OTS

JPRS: 16,238

19 November 1962

TRANSLATIONS FROM USPEKHI SOVREMENNOY BIOLOGII

(Achievements of Modern Biology)

- USSR -

U. S. DEPARTMENT OF COMMERCE
OFFICE OF TECHNICAL SERVICES
JOINT PUBLICATIONS RESEARCH SERVICE
Building T-30
Ohio Drive and Independence Avenue, S.W.
Washington 25, D. C.

Price: $5.60

FOREWORD

This publication was prepared under contract for the Joint Publications Research Service, an organization established to service the translation and foreign-language research needs of the various federal government departments.

The contents of this material in no way represent the policies, views, or attitudes of the U. S. Government, or of the parties to any distribution arrangements.

PROCUREMENT OF JPRS REPORTS

All JPRS reports are listed in Monthly Catalog of U. S. Government Publications, available for $4.50 ($6.00 foreign) per year (including an annual index) from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.

Scientific and technical reports may be obtained from: Sales and Distribution Section, Office of Technical Services, Washington 25, D. C. These reports and their prices are listed in the Office of Technical Services semimonthly publication, Technical Translations, available at $12.00 per year from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.

Photocopies of any JPRS report are available (price upon request) from: Photoduplication Service, Library of Congress, Washington 25, D. C.

TRANSLATIONS FROM USPEKHI SOVREMENNOY BIOLOGII

[Following is a translation of selected articles from Uspekhi Sovremennoy Biologii (Achievements of Modern Biology), Vol XXX, No 2(5), Moscow, 1950, pp 234-257; 258-270.]

Table of Contents

The Development of Immunological Reactions and the Problem of the Incompatibility of Tissue Transplants

G. V. Lopashov and O. G. Stroyeva (Moscow)

The importance of the problem of tissue incompatibility for biology and medicine hardly requires special proof. Its significance for practice stems from the fact that for grafting surgery has had to use tissues of the same person; tissues of other individuals are used as temporary transplants, as substrates for replacement by the host tissues. With the expansion of the problems of restorative surgery it is becoming progressively more necessary to bring about permanent tissue acceptance of the transplantable tissues, by means of which we can hope to reach the point of regular restoration of lost organs. Herein is the significance of the problem for future restorative surgery; its theoretical importance is no less. Incompatibility usually comes from congenital hereditary differences between the tissues of different individuals, breeds and species. If it were possible to find means of overcoming the incompatibility, it would be shown how to change these differences, to control them, and, finally, to penetrate more deeply into the nature of them. The present article has the aim of solving this problem, as a first approximation, and outlining means of future research in this very interesting field.

1. The Cytological Basis of Immunological Phenomena

Immunological reactions constitute the most important groundwork of incompatibility phenomena. (The phenomena of incompatibility (like the problem of surmounting them) are naturally not based on humoral relations alone but include the other relations of the transplant and the rest of the animal's body as a complex, among which relations an essential part must be played by the regulatory influences of the nerve connections. However, the many-sidedness of the problem causes us to limit ourselves here to the group connection, the role of which comes out with particular evidence and with the clarification of which, therefore, we can hope with the greatest success to begin untangling the problem as a whole). This has been shown by Sokolov (1923, 1924) and confirmed by Medawar (1944, 1945, 1946) and Harris (1943, 1948) subsequently. In the specific form in which we encounter them in animal organisms the immunological phenomena constitute a protective adaptation. They serve for defense of the animal against destructive agents which have penetrated into it through its outer defenses; this applies chiefly to bacteria and other foreign bodies of organic origin, that is, to those the basis of which is made up of proteins. These defense reactions extend to include grafted foreign tissue transplants also. Defensive phenomena have evolved regularly within the limits of the animal world. It is well known, beginning with the classic studies of Mechnikov, that in lower multicellular animals only the primordia of them exist in the form of phagocytic activity with

respect to foreign bodies. With increase in structural complexity and particularly with the development of the vascular system, another, more perfect type of defensive phenomenon, the immune reactions proper, acquires greatest importance along with the phagocytic type. They consist of the formation of accessory (protein) substances which, on entrance of foreign bodies (with a protein-base structure) into the body, combine with them and deprive them of activity. The former are called "antigens"; the latter, "antibodies." The fact is essential that these antibodies, by and large, do not arise at the site of entrance of these bodies (bacteria and others) but rather in special sites in the animal, and they are formed not only during the entrance of foreign bodies, in response to their presence but also are produced later throughout the life of the organism, continuing to protect it against repeated invasions of this type. With the entrance of each successive portion of bacteria or proteins this immunological activity is enhanced or bursts out with new vigor. However, for us such an ordinary interpretation of the immunological phenomena as a defensive adaptation against bacteria, prevalent in medical immunology, is inadequate. Numerous investigations (Zil'ber, 1948; Komikov, 1948; Boyd, 1949; Landsteiner, 1946 and others) have shown that such immune reactions occur also in case the most varied substances, having proteins as their biochemical basis, are introduced into the body. Examples are blood sera and other proteins of different animals and plants, blood cells of animals and other cells, and, finally, various purified proteins. Thereby, the specificity of these reactions is associated not only with the proteins themselves but so with the accessory chemical groups on the protein base; these groups are called "determinant" groups or "haptenes" (Landsteiner, 1946). Haptenes alone without a protein base cannot evoke the formation of the corresponding antibodies. The latter belong to a certain class of protein bodies, namely to the group of gamma-globulins.

The specificity of the immune reactions for foreign proteins is striking; they constitute the finest of the biological reactions, making it possible to distinguish various organic substances where all the biochemical methods are ineffective. Immunologically, blood sera and blood cells of related species and breeds are different from one another; on this the method of detecting kinship of animals and of plants is based (Taliyev, 1940; Landsteiner, 1946). The most detailed studies along this line have been made on pigeons, cows, ducks, chickens, rats, fish, infusoria and other animals. In these works the comparison of the immunological tests of related forms, of their hybrids and progeny can constitute the demonstration of the accuracy of the method. By precipitating the antibody-producing serum of a rabbit with the serum or blood cells of one of the parents (saturation reaction) it is possible to show a number of separate antibodies in the residual serum which distinguish both parents (Irwin, 1949). Another example of the accuracy of the method is the fact that the serum obtained against "male" sperm cells [that is, containing the "Y" chromosome] affects these cells selectively and does not affect the "female" sperm cells, and vice versa (Shreder, 1945). Finally,

as a result of immunological reactions substances are produced in the body of an unrelated species which are similar to those formed in the species from which the antigens are obtained. In the ova and sperm cells of sea urchins there are complementary substances--fertilisin and antifertilisin--which combine with each other in fertilization; substances like them can be obtained immunologically: the injection of one of these substances into the blood of a rabbit causes the formation of substances in the latter which give the reactions of the opposite substance and which cause precipitation of the former (Tyler, 1947).

All these data show that underlying immune reactions is the capacity of the bodies of higher vertebrates to react with the formation of accessory, paired substances to any proteins "unknown" to the organism. These reactions lead to the formation of protein substances the specific nature of which is determined by the characteristics of the substance to which they react rather than by the specific features of the organism itself. The unusual breadth of these reactions, their fineness (specificity) and, finally, the fact that they can lead to the formation of antibodies similar, in a number of respects, to the specific substances of an unrelated species indicate the fact that we should not consider the immune reactions a simple defensive adaptation occurring as such. Rather, their protective role can be represented as a specific evolutionary expression of a more general mechanism, deeply underlying vital phenomena. (The considerations presented below in this chapter represent a brief statement of the ideas of the evolution of structure and metabolism of cells in relation to a number of other basic vital phenomena, particularly ontogenesis, developed by one of the authors).

It seems probable that underlying the immune reactions are general phenomena of evolution of metabolism and structure of the cell. If we turn to methods of development and heredity in unicellular organisms (Lopashov, 1949), we see the following characteristic features of them. A more or less direct transition of a number of substances and separate structures or their precursors from generation to generation; at the same time, their variety is relatively low within the limits of the cells organisms. Therefore, in unicellular animals there is no need for the regular occurrence of a large number of different structures or substances for creating them during the course of formation of the individual. The ontogeny of mammals is a different matter. In contrast to unicellular animals, in them the occurrence of a colossal number of absolutely new substances and structures becomes necessary; these cannot be transmitted through the cells from which their development begins (gametes). This need for a colossal new formation and complexification increases as we go to progressively more highly organized animals, reaching a maximum in mammals and man with their complexly constructed brains.

This must have led to certain rules and regulations of cell evolution depending on the increase in the degree of complexity and the structural variety of multicellular animals. This evolution must have proceeded along the line of formation of cells which would possess a universalized structure and metabolism capable of leading

to the formation of the most varied substances and structures. In other words, the more or less immediate precursors of various substances and structures existing in the unicellular animals must have gradually disappeared from the cells; the minimum number of "generalized" structures and substances, which, first of all, were capable of going from cell to cell during the course of their multiplication and, secondly, of serving as the basis for the most diverse biochemical and structure-forming processes leading to the construction of complex organisms during the course of development, must have been preserved.

We can point to a number of components of the metabolism and structure of cells of animals serving for the formation of the most varied structures; for example, the nucleic acid metabolism of cells, going from the thymonucleic acid of chromosomes to the ribonucleic acid of cytoplasm, in which the latter participates in the formation of various proteins; a similar route of transformation of substances occurs in all cells. Here we have also the very tiny granule-like structures (microsomes and mitochondria) which constitute the basis of various synthetic processes; they are apparently capable of division, and during cell division various proportions of them are allotted to their progeny. Various morphogenetic processes are impossible without a certain initial minimum of structures, on which the subsequent ones "are based." With a great measure of probability it may be supposed that this original structural minimum exists in animal cells in a very generalized form. Concentric (and, therefore, divisible) structures of the outer layers of the germ cells, composed of dense ectoplasm and its internal fluid layer and an outer thickened endoplasmic membrane probably constitute an essential part of these original structures for the most varied acts of morphogenesis. These structures, being transformed, participate in the creation of the original forms of all the main cell types (erythrocytes, lymphocytes, muscle, nerve, epithelial, mesenchymal and pigment cells--Holtfreter, 1946, 1947). It is very probable that the granule-like structures mentioned above and their filament-like aggregates also play the part of a groundwork for other intracellular structures. The list of these generalized components undoubtedly is still incomplete, and their interrelationships in the course of metabolism are inadequately clear; however, on the other hand, it may be considered that the majority of them is already known, and, by gaining an idea of their significance, it becomes easier to define the others. Evolutionary changes along the line of "generalization" probably did not proceed uniformly for various cell components: some lost their specific type of succession sooner; others, later. However, this process had a single tendency leading to a generalization and coordination of the various constituent structures of the cells.

One of the components of this generalized arrangement consists of processes of protein synthesis, the specific expression of which is constituted by the immunological reactions. Processes of antibody production are apparently nothing other than the formation of protein molecules, paired (complementary to) with antigen molecules in their configuration, as a result of which they can be bound by

bound by them, sticking together tightly at their surfaces. The production of proteins with complementary configurations is apparently not limited to antibody formation but also plays a part in a whole series of phenomena of protein synthesis in cells. For example, we have the fertilisin and antifertilisin in the sea urchin ova mentioned above, in which the former is in the outer layer, concentrically surrounding the other, which lies under it. Both substances are related to each other, being complementary, and apparently are formed in connection with each other. It is very probable that a number of other phenomena of protein formation in cells proceed according to the same principle. The widespread nature of two-layeredness of the outer layers of cells, beginning with egg cells, permits us to assume that configurational complementarity relations may exist between them; in those cases where they are stuck together it may be supposed that this adherence is based on phenomena similar to immunological bonds. Apparently, the formation of proteins which in their configurations are complementary to each other is generally of this type.

However, it should not be thought that this is the only means of protein synthesis possible. If we assume that protein synthesis occurs in two phases--first, the formation of a chemically similar protein molecule along a line on which amino acids lie against corresponding amino acids of the protein molecule and combination of them in that order, and then the adoption of a certain spatial configuration by this molecule--this latter molecule must always be complementary in its configuration to some other protein molecule. However, undoubtedly, while there is a grain of truth in this viewpoint (Pauling, Campbell and Pressman, 1943; Talmud, 1944; Haurowitz, 1949), it lies in the fact that the protein molecules are capable of assuming a configuration dependent on the conditions under which they are formed but not at all in the idea that they must always be complementary to molecules already existing at the time of formation of the new molecule. Probably, in cases of protein synthesis in cells other possibilities are also realized: the molecules formed take on a configuration like the preexisting molecules, or, finally, acquire altogether new configurations depending on changing conditions in the cells and reflecting these changes.

The formation of proteins similar to those preceding them must, of necessity, occur in the phenomena of growth and multiplication of cells, when an increase occurs in the number of molecules of certain types. Therefore, attempts to generalize the concept of protein synthesis of the "immune" type, that is, of the paired protein formation type, to include any cases of protein synthesis in cells (Tyler, 1947) should be considered an unlikely generalization of a specific variant to include all possible cases. The idea of the formation of only complementary configurations, which follows from the antibody production theory of Haurowitz and Pauling, requires for antibody synthesis that antigens be preserved and multiplied in the organism from which antibodies would continue to be "printed"; it is an admissible but not the only possible means of their formation. It may be supposed that protein synthesis can have different types of

structure determination depending on conditions as yet unknown in the cells. In the event of formation of paired proteins, which takes place in antibody formation, the possibility is not ruled out that proteins of cellular origin which have acquired a configuration complementary to or like antigens continue to be preserved and multiply along with them in the cells themselves. Antibodies would then be, respectively, the multiplication product of molecules with complementary configurations or the result of the action of molecules with an antigen-like configuration on the synthesis of molecules with a complementary configuration.

