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BASIC TRENDS IN THE RESEARCH OF THE LABORATORY OF ELECTRON MICROSCOPY OF THE DEPARTMENT OF BIOLOGICAL SCIENCES, ACADEMY OF SCIENCES USSR

- USSR -

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At the end of 1946, by decision of the Presidium of the Acad. Sci. USSR, an Office of Electron Microscopy was established at the Department of Biological Sciences. It was created as an inter-institutional organ called upon to work in contact with the biological institutes and laboratories of the AS USSR which are interested in the use of the method of electron microscopy.

It must be noted that this office, shortly afterward reorganized into a Laboratory of Electron Microscopy at the Department of Biological Sciences AS USSR, became in our country one of the pioneers in the field of the use of the electron-microscopic method for the development of biological problems. The Laboratory of Electron Microscopy became a sort of school for the groups of biologists emerging from the scientific institutions of the Soviet Union and striving to use electron microscopy in their research. Within a short time following the organization of this Laboratory, various specialists in the field of biology began to visit it, familiarize themselves with the studies, and learn the experience of its work, especially the methods of preparation of biological specimens for electron microscopy worked out by the Laboratory.

The Laboratory rendered extensive consultative aid on the organization of works with an electron microscope to the affiliates of the AS USSR, to the academies of sciences of the allied republics, to the Moscow and Leningrad institutes of the Acad. Med. Sci. USSR, and to other scientific research institutions of the country.

At the present time the Laboratory of Electron Microscopy, in the extent and volume of its studies, in the results of the method of application of electron microscopy, and in

the character of scientific systematic aid rendered to other scientific research institutions, constitutes a leading scientific institution in the field of the use of electron microscopy for biological studies.

One of the examples which illustrate this statement may serve to point out that about 50 percent of the experimental works on electron microscopy in the field of biology published in the USSR was issued from the Laboratory of Electron Microscopy.

The Laboratory of Electron Microscopy submitted six reports at the international congresses on electron microscopy in Stockholm and Tokyo. These reports were published in the Works of these congresses, though not all submitted reports were selected for publication.

At the very start of the research work of the Laboratory the defects of the existing methods of purification of biological preparations for electron microscopy became obvious. As is known, the purification of biological objects, particularly, microbiological and virological ones from admixtures (the components of the culture medium and products of cellular metabolism and disintegration) is an essential condition of electron-microscopic studies, since, upon desiccation of the preparations, these admixtures may disguise the object itself.

The Laboratory faced the task of the development of new methods which would permit the purification of the object without impairing it.

Such methods have now been developed. The principle of one of them consists of the use of a supporting colloidal film as a dialyzing membrane. This method, designated as the method of drip dialysis, not only insures the purification of biological preparations but is, in addition, of great help in the electronic optical study of various stages of the individual development of cells. It facilitates the electron-microscopic studies of the processes of changes in cells under the effect of physical, chemical, or biological agents, and it permits the follow-up of the dynamics of the influence of various factors on the biological objects.

S. B. Stefanov developed a method of diffusion-purification of virus preparations, according to which the purification takes place under conditions of complete physical rest of the particles with a gradual substitution of the initial medium with distilled water. In contrast to the drip dialysis, diffusion-purification also permits the removal of large molecules, since there is no semipervious membrane between the purified and the purifying liquids.

However, the diffusion-purification, as well as all other currently known methods, possess the same basic

defect -- that the object is transferred from the natural medium to the medium of distilled water unfamiliar to it. Changes in the form and structure of the viral particles are possible under these circumstances, but it is impossible to observe them directly. In order to avoid these changes, S.B. Stefanov suggested a method of a non-rinsing preparation which permits the desiccation of viral particles on the carrier-film directly from the original medium.

Of substantial importance to electron-microscopic histology is the method developed by V. P. Gilev (1957) of employing gelatine as the embedding material for the preparation of ultra-fine microscopic sections of biological objects. This method, in contrast to methacrylic embedding, enables one to preserve separate details of the tissue in the least altered form, since it eliminates the use of organic solvents. In particular, the tissues are not subjected to such considerable shrinking, as in the use of strong alcohols, while the fat - and lipoid-containing tissue structures are well preserved.

On the ultramicrotomes it is possible to obtain gelatine slices of 200A thickness. They possess a higher resistance to the electronic beam than the methacrylic ones.

The method of gelatine embedding of V.P. Gilev (1957) was reported at the Conference on Electron Microscopy in Stockholm and created great interest.

