. Induction of differentiation

in tumor tissue may be a new biological method for the treatment of malignant tumors.

The question arises as to the nature of the active principle in the inducer, under the influence of which the malignant cells may differentiate and lose their malignancy. This question has not yet been sufficiently studied, even less so than the problem of the possibility of induction of differentiation in tumor tissue. Everything concerning this is new, and necessitates further investigation. Certain data on this subject, however, may be quoted.

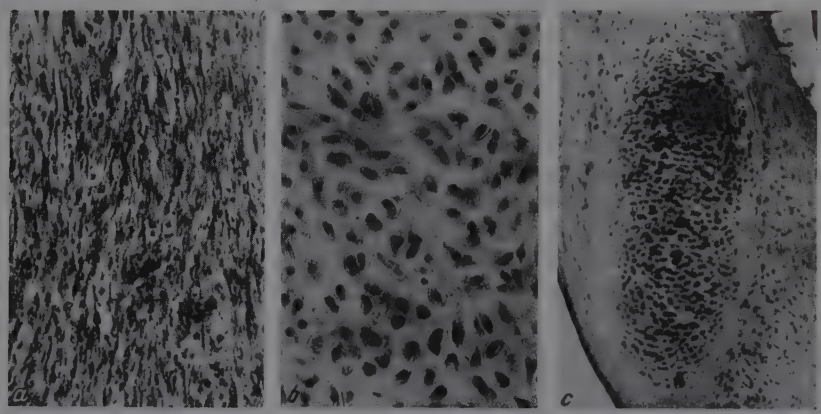


FIGURE 121. Chondrification in vitro of a tumor induced in a chicken by dibenzanthracene. The tumor which appeared in the muscles of the shin gave metastases into the lungs, liver, and kidneys, and resulted in death (according to Seilern-Aspang and Kratochwil, 1963b):

a – sarcoma cells after 18-day cultivation on the tunica vaginalis of rat testes; b – sarcoma after 18 days of cultivation on the tunica vaginalis, and addition of a cord of a 5-day-old chick embryo; c – the same as in b, lower magnification. The cartilage anlage in the form of a column.

An interesting and detailed analysis of the role of nucleic acids in differentiation and malignization was carried out by Erenpreis (1963). We cannot here dwell on these data, but we shall point out the following. At present we may consider proven the finding that proteins of malignant tumors differ from proteins of the same type of normal tissues. A number of investigators have also shown that numerous tumors may be induced by RNA isolated from tumors. Erenpreis (1963) reached the conclusion that "malignization can be defined as the acquisition by the RNA-template of the cell of the capacity for self-reproduction" (p. 124). The capacity for malignization is predetermined by the most general properties of the nucleic acids.

In this connection the studies of Niu (1956, 1958, 1958), who ascribes the role of the active principle in the inducer, causing differentiation in the embryonic material, to its RNA, are of great interest. These data are contested by certain investigators (see Saxén and Toivonen, 1963). The data of Niu and co-workers, however, are quite definite (Niu, Cordova, and Niu, 1961), showing that if RNA isolated from a normal liver is administered

to ascites tumor cells before their implantation in mice, there is a drastic reduction in the frequency of appearance of ascitic tumors, and the tumor cells differentiate into cells which are similar to liver cells, being devoid of malignancy and of the capacity for synthesizing serum albumin. In other words, under the influence of a tissue-specific RNA the malignant cells are transformed into normal cells.

Inhibition of the formation of Novikoff's hepatoma and of a mucous tumor in the liver under the influence of RNA was also observed by other investigators (Decarvalho and Rand, 1961; Aksinova, Bresler, Vorobyev, and Olenov, 1962).

Somewhat later, Niu (1964) showed that treatment with RNA isolated from liver nuclei reduces quite strongly (in 32% compared with 96% of cases in the control) the frequency of formation of Erlich's ascites tumor.