Finally, we should not overlook a third possibility--the formation of protein configurations in accordance with changing cell conditions. Such an instance is probably realized in the neurons and serves as one of the bases of memory and mental activity; here the protein configuration probably depends on the courses of the nerve impulses and serves as a kind of record of their changing course, which is put together to make a definite picture. The adaptation to such a determination of protein configuration during the course of their production apparently is still another variant of change in the cell structure, in contrast to those which lead to the formation of paired and like proteins. Therefore, the most varied aspects of cell phenomena are closely connected with one another: the rules and regulations of protein syntheses in the cell, of cell differentiation, antibody formation and nerve activity. These aspects have evolved together, and those changes in the capacity of producing the most varied protein structures which lead to an increase in the power of nervous activity also lead to an evolutionary increase in the ability to react with antibody formation to the most varied proteins in the developing immune systems of the higher vertebrates. These transformations apply, certainly, not only to proteins but also to the entire cell structure, the various components of which evolve together, interrelatedly, and in conjunction with the evolution of the interrelationships of the parts of the organisms themselves created by these cells. However, such phenomena are latent in the majority of cells, except perhaps the earliest stages of embryonic development. In the majority of cells the differentiating outer layers connect metabolic processes occurring on the level of the more complex and high molecular-weight substances, such as proteins, within the cells. These characteristics of metabolism are related to the appearance of blood as a common source of nutrition of the various cells, both historically and ontogenetically. Blood goes to all the cells of multicellular animals as a carefully adapted and generalized source of nutrition from which they can directly build substances necessary for their maintenance and growth. The splitting of food into the simplest components in the digestive tract is inseparable connected with the formation of blood as a generalized source of nutrition of variously differentiated cells; it serves as the first step in the transformations of substances which convert them into the generalized form in which they exist in the blood.

In this process of assimilation of the original food substances

to the substances of various parts of the body, various stages of this process probably do not occur simultaneously or equally. First of all (speaking about protein metabolism only), even in the blood certain amino acid interrelationships are established, whereby the set of them needed for nutrition of the animal by means of feeding is differentiated from the set needed for maintaining the life of the tissue cells and which circulates in the blood (Fischer, 1947). This change undoubtedly represents the first step on the way to formation of special proteins characteristic of the tissues of the given animal. However, a number of proteins are included in the blood which also serve as nutrient components for tissue cells. The mechanism of their assimilation is not yet entirely clear, but it is evident that these proteins can be assimilated by the cells without preliminary splitting into amino acids (Kaplanskiy, 1946). Blood proteins are produced by cells of the hemopoietic system, and as early as when they come out into the blood they possess specific individual characteristics. Not all the elements in the formation of proteins from amino acids are included in the bodies of animals, in their tissue cells. Part of the proteins is created before this in specially differentiated cells and reaches the other body cells in the form of proteins (globulins, albumins, fibrinogen, and others) included in the combination of blood nutrient substances as an essential component; as might be supposed, the assimilation of the various proteins must proceed unequally, depending on the need for correlation between the specific characteristics of these proteins and the tissue cells.

Therefore, if the considerations presented are correct, the main mass of phenomena of protein synthesis and their specific form, the formation of complementary proteins (reactions of the immune type), is hidden within the cells and is not demonstrable outside them. However, they exist in all the cells of the blood sources (of its proteins and its cells): in the liver, spleen, bone marrow and other hemopoietic organs which form the protein substances of the blood, including the antibodies. Specifically which cells within the limits of this group of organs are capable of producing antibodies is not yet entirely clear. It has been shown for the spleen that within its limits the main source of antibodies is constituted by the immature plasma cells; with injection of antigens a mass transformation of reticular into plasma cells occurs in the spleen, and specifically in the course of this transformation the cells produce antibodies (Fagraeus, 1948). Apparently, various types of cells, such as plasma cells, macrophages and lymphocytes, Kupffer cells of the liver and others are immunologically active (with different degrees of activity). It is significant that cells capable of producing antibodies possess the power of absorption and accumulation of various protein particles and are distinguished by the arrangement of their outer layers. Particles are continuously separating from their surfaces which are included in the blood in the form of its proteins (Sabin, 1939); by this means, apparently, the antibodies are formed (apparently, this process of separation of the surface portions of the cells can occur by several different routes in different cell forms).

The same cells in the foci of hemopoiesis serve as the common source of origin of antibodies and the wandering defensive cells of the blood (phagocytes). Because of this, the functions of defense against foreign proteins which have entered the blood are conferred on both antibodies and phagocytes, giving rise, respectively, to humoral and cellular immunity (Zil'ber, 1945). Whereas the other cell types of animals with an "enclosed" metabolism cannot react to external protein groups, these cells for their entire lives maintain the power to include in their metabolic apparatus the traces of effects of protein substances from without, which in evolution is connected with their defense function. The basis of these defense functions is nothing which arisesde novo with them but rather is the transformation of the phenomena of synthesis of proteins which play a much greater general part in the lives of the cells.

2. The Ontogenesis of Incompatibility Phenomena and their Relation to the Taxonomic Positions of Animals

If the general considerations presented above are correct, it must be expected that the defensive nature of the immunological reactions to foreign protein substances is not originally present in the cells. In embryogenesis, on the basis of protein syntheses of the same types as immune reactions, a number of protein structures arises which then remain permanently in the organism and are characteristic of it throughout its life. The universality of immune reactions shows that they are of the nature of a general reaction to proteins, of the same type as the synthesis of proteins with complementary configurations. From these facts it follows that they initially cannot be of a defensive character with respect to protein substances; therefore, it is more probable to suppose that this defense character of the reaction to foreign proteins must have arisen, like the other properties of organisms, during their development, as an adaptive modification of the phenomena of protein synthesis. It might be expected that the appearance of the defensive immune reactions would be associated with those stages of development at which this form of defensive adaptation becomes necessary as an addition to or replacement of the then existing defensive facilities of the developing animals.

The facts are in agreement with these considerations. At the same time, they show the relationship between the build-up of defensive immune reactions to foreign cells and the increase in complexity and variety of the structures of the organisms and the degree of differences between them. This is expressed in three lines of phenomena. First of all, in the fact that during the course of the individual development of organisms the incompatibility phenomena appear at a certain stage and then are increased; secondly, in the fact that such reactions are the more pronounced the more highly organized the animals are; and, thirdly, in the fact that their intensity depends in a certain way on the degree of kinship between individuals or tissues which are growing together. In evaluating the results of experiments, however, a number of facts should be kept in mind.

First of all, the fact that the phenomena of necrosis of transplants in experiments may depend on interruption of nutrition, respiration, and excretion of the transplantable tissue or organ as the result of interruption of the blood supply, which can lead to partial or complete necrosis (Lapchinskiy, 1940, 1941). This fact should always be given consideration in evaluating experiments; it markedly distinguishes transplants at the early embryonic stages, before the circulation begins, from transplants at the later stages; and transplants of embryonic and larval tissues of the later stages are less sensitive to interruption of the blood supply than the tissues of adult animals, which are highly sensitive to it. Secondly, the fact should be taken into consideration that the very phenomena of incompatibility are heterogeneous in their nature; other phenomena beside immune (and phagocytic) phenomena participate in them, and the role of the various phenomena changes as we go from one group of animals to the next.

Within limits of the vertebrates, investigations which have been made give us the following picture. In Urodela the incompatibility phenomena are the weakest and usually are practically unnoticeable. An infinite number of transplantations between different individuals, species and genera at the embryonic stages shows that the transplanted cells in the majority of cases are preserved for an indefinitely long time, going through metamorphosis. Thus, the pigment cells of species of Amblystoma transplanted to tritons regularly change the embryo, larva or adult triton to the color of the donor, which is persistent (Twitty and Bodenstein, 1939). The eyes, heart, extremities, auditory vesicles, skin and other organs and tissues of unrelated species "take" without any signs of degeneration (Harrison, 1936); the anlage of the entire entoderm, replaced in one species of triton by the entoderm of another, is preserved and functions in the adult animals (Mangold, 1949). No less successful are the transplantations at later larval stages, when the vascular system is developed and the animals feed actively, which is seen clearly from the numerous experiments of transplantation of eyes in Urodellarvae. In adult triton after such eye transplantations a degeneration of the retina and lens occurs, but this does not signify signs of incompatibility but rather is the result of an interruption of nutrition. Such eyes, when transplanted from individual to individual after a seven-day period of preservation in the cold or between different species, newly regenerate the retina and lens, and after restoration of the nerve connection with the brain they see well (Stone and Zaur, 1940; Stone, 1948) and are not subject to absorption phenomena. The ability to "take" without signs of incompatibility distinguishes Urodela among the other vertebrates just as characteristically as their great power of regeneration. However, despite the fact that in a number of experiments the embryos made up of halves belonging to different species of Urodela survived well without signs of degeneration, like parabionts of different species (embryos which are grown together in toto), in some combinations other pictures have been observed. As early as at the early stages the embryos began to show signs of

necrosis (or paralysis) of the tissues of one of the species. Detailed studies (Twitty, 1937; Humphrey and Burns, 1939; Roth, 1949) have shown that here various signs of direct intoxication of the tissues of one species by the tissues of the other occur, frequently even at the early stages but in some cases later also. Such an intoxicating effect, depending on its strength, may be exerted by comparatively small transplants also; sometimes, only the intact embryo can intoxicate the transplant but not vice versa, and the transplant in the reverse combination is in these cases capable of surviving with interspecies grafting. Such intoxications are the reverse of immune phenomena--the animal organism does not block the injurious substances but rather is killed by them, which may be connected with the fact that the immune reactions in these forms are expressed very weakly; these phenomena represent another aspect of the high degree of fusibility of those species of Urodela which do not exert an intoxicating effect on one another. A distinctive feature of the phenomena of such intoxication is the fact that tissue degeneration occurs by means of histolysis (dissolution of tissues) rather than by means of phagocytosis, as in the typical defense phenomena. The possibility of such toxic phenomena (intoxications) should always be kept in mind in evaluating the results of transplantations in order not to confuse them with death of the transplants from defense reactions and in order not to befog the pictures of changes in the defensive measures of organisms from one group of vertebrates to the next.

The picture changes even when we go to the Anura. At the early stages they possess just as great compatibility as the Urodela (with the exception of the phenomena of direct intoxication, which are encountered in a number of combinations, particularly with the action of toad tissues on the tissues of other members of the Anura). Special studies made on this problem show that the eyes (investigated in the greatest detail), transplanted in embryos and early tadpoles from one species of frog to another, live in the new site only a limited time. At the late tadpole stages with the growing legs, signs of necrosis of the cells and infiltration of the eyes with leucocytes begin, regardless of the stage of the transplant, but in various animals these phenomena do not occur simultaneously; in some, they unfold even after metamorphosis is complete. First the retinal cells are involved; those of the iris are affected last. The same pictures are observed in intraspecies (homoplastic) grafts, but they occur more slowly, and the majority go through metamorphosis with normal eyes; in the case of transplantations within the limits of the progeny of the same clutch, necrosis of eye tissues was less than in the case of transplants between unrelated animals (Schwind, 1937). Schwind's attempts to make the eyes of different species similar by means of growing embryos of different species together (parabiosis) and subsequent transplantation of the eyes from one of the members of the pair to tadpoles of another species did not lead to any results--these eyes behaved just as in simple interspecies (heteroplastic) transplantations.

Transplantation of different tadpole tissues within limits of

the same species and between different species led to the same result, but it is very significant that death of the different tissues did not occur simultaneously. A number of other studies on transplantations in anurans also show different times of death of the various tissues, whereby some can live for a long time in interspecies transplantation, such as the hearts of adult frogs, which function 12-16 months (Sinitsyn, 1948). It is possible approximately to establish the order of deathof the tissues: brain cells > eye cells > epithelium of the labyrinth > epidermis > pigment cells > heart > cornea > cartilage. The main period of degeneration in the case of interspecies transplantations is before and during metamorphosis; in intraspecies transplantations it occurs after metamorphosis and much more slowly.

The last group of experiments on the Anura is that of transplantations of the embryonic anlagen to adult frogs and tadpoles. Detlaf (1937, 1940), based on ideas of the greater possibility of adaptation of embryonic tissues to an adult organism, transplanted tadpole limb buds subcutaneously to adult Bombinans and brought about the formation of normal small extremities in them, which were preserved up to two years. However, once again various anlagen in such operations are preserved unequally: transplantations of parts of the gastrula to tadpoles showed that the majority of the structures formed afterwards underwent degenerative changes. This indicate that although the embryonic tissues are better accepted than the tissues of the later stages it is not because incompatibility phenomena fail to develop with respect to them; those tissues and organs which survive better in transplantations between tadpoles survive better also in transplantations from embryos to adults and in all other cases. "Assimilation" of the tissues of two individuals to overcome their incompatibility (including immune and phagocytic phenomena) cannot be accomplished by such a simple method; it requires a more profound knowledge of the nature and development of incompatibility phenomena.

Numerous transplantations made recently between embryos of the Anura and Urodela with the aim of studying tissue survival give us a picture similar to the result of interspecies transplantations in anuran embryos. If we consider the phenomena of direct intoxication, it turns out that the tissues preserved from these effects degenerate in the same order and with only slight time differences compared with the combinations between anurans, chiefly also during the period of metamorphosis (Eakin and Harris, 1945; Roth, 1949).

Data already presented on the amphibia permit us to draw some basic conclusions which have been confirmed for other classes of vertebrates also. Incompatibility reactions are inhomogeneous in nature; they include primarily the defensive immune and phagocytic reactions, closely associated with one another, but can also be based on toxic properties of the tissues of one species for another. In amphibians the latter is a not infrequent phenomenon and is not directly related to the distance of the species from one another; as has been mentioned, these phenomena of tissue necrosis are based on phenomena opposite to those in cases of death from the operation of defense mechanisms.

In accordance with the original considerations, the defense mechanisms which lead to incompatibility arise during the course of development. Appearance of them is associated with a certain stage of development and to different degrees coincides with the metamorphosis of Anura, but the stage at which they appear here is not so distinctly expressed; for a long period of time a build-up of them occurs. The defense mechanisms differ in their degrees of expression in different Amphibia. In the case of transplantations between Urodela they are not manifested, for practical purposes. In the Anura they are expressed but weakly within the limits of the same species, but here there are further differences between the members of the same clutch and unrelated individuals. Interspecies transplantations lead to distinct incompatibility phenomena, but thereby no great difference is seen between species of the same genus or distant genera. The most marked build-up of the incompatibility phenomena occurs in going from intraspecies to interspecies differences.

The rate of necrosis as the result of the incompatibility reaction is very different for different tissues, which can lead to apparent contradictions between the data of different studies in which different tissues are used. The brain, eyes, and labyrinth survive most poorly. Thereby, within the limits of eyes differences are also clearly outlined between the various cell forms: most sensitive is the retina; the iris is preserved longer, as is also the sclera. These differences show that first the most complexly differentiated cells, possessing a well-developed blood supply and more active metabolism (Konikova, 1948), are destroyed first. The fact that the transplants with inhibited growth survive longer than those growing at a normal rate as well as the fact that the occurrence of the incompatibility phenomena depends on the age of both host and donor--in transplantations to older hosts these phenomena occur after a certain time, when the transplanted tissues reach an adequate degree of differentiation--are in agreement with this.