Kh. Fernandez-Moran (Fernandez-Moran and Finean, 1957), known through his invention of an ultramicrotome with a diamond knife, had already employed the method of pouring-in gelatine in his studies. He points out that this method permits the preservation of the laminated structure of the myelin sheath of the nerve in a less altered form than when methacrylates are used as embedding material.

In passing now to a brief analysis of the basic trends of the work of the Electron Microscopy Laboratory, it is necessary to note that its problems include the most important problems of histology, crytology, virology and bacteriophagy, the solution of which is impossible without the use of methods of electron-microscopic and electronographic studies.

One of the sections of histological research in our Laboratory is the study of the ultrastructure of muscular tissue.

Foreign researchers, occupied with the problem of muscle fiver contraction, concentrate their entire attention only on the changes in the myofibrillary structure during its process of contraction. However, in the studies of V.P. Gilev (1956) there were detected regular changes not only in the myofibrils, but also in the protoplasm of the muscle fiver - - the sarcoplasma. During the contraction of the muscle fiver the sarcoplasma drastically changes its structure; there

appear in it large cells resembling vacuoles (Fig. 1). It is possible that in these "vacuoles" or "cisterns" there are stored substances needed for the contraction of the muscle.

The use of the method of pouring gelatine over the tissues enabled us to obtain microscopic sections of a large area, which ensured the possibility of observing in one field of vision a large section of the muscle tissue and, in particular, all stages of myofibrillary contraction. The pictures of transition from the stage of contraction to the stage of relaxation have up to the present time, not as yet been described.

The method of gelatine embedding is very useful also in the study of the nucleus of muscle fibers. It was observed that in the nondividing nuclei there are encountered against the background of fine granularity some peculiar little clumps, rods, and loops (Fig. 2). Judging by their strong colorability with osmium, these structure are, presumably, of a lipoprotein character. These data are of interest in regard to the study of the structure of the cellular nucleus. These structures are not elicited on preparations of muscle tissue poured into metacrylates, a fact which is, undoubtedly, connected with the processing of these preparations with strong alcohols and ethers.

Also of interest are the data on the muscular tissue structure during the process of its development. In the protoplasm of muscle cells which had not as yet formed, there were detected very fine tubules extended along the cells. There are reasons to believe that these tubules participate in the formation of the initial fibrils -- the basic elements responsible for the contraction of a muscle.

Special works in the Laboratory are devoted to the study of so-called ergastoplasmatic structures to which a great importance is attached in the biochemical cellular activity. I.B. Tokin (1958) detected in the genital cells of ascarids, parallel with vesical-like and sac-like formations described in the literature, long canaliculi the diameter of which varies much; bulges in the form of ampules or tiny bags are frequently encountered. We also succeeded in demonstrating that the membranes connecting the walls of the strands of the ergastoplasmatic network are double.

Recently, interesting data were obtained in the Laboratory on the structure of the protoplasmic layer of the bacterial cell. V. I. Biryuzova detected on a large number of ultrafine bacterial slides thin canaliculi in the cytoplasm.

In Fig. 3 are shown ultrafine slides of the soil bacterium, Bac. mycoides. The canaliculi pass along the long axis of the cells and are somewhat curved. Their diameter is approximately 100 to 200A. These canaliculi are observed

in bacterial cells of various ages.

This structure of cellular protoplasm resembles the structure of the ergastoplasmatic structure of higher organisms. As is known, the structure of the ergastoplasma ensures the needed spacial arrangement of the well-regulated work of cellular enzymes, the activity of which controls the assimilation-dissimilation processes in the cell.

Future studies must show whether the functional role of the canalicular systems in the bacterial cell can be explained from this point of view. At any rate, the discovery of this system offers new perspectives for the functional-cytological study of the bacterial protoplast.

A great deal of attention is concentrated in the Laboratory on the problem of bacteriophage structure.

The structure of the bacteriophage occupies at present many foreign researchers. As is known, a bacteriophage partic' resembles, in an electron microscope, a spermatozoid with a head and tail. There is a general idea, chiefly on the basis of American works, that the head is a rounded or hexahedral formation possessing a compact membrane and filled with desoxyri bonucleic acid. From the head issues a tail with a tubular canal. There is a bulge at the end of the tail containing an enzymic system which plays a part in the interaction of the phage corpuscle with the abcterial cell. According to these concepts, the phage corpuscle, notwithstanding its small size (50 to 100 micromicrons), has a structure similar to a cellular one.

Detailed studies carried out in our Laboratory by means of various methods offer no basis for concurring with these ideas, which are largely speculative. The study of several thousand preparations of a bacteriophage showed that the spermatozoid-like figure of the phage corpuscle is formed by a spiral twisted thread, the free end of which represents the tail. This thread represents a long chain of round particles, of 15 to 25 micromicrons each.