All these studies are now in progress and promise to yield interesting results. We point them out in particular because the important role of RNA in processes of regeneration has already been established. The assumption inadvertently suggests that tissue-specific RNA may be a factor of differentiation of malignant tumor cells and of their transformation into normal tissues.

Results of some of our own experiments

We shall now briefly dwell on the results of our studies related to the role of destruction and dedifferentiation in regeneration, and to some pathological phenomena similar to malignant neoplasia.

Studying the rule of loss and restoration of the regenerative capacity of extremities in tailless Amphibia, we carried out transplantations of extremities from tadpoles of the grass frog (Rana temporaria) which were at the critical stage of loss of regenerative capacity (Stage IIa) to tadpoles at a later stage of development (Stage IIIa), which had already lost the regenerative capacity, and had an altered internal humoral milieu (Polezhaev and Gintsburg, 1939; Polezhaev, 1940, 1946, 1948). It proved that, under the influence of the internal humoral milieu of the animals at later stages of development, the regenerative capacity of the transplanted extremities is lost. In some cases the extremities did not regenerate despite the deep destruction and dedifferentiation of their component tissues of mesodermal origin (Figure 122 a, b). From the dedifferentiated muscles, connective tissue, and cartilage of the skeleton, a mass of cells was immediately released and those became rounded, decreased in size, and became similar in shape to lymphocytes. No blastema was formed. Sometimes these cells infiltrated deeply into the recipient's tissues. Some of the animals died with manifestations of toxicosis. In the control, after amputation of the extremities without transplantation, the tissues of the residue of the organ differentiated normally, and in some cases, blastemata developing into regenerates appeared on the wound surfaces (Figure 123 a). The blastemal cells were large and looked like embryonic-type cells (Figure 123 b). Destruction and dedifferentiation of the tissues of the stump of the organ took place. Consequently, tissues of organs at the same stage of development and in the same animal species, when being

destroyed and dedifferentiated, form a regenerate which grows normally and differentiates in some cases, but in other cases (as transplantation under the influence of the internal humoral milieu of animals at a later stage of development), the tissues are qualitatively altered, do not regenerate, and result in death.

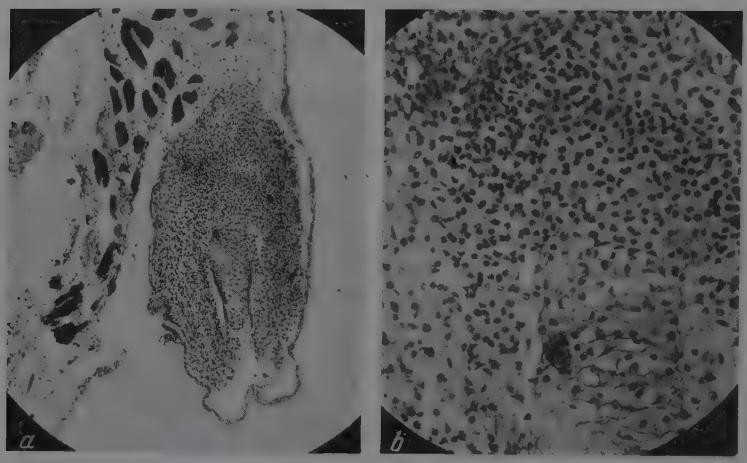


FIGURE 122. Section of an extremity of a tadpole of a grass frog at Stage IIa, transplanted to a tadpole of Stage IIIa. Destruction, dedifferentiation, and transformation of tissues in the absence of regeneration of the organ:

a – general appearance of the organ; b – higher magnification.