Birds. In these warm-blooded animals with active metabolism and a high degree of structural complexity the incompatibility phenomena are very similar to the phenomena which are most important in practice and which have been best studied in mammals. Transplants of skin in adult chickens (Kozelka, 1933) are well accepted only in cases of transplantations from the same individual (autotransplantations). In other grafts the transplants remain from two to 12 weeks, depending on the degree of similarity between recipient and donor. With the development of the technique of embryonic transplantation it was extended to transplantation of anlagen (of extremities, eyes, sources of chromatophores) in the eggs of the birds. The wings and legs, transplanted in the form of buds from ducks, quails, partridges or guinea hens to chickens, develop well; but on the 10-14th day after hatching incompatibility phenomena of different degrees of expression occur (falling out of feathers, areas of necrosis, stoppage of blood flow), which terminate in death of the extremities. Thereby, the incompatibility phenomena occur no sooner to the duck extremities than to other, closer species. In the case of intraspecies transplantation

in chickens the same phenomena occur but much later, after two-five months (Eastlick, 1941). Transplanted bud anlagen from 9-24-day duck embryos to freshly hatched chicks do not grow more than eight days, after which they are rapidly destroyed; this is close to the time of death of transplanted extremities. At the same time, in numerous experiments on transplantations of various tissues of the same and other species, including mammals up to man, to the chorioallantois (Studitskiy, 1937), they live without signs of degeneration, but these transplants are limited in their activity to the embryonic life of the recipient. The neural crest of the mouse, transplanted to a chick embryo, survives well, producing pigment cells, and hair in epidermal areas; it dies approximately a week after the host hatches (Rawles, 1947). In contrast to all these tissues, the melanophores of one breed of chicken are preserved for a long time in the cutaneous area of another breed, coloring the feathers of subsequent generations (Rawles, 1945).

Therefore, in birds the same rules and regulations are demonstrated as in amphibians but in a more distinct form: 1) heterogeneous incompatibility of different tissues; 2) the occurrence of incompatibility phenomena at a certain stage of development (a certain time after hatching); 3) increase of incompatibility phenomena within the species, with similarity between the defense reactions to tissues of other species, both nearer and more distantly related.

Mammals are of the greatest importance for the problem being analyzed, because, based on them alone, the question of utilization of the conclusions obtained in medicine can be put. Specifically here, at the same time, it is possible to bridge the gap to immunological data and regard the incompatibility phenomena in close connection with the nature of the phenomena participating in them. Without going into a detailed discussion of the problem, we should like to note only that modern human surgery does not use homoplastic grafts, with the exception of cornea and cartilage, for the purpose of obtaining regular and permanent acceptance of a transplant. Transplantation of the tissues of other individuals (usually cadavers) is used for the creation of temporary transplants, which serve as the substrate for replacement by the host tissues; with this aim in view, frequently killed tissues are used. Among transplantations of this kind blood transfusions may be mentioned. When it is necessary to obtain a permanent transplant tissues of the same person are used. Thereby, usually these transplantations are made without interruption of the blood supply, because it might cause the death of the transplant; however, direct skin grafts taken from the same person are also successful (for example, Lapchinskiy, 1948), so that once again the relationship should be emphasized between the development of such direct transplantations and improvement of operative technique; once again the need for a distinct accounting of its significance for the evaluation of the role of incompatibility phenomena proper should be emphasized.

Numerous experiments of transplantations in mammals basically appear to confirm this idea. Only transplants from the same animal or from one member of an inbred line to another "take" well (Nemilov

1940; Loeb, 1945). Accessory hearts, transplanted in different mammals, live and function about a week (Sinitsyn, 1948). Skin transplants, which grow well at first, then undergo degeneration and die, being replaced by skin of the recipient, whereby the speed of death is proportional to the size of the transplant (Medawar, 1945). The death of various tissues in the case of transplantations in mammals occurs at different rates.

Incompatibility phenomena develop also in the event the transplantations were performed without interruption of the blood supply. Shipachev (1946), using the method of temporary parabiosis, transplanted toes to the hand and fingers from hand to hand in people. The same results were obtained in three pairs of parabionts. The initial "take" was good. Fifty days after the operation the first third of the flap connecting the digit to the donor was cut off; after 52 days, the second third, and after 55 days, the last third. When the first two third were cut no perceptible changes occurred in the transplanted digit, even though the main nutrient blood vessel had been cut off. Cutting the last third of the flap led to death of the digit and complete separation of it after 12 days.

There are a number of data, however, which speak for the opposite possibilities. Schwind (1938) and Lapchinskiy (1940, 1941), using the same method of temporary parabiosis, were able to "transfer" legs from one young rat to another, after which these legs survived on the new host for a long time, up to 18 months. There have been successful cases in transplantations of ovaries in rabbits (Krymskaya and Lopyrin, 1939). In these experiments 61 percent of the rabbits operated on subsequently bore young. In white rats the ovaries survived and functioned more than three months without signs of degeneration (Harris and Eakin, 1949). Thereby, it was made clear that if the transplanted ovaries are put under functional stress (in the event of removal of the host's own ovaries), they survive almost as well as autotransplants and resist the destructive actions of the host much better than ovaries which are not put under such stress. Finally, probably the survival of thyroid glands occurred in some transplantations in people (Bogoraz, 1948) and once again, in such cases, when the transplants were put under functional stress because of underdevelopment of the recipient's own glands.

Of the greatest importance in surgical practice are the noteworthy studies of V. P. Filatov on corneal transplantations in man (1945). The success of these operations does not imply the inevitable preservation of the transplanted corneas however. The possibility cannot be considered completely ruled out that after grafting pieces of cadaver corneas in a number of cases a gradual replacement of them occurs with the host's residual cornea. The fact that "takes" of corneas and sometimes even preservation of transparency were obtained in those cases where formalin-fixed cornea was used instead of fresh cornea or cornea preserved at low temperature (Shereshevskaya) attests to this possibility; to a certain degree this possibility is also indicated by the fact that when the entire cornea was replaced the transplant always opacified.

Transplantations of cartilage (Mikhel'son, 1939, 1946; Bogoraz, 1949) throw some doubt on the regularities of tissue acceptance--this doubt is based on the fact that transplants not only of fresh cartilage but also of that which had first been kept in alcohol were successful. Cartilage here may have played the part of a substrate for replacement. Therefore, the question of permanent acceptance of corneas and cartilage should be studied with greater precision than it has been to date.

A great service of the late D. P. Filatov and his coworkers was the development of investigations on the transplantation of embryo anlagen to adult animals; the aims of these studies were presented in a special article (1940). The transplantations made by Detlaf in amphibians along this line have already been mentioned. The greatest progress here has been made by Soviet investigators. Anlagen of teeth, transplanted to rats and dogs and developed into teeth, remained for a year, until the animals died (Lapchinskiy and Malinovskiy, 1940a, b, and c; 1943). In some cases anlagen of the thyroid gland, transplanted subcutaneously to adult rats, were preserved and functioned for a long time (Revzina, 1939). Increased function as the result of removal of the thyroid gland of the host improves the tissue acceptance of the transplants in such cases (May, 1933). Transplants from the skin of rat embryos to the eyes of adults are maintained for several months and change their route of development in the direction of transformation into cornea after such grafting (Popov, Bednyakova and Belyayeva, 1950). It has been possible also to observe complete differentiation of embryonic cartilage grafted to the body of an adult host in rodents (Volodina, 1944), and with grafting of the primordia of the testicles to adult white rats there was complete spermatogenesis (Ugarov, 1938). Finally, Okulova (1950) succeeded in bringing about the prolonged acceptance of embryonic skin at the sites of skin defects in adults in man. With repeated transplantations to the same patient the grafts "took" more poorly and, finally, degenerated with the simultaneous breakdown of pieces of skin which had previously been accepted.

These successful cases should not lead to the opinion that grafts of embryonic tissues give qualitatively different results compared with the transplantation of adult tissues. Here, those tissues and primordia which possess the lowest metabolic rates and show the best compatibility have the best tissue acceptability; the same applies to all other cases described. Here, for example, we have cartilage, cornea and teeth. Transplantations of eyes from 15-day embryos to adult rats (Tansley, 1946) lead to the rapid occurrence of incompatibility--cell death and phagocytosis. They include the eyes in the same order as in amphibians and occur in members of the same strain of rats at about the 20th day; in the case of transplantations between different strains, as early as after a week. The incompatibility phenomena develop also with transplantation of a number of embryonic anlagen to the omentums of adult rabbits; here, the reactions of incompatibility are manifested to a lesser degree. Thus, the embryonic tissues in mammals give more frequent and more permanent "takes" than adult tissues, and the incompatibility reactions to the former occur less readily. This

means that in transplantation of embryonic tissues the differences from adults are more of a quantitative than a qualitative nature.

In sum, as we go to the higher vertebrates regular changes occur in the incompatibility phenomena. They increase sharply from amphibians to mammals, increasing particularly in man. Complete compatibility of tissues of genetically different animals is limited to the embryonic stages in birds and mammals, but for these stages tissue compatibility is possible in very distant forms. The same heterogeneity occurs in the survival of various tissues as in the Amphibia, and those tissues which survive better in transplantations from adult animals also survive better in the case of transplantations from embryos. There is an increase in the significance of the relatedness of tissues being grown together, whereby the importance of the intraspecies degrees of kinship increases particularly. Tissues of distant species are capable of surviving in a foreign host for approximately the same time as the tissues of closely related species; a difference in the time of survival up to the point of permanent "take" is observed with increase in the degree of kinship within the species. Tissues of other species of various taxonomic degrees of relatedness and their derivatives are apparently perceived by the host as "foreign proteins", which give rise to pronounced defense reactions. In all cases only the autotransplants and tissues of the members of the same inbred line are accepted. In complete accordance with this is the fact that apparently the semm of each person possesses individual immunological characteristics which to different degrees differ from the characteristics of other individuals (Cumley and Irwin, 1943).

At the same time, in transplantations in mammals there are no appreciable toxic phenomena. Incompatibility phenomena refer to the defense reactions proper of the organisms; they are of a more uniform nature. Increase of them is in complete accordance with the gigantic build-up of immunological activity from lower to higher vertebrates (Poltev, 1947). Evolutionary changes refer chiefly to the "reactive" defense system of the organism specifically, which develops on the basis of the hemopoietic organs.

These evolutionary changes in the immunological phenomena occur in close connection with other changes in the basic properties of the taxonomic groups of vertebrates. These are: a) complexification and intensification of metabolism, particularly in connection with the occurrence of warm-bloodedness; b) complexification of the structure of animals, particularly of the brain; c) limitation of the power of regeneration, associated with the transition of tissues from one cell type to another, to the early stages of development only. Such a relationship probably is not chance. It is an expression of the necessary transformations to which the rules and regulations of animal development have been subjected in connection with the transition to the construction of progessively more complex and varied beings. Thereby, the cytological basis of developmental processes has also changed, which is particularly clearly seen: 1) in the gigantic increase in precision and variety of formation of substances in reactions of the immune type, that is, the formation of structurally complementary

substances; 2) in the creation of special cell differentiations in the system of hemopoietic organs which are associated with the appearance of a structure in the cells which permits the egress of the complementary substances outside the cells, in contrast to the usual "enclosed" metabolism in differentiated cells of animals.

3. The Significance of Various Parts of the Organism in the Phenomena of Tissue Incompatibility

Comparison of the various forms of animals with regard to the extent of incompatibility phenomena in them is inadequate to give a detailed concept of this subject. In order to gain a deeper idea of the sources of tissue incompatibility it is necessary to take into account and compare the significances of the various processes developing in the body of a single animal and playing a part in the incompatibility. They are related to differences in the different cells and their components with regard to incompatibility phenomena. Therefore, in this section we shall return to the cellular basis of incompatibility but now from a different aspect than at the beginning of the work; not from the aspect of the general evolutionary basis of incompatibility but rather from that of the detailed roles of the various cells and their components as demonstrated by means of a comparison of the factual data.

A. Tissue Compatibility with Regard to the Source of the Immunological Reactions. A number of specific data speak for the fact that incompatibility phenomena are associated in mammals primarily and specifically with the particular system of cells which accomplishes its defensive function in the entire organism rather than with the toxic effect of heterologous tissues. Mixed cultures of various mouse, rat and even chicken tissues, where the cells are mixed with one another, live for a long time together without the slightest signs of necrosis (Harris, 1943; Grobstein and Youngner, 1949). Tissue cultures live in the blood sera of other species, and very distant ones at that (birds-mammals), being only somewhat delayed in their growth rates; the addition of chick embryonic extract has a beneficial influence on the growth of mammalian tissues; some other stimulators also possess species specificity (Khlopin, 1940; Khrushchov, 1945). All this speaks for the fact that the incompatibility reactions are associated with a particular system of cells in the organism; it cannot be otherwise if true immune reactions underlie the incompatibility phenomena.

Aside from the data which we have mentioned in other places, the nature of the functions of cytotoxins (antibodies to tissues of another species), discovered and first studied by Mechnikov (1901) and his coworkers, speak for this. They arise from the injection of tissues of another species; after the breakdown of the grafted tissue the destructive influence of the animal upon the tissue of this species increases with subsequent grafting, as does also the effect of the animal's serum on the tissues of this species. The species specificity of the cytotoxins is proved also by the fact that the absorption of

such sera by tissue extracts of the species against which the cytotoxin was obtained eliminates their injurious effect (Harris, 1948).

The same phenomena of immunization and antibody formation occur in intraspecies transplantations, where, as has already been mentioned, death of the grafted tissues also occurs. The difference lies in the fact that immunization, like destruction of the transplants, occurs much more slowly here. However, here also repeated transplantations from the same donor, grafted after the breakdown of previous transplants, die in a shorter time. Thereby the corresponding antibodies to the transplanted tissues are found in the blood of the animals (Sokolov, 1923a). The time of death of second transplants, grafted on the same place as the first, was almost the same as that for those which were simultaneously transplanted to some distant place in the body. This shows that the decisive part in the incompatibility phenomena is played by general immunity; local immunity occupies a very small place, giving only an insignificant addition to the action of the general immunity (Medawar, 1944).