Thus, on the example of the bacteriophage, we come across a new type of supramolecular structure of protein bodies. In contrast to actin, the structure of which is characterized by a linear type of an aggregate of globular proteins, the phage corpuscle represents a spiral-like type of an aggregate of protein macromolecules.

Upon exerting high pressures (a few thousand atmospheres) on a liquid in which a large number of phage particles are concentrated, there appear in it threads which, apparently, originate as the result of the unwinding of the spermatozoid-like particles, because we can trace transitory forms among them. These threads consist of round particles arranged linearly. A more prolonged effect of high pressures

causes breaking-up of the phage corpuscles into small (25 to 15 micromicrons) globe-like particles.

A. S. Tikhonenko (1958) demonstrated that, under the effect of such denaturating and disaggregating substances as urea and quaternary-ammonium bases, there takes place a breaking-up of the bacteriophage head into round particles of 15 to 25 micromicrons. One can observe in certain instances how these particles retain their bond with each other and form the familiar figure of a phage head (Fig. 4).

These studies are not only of interest to the problem of bacteriophgy but, also, are of undoubted importance in learning the structure of the perimolecular system of protein bodies.

Interesting data have been obtained in our Laboratory in regard to the stages of formation of viruses which affect plants. As is known, the classical object of plant viruses study is the virus of tobacco mosaic (TMV). Until recently the elementary TMV particle was considered a rod of 280 to 300 micromicrons in length and 15 micromicrones in diameter. It is generally accepted that a TMV rod has a bi-component structure; a protein sheath and an RNA stem.

It is perfectly obvious that a rod-like TMV particle of such complex structure must be formed of some smaller units, and must have its developmental history; however, there are no foreign works known to us which attempt to clarify this process.

A few years back, V. A. Smirnova (Smirnova, 1956, 1958) observed that, upon intensive reproduction of the virus in the extracts from a diseased plant at the early stage of the disease, there are present, parallel with rod-like particles, a large amount of minute round corpuscles comparable in their diameter to that of a virus particle. At the latter stages of development of the infectious process the number of round corpuscles decreases, while the number of rod-like particles increases.

It was easy to assume that these round corpuscles are the forerunners of rod-like particles. However, all attempts to elicit the transitional forms in the preparations from the extracts proved unsuccessful. This fact induced us to switch to the study of the initial stages of the virus within the cell. For this it was necessary to select a tissue with a large number of newly diseased cells. Such tissue was found in a small area of a very young leaf of a diseased tomato. In this zone, bordering on an embryonal one, an infection of young cells is caused by the virus brought in from the lower-situated affected plant tissues.

In studying the cells of this zone of the leaf, we were able to detect a great diversity in the shape of particles,

which must be considered as the early stage of virus development, since they are not encountered in the analogous areas of healthy leaves.

Ovaloid elements, chains, and curved, long particles were seen on the microscopic section of the cell (Figs. 5,6). After everything had become systematized, various shapes were formed covering every transition from a spherical corpuscle to a rod-like particle.

It is possible that the observed spherical corpuscles, from which rods originate, are not even the minutest units constituting the structure of the mature viral part. It is very likely that these elementary units lie beyond the reach of our microscope. Nevertheless, the pictures of the gradual transition of a virus from more minute forms to a completed rod which had been obtained in our Laboratory are the first visual observations of the process of formation of the rod-like particle of tobacco mosaic virus which hitherto had been considered elemental.

As far as the animal viruses are concerned, there are already many facts available at present which attest that these viruses pass, in the process of proliferation, through a series of forms somewhat distinct from the typical viral particles. However, in addition to such forms, the electron microscope reveals corpuscles which do not resemble the viral particles externally but which accompany them regularly in the cultures.

One of the outstanding examples of such particles are the filiform and spheroid bodies in the cultures of influenza and bird plague viruses now being studied in our Laboratory. There are experiments described in the literature which attest to the fact that the filiform corpuscles possesses certain viral properties.

The abundant data collected in our Laboratory by S. B. Stefanov (Draganov, 1956, 1957) permit the assertion that the filiform corpuscles are of complex structure. Not infrequently there is a longitudinal (Fig. 7) and transverse (Fig. 8) striation revealed in the filaments. One can also observe how the thread is extended directly into a chain of oval particles which have the size of elementary corpuscles of the grippe virus (Fig. 9).

Under definite conditions of processing these filaments with distilled water, there are formed in the majority of filaments bulges of characteristic shape and complex structure (see Figs. 7 and 8). This points to the nonuniform structure of the filament in its various sections.