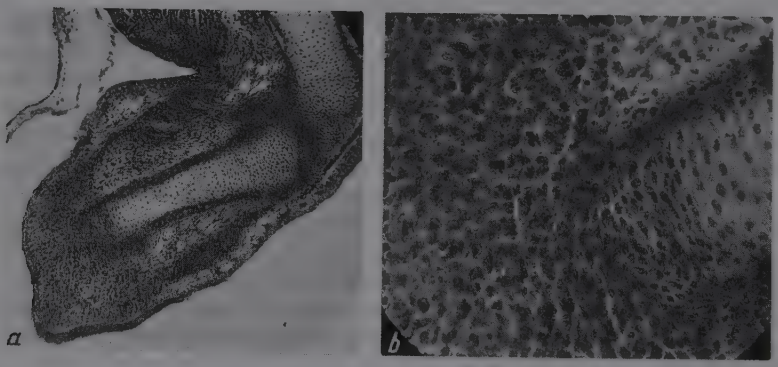


FIGURE 123. Control. Regeneration of extremities of tadpoles of a grass frog at Stage IIa. Destruction, dedifferentiation of all tissues, and formation of embryonic type cells of regeneration blastema:

a – general appearance of the organ; b – higher magnification.

At our suggestion, Gintsburg (1951, 1951a) continued these studies. He transplanted hemoplastically extremities of tadpoles of Stage I to the back and to the extremities of adult Rana temporaria frogs.

The transplants were greatly destroyed, dedifferentiated, ulcers appeared around them, and the animals died in 72% of cases, 10—20 days after operation. Numerous cells of the lymphocyte and erythrocyte type appeared in the mesodermal tissues of the transplants (connective tissue, muscles, cartilage). Autotransplantation of a toe of an adult frog was never accompanied by such phenomena. Transplantation of extremities from young tadpoles at Stage I to old Bombina led to the transplants' "taking," and to their growth and differentiation. Destruction, dedifferentiation, and transformation of the tissues of the transplants, formation of ulcers, toxicosis, and death of animals was not observed.

The reasons for these phenomena of transformation of tissues from the extremities of tadpoles transplanted to adult frogs may be: 1) age incompatibility of tissues, 2) effect of the adult animals' internal humoral milieu on the transplant, 3) denervation of the transplants, 4) infection.

Recently (1964 and 1965) we repeated the experiments just described, transplanting extremities and segments of extremities from tadpoles at Stage I onto the back of adult grass frogs. To our amazement, the transplants took in all the cases, and the transplanted extremities did not regenerate, but the wound surfaces healed smoothly. The tissues of the transplants differentiated into cartilage, bone, muscles, and connective tissue. There was no formation of ulcers, or toxicosis, or death of animals. These facts, which had also been observed for a number of years in our previous experiments (1938, 1946—1949), were due to infection which was absent in the experiments performed in 1964—1965.

We may draw the following picture of sequential changes arising during the ontogenesis of tailless Amphibia, which are the basis for these transformations. In the process of metamorphosis the metabolism, physiology, and entire structure of the animals, their capacity for reparative regeneration and immunity, undergo regular profound changes. The changes determine the appearance of incompatibility between the semi-embryonic, semi-differentiated tissues of the extremities of tadpoles at early stages of development transplanted to an adult frog and its differentiated tissues and internal humoral milieu. The tissues of the transplant are subjected to intensive destruction and dedifferentiation, and lose their nervous connections. However, the dedifferentiated cells, under the influence of the new humoral milieu, lose the capacity to form regeneration blastema. If they are subjected to some infection, possibly viral, they are, so-to-say, malignantly transformed into round cells of the lymphocyte type, which, similarly to cells of malignant tumors, infiltrate the tissues of the recipient, destroy them, and cause toxicosis and death of the animal.

The appearance of "malignancy" of these cells is in this case based on a number of prerequisites: the presence of semi-embryonic cells in an adult organism with its humoral conditions and its internal humoral milieu; impairment of nervous connections; intensive destruction, deep dedifferentiation; and, finally, infection of the transplant tissues. Each of these factors is of great importance for eliciting the changes or malignization of the cells. It suffices to mention the appropriate theories of the formation of malignant tumors. At the same time, we see that the action of a single factor is not sufficient for malignization of cells. In the case of autotransplantation of a toe from an adult frog, transformation

of tissues is not obtained. In this case there is denervation, infection, destruction, dedifferentiation, and the internal humoral milieu of an adult animal, but the transplant consists of differentiated and not of semi-embryonic tissues. For a malignant transformation of tissues to take place, a complex series of conditions is essential. If one of them is missing, no transformation occurs.