Specifically because of the immunological defensive nature of the incompatibility phenomena they are found between tissues only in the intact organism. This "integrity," to which many like to refer without interpreting its nature, can be adequately understood here as a first approximation. It is conditioned by the fact that only in the intact organism is there a system of specially constructed cells which produces antibodies in response to the influx of foreign proteins. Further, only in the organism do conditions exist for the activity and multiplication of these special cells, owing to which they can produce large numbers of antibodies necessary for the occurrence of the incompatibility reaction. Finally, with transplantation to an intact organism all the conditions are present for the prolonged effect of the transplant (of its products) on the cells of the recipient's hemopoietic system, which is also necessary in order for it to begin producing considerable quantities of antibodies. At the same time, a characteristic feature of living transplants can be demonstrated from this aspect; in contrast to the injection of various cell components and substances, transplants continuously produce the substances which serve for the increase in antibody production against them. The absence of a direct spatial relationship between the transplant and foci of hemopoiesis leads to the need for a connection between them through the products of transplants which enter the blood stream. Naturally, a certain time is needed for an adequate quantity of these substances to come to the hemopoietic system, and this time should be less the larger the transplant and the more active its metabolism and the closer its connection with the blood stream. This is in agreement with what actually occurs. The data presented show that tissues with a higher metabolic rate die more quickly in the transplants and that the survival time of the skin transplants of different sizes is inversely proportional to their sizes.

At the same time, all these data also speak against the fact that death of the tissues is based on toxic phenomena, as Loeb, for example, believed (Loeb, 1945). The phenomena of direct intoxication

observed in many experiments on amphibians occur immediately after the grafting (including in explants deprived of a vascular system--Lopashov, 1948). Incompatibility in mammals devlops gradually and is for the most part associated with immunological phenomena, after which comes destruction of the injured cells by the phagocytes of the recipient. Apparently, the phagocytic leucocytes which occur in the same hemopoietic system as the immune bodies possess specific activity with respect to those foreign cells the products of which have acted immunogenically on their source.

B. The Significance of the Various Parts of Cells in the Creation of the Immunological Basis of Incompatibility. The ideas presented above on the connection between transplants and the immune system through metabolism cause us to put the following question: which cells components and derivatives are accessible to the system of hemopoietic cells and responsible for the production of the immune reactions? Since the antibodies which arise are then bound to the cells of the transplants, blocking their activity and causing their death, it is clear that here we cannot speak of simple products of normal cell metabolism but rather of substances which are themselves always included as necessary structural components in the composition of the cells; this does not mean, however, that all the cell components must participate in phenomena of immunization. In complete agreement with this condition is the basic fact that only protein substances are antigens. Various simpler substances, products of cell catabolism, cannot serve as a means of communication between the cells of the body andof the hemopoietic system. The question arises as to whether in the cycle of matter in the blood not only substances serving for cell nutrition and not only products of cell catabolism but also more complex proteins, which are the typical constitutents of tissue cells and which are included in their normal biochemical characteristics might not participate. Which characteristic proteins can come out of the cells, under what conditions, and with what parts of the cells is their origin associated?

In this connection, we must turn to the basic features of cell structure; as a schema of the general structure of cells it may be accepted that they have two outer layers and internal endoplasmic structures, among which microsomes, mitochondria and other granular components may be distinguished, possibly included as elements in the fibrillar network, made up of filaments of a linear nature. With differentiation of cells numerous specialized proteins arise in them, different in cells of different types and different organs. Here, for example, we have melanin, collagen, myosin, keratin, neurokeratin, hemoglobin and other substances of limited distribution in the body. Unfortunately, with respect to the connection of the immune reactions with these proteins only anatohy information exists. This is to a considerable degree based on the biologically limited methods with which the majority of immunologists have worked who studied the antigenic properties of organs and tissues and their development in the course of ontogeny. They ground up the investigated tissues in a mortar and studied

the antigenic properties of the mixture of different substances and cell components obtained. However, the studies in which the functions of the parts of the cell were distinguished permit us to speak of the fac that their immunological roles are different. In Tyler's experiments (1947), where the outer and inner layers of sea urchin ova were investigated, the outer layer contained fertilisin (or consisted of it) and the underlying layer contained antifertilisin. When injected into the blood of a rabbit they induced the formation of antibodies which were like the opposite substance--antifertilisin gave rise to fertilisin, and vice versa. Different strains of Paramecium bursaria infusoria, after being injected into the blood of rabbits for a long time, induce the formation of antibodies which combine with the cilia and outer membrane but do not affect the inner cytoplasm or nucleus. In the same way, antigens exist in bacteria which are localized in the capsules and flagella the antibodies to which do not affect the inner parts of the cells. Finally, in erythrocytes it has been possible, by the method of separating the outer layers by freezing, to show that their main antigenic properties are associated with the inner stromata of these cells.

All these data indicate that different cell components are actually antigenically different. The impression may be created that in the intact state the antigenic role must be played chiefly by the substances of their outer layers. However, studies of the immunological properties of microsomes made shortly after their discovery (Kidd and Friedewald, 1942; Kidd, 2946) showed that the antigens are not limited to the cell membranes. Microsomes isolated from the cells of a number of organs (and malignant tumor) and injected into the blood of an animal of another species induce antibody formation, and these antibodies are capable of suppressing the growth of the tissues of the animal and of the tumor strain from which the microsomes were extracted. In the blood of the same animals there is a natural antibody to microsomes of the same individual. This speaks for the fact that the intracellular components participate, possibly, in antibody production and are accessible to their effects. The absence of organ specificity in the action of antibodies on microsomes and pronounced species (and strain, in the case of tumors) specificity speak for the fact that they may be only partial agents in antibody formation from transplantations.

All these data, however, which show that the various cell components can serve as different antigens do not give us any idea of the actual means of action of transplants or what specifically is the immunization agent in grafting. In all experiments of injection of different cells and their components, namely, bacteria, infusoria, erythrocytes, erythrocyte components, microsomes, and others the agent injected can itself come into the area of antibody formation and come into direct contact with the cells forming them. In the case of transplants the question again arises: specifically what comes from them to the foci of immunologically active cells? That such production of proteins by tissue cells does occur is shown by experiments of cultivating chicken fibroblasts in a medium of rabbit plasma. After the growth of the cultures in this medium substances were found in it

which gave immune reactions with chicken plasma antibodies (Landsteiner, 1946).

It would be most probable to suppose that, depending on the conditions, various protein components of the tissues come out into the blood stream. In all cases normal protein products of metabolism are given off which are distinguished by species specificity (and which do not produce immune reactions in the body of the same individual). In cases of injury or of conditions of atypical metabolism (with inadequacy of nutrition, respiration and disorders of excretory processes) under which the grafted tissue comes, other protein components of the cells or complex products of protein metabolism which normally do not come out of the cells may be given off into the blood. Therefore, the more typical the metabolic conditions (chiefly, blood supply) under which the transplant has come and the less its requirements on these conditions the less will be the escape of substances which it normally does not excrete.

C. Organ Specificity of Antigens, its Ontogeny and Rules and Regulations. In intimate association with the problems analyzed and representing their final link is the problem of how specific are the immunological properties of tissues of different organs and with what cell components is such specificity connected. The existing data make a quite definite picture. However, it should not be forgotten that the data existing here are very inexact: first of all, because of the methodological heterogeneity of the majority of studies already noted; secondly, by virtue of the fact that the majority of organs includes very different cell types and they are frequently very little related to one another: their antigenic properties may be different, but still they can all be mixed together. Therefore, it is probable that some characteristics of the data on organ specificity of antigens owe their existence specifically to this inaccuracy of the methods of investigation. The degree of specificity of the antigens obtained from various organs might be greater if the various components of homogeneous cells or whole living cells were used; it would perhaps be higher also where this condition has been unintentionally observed by the investigators.

Among the organs investigated the lens possesses the greatest organ specificity; at the same time, its antigens do not possess pronounced species specificity. This is expressed in the fact that antibodies to the lens do not give reactions with the material of other organs of the same species but react with the lens material of other species (Loeb, 1945). The cornea is similar in its properties to the lens. Antibodies (cytotoxins) to the lens injure it specifically. Antigens of other organs induce antibody formation, which react not only to the given but also to other organs and tissues. These are the kidneys, liver, spleen, leucocytes and others. This occurs also after the absorption of immune sera by the material of other organs for the purpose of removing organ-nonspecific antibodies encountered in the latter (Burke, Sullivan, Petersen and Weed, 1944; Harris, 1948). Thereby, the antibodies show species specificity poorly, or do not react at all with the material of the same organs of other

species of animals. From different studies of tissues it has been found that the most specific reactions are given by bone marrow, the antibodies to which react to a lesser degree to the material of other organs than to their own. Antibodies to the brain give cross reactions with antigens of the testicles, ovaries and kidneys. The absorption of an antigen to one organ by the material of others, which give cross reactions, considerably lessens the reaction to its own material. This causes us to suppose that in immunization with these organs their material introduces a number of antigens, which induce the production of several antibodies, and that these antigens have different distributions among various organs of the animal.

The isolation of a number of biochemical components of the cells of various organs permits us to go somewhat deeper into this picture. Different proteins present in the blood plasma--fibrinogen, globulins, albumins, and hemoglobin of erythrocytes--taken from the same species behave like different antigens. Collagen is also distinguished immunologically from the plasma proteins of the same animals. However, these proteins, at the same time, possess a certain species specificity, since antibodies to them react with the corresponding substances of closely related species only. Thyroglobulin possesses less species specificity. Finally, proteins isolated from the lens and keratin possess the greatest individual specificity and, at the same time, are almost devoid of species specificity; antibodies to these substances give reactions with the corresponding substances of representatives of even some other classes of vertebrates.

The division of substances used as organ antigens into separate fractions has made it possible to show that their species and organ specific properties are actually associated with their various components. The brain can be divided into protein and lipoid fractions. Of these, the lipoid fraction is organ specific; it reacts with antiserum against the brains of all species of mammals. The protein fraction possesses species specificity; it reacts only with antiserum obtained to the brain of the same species of animal. The antigenic properties of the posterior lobe of the hypophysis are divisible in the same way. Similar fractions have been isolated also from the microsomes already mentioned, the species specificity of which was also found to be connected with the protein fraction. The low species specificity of thyroglobulin is probably connected with the presence of thyroxin and diiodotyrosine in it in all species. Finally, in the lens it is possible to isolate no less than two antigenic components, one of which, an alpha-globulin, is encountered in all vertebrates.

The conclusions which these data permit us to draw are that the material of the majority of organs possesses slight antigenic specificity, and it is overlapped by species specificity. The organs, mainly the lens, which possess a high degree of organ specificity do not possess species specificity, so that the organ and species specificity are in reciprocal relations. It is significant that during the course of development the lens antigen undergoes a number of changes and that antiserum to lenses of 160-hour chick embryos reacts also with other organs, killing the embryos injected with it, whereas

cytotoxins to adult lenses selectively injure the lenses of the chick embryo in the egg; in the course of development the quantity of specific antigenic substance in the lens increases; the antigens nonspecific for the lens disappear. Species specific antigen is present first in the lens, but then it is completely replaced by organ specific antigen (Burke, Sullivan, Petersen and Weed, 1944). Organ specificity increases in the course of development.

The data presented permit us to consider, in a tentative form, that in the tissues of various organs there are a number of antigens rather than a single one; of these, one is more associated with species and the others, with tissue differences. Organ specific antigens can be detected only in experiments in which the cells are destroyed. Normally, they apparently do not go outside the cell limits. The fact that lens material injected into the blood can cause antibodies to be formed against the lens even in the same individual--consequently the hemopoietic system has not been "adapted" to the lens antigens--indicates this. In other organs also there are antigens which induce the formation of antibodies when their cells are destroyed which to varying degrees act specifically on this organ; the same results have been obtained with the phenomena of organ degeneration resulting from ligation of its main vessels (Sokolov, 1923a). However, apparently, the lower the percentage of organ-nonspecific proteins participating in the construction of a given organ, the higher the percentage of organ specific proteins and, accordingly, the greater the capacity of the organ for inducing antibody formation in the organism of which this organ is a part. However, despite the interest of these data, they do not permit us to find out the general rules and regulations underlying immunity phenomena with respect to various cell components and the material of different organs and, hence, of incompatibility phenomena. This cannot be done without considering the manner in which the immune system is formed during the course of development of the animal, by regarding the animal producing the antibodies as a simple supplier of them without regard for the history of the creation of the defense capacity in the course of ontogeny.

4. The Ontogenesis of Immunological Phenomena and Means of Changing Them

The material presented above and the direct conclusions from them permit us to proceed with certain theoretical constructions. The following conclusions are most significant for these constructions. Immunological phenomena directed at the formation of antibodies in the blood of animals develop regularly in the course of ontogenesis. Their occurrence is associated with: 1) the formation of a certain tissue system, namely, the blood system in the broad sense of the word, which occupies a special position in the metabolic system of the organism, contrasting, by having a nutrient function, with the other tissue systems; it is present everywhere in the body and is the source of the blood proteins, antibodies and phagocytes; 2) the stage of functional change in this system, when it develops the power of antibody formation, which it did not have theretofore. In the stage of the developed organism this

system forms proteins in relation to other, the most varied proteins external to and unknown to the organism. The proteins created, antibodies, against these external substances are complementary, paired substances; these antibodies are neither a part of the organism nor of the heterologous cells which have been introduced into it and have induced antibody formation; herein lies their difference from the ordinary proteins of the body which are included in its cells and intercellular structures. While the proteins of various higher vertebrates differ in composition to different degrees, their configurational properties, apparently, are almost indistinguishable. This is natural, since in various species they have changed over the course of history on the road to reinforcing one power or another of adaptation to environmental conditions, chiefly to outside protein structures. Therefore, with evolution toward the higher vertebrates another universal tendency becomes progressively more distinct, the tendency toward functional assimilation of various proteins to one another in accordance with their capacity of creating different configurations which make up for constitutional differences.

The first things that the tissue system capable of immunological reactions must come up against in the course of ontogenesis are the proteins (and protein complexes) of the same organism, the products of its organs and cells. The universal reactivity of the immune system does not permit us to expect that it would possess a predetermined power of reacting only to foreign proteins and not to its own, that it would have an innate power of distinguishing between its own and foreign proteins and that this power be manifested outside of its ontogenetic development.