Of great interest also are the spherical corpuscles in the cultures of influenza and bird plague viruses. The dimensions of the spherical corpuscles may exceed by many

times the dimensions of typical viral particles of spherical form. The spherical corpuscles have a complex and diverse inner structure (see Fig. 9).

The interest in the large globes increased particularly when S. B. Stefanov succeeded in detecting in the virus cultures of type D influenza large globes disintegrating into fine threads (Fig. 12) and, subsequently into minute granules (Figs. 10 and 11).

We cannot as yet judge the nature of these globes, especially the largest ones. But the mere fact, new and previously never alluded to in the literature, of the presence of giant globes in viral cultures demands close attention. In the control preparations, not infected with the grippe virus, no such formations have been demonstrated up to the present time.

In conclusion it is necessary to discuss briefly the electronographic studies which have been started in our Laboratory.

As is known, the electronographic method of investigation is still only slightly used in the study of the structure of biological objects by the method of electronic diffraction possesses, in a number of cases, advantages over roentgenographic method.

In our Laboratory Ye. M. Belavtseva (1957) obtained for the first time electronograms of beta-carotin, chlorophyl a+b, gramicidin C, and certain other objects.

It became clear in the course of these studies that the distinct rings on the diffraction images (Fig. 12) can be obtained only under conditions of a definite performance of the electron microscope and electronograph. If a certain definite threshold of intensity of the electronic beam is crossed, the distinct rings of the same objects on the electronograms change into diffuse rings (Fig. 13). It indicates a change in the objects during their exposure to the electronic beam, their transition from a crystalline into an amorphous state.

Further studies by Ye. M. Belavtseva showed that heating, caused by the action of the electronic beam and the ionizing effect of the electrons, are responsible for the disorganization of the crystalline structure of the organic substances of biologic origin during the electronographic study of these objects. There is a basis to assume that radiation effect in an electron microscope causes very strong alterations in the molecular structure of organic tissues.

This concludes the brief review of the basic trends which are being developed in the Laboratory of Electron Microscopy. We did not mention the works carried out by the

biological institutes of the Acad. Sci. USSR jointly with our Laboratory, since these studies are directly connected with the problems which are being studied by the corresponding institutes.

There is no doubt that, at the present stage of development of the method of electron microscopy, the possibilities of its use in biological science are far from exhausted, and that there is a need for the intensification of biological studies based on the utilization of this important method.

There is a definite need to coordinate the efforts of physicists and technicians in further raising the clarifying and "piercing" properties of the electronic beam, and facilitating the use of the electron microscope in its application to biological studies. A close contact between the biologists and physicists and technicians is essential, because it will contribute to the concretization of the specific requirements of the biologists in regard to the electron microscopy method and, thus, stimulate further inculcation of this method in biological sciences.

Fig. 1. Section of a contracted muscle fiber of an axolotl. Cells resembling vacuoles are seen in the sarcoplasma.

Fig. 2. The interkinetic nucleus of the striated muscle tissue of an axolotl. Clumps, rods, and loops are seen in the nucleus.

Fig. 3. Bac. Mycoides cell from a 24-hour culture on a meat-pepton agar. One can see slightly curved canaliculi (indicated with arrows), situated along the long axis of the cell.

Fig. 4. Disintegration of phage-ends of Bac, mycoides into round particles following processing with four percent trimethylphenylammonium.

Fig. 5 and 6. Microscopic sections of a young leaf of a diseased tomato. Various formation stages of a particle of tobacco mosaic virus: a -- globe-like elements; b -- chains; c -- curved elongated particles.

Fig. 7. Chorioallantois culture of influenza virus, type A_1; longitudinally striated filaments with a bulge.

Fig. 8. Chorioallantois culture of influenza virus, type A_1; one transversely striated filament with a bulge.

