FAO/UNEP/USSR

International Training Cours

«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

I. A. MOROZOV

MORPHOLOGICAL METHODS IN THE STUDY OF MYCOTOXINS

Centre of International Projects, GKNT

Moscow, 1985

MORPHOLOGICAL METHODS IN THE STUDY OF MYCOTOXINS

I.A. Morozov

Morphological methods of research are capable of resolving independent tasks, nevertheless more often than not, they are applied jointly with other (functional) methods. Morpho-functional methods of research allow for a fuller, deeper and more perfect study at all levels of structural organization of intricate biological systems—cells, tissues, organs of animal and man, both normal and pathologic. These methods include light and electron (transmission and scanning) microscopy, light and electron histochemistry (qalitative and quantitative), immunohistochemical, ultraviolet, luminiscent microscopy, light and electron autoradiography, the method of X-ray-structural analysis, the method of stereological analysis of ultrastructures, etc. All these methods are widely applied to scientific studies and to practical pathological anatomy.

The more recent methods of morphological studies have made it possible to consider structural and functional disorders in their unbreakable dialectical unity, opened up the ways towards a more varied and subtle understanding of living matter. There is a trend towards an integrated application of methods mutually complementing and broadening the possibility of understanding the observed phenomena. We should first of all note the integrated application of electron microscopic studies, morphological analysis of ultrastructures, histochemistry and autoradiography which offer an idea about the three fundamental parameters of the intracellular process: its localization, nature and intensity, i.e. its dynamics. Integrated application of methods opened up a wide prospect of mate-

rialization of functions, determined the trend of modern medicine towards the understanding of normal and pathological processes as a structural-functional entity.

All methods of morphological analysis without exception are applicable to the study of mycotoxin action upon an animal organism. In this lecture, we shall not discuss specific results of morphological studies involving the action of various mycotoxins on an organism. We shall merely demonstrate the possibilities of individual methods and the necessity of their application for resolving tasks of mycotoxicology.*

Notwithstanding the fact that light microscopy is in many ways inferior to electron microscopy, it has not lost its importance for the study of the organotropic influence of mycotoxins and for the determination of their tissue specific action. The method of light microscopy is a sine qua non of morphological research in mycotoxicosis specifically when the origin of the mycotoxin is unknown.

An overall study of organs and systems in experimental mycotoxicosis makes it possible to determine target-organs which are particularly subject to the action of toxin. This includes the determination not only of organotropy but the character of pathology. True, this calls for the application of aggravated doses of the toxin (LD_{50}), which are incomparable with those received by the organism when ingesting products affected by microscopic fungi.

---

* Specific methods of processing experimental material may be found in numerous handbooks published in a number of languages.

The nature of the pathology in those instances might also be at variance. It can be identified using small doses of the toxin during the subacute course of toxicosis.

Being aware of the target-organs, which are specifically damaged by the studied mycotoxin it is possible to undertake a functional study making use of a variety of histochemical methods. Light-optical histochemistry makes it possible to identify the localization and activity of enzymatic systems of organs and tissues. The histochemical identification of enzymes is based on their specific action upon substrates resulting in an insoluble product. If the latter is colourless then additional staining is used. Most widely used in histochemistry are the methods of identification of the activity of oxidoreductases, transferases and hydrolases.

More than eighty known enzymes belong to oxidoreductases which act as catalysts for oxidation-reduction reactions. Reliable methods have been elaborated for the determination of the activity of such enzymes as lactatedehydrogenase, glucose-6-phosphatedehydrogenase, 6-phosphogluconatdehydrogenase and many others. Particularly important among the enzymes which act upon the CH-CH-group of donors is succinate dehydrogenase and several adequate methods have been elaborated for its identification. There are reliable techniques for the identification of the enzyme which act upon the $CH-NH_2$-group of donors (monoaminoxilase), oxidoreductases which act upon the C-NH-group of donors (tetrahydropholate-dehydrogenase and dihydropholatedehydrogenase), oxidoreductase which acts upon the groups of hemodonor (cytochromoxidase), and also oxidoreductases which are applied as the acceptor of hydrogen pero-

3018

xide (peroxidase and catalase).

Most frequently identified enzymes among transferases are those which carry phosphocontaining groups (various ATP-ases). Besides these, there are reliable techniques for the identification of acetyltransferases (cholineacetyltransferases) and transferases which transfer O-residues (acetyl-serotonintransferase).

Investigators engage rather frequently in the study of the activity of hydrolases, such as acid phosphotase, alkali phosphotase, acetyl-cholinesterase, β-galactosidase, enterokinase.

As a rule, the method of identification of activity and localization of a definite enzyme has several modifications the application of which depends upon the specific tasks facing the investigator.

Radiography is acquiring increasing importance in medico-biological studies. It combines the fundamentals of physical, biochemical and morphological analysis. Unlike the physical method of registration of radiactive emission autoradiography is highly sensitive since it gives the possibility of recording such amounts of radioactive substances in tissues which are not determined by dosimetry. Besides, autoradiography makes it possible to accurately localize sites with higher or smaller accumulation of radioactive compounds, regulate the intensity of radioactivity in cells and intercellular areas.