Therefore, it is most probable to suppose that contact of the immune system with the products of the rest of the body does not occur without leaving some trace. Subsequently, the immune system does not produce complementary substances (antibodies) to the tissues of the same organism, and the phagocytes coming from it do not destroy these tissues. Therefore, it may be supposed that the effect of the protein substances of the body on its own immune system lies in creating compatibility in the latter to its own proteins, permitting their subsequent coexistence.

Of what does such a manner of creating compatibility consist? We may assume that the hemopoietic cells, coming into contact with proteins circulating in the blood during the course of their development, begin to produce proteins themselves which correspond more precisely to the proteins formed by the tissue cells than was possible with the original degree of similarity of the egg proteins. In turn, the protein composition of the latter may be modified in the direction of correlation with its nutrient substances carried by the blood. As a result of this reaction, the proteins formed by the hemopoietic organs not only fail to show immune reactions with the tissue cells, but, conversely, are made like them to the highest degree. Probably, the individually specific blood protein composition created in this way is, in one way or another, put together in accordance with the changing metabolic, particularly

nutritional conditions during the course of development of the animal, each time producing something new by comparison with the other individuals.

Therefore, during the course of ontogenesis an "acquaintance" of the hemopoietic system occurs with the proteins of the same organism, with an "adaptation" of it to them. Then comes the stage in which the capacity of defensive immune reactions is created. It occurs as an adaptation needed for the transition to changing environmental conditions—emergence from the state of the protected embryo--in birds and mammals; in amphibians its occurrence is not so clear-cut and is related approximately to metamorphosis. After it occurs the immune system begins to react to the intake of new "unknown" protein substances with the formation of complementary, paired substances which serve as antibodies; the intake of the "unknown" proteins only intensifies the production of proteins which are to be assimilated by tissue cells or remains without effect, since the very creation of the properties of the hemopoietic cells occurred in interaction with them.

This succession of stages is readily substantiated by data on transplantations and immunological reactions. In birds the phenomena of death of heterologous transplants occur shortly after hatching; before this, transplants of other species, including even mouse tissues, survive readily in the bodies of their embryos. In mammals, immediately after birth there are no immune reactions and, in accordance with this, there are no gamma-globulins in the blood; the immune reactions are associated with their appearance. Probably, the occurrence of this new defensive function of the body is associated with the beginning of activity of a tissue system capable of producing antibodies and which was in an undifferentiated state prior to this stage.

It is possible to conceive of a number of specific mechanisms for this succession of reactions of the hemopoietic system to protein substances. These mechanisms are associated with transformations of the cell structure, particularly the outer layers, and with its capacity of forming proteins with correlative and complementary configurations. However, the actual significance of one variant or another of these ideas can be learned only in complete association with a number of phenomena of protein metabolism, which themselves are unclear as yet, for which reason it would be premature to present these variants.

The ideas presented permit us to explain a number of phenomena. During the course of development the synthetic properties of the hemopoietic system are probably put together in accordance with the protein substances which: a) enter the blood in considerable quantity, and b) are common to various organs in their antigenic properties. These very common substances and the blood proteins created in relation to them appear to be general proteins which are specific for the species and the individual. The hematopoietic systems of the adult animals have already adapted to the characteristics of the very common substances produced by various organs and entering the blood during the course of their development, but they have not adapted to

those body substances which do not enter the blood but rather go through their transformations inside the cells. If different antigens are injected a reaction occurs first to substances which distinguish individuals and particularly species, since they were not "known" to the hemopoietic system. However, this reaction occurs also to those substances of the same organism which have not entered the blood stream. Even in the same organism not all the proteins are compatible with its immune system; probably only those which normally enter the blood stream.

Therefore, the hypothesis presented conceives of antibody formation in connection with the entire development of the nutrient system of the organism. The defensive reactions to "unknown" proteins are not congenital; they become possible as another aspect of adaptation of blood proteins to the nutrition of tissue cells during the course of development. If these ideas are correct enough, we should expect the existence of new, still unstudied relations between the hemopoietic system and the other organs of animals in connection with the distinctive features of their positions in the metabolic system of the body.

The fates of heterotransplants, including embryonic, and the means by which we can hope to change their fates intentionally in the direction of permanent preservation are getting to be understood. The immune system of the animal is oriented in its defensive actions to what it "did not know" in the course of ontogeny. Incompatibility phenomena, appearing at a certain stage of development, lead to the displacement of the heterografts in different cases for several different reasons. The transplanted tissues survive in those cases where the blood supply in them is rapidly restored, owing to which their metabolisms are not impaired (or are impaired to the least degree) and their activity is not reduced. In homografts, even with a good initial "take" the transplant subsequently breaks down in the majority of cases. Probably, the initial factor in the fates of such transplants is the fact that the absence of the necessary minimum of blood protein components, adapted within individual limits, reduces the activity of the transplant. This leads to a metabolic disorder (which is evidenced, for example, by mitotic arrest in the cells of skin transplants) and to the fact that: a) metabolism is either incomplete in the transplant cells and proteins come out into the blood which normally do not enter it; b) or there is a disorder of permeability of the outer layers of cells, which also leads to the entrance of proteins into the blood. These proteins affect the immune system, and the antibodies produced increase the destruction of the weakened transplant, which then submits more readily to their influences. As a result, this process comes to an end, because of its intrinsic contradictions; there is complete destruction of the transplanted tissue.

Thus, these contradictions which underlie the incompatibility phenomena are based on the following relations: 1) metabolic disorders of the transplanted tissues because of inadequate metabolic conditions (associated with the technique of grafting or lack of correlation of the blood protein components with the transplant cells); 2) immune reactions of the recipient occurring on the base of entrance of the protein products of the transplant into the recipient's blood.

These relations are associated with the entire combination of conditions which contribute to or prevent the onset of development of incompatibility phenomena. Embryonic transplants survive better, probably because they are less demanding of metabolic conditions in the initial period of the transplantation. Transplants with lower metabolism survive better for the same reasons. By contributing to normal metabolism of the transplanted tissues, functional stress reduces the probability of immunization of the recipient with products of incomplete metabolism. The more the donor and recipient are like each other the greater the part which is played by emergence of protein products into the blood which do not normally come out of the cells in the incompatibility phenomena resulting from impairment of the blood supply. In the case of more distant, particularly interspecies transplantations, the inadequacy of the blood composition of the recipient for nourishment of the transplant is added to this, which accelerates the metabolic disorders of the graft and the resulting antibody production against it and its death. By modifying various conditions in this complex, we may hope to increase tissue compatibility considerably in transplantations.

Transplants to adult animals are made at a time when the protective system has already been developed, and the transplant becomes the source of the "unknown" substances. Then, the larger the transplant and the more active its metabolism and the less its protein composition has in common with that of the recipient the sooner it is destroyed. In cases of transplantations to embryonic stages, on the other hand, before the development of the immune system, the subsequent displacement of the transplant is conditioned by the fact that the mass of recipient tissues is usually incomparably greater than that of the transplant and for this reason the adaptation of the immune system proceeds in accordance with the recipient rather than the transplant, which is later crowded out, as usual. If the mass of recipient tissues were about the same as that of the transplant it might be expected that the immune system would adapt itself to both of them.

Certain facts in addition to those already mentioned are in agreement with these ideas. First, even in the old experiments of Burn and Harrison embryos of different species of frogs, joined together in halves, went through metamorphosis in some combinations, maintaining their viability, whereas small transplants in the same combinations undoubtedly died at a certain stage of development. Obtaining such results in amphibians is difficult because in many combinations signs of direct intoxication of tissues of one individual by those of the other occur to different degrees. Secondly, data on twins in cows obtained from different fathers speak for the truth of our hypothesis. It has been shown that if their blood streams are anastomosed the antigenic compositions of their erythrocytes is mixed and becomes the same in both individuals. Apparently, during the course of development the calves exchange stem cells and, in this way, each receives primitive cells half consisting of the cells of one individual and half, of the other. (This speaks for the fact that not only the vascular endothelium cannot come from

the local connective-tissue cells (Khlopin, 1944) but also that the cells of the original sources of blood (liver, spleen, bone marrow) do not arise from local cells of the anlagen of these organs but rather from wandering primordial derivatives of embryonic blood). This does not prevent such hemopoietic stem cells, coming from different breeds, from living their entire lives in the bodies of bulls and cows, without showing any signs of incompatibility with them and without affecting the viability of the recipients (Owen, 1945). Undoubtedly, however, special experiments are needed for proving this hypothesis.

Essential in our approach, it seems to us, is the fact that the phenomena of incompatibility are not regarded separately but rather in their specific association with a larger group of vital phenomena and the evolution of the latter. This approach is demonstrated even in the analysis of incompatibility as a function of ontogenesis of the entire hemopoietic system, in close connection with its changing conditions. Many authors who have transplanted embryonic tissues to adults have expected that they would "adapt" more easily to the host because of their greater plasticity, by analogy with plant grafts. As a matter of fact, however, the actual picture in transplantations in higher animals is exactly the opposite: it is not the transplant which adapts itself to the host but, conversely, the host which adapts itself to destroying the transplant, by defense reactions which have been created during the course of evolution. If we do not seek to understand and master the regularities of creation of this defensive capacity, the expectations of "accustomation" of the transplant in animals will be perfectly arbitrary; they will be based essentially on the conception of adaptability as a universal property of the living, manifested without relation to the specific conditions of evolution. As a matter of fact, this capacity of defense against foreign bodies is an expression of evolutionary changes in the phenomena which in other cases lead to adaptation to environmental conditions, the phenomena of plasticity of proteins in their various relations in the cells.

Bibliography

1. Bogoraz N. A. 1948/49. Vosstanovitel'naya Khirurgiya (Restorative Surgery), I, II, Medgiz, Moscow.
2. Boyd W. 1949. Fundamentals of Immunology. Inogiz. Moscow.
3. Volodina Z. S. 1944. Byull. Eksp. Biol. i Med.(Bulletin of Experimental Biology and Medicine), 13, 6-8.
4. Harrison R. H. 1936. Geteroplasticheskiye Peresadki v Embriologii. (Heterotransplantations in Embryology). Medgiz. Moscow.
5. Detlaf T. A. 1937. Arkh. Anat., Gist. i Embr. (Archives of Anatomy, Histology and Embryology), 16, 247-255.--1940. Trudy In-ta Morfogeneza (Works of the Institute of Morphogenesis), MGU [Moscow State University], 7, 15-25.
6. Zil'ber L. A. 1945. Uspekhi Sovr. Biol.(Progress of Modern Biology), 20, 6-19.--1948. Osnovy Immuniteta (Fundamentals of Immunity), Medgiz. Moscow.
7. Kaplanskiy S. Ya. 1945. Uspekhi Sovr. Biol., 19, 324-338.

8. Konikov A. P. 1948. Soveshch. po Belku. (Conference on Protein). Published by the Academy of Sciences USSR, 102-117.
9. Konnikova A. S. 1948. Soveshch. po Belku. Published by the Academy of Sciences USSR, 225-238.
10. Krymskaya M. K. and Lopyrin A. I. 1939. Probl. Endokrinol. (Problems of Endocrinology), 4, 39-53.
11. Lapchinskiy A. G. 1940. DAN SSSR (Reports of the Academy of Sciences USSR), 26, 725-729.--1941. Trudy In-ta Tsitol., Gist. i Embr. (Works of the Institute of Cytology, Histology and Embryology), 1, 183-207. --1948, Khirurgiya (Surgery), No 9, 14-21.
12. Lapchinskiy A. G. and Malinovskiy A. A. 1940a. DAN SSSR, 26, 730-732. --1940b. Ibid, 28, 751-754.--1940c. Ibid., 29, 269-272.--1943. Ibid. 41, 188-190.
13. Lopashov G. V. 1948. DAN SSSR, 60, 1281-1284.--1949. Uspekhi Sovr. Biol. 27, 345-368.
14. May R. 1933. Uspekhi Sovr. Biol., 2, 179-184.
15. Mechnikov I. I. 1901. Russk. Arkh. Patol., Klin. Med. i Bakteriol. (Russian Archives of Pathology, Clinical Medicine and Bacteriology), 11, 101-118.--1947. Lektsii po Sravnitel'noy Patologii Vospaleniya (Lectures on the Comparative Pathology of Inflammation). Medgiz, Moscow.
16. Mikhel'son N. M. 1939. Khirurgiya, No 10, 29-34.--1946. Sov Med. (Soviet Medicine), No 1-2, 17-18.
17. Nemilov A. A. 1940. Osnovy Teorii i Praktiki Peresadki Tkaney i Organov (Fundamentals of the Theory and Practice of Transplantation of Tissues and Organs). Medgiz, Leningrad.
18. Okulova A. N. 1950. Khirurgiya, No 4, 52-58.
19. Poltev V. I. 1947. Uspekhi Sovr. Biol., 23, 289-296.
20. Popov V. V. 1938. Uch. Zap. Gor'k Gos. Un-ta (Scientific Notes of Gor'kiy State University), 8, 24-87.--1948. Sborn. Pam. Akad. Averbakha (Collection in Memory of Academician Averbakh). Published by the Academy of Sciences USSR, 119-138.
21. Popov V. V., Bednyakova T. A. and Belyayeva T. G. 1950. DAN SSSR, in the Press. ["v pech." is written here which means "in the press"; "v per." may have been intended, which means "bound"].
22. Revzina S. I. 1939. Byull. Eksp. Biol. i Med., 8, 521-524.
23. Sinitsyn N. P. 1948. Peresadka Serdtsa kak Novyy Metod v Eksperimental'noy Biologii i Meditsine.(Transplantation of the Heart as a New Method in Experimental Biology and Medicine). Medgiz. Moscow.
24. Studitskiy A. N. 1937. Sborn., Posv. Akad. Nasonovu (Collection Dedicated to Academician Nasonov). Published by the Academy of Sciences USSR. 441-569.
25. Sokolov N. V. 1923a. Kazan. Med. Zhurn.(Kazan' Medical Journal), 19 (4), 3-12.--1923c. Vestn. Mikrob. i Epidemiol. (Herald of Microbiology and Epidemiology), 2 (3-4), 64-70.--1924. Trudy 16-go S'yezda Russ. Khirurgov (Works of the Sixteenth Congress of Russian Surgeons), 196-198.
26. Taliyev D. N. 1940. Trudy Bayk. Limn. Stants. (Works of the Baikal Limnological Stations), 10, 241-355.
27. Talmud D. L. 1944. Uspekhi Khimii (Progress of Chemistry), 13, 35-47.