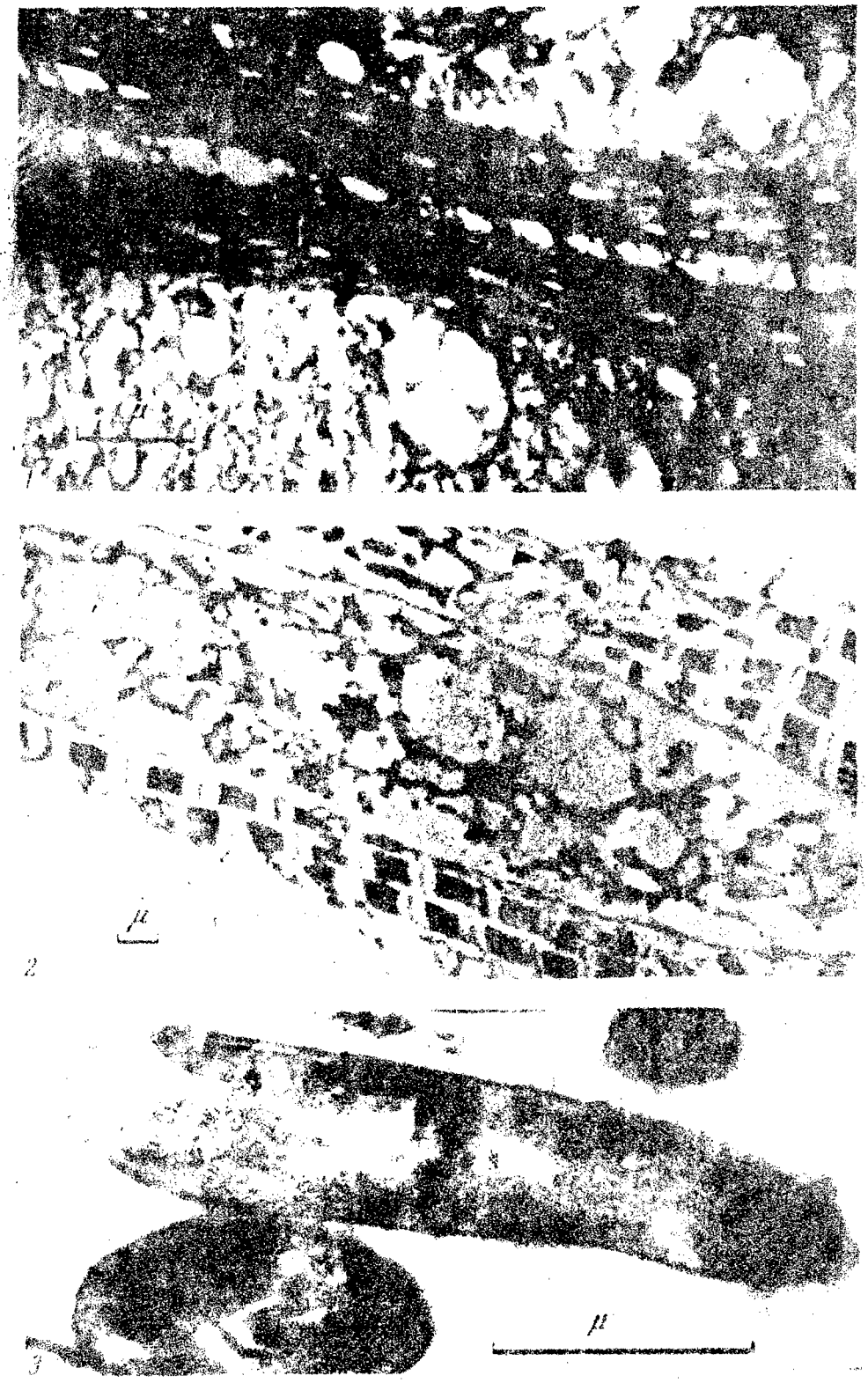
Fig. 9. Chorioallantois culture of grippe virus, type A_2; a filament extending into a chain of oval elements; also, two giant spheres are seen.

Fig. 10 -- 11. Chorioallantois culture of grippe virus, type D. Giant spheres breaking up into thin filaments and minute grains.

Fig. 12. An electronogram of beta-carotin crystals obtained at

low electron-beam intensity.

Fig. 13. An electronogram of beta-carotin which had been altered as a result of increase of intensity of the electron beam.