It may be proposed that a similar thing happens during the process of malignization in human beings and animals.

The foregoing analysis makes it clear why destruction and dedifferentiation caused by damage of tissues in some cases result in regeneration, while in others they lead to malignization.

We emphasize, however, the fact that a viral infection is not always essential for malignization. Malignization also occurs under known sterile conditions, e. g., cultivation of the tissues or implantation of embryonic tissues into the brains of mammals (Lavrov, 1960). In these cases, embryonic material is present (embryonic extract or embryonic cells) and there is tissue damage. The presence of these conditions may be sufficient for malignization of tissues to occur.

From what has been said in this chapter, the following conclusions may be made:

1. Malignization of tissues is connected with the existence of a certain complex of conditions and a given external effect (damage, viral infection, foreign RNA from cells of malignant tumors, denervation, etc.).
2. Dedifferentiation of tissues occurs during malignization.
3. The capacity for reparative regeneration of organs and the capacity for tumor formation are inversely related to each other.
4. In order to prevent malignization the normal, healthy state of the body must be maintained and its regenerative capacity increased.
5. One way to fight malignant tumors may be the elaboration of methods for the induction of differentiation in the tumor tissue.

Chapter V

CONCLUSION

Until recently, the following 3 basic rules were the leading ones in the theory of regeneration; the first 2 of them already formulated by Charles Darwin (1868), while the 3rd was formulated by histologists and pathologists (Kelliker, Walderer, and others): 1) the capacity for reparative regeneration of organs decreases with the increase in organization in a comparative series of animals; 2) regenerative capacity decreases during ontogenesis with age, and with increase of the degree of differentiation of tissues, and 3) each tissue produces one like itself during regeneration.

These rules generalize the major part of data known at that time. However, they provided no indications as to whether, and in what way, regeneration can be caused in mammals and man, in which the regenerative capacity is very low. During the last 100 years, many exceptions to these rules accumulated in studies on regeneration. In addition, the general level of development of biology increased, and this provided the possibility for a new approach to life phenomena. In this connection, two new directions appeared in the theory of regeneration which determined its transition to a new, higher level. The first new direction, chosen by Professors Vorontsova, Liozner, and Studitskii, showed that reparative regeneration of organs and tissues in mammals is not so limited as had been assumed earlier; that it is not reduced to mere scarification of wounds. The second new direction chosen by me, and also by Professors Umanskii, Rose, and others, showed that the weakened or lost capacity for reparative regeneration of organs can be restored in various groups of animals, even in mammals.

Equating the phenomena of reparative regeneration and hypertrophy, Vorontsova and Liozner reached the conclusion that mammals are capable of regeneration of all the organs: liver, spleen, kidneys, lungs, etc. Studitskii concluded that the regenerative capacity of organs and tissues is higher in mammals than in lower animals. According to Liozner (1962), a unitary rule for regeneration of organs in animals cannot be established. According to Studitskii (1959, 1963) the rule is: the capacity for regeneration of organs in animals increases with the increase in organization of the animal, and with the level of its metabolism. The positions of these investigators do not permit the statement of any rule of the loss and restoration of regenerative capacity of organs in animals, since all the organs can regenerate. The investigator can only empirically choose conditions for the stimulation of the spontaneous processes of regeneration.