28. Ugarov L. A. 1938. Byull. Eksp. Biol. i Med., 6, 714-717.
29. Filatov V. P. 1945. Opticheskaya Peresadka Rogovitsy i Tkanevaya Terapiya (Optical Transplantation of the Cornea and Tissue Therapy). Medgiz, Moscow.
30. Filatov D. P. 1940. Tr. In-ta Morfogeneza, MGU, 7, 7-13.
31. Shipachev V. G. 1946. Rekonstruktsiya Pal'tsev Ruki (Reconstruction of Fingers). Medgiz, Moscow.
32. Shreder V. N. 1945. Izv. AN SSSR, ser. biol. (News of the Academy of Sciences USSR, Biology Series), 404-418.
33. Khlopin N. G. 1940. Kul'tura Tkaney (Tissue Culture). Medgiz. Leningrad.--1944. Zhurn. Obshch. Biol. (Journal of General Biology), 5, 43-55.
34. Khrushchov G. K. 1945. Rol' Leykotsitov Krovi v Vosstanovitel'nykh Protsessakh v Tkanyakh (The Role of Blood Leukocytes in Recovery Processes in Tissues). Published by the Academy of Sciences USSR. Moscow.
35. Burke V., Sullivan N. F., Petersen H. and Weed R. 1944. J. Inf. Dis., 74, 225-233.
36. Cumley R. W. and Irwin M. R. 1943. J. Immunol., 46, 63-70.
37. Eakin R. M. and Harris M. 1945. J. Exp. Zool. 98, 35-64.
38. Eastlick H. L. 1941. Physiol. Zool., 14, 136-144.
39. Fagraeus A. 1948. Acta Med. Scand. 130, suppl. 204, 1-122.
40. Fischer A. 1947. Biol. Rev., 22, 178-194.
41. Grobstein C. and Youngner J. S. 1949. Science, 110, 501-503.
42. Harris M. 1943. Anat. Rec., 87, 107-117.--1948. J. Exp. Zool., 107, 439-453.
43. Harris M. and Eakin R. M. 1949. J. Exp. Zool., 112, 131-163.
44. Haurowitz F. 1949. Quart. Rev. Biol., 24, 93-101.
45. Holtfreter J. 1946. J. Morphol., 79, 27-62.--1947. J. Morphol., 80, 57-92.
46. Humphrey R. R. and Burns R. K. 1939. J. Exp. Zool., 81, 1-42.
47. Irwin M. R. 1949. Quart. Rev. Biol., 24, 109-123.
48. Kidd J. G. 1946. Symp. Quant. Biol., 11, 94-110.
49. Kidd J. G. and Friedewald W. F. 1942. J. Exp. Med. 76, 543-578.
50. Kozelka A. W. 1933. Physiol. Zool., 6, 159-182.
51. Landsteiner K. 1946. The Specificity of Serological Reactions. Harvard Univ. Press.
52. Levander G. 1945. Nature, 155, 148-149.
53. Loeb L. 1945. The Biological Basis of Individuality. C. Thomas, Springfield and Baltimore.
54. Mangold O. 1949. Naturwiss. 36, 112-120.
55. Medawar R. B. 1944. J. Anat. , 78, 176-199.--1945. J. Anat., 79, 157-176.--1946. Brit. J. Exp. Pathol., 27, 9-24.
56. Owen R. D. 1945. Science. 102, 400-401.
57. Pauling L., Campbell D. H. and Pressman D. 1943. Physiol. Rev., 23, 203-219.
58. Rawles M. E. 1945. Physiol. Zool., 18, 1-16.--1947. Physiol. Zool. 20, 248-256.
59. Roth H. 1949. Rev. Suisse Zool. 56, 291-298.
60. Sabin F. R. 1939. J. Exp. Med., 70, 67-82.

61. Schmid J. L. 1937. J. Exp. Zool., 77, 87-107.--1938. J. Exp. Zool., 77, 337-346.
62. Stone L. S. 1938. Ann. N.Y. Ac. Sci. , 49 (5), 856-865.
63. Stone L. S. and Zaur I. S. 1940. J. Exp. Zool., 85, 243-269.
64. Tansley K. 1946. J. Exp. Biol., 22, 221-224.
65. Twitty V. C. 1937. J. Exp. Zool., 76, 67-104.
66. Twitty V. C. and Bodenstein D. 1939. J. Exp. Zool., 81, 357-398.
67. Tyler A. 1947. 6th Growth Symp., 7-19.

The Process of Organ Regeneration in Animals

L. V. Polezhayev (Moscow)

I

At the present time the principal task in the regeneration problem is the elaboration of methods which make it possible purposefully to regulate processes of restoration of lost organs (or tissues) of the body and to restore to normal their weakened or lost power of regeneration, based on the aims promulgated by the practice of Soviet socialistic public health and the national economy. For the purpose of controlling regeneration processes a theory is needed; it is necessary to know how, by what method the processes are accomplished which underlie them. In connection with this, it is very important to know the mode of formation of the regeneration primordium. Ideas about the essence of the regeneration processes have changedcontinuously.

In the field of the study of regeneration there has long been a bitter conflict between idealism and materialism. Driesch (1915) proceeded along the line of frank idealism, vitalism, declaring that the regeneration processes are controlled by an entelechy, a non-material metaphysical factor. Morgan (1907) declared that the power of regeneration is a primordial property of animals, not related to their adaptation to environmental conditions or natural selection. Morgan's idealism, therefore, is expressed in the assertion that there is a property of original expediency intrinsic in living organisms. His approach is metaphysical. He regards adaptation and the regenerative power of living matter to be separate, seeing only the opposite nature of these phenomena rather than their unity.

Weismann (1892), on the other hand, declared that regeneration is an adaptation, the result of natural selection, and is not a self-reproductive property of animals. Weismann, like Morgan, metaphysically separated and opposed adaptation and the self-reproductive capacity of living matter, without seeing their unity. Weismann's idealism,despite his verbal acceptance of natural selection and phylogenesis, consists of the fact that the development of the power of regeneration is explained by a miracle, by chance, and does not depend on the properties of organisms; therefore, it requires the assumption of a particular expediently acting principle.

All these thoroughly reactionary idealistic evasions led to the complete degeneration of research on regeneration abroad. Characteristic of all of them are an antihistorical approach and the analysis of the organism outside of its relationship with the

environment. The route of their development is characterized by formulations of the ideas of Spemann (1924-1936) of "organizers" and the theory of "fields" by Weiss (1929) and other foreign scientists. "Organizers" are parts of a developing organism to which the significance is ascribed of being factors accounting for the development of other "organizable" parts and which do not depend on them. The development of the organism, according to Spemann, is a chain of reactions of passive, unorganized material to the influence of organizers. Where does the first organizer come from? Spemann gives no answer to this question. His antihistorical approach logically leads to an act of creation, that is, to idealism. The "fields," according to Gurvich, Weiss and their followers, are dynamically preformed entities of unknown origin accounting for the development of the organism and not related to or depending on its material. The preformistic autogenetic idealistic significance of the field theory is seen very clearly.

The most progressive of the foreign regeneration theories is Child's physiological gradient theory (1948), according to which development is determined by environmental conditions which bring about first physiological quantitative differences in the metabolic rates in the initially homogeneous living material, which then leads to qualitative changes, changes in shape, and differentiation. However, this theory is limited and antihistorical, regarding ontogeny, phylogeny and heredity separately, independently of one another.

Darwin's materialistic concept, fundamentally correct, should be contrasted with the idealistic view of the nature of regeneration by Weismann, Morgan and their followers. He regarded the power of regeneration, on the one hand, as a property of animals, as one of the forms of self-reproduction of their organs or parts of the body essentially identical with the phenomena of asexual multiplication (budding and fission) and the phenomena of growth and even sexual multiplication, and, on the other hand, as an adaptation of animals to their existential conditions. He approached regeneration from a historical standpoint, implicitly regarding these phenomena in their unity. Fundamentally, his view of regeneration is correct and requires further development.

We may define regeneration as one of the adaptive forms of self-reproduction of living matter. Underlying regeneration is metabolism -- anabolism and catabolism. Metabolism is the primary property of living matter and, at the same time, it is an adaptive process. This may also be expressed in a different way: adaptation is a primary property of living matter, because underlying the adaptation is metabolism of the organism which is in unity

with its existential conditions. If these conditions are changed there will be an adequate change in metabolism, that is, the organism will adapt itself to the new conditions, and hence there will also be a change in the power of regeneration and form of the organism. However, adaptation, that is, an adequate change of the organism and of its metabolism to environmental conditions, will not always be expedient for the life of the organism and can ultimately lead to the death of the animal. In the development of living nature, expediency and particularly the phenomena of regeneration is not determined by adaptation but rather by natural selection -- by heredity, variation and survival. An example is seen in the regeneration of the extremities of lower vertebrates (axolotl, triton), an expedient adaptation which contributes to their viability; in the higher vertebrates this is absent, because under the conditions of their evolutionary development and their adaptation it would have led to a reduction in their viability, that is, it would have been inexpedient. N. V. Nasonov (1941) justifiably points out that the presence of a delicate blastema on the wound surface of an extremity in mammals would be a source of trauma and a portal of infection and would lead to their deaths. During the course of evolution some forms of adaptation are replaced by others which are more advantageous for the organism, according to its existential conditions. In a corresponding manner the development of the power of regeneration occurs in different forms: in some animals the power of restoration of an entire individual from a part of it is preserved; in others only organs may be regenerated; in still others, only physiological regeneration and wound healing occur. A similar phenomenon is observed in the same organism: some organs possess the power of regeneration in it; others do not.

II

There has been and continues to be a bitter controversy also on the subject of the mode of regeneration. Initially, there was a conflict between preformistic and epigenetic trends. Then, scientists who took the viewpoint of regeneration as a transformation began to oppose the preformists and epigeneticists. In other words, development began to be conceived as a transformation, that is, the occurrence of something new on the basis of a qualitative change in the old under new environmental conditions, under new existential conditions, rather than a preformation, the development or growth of pre-existing elements and rather than a new formation. The specific ideas of the course of regeneration have changed progressively.

The preformistic virchowian cellular theory with its main principles of "all cells only from a cell", "nothing new arises in the development of anything," "the cell is the ultimate element of living matter," and others received particular impetus specifically in the study of regeneration. Waldeyer, Thiersch and Bard extended the virchowian dogma further, declaring: "Any tissue regenerates only the same kind of tissue." These ideas were extended in biological and pathological research on regeneration. Regeneration was conceived as a reorganization, as a growth of pre-existing tissues in the area of the wound, as growth occurring by means of active cell multiplication of wounded tissues. Morgan (1907) wrote this directly: ". . . regeneration is nothing other than the repetition of growth which has temporarily stopped."

This theory was given the name of "the theory of tissue outgrowth in regeneration." The frank metaphysical character of this theory, the idea of regeneration as growth rather than development, and the platitudinous view of morphogenetic processes require no particular analysis. The view of the behavior of separate tissues was also metaphysical: they were conceived as regenerating independently of one another and for this reason even coming into conflict with one another. Thus, according to Tornier (1906), in the regeneration of the triton tail "growth along the length of the regenerate is stimulated and controlled by the central portion of the regenerate and, specifically. . . in the final analysis by the tail skeleton regenerate," while the skin inhibits growth of the regenerate. In this way he formulated the theory of mechanical conflict of tissues in regeneration. However, at the same time and even in the studies of the same scientists, facts began to be accumulated which were in direct contradiction to their theories. Thus, it was found that in the regeneration of extremities of lower vertebrates the skeleton in the regenerate appears as a new formation, similar to its development in the course of typical ontogeny (Goethe, 1875; Freis, 1885; Barfurt, 1891). Somewhat later, through histologic studies it was very firmly established that regeneration, for example, of the extremities of salamanders and tritons, is not accomplished by means of a simple growth of pre-existing tissues but rather by means of the formation of a blastema, that is, the primordium of the organ, like the embryonic primordium. The blastema consists of epithelium and subepithelial tissues formed from cells of mesenchymal or embryonic type, initially similar to leucocytes (Fritsch, 1911). The homogeneous cell mass of the blastema grows rapidly and forms a bud, which is converted into a regenerate by means of further growth and differentiation. This fact of regeneration by means of the formation of a blastema was

confirmed subsequently by a number of investigators. In this way the "theory of regeneration by means of new formation of a blastema" was created. In the field of pathology, for example, in healing of a wound, the element similar to the blastema is granulation tissue.

The fact established was correct, but investigators who were in the realm of metaphysical thinking and virchowian ideas, became confused in their theoretical generalizations, and, in the final analysis, the majority of foreign scientists proceeded along the line of idealism.

The theory of regeneration by means of new formation of blastema required an answer to the following basic questions:

1. From where do the blastema cells come?
2. How does growth of the blastema occur?
3. How is the development of the blastema accomplished and on which factors does its quality depend?

The first question in their theory, supported by such scientists as Weiss (1922-1930), Guyenot (1922-1930) and others, was, for the most part, answered in the same way as Weismann answered it, by assuming the existence of special reserve cells, of the nature of the idioplasm or germ plasm, from which the blastema elements arise. The insolvency of this view stems from the fact that in the animal organism there are no such reserve embryonic cells. In vertebrates there is, for example, connective tissue which histologically is poorly differentiated, but in its properties it is not at all identical with the cells of embryonic tissue. On the other hand, it was shown that in regeneration of both the lower (for example, hydras) and higher animals (for example, vertebrates) all the tissues participate, even though they are fully differentiated and can in no way be classified as reserve tissues. During the course of regeneration the differentiated tissues regularly change - - they are destroyed and dedifferentiated

The investigators answered the second question generally in the same way as did the representatives of the "outgrowth" theory; specifically, they considered that the source of regeneration is cell multiplication of the injured tissues in the area of the wound. This idea was based on the theory of "wound hormones," data on "mitogenetic radiation" as a source of cell multiplication, and others. Whatever the kinds of cells in the wound area -- whether "reserve" or old tissue cells -- they believed that multiplication of them constitutes the main regeneration process of the organ. The logical consequence of this idea was the conclusion that in order to stimulate the regeneration process in organs or tissues it is necessary to produce or stimulate the cell multiplication process. This idea unified scores of various investigations attempting to work out methods of stimulation of cell multiplication and hence, wound healing. The

principle became generally accepted that stimulating wound healing or processes of regeneration means stimulating cell multiplication in the area of the wound. However, a strong blow was struck against this idea, because proof was shown that regeneration does not begin with cell multiplication and that it far from always plays the main part in regeneration.