Figures 1-3

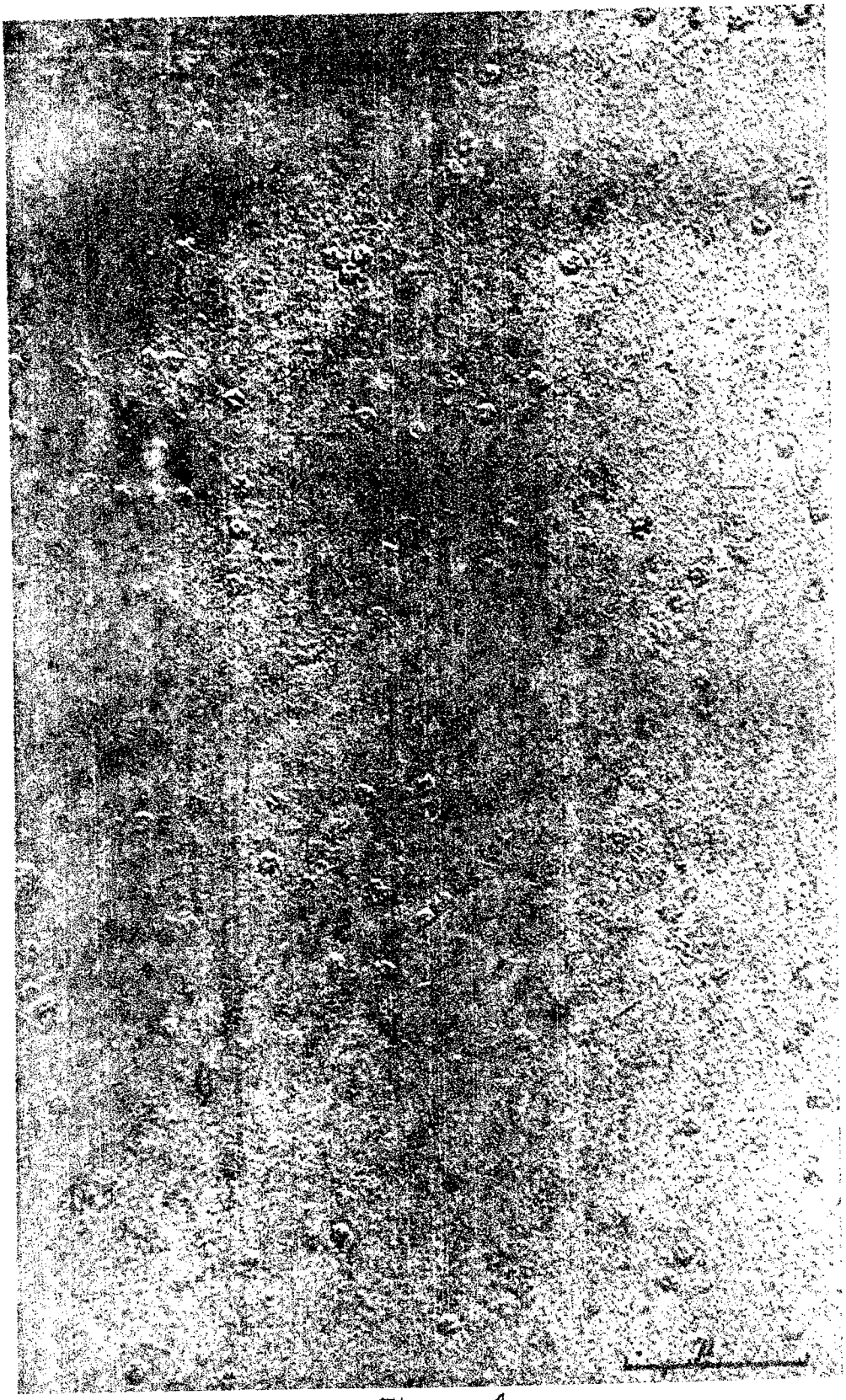
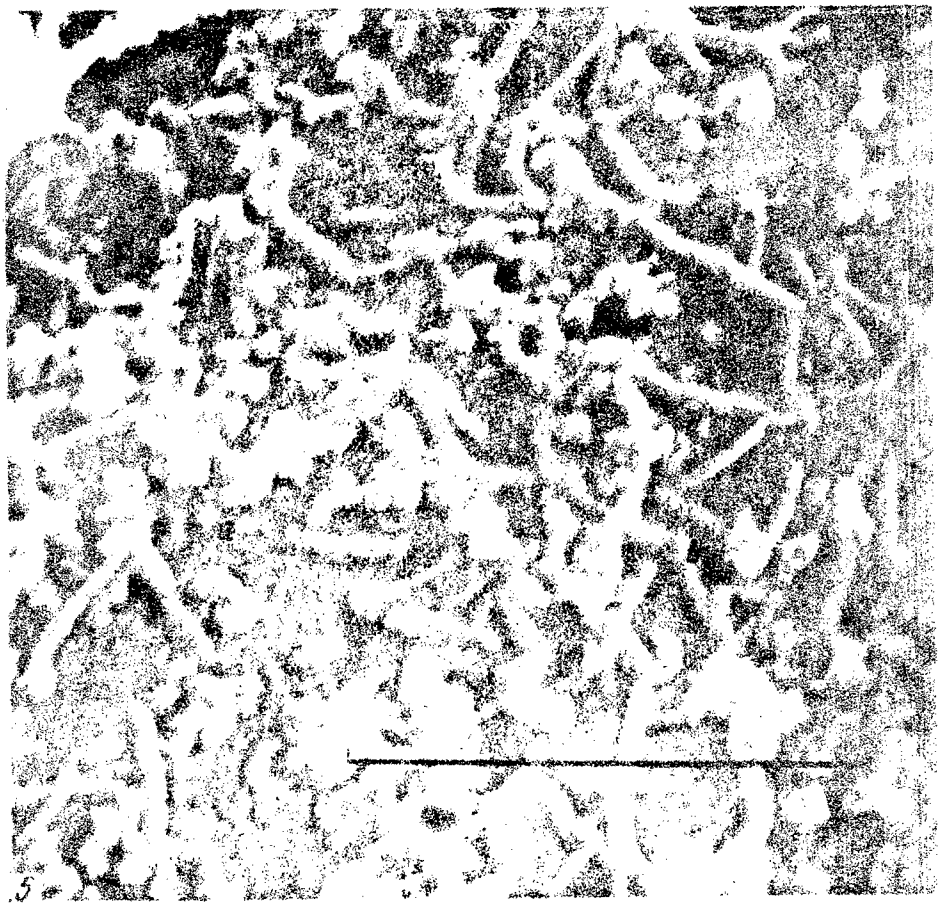