It is difficult to agree with these assumptions. Qualitatively different phenomena, such as regeneration and hypertrophy, cannot be identified with each other since they obey different rules. Such an identification leads to a serious error, to the denial of the existence of a unitary rule governing regeneration. The assumption that the capacity for reparative regeneration increases with an increase in organization of the animals also does not correspond with reality. Man is the highest animal, but this capacity for reparative regeneration of his organs is the lowest. There is no Weismannism in this fact, and the knowledge of this fact does not disarm investigators, but forces them to search for methods of restoring lost regenerative capacity in higher animals.

Analysis of the old and new data obtained in studies on regeneration, taking into account new data and principles of biology, allowed us to formulate the following 2 rules:

1. The capacity for reparative regeneration of organs as one of the adaptive forms of self-reproduction of living matter is based on metabolism, and is due to damage of living matter; it changes regularly during evolution and ontogenesis, under pathological and experimental conditions, depending on changes in the living conditions of the animals.

2. Loss of the capacity for reparative regeneration of organs during ontogenesis and phylogenesis of animals is due to a decreased capacity for destruction and dedifferentiation of the basic tissues comprising these organs, and its restoration may be caused by a strong enhancement of these phenomena.

The first rule, which we call the main rule of regeneration, includes all 3 old rules of regeneration and all the exceptions to these rules. Starting from Sechenov's principle of unity of the organism and the environment, this rule emphasizes the dependence of regeneration and regenerative capacity of organs on prevailing conditions. This assumption allows the formulation of a unitary rule of regeneration. On the other hand, it is effective, it contains indications how to change in a controlled manner the course of regeneration and the regenerative capacity of organs and tissues in any animal species or in man.

The second rule indicates how to approach concretely the problem of restoration of regenerative capacity of organs lost or weakened during ontogenesis and phylogenesis of animals, and under pathological and experimental conditions.

Two principles lie at the base of this rule.

1. The principle of stages, according to which in any process of regeneration there are at least 2 physiological stages differing in type of metabolism: 1) the stage of destruction and dedifferentiation, which is physiologically characterized by proteolysis and glycolysis, reduction of the nucleic acid content and of pH, and is morphologically characterized by accumulation of undifferentiated cellular material in the focus of damage, and 2) the stage of growth and differentiation which is physically characterized by prevalence of protein synthesis over its degradation by aerobic respiration, increased content of nucleic acids and high pH, and is morphologically characterized by growth and differentiation of the regenerative cellular material in the focus of damage.

2. The principle of organospecificity, according to which the same organs in different species of animals are biochemically related and contain some common specific substances which can affect the growth, development, and regeneration of the respective organs.

The stage of destruction and dedifferentiation may be enhanced or weakened if certain links of metabolism using organospecific substances are acted on. This allows restoration of lost regenerative capacities of some organs in vertebrates, including mammals.

The knowledge of these rules and principles of regeneration allowed us (the author and his co-workers), and also a number of other investigators, to obtain restoration of lost regenerative capacity of the following organs and tissues in vertebrates:

1. extremities in tailless Amphibia, lizards, and also young rats, and toes in young rats;
2. bones of the cranial vault of dogs, rats, mice, and sheep, which allowed us to suggest new biological methods for the replacement of cranial defects by regenerating bone;
3. dental tissues in dogs;
4. cardiac muscle in rats, rabbits and dogs, which allowed us to normalize the disturbed physiology of an injured heart;
5. to stimulate greatly the physiological regeneration of nervous tissue in the cortex of the cerebral hemispheres of the brain in rats, which allowed us to change the interrelationship between the processes of stimulation and inhibition in the central nervous system; and
6. to restore lost regenerative capacity of the extremities of axolotls, suppressed by X-ray irradiation, and, therefore, prevent the formation of X-ray ulcers which do not heal for long periods of time, and which may be transformed into malignant tumors in man.

The data obtained showed that it is possible to restore lost regenerative capacity of other organs and tissues in mammals and man.