To the third question -- on which factors the quality of the blastema depends -- the epigenetic theory of regeneration gave an unsatisfactory reply. Under the influence of Spemann's and Gurvich's ideas foreign scientists -- defenders of the epigenetic theory of regeneration -- began to conceive of the blastema as devoid of its own organizational powers, as being "nullipotent" (Weiss) and determined under the influence of a particular specific factor of the whole, a "field" or "organizer" intrinsic in the remnant of the amputated organ. Therefore, the epigenesis of Spemann, Weiss and their followers is preformism inside out and does not explain but rather denies development, denies the occurrence of a new quality during the course of development of the regenerate.

The vicious weismannistic-virchowian "epigenetic" theories of regeneration of foreign investigators were severely rebuffed and exposed to decisive objections by Soviet scientists, who, on the basis of new facts, arrived at a number of new principles and the formulation of the theory of regeneration by means of transformation of the tissues of an amputated organ.

III

During the period from 1930 to 1950 Soviet investigators established a number of essential facts and drew some very important conclusions concerning the method of formation of the regeneration primordium. The main attention was given to the problem of the initial stages of regeneration, which determine the entire subsequent course of the process and, by regulating which it was possible considerably to alter the course of the entire process. In this respect the main conclusion consists of the establishment of two phases in the course of regeneration: 1) the phases of initial accumulation, when the regeneration primordium is formed basically without cell multiplication, and 2) the phases of secondary accumulation, at which time the regeneration primordium is formed basically by means of cell multiplication. This conclusion is based on three groups of facts (Polezhayev, 1945):

1. Young regeneration blastemas of an extremity or tail of an axolotl are incapable of growth when transplanted to another part of

animal's body, for example, to the dorsal region. If cell material taken from the subepithelial portion of 4-10 such young blastemas is added to it for the transplantation active growth of the transplants occurs, and appropriately, an extremity or tail-like organ is formed from them. Hence, two conclusions follow: 1) young regeneration blastemas are not "nullipotent," but rather possess their own organizing potentialities which are not expressed because of the stoppage of growth of the young blastema when it is separated from the old tissues of the regenerating organ, and 2) up to a certain stage the blastema does not grow by means of cell multiplication (mitotic or amitotic) but rather by means of the influx of cells to it from the wounded tissues, for the most part without proliferation.

2. At a certain stage of ontogeny extremities of tadpoles lose the power of regeneration, and after amputation of the organ the wound surface heals smoothly. The tissues of the extremity thereby differentiate progressively and are very little destroyed after the amputation. In them quite a number of mitoses are observed. If the tissues of an amputation wound surface of an extremity are injured severely mechanically or chemically they are subject to considerable lysis and deep-seated dedifferentiation. Thereby, the number of mitoses increases little. The cells lose their tissue connections and create a blastema, which grows and forms a typical regenerating extremity. Therefore, loss of the power of regeneration of extremities in anurans has as its immediate cause the loss of the power of the tissues of the organ to be lysed and dedifferentiated, thereby liberating cell material without proliferation, rather than a loss of the power of multiplication by the cells (mitotic or amitotic). Therefore, for the purpose of restoration of the lost power of regeneration of an organ it is necessary to produce destruction and dedifferentiation of tissues in the wound area rather than proliferation.

3. A count of the number of mitoses showed that the mitotic index in the subepithelial area of the blastema changes regularly at successive stages of regeneration of the extremities in tadpoles, young and adult axolotls and tritons: the initial stages of formation of the blastema occur practically without mitosis or amitosis; at later stages, the number of mitoses increases sharply; still later, this number is reduced. This means that initially regeneration proceeds without cell multiplication, which can begin only when the primordium reaches a certain size, after a certain minimum number of cells has been produced in it. Then, regeneration proceeds basically by means of mitotic multiplication. Later, growth proceeds by means of an increase in the metaplastic, acellular masses of organic matter.

The theory of the biphasic course of regeneration, which has

been proposed against the current idea that regeneration is a simple growth, accomplished by means of cell multiplication stimulated by "wound hormones," mitogenetic rays and other factors found no sympathy among investigators. For a long time it was simply overlooked, and then, "after being noticed," it was stated that it was incorrect, that at the initial stages of formation of the blastema cell division does occur but it proceeds so quickly that it is impossible to see it with ordinary fixation of the material. Then, this theory began to be accepted, but some authors (Luscher, 1946; Forsyth, 1946 and others) considered it their own discovery, although their observations were made 12 years after its formulation and even though they were very incomplete; other authors declared this theory to be obvious and a long-known fact. Vorontsova (1949), who knows the literature and the entire history of the subject excellently, in his book for some reason ascribes this theory to an American investigator, Litwiller (1939) and to Basina (1940); doubting the occurrence of the first phase of formation of the regenerate without mitoses, through the destruction and dedifferentiation of tissues, she writes that on the basis of the existing data "it is impossible to gain a complete idea of the sources of growth of the regenerate. Since the duration of the various mitoses remains unknown, it is difficult to take into consideration the role of the latter in increasing the mass of the regenerate." "We believe it is still premature to distinguish phases of primary and secondary accumulation in the formation of the regenerate." These doubts, however, do not prevent her later from accepting what she had just denied; she writes: "Strictly speaking, only the sharp separation of the various periods of formation of the regenerate is objectionable. Undoubtedly, dedifferentiation of cells which have entered the blastema occurs basically at the beginning of the regeneration process, and the most active proliferation occurs at the late stages of development of the regenerate." It is characteristic that Vorontsova like other authors, touching on the question of the biphasic nature of regeneration, continues to "overlook" three groups of proofs presented and discusses and criticizes only one of them -- that of counting the number of mitoses at the successive stages of regeneration. However, whatever critics say or write, they will be forced to reckon with the existing proofs and the theory which has been formulated, because it more correctly depicts the course of the regeneration process than previous theory, and because on the basis of it alone it is possible to understand the course of this process and to seek out effective methods of controlling it.

According to the old concepts, regeneration is the secondary growth of an organ based on cell multiplication. Therefore, in order to produce regeneration of a non-regenerating organ, it is necessary

to stimulate the process of cell multiplication in injured tissues. According to the theory of the biphasic course of regeneration, regeneration is not at all a simple growth but rather is a developmental process with qualitatively different stages in it; in order to produce regeneration where it is not occurring it is necessary to stimulate destruction and dedifferentiation rather than proliferation. In other words, the approach to the study of regeneration and its control should be different in principle from what it has been previously. So far this new approach has justified itself, because it has made it possible to work out methods for restoring the lost power of regeneration of extremities in lower vertebrates (Polezhayev, 1933-1948).

The main significance of the data on the biphasic nature of the course of regeneration lies in the fact that they permit a determination of the stage nature of this process. Thereby, by stages we do not mean morphologically but rather physiologically different stages of regeneration on the basis of which these morphologic changes occur and which, in turn, represent an expression of some biological stages in Academician T. D. Lysenko's sense, that is, stages arising during the course of evolution and ontogenesis as an adaptation to changing environmental conditions. During the course of regeneration we can determine two successive, qualitatively different physiological stages:

1) the stage of destruction and dedifferentiation of injured tissues of the remnant of an organ and 2) the stage of growth and differentiation of the regenerate.

The first stage is characterized by a certain type of metabolism: by the predominance of breakdown of tissues proteins over synthesis of them, by the predominance of anaerobic respiration (glycolysis) over aerobic respiration, by a shift of the active reaction of the medium to the acid side and others. The second stage is characterized by the predominance of protein synthesis over analysis, by the predominance of aerobic over anaerobic respiration, by a return of the active reaction of the medium to normal, and others. On the basis of the first stage epithelialization of the wound and the formation of the blastema (granulation tissue) occur by means of the primary accumulation of cells, that is, basically without cell multiplication. On the basis of the second stage growth and differentiation of the blastema occur. These two physiological stages can be determined not only in the regeneration organs but also in tissue regeneration, in wound healing (see Leytes, 1945). Therefore, these stages characterize all the processes of regeneration.

Naturally, by acting on the metabolism typical of the first or the second stage in different ways, it is possible to influence the course of the process differently. For example, artificial increase of

oxidative processes should contribute to the occurrence of the second rather than the first stage, of proliferation rather than dedifferentiation. This is the way it actually occurs. The thyroid hormone stimulates proliferation, stimulates smooth wound healing in mammals and amphibia but shortens the stage of destruction and dedifferentiation and, by the same token, inhibits the regeneration of extremities in tadpoles.

Loss of the power of regeneration of extremities during the course of metamorphosis of anuran amphibians is the result of their switching from aqueous conditions of existence to terrestrial conditions, is the result of adaptation of the animals, whereby there is a qualitative change in their types of metabolism; this change, associated with an increase in the oxidative processes, leads to a reduction of the first stage of regeneration: after amputation of an extremity the tissues of the organ remnant are poorly destroyed and dedifferentiated; primary accumulation does not occur, no blastema forms, and the organ does not regenerate.

Artificial intervention (injury, chemical treatment and others) produces a type of metabolism in the organ characteristic of the first stage of regeneration, brings about destruction and dedifferentiation of tissues and, hence, the formation of a blastema and regeneration of the organ.

Therefore, phylogenetic and ontogenetic loss of the power of regeneration by organs in animals, according to our ideas, is the result of their adaptation to new existential conditions, is a change in the type of metabolism in an individual by virtue of which there is a reduction of the first stage of regeneration. This is a more advantageous adaptation for the animals than previous ones; this explains why in a number of cases the power of regeneration of organs disappears during the course of evolution and is replaced by the power of more rapid, smooth wound healing.

The stage nature in regenerative processes is an expression or recapitulation of some phylogenetic processes, according to the so-called biogenetic law. An analysis of the type of metabolism characteristic of two stages analyzed confirms this conclusion: characteristic of the older forms of animals is greater lability, destruction of proteins; glycolysis rather than aerobic respiration is characteristic. Therefore, it may be said that in those cases where the organs regenerate the conditions exist for adequately complete recapitulation of the first stage of regeneration; where organs do not regenerate these conditions are absent. Hence, it is clear that if we find and create these conditions it is possible to produce organ regeneration in places where they usually do not

regenerate.

IV

In the previous section of the article we noted the insolvency of the criticism directed against the theory of the biphasic nature of the course of regeneration. The viciousness of this criticism amounts basically to the fact that it attempts to drag the investigation back and, by the same token, drives it out of its way and interferes with its progress. However, by way of "undermining ourselves," we can, on the other hand, criticize this theory, showing in it contradictory features which it has not resolved. This type of criticism, by destroying the theory, at the same time will develop it further, that is, it should contribute to progress of research on regeneration.

The two following arguments can be advanced against the theory of the biphasic course of regeneration: 1) it is not always possible to reduce the phase of primary accumulation to an influx of cells into the blastema as a result of tissue dedifferentiation of the organ remnant; 2) it is possible to obtain regeneration of an entire organ, thereby practically excluding processes of cell multiplication.

After the amputation of extremities in young tadpoles signs of destruction and dedifferentiation of the tissues of the organ remnant are exceedingly severe, they include a very large distal portion of the organ, whereby the muscle anlagen and the connective tissue are converted entirely into a multitude of isolated mesenchyme-like cells, while the cartilaginous skeleton is converted into prochondral tissue and sometimes also into a mesenchyme-like mass of cells. From this dedifferentiated mass of tissues and cells the regeneration blastema arises. Thereby, a marked shortening of the remnant of the amputated organ is characteristic, which in all probability if related to greater consumption of its cell material, which goes into formation of the blastema. At this time there are practically no mitoses either in the blastema or in the organ remnant.

In axolotls and tritons signs of destruction and dedifferentiation of tissues of the amputated extremity are quite well expressed but they include a smaller tissue thickness. Therefore, the influx of cells from the organ remnant into the blastema in them is more limited than in tadpoles. Basina (1940), studying the mitotic index in regeneration of the triton extremity, established the fact that up to the age of six days there are no mitoses either in the blastema or in the adjacent tissues of the organ remnant; in the blastema (without epithelium) there are, on the average, 180,000 cells; in a transverse section of the adjacent tissues of the extremity an average of 1,500 cells is counted. Therefore, in the blastema an unusually rapid,

active multiplication of cells occurs and, thereby, without mitosis. From where are these cells taken? In Basina's opinion, from amitotic division and the movement of cells from the proximal portion of the organ rather than from the area of the old tissues adjacent to the blastema, because for this purpose the cells in a 1.2-millimeter thickness of tissues would be required.

We can not agree withthis opinion: no amitotic cell division is observed in regeneration of amphibian extremities; it is a rarity; in addition, if the blastema arose from this cell division, it, the young blastema, would be able to grow even after its isolation and transplantation, but this does not occur; further, it is perfectly incredible that in the proximal portion of the organ remnant, where the tissues are not dedifferentiated but are structured, the cells could be liberated from their tissue connections without being noted by the investigator's eye, drive through the thickness of tissues of the distal portion of the organ remnant and form a blastema. At the same time, it is well known that formation of the blastema is associated specifically with the distal portion of the organ remnant. The fact that consumption of a considerable tissue thickness of the distal portion of the organ remnant, equal to 1.2 millimeters as calculated by Basina, is required for the formation of the blastema should not disturb us -- it occurs in this way: in regeneration a certain shortening of the organ remnant is always observed. Nevertheless, there are cases, for example, in the transplantation of the thin (1-2-millimeter in thickness) extremity disks to the back of an axolotl where regeneration has the transplant as its source, and the number of cells in it is less than in the blastema, particularly keeping in mind the considerable absorption of the transplant tissues after grafting. The question arises, from where do the blastema cells come in this case?

Here, the idea is possible that the cells are formed anew in the blastema from organic matter occurring from destruction of the transplant tissues. Checking on this assumption requires careful cytological and histological study of the successive elements of the initial stage of regeneration and the performance of special experiments.