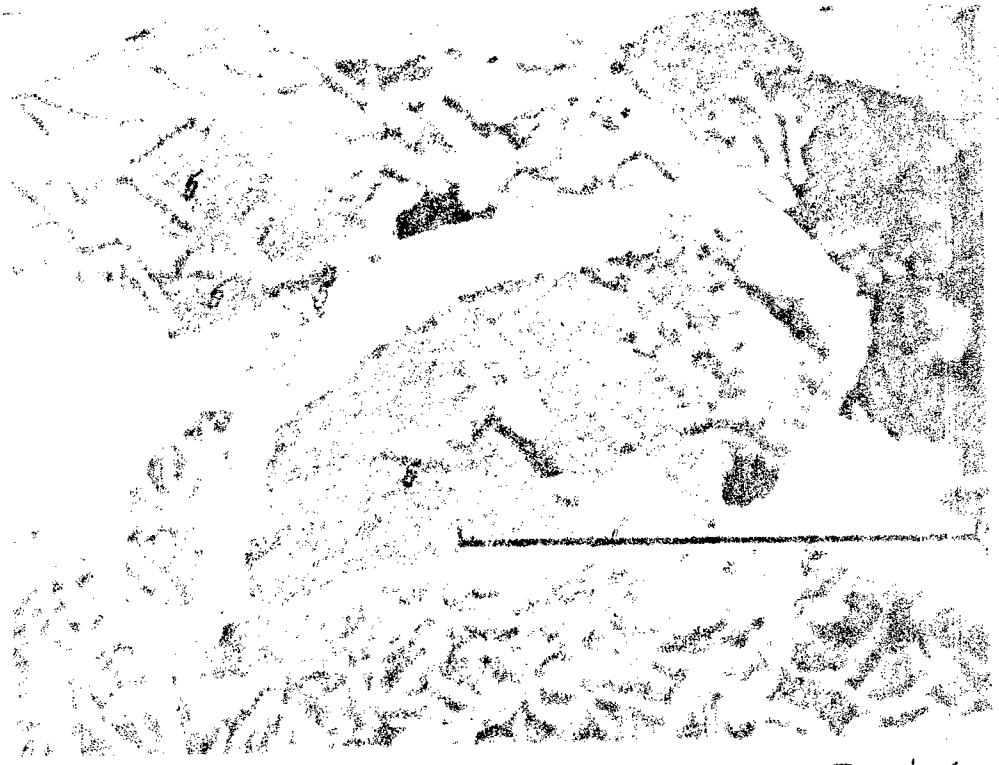
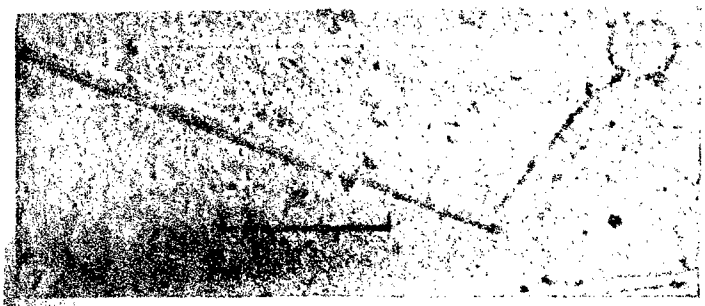