Work on the problem of loss and restoration of regenerative capacity of organs and tissues in mammals made possible the establishment of a new method of regeneration, regeneration by induction. From the time of Morgan (1901) until recently, 2 main ways of regeneration were distinguishable: morphallaxis, i. e., regeneration by rearrangement of old tissues; and epimorphosis, i. e., regeneration by proliferation of tissues from the amputation wound surface —regeneration by induction, restoration in which the plastic cellular regenerative material is formed not by proliferation, but under the inducing effect of the adjacent old tissues. The discovery of the phenomenon of regeneration by induction allows establishment of a new rule in the course and methods of regeneration, and permits suggestion of a way for elaborating concrete methods for the restoration of lost regenerative capacity in mammals. This has been demonstrated in the example of regeneration by induction of cranial bones, dental tissues, and cardiac muscles in mammals.

The new approach to the study of regeneration made possible the finding of other new approaches to a number of important biological phenomena. Methods for the stimulation of physiological regeneration of tissues of organs which are not capable of reparative regeneration, for example of nervous tissue in the brain and of other internal organs, could be proposed. On the basis of the new principles of regeneration, it was seen that it is possible to find an approach to the restoration or enhancement of lost or weakened physiological

functions of various organs of animals and man. This fact can be of practical importance in medicine, veterinary medicine, animal husbandry, and in our national economy. A direct relationship between the phenomena of immunity and regeneration could be established. Finally, the connection between regenerative capacity and tumor formation could be demonstrated. The knowledge of the fact that the higher the former, the lower the possibility of the appearance of malignant tumors, permits finding of new ways for their prophylactic treatment. The demonstration of the possibility of proposing methods for causing differentiation of cells in malignant tumors allows outlining of new ways of tumor treatment.

From all this, it is clear that the elaboration of the new direction in the study of regeneration is highly important both theoretically and practically. Its elaboration poses a number of new, highly important problems and opens up wide new prospects for study.

The theory of regeneration is one of the chapters of experimental morphology of animals. Its transition to a new higher level also produces changes in the entire field of experimental morphology. While earlier in the development of experimental-morphological studies, as correctly pointed out by Filatov (1939), the leading role was played by the subject which determined the purpose and methods of study, now the situation has changed entirely. The investigator approaches the experimental-morphological investigation with a defined goal, namely to change morphogenesis in a directed manner during ontogenesis, to restore regenerative capacity of organs and tissues, lost during ontogenesis and phylogenesis of animals. The formulation of this goal allows him to define in a concrete manner the tasks of the study starting from the knowledge of certain subjects and methods. In this case the study can no more be confined within the framework of one biological discipline, as was the case earlier, but requires a complex application of methods from different disciplines. The elaboration of the problem of loss and restoration of regenerative capacity of organs in animals requires the use of methods from experimental morphology, histology, histochemistry, biochemistry, physiology, surgery, and knowledge of certain chapters of the theory of evolution, genetics, pathology, cytology, immunology, etc. The investigation cannot be carried out solely on the basis of an experimental analysis of phenomena, but it also must be at the same time, a synthetic study. The result of this kind of situation and approach is that the discovery of new factors in the field of regeneration inadvertently acquires an important meaning for a number of biological and medical disciplines. The problems of regeneration of extremities, cranial bones, dental tissues, cardiac muscle, nervous tissue of the brain, and of restoration of regenerative capacity suppressed by X-ray irradiation, already touched upon in the present book, necessarily require a concrete solution of problems in evolution, ontogenesis, and the theory of heredity. This is a theoretical problem of immense importance. On the other hand, these problems directly touch upon the interests of a number of theoretical and practical disciplines: histology, embryology, cytology, histochemistry, biochemistry, physiology, surgery, therapy, stomatology, neuropathology, psychiatry, pediatrics, radiobiology, oncology, and immunology. This situation is a reflection of the contemporary state of science which allows us to formulate tasks most important for humanity, as the conquest of space, and to accomplish them by using data and methods from a number of widely different scientific areas. There is no doubt that this way will allow us to solve a number of most important scientific problems.

General Problems of Regeneration

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