This idea appears to me to be probable to the highest degree in connection with the brilliant work of O. B. Lepeshinskaya (1950), who showed the possibility of new formation of cells after their complete destruction in the hydra and in the healing of skin wounds in mice. The cells thereby are not formed from cells but rather, in spite of Virchow's dogma "all cells only from a cell," they are formed from the organic matter of an acellular structure. "Organic

matter is a mass of protoplasm which does not have the form of a cell, which contains in itself nuclear substance in one form or another but does not have the form of a nucleus, and which is in the protoplasm in a dispersed or diffuse state; organic matter must, of necessity, possess the power of the type of metabolism which is an essential condition for their existence" (1950, page 77). "We must recognize the existence of living molecules. . . undoubtedly, those molecules should be considered living which possess the power of metabolism which leads not only to their preservation, rather than death, but also leads to their multiplication through a transitional state of growth" (1950, page 79).

O. B. Lepeshinskaya's discovery permits biology to take a colossal step forward. The author has formulated a new theory of the structure of living matter, a new cell theory, according to which the ultimate element of the living is not the cell but rather the living matter of acellular structure, from which at a certain stage in its development cells arise when the appropriate conditions are present. Undoubtedly, there is a great future for this very great discovery, which has revolutionized all of biology, which has made necessary a reorganization of cytology, histology, embryology, physiology, experimental morphology, pathology, microbiology (including virology) and other disciplines. Undoubtedly, it will also bring about a revision and a great advance in the study of regeneration.

O. B. Lepeshinskaya's principles of living matter is the standpoint of michurinist biology, which states that in the body not only the cell but also every droplet, every molecule is capable of metabolism, is alive and possesses the property of heredity. Therefore, her theory opens up new specific routes and approaches to investigation on the basis of the principles of Michurin's doctrine for a number of divisions of biology, including the study of regeneration.

At the present time, we can present the following data from our observations on the course of regeneration of extremities in amphibia.

During the first few days after amputation of an organ a large quantity of organic matter accumulates in the area of the wound containing small granules of broken down cell nuclei, the so-called detritus, which comes from local tissues and blood cells. This matter or these breakdown products participate actively in metabolic processes, that is, they are alive rather than dead. Further, a large number of large granules, which stain with nuclear stains but which do not have the shape of typical nuclei, appears in this substance. Shortly after, in place of them a progressively increasing number of "regeneration" cells like the

embryonic mesenchyme of blastema cells appears. These cells, the origin of which has been discussed for a number of decades and has not been finally decided in one way or another, are homogeneous and initially are not directly connected to the old tissues of the organ remnant. It is interesting that initially, according to the observations of N. F. Barakina in our laboratory, they are abnormally small, and a multitude of small growing cells is encountered along with the large cells. (Data on the origin of cells in regeneration will be published in the special works of our laboratory). These facts afford the basis for the supposition that at least the beginning of the phase of primary accumulation is brought about by the new formation of blastema cells from living matter. This supposition gives us the clue for explaining a number of phenomena observed in regeneration: the absence of cell multiplication in the phase of primary accumulation, the embryonic type of blastema cells, their morphologic homogeneity, different sizes and others.

The phenomenon of new formation of cells in regeneration does not negate the significance of the phenomena of destruction and dedifferentiation of tissues in the organ remnant or their necessity for regeneration. Part of the blastema cells undoubtedly arises from the dedifferentiation of old tissues, but another part of them is newly formed. On the other hand, destruction and dedifferentiation of tissues constitute an essential condition for the new formation of cells, because thereby living matter is formed, and conditions are created for the new formation of cells. The process of new cell formation in regeneration proceeds on the basis of the stage of destruction and dedifferentiation; the conditions for this new formation are the same conditions which occur at this stage in the process of recapitulation: increased proteolysis, glycolysis, acidification of the medium and others. Living matter arises through the breakdown of protoplasm and of the nuclei of local tissues and blood cells.

Let us return to the position noted at the beginning of this section that in some cases regeneration of an entire organ is observed without mitotic division of cells. From previous studies (Guyenot, 1927) it is known that in amphibians the extremities regenerate by means of the formation of a blastema; the tails, by means of the outgrowth of old tissues. In other words, this means, according to the theory of the biphasic nature of regeneration, that the processes of cell multiplication play a greater part in regeneration of the tail than in regeneration of the extremities in amphibians. We performed the following experiment. In young grass frog tadpoles the hind extremities and the tails were amputated simultaneously, and the animals were treated with colchicine solution, which stopped mitosis

in metaphase. This treatment, therefore, must have stopped the process of mitotic cell division during the course of regeneration (Polezhayev, 1945-1948; Polezhayev and Gurvich, 1948). It might be expected that despite the treatment the phase of the primary accumulation would occur. However, the result surpassed our expectations. It was shown that although the phase of primary accumulation occurred in both cases the tails could not regenerate further, and the extremities could not regenerate completely, even though they did regenerate in a somewhat retarded manner. Cytological study showed that in the tissues of the regenerate there was a multitude of cells division of which had been stopped in the stage of metaphase. No amitotic figures were observed. Single telophases were observed only at the very end of the regeneration process, when the extremities had already been formed.

How did regeneration of extremities occur in the case described if the mitotic division process was completely arrested, while the number of cells in the regenerate increased continuously? There could have been two sources here: 1) the cells were liberated from their tissues connections because of tissue dedifferentiation, and 2) the cells were newly formed. The first source alone could hardly provide for the entire process of regeneration of the extremities, since the total living mass and the total number of cells in the organ increased considerably during regeneration. Therefore, the second source is very probable. Further experiments should clarify the mode of regeneration in these cases.

V

The question arises, do we change anything if we adopt the position of O. B. Lepeshinskaya concerning the new formation of cells in regeneration? Yes, very much is changed. The approach to the phenomena of regeneration and methods of controlling it are changed. While previously attempts were made to stimulate or produce regeneration by methods contributing to cell multiplication, the theory of the biphasic nature of regeneration required a different approach: in order to produce or stimulate regeneration it is necessary to cause destruction and dedifferentiation of tissues of the organ remnant rather than proliferation. Now, assuming the possibility of new formation of cells, we proceed even further. Now, we can say that in order to produce or stimulate regeneration it is necessary to create conditions essential for new cell formation. The phenomena of destruction and dedifferentiation are included in these conditions, but these are not all. Specifically, the blood plays

a part, the significance of which for regeneration has been very well shown by O. B. Lepeshinskaya. By intentionally creating all these conditions it is possible to count on producing regeneration where it does not occur.

Therefore, changes in ideas on the mode of regeneration absolutely change the approach to the study of it and the methods of controlling this process. However, this does not exhaust the subject. O. B. Lepeshinskaya's theory of new cell formation and the role of living matter in the development of organisms makes it possible, or more accurately, makes it necessary to create new ideas concerning the method or nature of development of a number of other biological processes and, therefore, opens up new lines and prospects for studying them. We should like to present two examples: elsewhere (Polezhayev, 1950), the idea has already been developed that based on th michurinist doctrine of heredity, of the predominant significance of physiology and metabolism in the development of organisms, the recovery not only of lost organs (or tissues) but also of the functions of the body should be classified among regeneration phenomena. In this latter case, the tissues and cells of the organ may not be lost but living matter and metabolism in them is, of necessity, altered. Therefore, with the idea that regeneration of organs and functions are primarily and necessarily associated with the conditions under which living matter arises it becomes clear that for the purpose of restoring lost functions it is necessary to create conditions for the formation of living matter and specifically to alter the metabolism of the organ with the impaired function. Thereby, we can apply this to any organ, internal or external, or any function of it. Specifically, it is possible to restore or intensify the protective function of the living organism, its defense forces in the control of infectious diseases. Along this line in recent years we have attained certain positive results, and the problem is being worked out further.

Another example. O. B. Lepeshinskaya's theory of the role of living matter in the origin of cells and in the development of the organism uncovers the broadest prospects for working out the problem of malignant tumors and methods of controlling them. There are a number of factors in common in the development of malignant tumors and the phenomena of regeneration, although these processes are qualitatively different. They have the following in common: both processes begin on the basis of the stage of destruction and dedifferentiation with its specific type of metabolism (increased proteolysis, glycolysis and others); in both cases de-differentiation and embryonization of the cells occurs; in both cases

the changes undoubtedly begin with much earlier changes than those visible under the microscope, changes invisible under an ordinary microscope, changes in the physiology of metabolism and the structure of living matter. The attention of investigators should be drawn specifically to these very important changes in the organism primarily. The occurrence of malignant cells is a secondary phenomenon. It must be supposed that malignant cells, like "regeneration" cells are newly formed from living matter, but under particular conditions. It is possible that this specifically explains the fact that in rapidly growing tumors there are very few mitoses and that a number of very small cells are encountered there. Among the essential differences between the phenomena of malignant neoplasia and regeneration we should like to note chiefly the following: in the case of regeneration, following the stage of destruction and dedifferentiation the stage of growth and differentiation occurs, of necessity, with the type of metabolism characteristic of us (increased protein synthesis, aerobic respiration and others), while in the development of malignant tumors this stage is absent and is replaced by the process of tumor necrosis. Therefore, in order to eliminate the development of a malignant tumor it is necessary to inflict the main blow against the first stage, primarily eliminating the conditions for the formation of living matter as a source of the neoplastic malignant change. Naturally, the characteristic features of the problem are not at all exhausted by the few statements made here. For us it was important only to show how one might approach the problem of malignant tumors, based on the ideas of the nature of regeneration phenomena and the role of living matter in it.

From what has been stated in the present article it follows that the problem of the mode of formation (the nature of development) of the regeneration primordium is of great importance for working out effective methods which would make it possible to control the regeneration process of organs and functions, intensify or reduce them, suppress a process which is occurring or produce a process where it is not occurring. This problem is not only of theoretical interest but is also of practical significance for the socialist national economy and public health. This problem can be understood only on the basis of revealing the bitter battle between idealism and materialism which has occurred during the entire time of development of the study of regeneration, on the basis of elimination of reactionary idealistic and metaphysical concepts of Weismann and Virchow and on the basis of development of the progressive materialistic principles of the I. V. Michurin-T. D. Lysenko teaching and the new cell theory of O. B. Lepeshinskaya.

Bibliography

1. Basina Yu. A. 1940. Study of Regeneration by the Method of Calculating the Mitotic Index. Byull. Eksperim. Biol. i Med. (Bulletin of Experimental Biology and Medicine), 10.
2. Weismann A. 1918. Lectures on the Theory of Evolution. A Translation from the Third German Edition. Petrograd.
3. Vorontsova M. A. 1949. Regeneratsiya Organov u Zhivotnykh. (Regeneration of Organs in Animals). "Sov. Nauka" Publishing House, Moscow.
4. Gurvich A. G. 1944. Teoriya Biologicheskogo Polya (Theory of the Biological Field). "Sov. Nauka" Publishing House, Moscow.
5. Driesch H. 1915. Vitalism, Its History and System. Translation by A. G. Gurvich.
6. Leytes S. M. 1945. Developmental Dynamics of the Regeneration Process. Khirurgiya (Surgery), 11.
7. Lepeshinskaya O. B. 1950. Proiskhozdeniye Kletok iz Zhivogo Veshchestva i Rol' Zhivogo Veshchestva v Organizme (The Origin of Cells from Living Matter and the Role of Living Matter in the Organism). Publishing House of the Academy of Medical Sciences USSR, Moscow.
8. Lysenko T. D. 1948. Agrobiologiya (Agrobiology), Third Edition, Sel'khozgiz.
9. Michurin I. V. 1948. Sobr. Soch. (Collection of Works). Sel'khozgiz.
10. Nasonov N. V. 1941. Dobavochnyye Obrazovaniya, Razvivayushchiyesya pri Vlozhenii Khryashcha pod Kozhu Vzroslykh Khvostatykh Amfibiy (Accessory Structures Developing from Putting Cartilage under the Skin of Adult Urodel Amphibians). Publishing House of the Academy of Sciences USSR.
11. Polezhayev L. V. 1945. Osnovy Mekhaniki Razvitiya Pozvonochnykh (Fundamentals of Developmental Mechanics of the Vertebrates). Publishing House of the Academy of Sciences USSR. -- 1948. Loss and Renewal of the Power of Regeneration of Extremities in Anurans. Tr. In-ta Tsitol., Gistol. i Embriol. AN SSSR (Works of the Institute of Cytology, Histology and Embryology of the Academy of Sciences USSR), Vol II, No 2. -- 1950. Some Principles in the Study of Regeneration. Zhurn. Obshch. Biol. (Journal of General Biology), 11, 4.

12. Polezhayev L. V. and Gurvich A. G. 1948. The Effect of Colchicine on the Regeneration of Organs in Anuran Amphibians. DAN SSSR (Reports of the Academy of Sciences USSR), LIX, 5.
13. Timiryazev K. A. 1948. Sobr. Soch. Sel'khozgiz.
14. Child C. M. 1948. The Role of Organizers in Developmental Processes. Foreign Literature Publishing House, Moscow.
15. Forsyth J. W. 1946. The Histology of Anuran Limb Regeneration. J. Morph., 79, 2.
16. Fritsch G. 1911. Experimentelle Studien über Regenerationsvorgange des Gliedmassenskeletts der Amphibien. Zool. Jb., Physiol., 30.
17. Guyenot E. 1927. Le Problème Morphogénétique dans la Régéneration des Urodèles; Determination et Potentialités des Régénerats. Rev. Suisse Zool., 34.
18. Litwiller R. 1939. Mitotic Index and Size in Regenerating Amphibian Limbs. J. Exp. Zool., 80.
19. Luscher M. 1946. Die Wirkung des Colchicins auf die an der Regeneration beteiligten Gewebe im Schwanz der Xenopus-Larve. Rev. suisse de Zool., 53, 31.
20. Spemann H. 1936. Experimentelle Beitrage zu einer Theorie der Entwicklung. Berlin.
21. Tornier G. 1906. Der Kampf der Gewebe im Regenerat bei Begunstigung der Hautregeneration. Roux' Arch., 22.
22. Virchow R. 1859. Die Zellular-Pathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre.
23. Waldever W. 1906. Handbuch der vergleich-experimentellen Entwicklungslehre der Wirbeltiere. Bd. I, T. 1.
24. Weiss P. 1926. Morphodynamik Abh. z. theoretischen Biologie, herausgegeben von Prof. J. Schaxel. Berlin.

END

1288
CSO: 7297-N