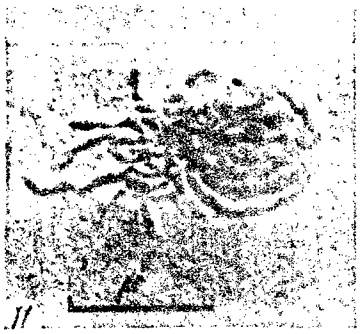
Figure 4

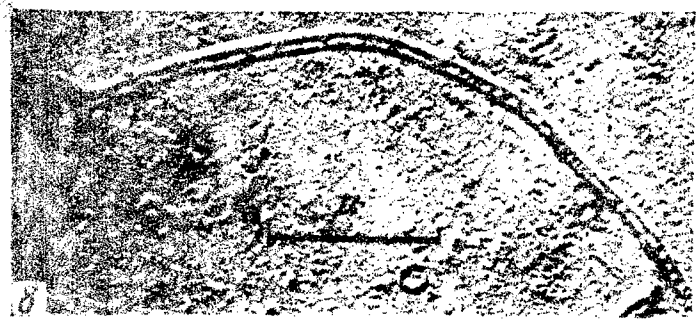


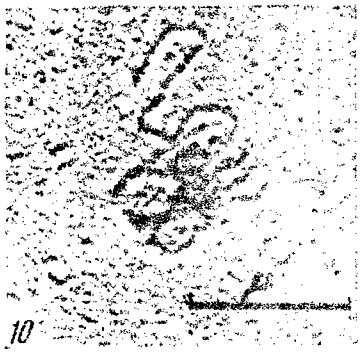


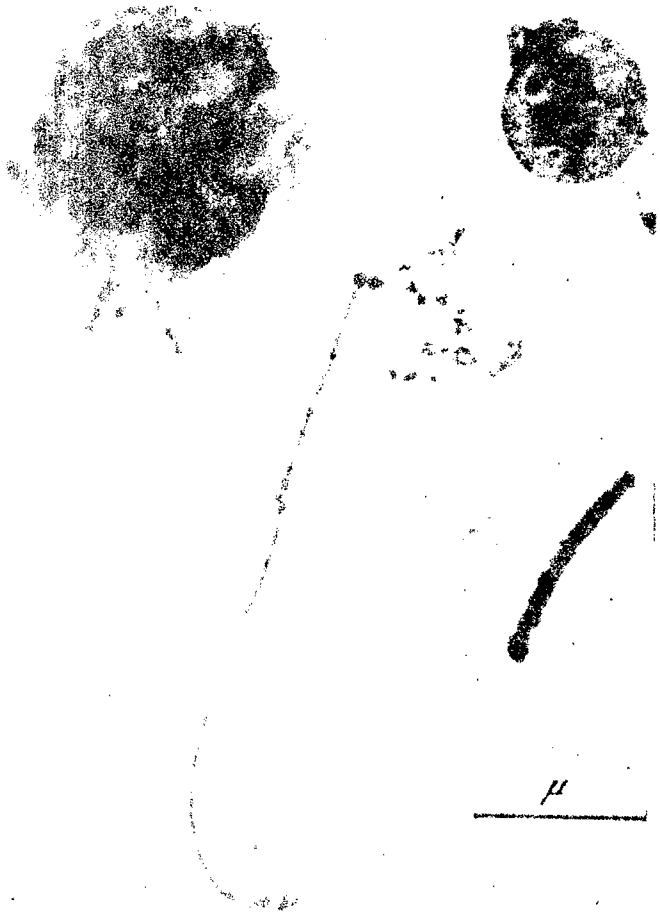
Figures 5 and 6

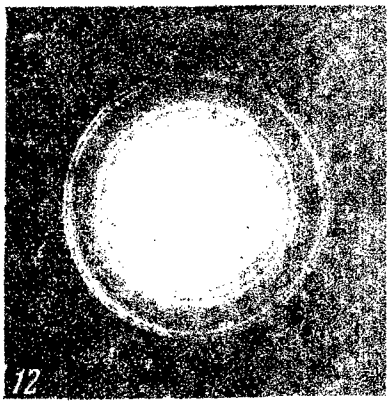


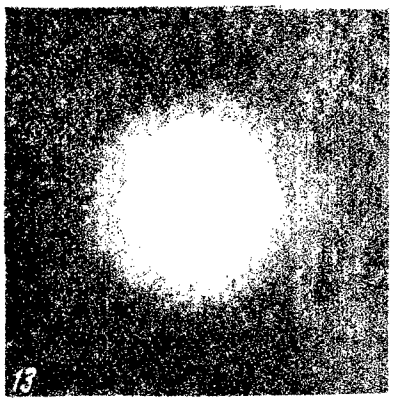












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