The distribution of radioactive compounds which arrive into the tissues of an organism in identifiable volumes is not random but reflects the peculiarities of their metabolism in every tissue.

This method is based on the ability of photographic material (films, photoemulsions) to record radioactive emission coming from the biological sample. This is attained by the contact of the histological section containing a radioactive substance with a highly sensitive photoemulsion. The emission energy acting upon the silver bromide of photoemulsion reduces it and following an appropriate photographic processing the metalic silver becomes visible in a microscope. The amount of reduced sections of silver bromide gives an idea about the radiation effect in cells, tissues and organs.

Hence it becomes evident that autoradiography may be used in mycotoxicology for two objectives. Firstly, using ^{3}H or ^{14}C labelled mycotoxins it is possible to reliably identify the target-organs. The dose of the toxin may be very low, practically without inducing clinical manifestations of the toxic action. It is, however necessary to use labelled mycotoxins with a high specific radioactivity. Secondly, making use of the ^{3}H-thymidine which is active in the synthesis of nuclear DNA it is possible to study the influence of mycotoxins upon the physiological renovation of tissues in the organism. Besides, making use of other precursors of the synthesis (^{3}H-uridine, ^{3}H-aminoacids, ^{3}H-glucose), it is possible to investigate the intensity of such processes as the synthesis of RNA, protein and polysaccharides and also the influence of mycotoxins upon these processes.

At the same time a reservation has to be made that we have not found in the world literature such morphological studies in which autoradiography could be applied for the above mentioned objectives. We can only hope that this precise and

modern method will find its followers among investigators engaged in the study of biological action of mycotoxins.

The interpretation of autoradiographic data calls for a quantitative consideration of the inclusion of radioactive substances into biological structures. A measure of this evaluation is the number of grains of reduced silver of the photoemulsion produced as a result of the action of β-particles of the radioactive compound. It has been experimentally determined that the number of grains of reduced silver is proportional to the number of β-particles which passed through the emulsion.

To obtain comparable results all procedures associated with the experiment, the conditions of making histopreparations and autographs should be the same. The counting of silver grains may be carried out visually or automatically. Though the latter technique is more progressive, the majority of investigators prefer the visual count of grains. This is due to the complexity of the recording systems and the need of counting small objects of differing size and shape.

In the recent 10-15 years, owing to the considerable improvement of instrument-making, electron microscopy has taken a leading place among morphological techniques. It was first believed that electron microscopy has but supplemented the range of morphological methods having joined the classical methods as a new and highly valuable method but just as the latter still being specifically descriptive. It is quite understandable and natural that in the opening years of electron microscopy investigators were carried away as if hypnotised by the extraordinary vision of most intricate intracellu-

lar architecture and its various changes under different actions. It is likewise natural that the description of these ultrastructural changes was accompanied by some or other hypothetical considerations regarding their physiological importance.

At present, the stage of "pure" electron microscopy is practically over since it is no longer satisfying those investigators who are eager not only to see some or other most subtle intracellular developments but wish to have precise rather than hypothetical ideas about their physiological meaning.

Numerous studies which were carried out alongside with biological and functional methods have helped to determine rather definitively the functional importance of various submicroscopic changes in intracellular organelles. As a rule, these changes are not specifically oriented. A specific response may be shaped by different combinations of damages done to the ultrastructure of an organelle and the intensity of manifestation of pathologies.

Changes of the nucleus are manifested in pycnosis, vacuolization or karyolization. However, such processes constitute an extreme response to a pathological action. Primary reactions of cell nuclei in case of subliminal doses of mycotoxins (or some other damaging agents) consist in the alteration of the nucleus shape which becomes more rounded, in the clarification of the karyoplasm, condensation of heterochromatin into puffs and in marginal localization of heterochromatin close to the nuclear membrane. Then comes the vacuolization of the central part of the nucleus. The DNA threads

which form heterochromatin become denser and are sharply basophilic. This process terminates on the disappearance of the nucleus so that only several small puffs of chromatin remain in its place.

The changes of the ultrastructure of the nuclear envelope are related to the pathology of a nucleus but conditionally since it represents a perinuclear sack associated with a system of the endoplasmatic network. The nuclear membrane by its responses to a pathological action belongs to this system. The two membrane leaflets which form the membrane may become more distant one from another or can get closer which leads to the alteration of the volume of the perinuclear space.

Changes in the surface of the cell can be recorded by a scanning electron microscope. However, this relates but to blood cells. Somatic cells which are incorporated as elements of tissues and organs practically do not alter their shape in case of subliminal influences. At the same time, one has to take into consideration that the cellular membrane performs an extremely important function, the transport of ions and small molecules of nutrients. Hence the necessity of studying the state of the plasmatic membrane and its responses under the action of different damaging factors, including mycotoxins. This task cannot be resolved by means of routine transmission electron microscopy. At the same time there is the method of electron microscopic tracers which helps to judge indirectly the state of permeability of the cell membrane. It has been reliably determined that any agent acting upon the cell cannot evoke profound changes in the cytoplasm without altering at the same time the cell membrane. These changes represented

by different diameter pores can be detected by introducing into intercellular (or extracellular) space such electron dense tracers as peroxidase (particle size 5-8 nM), ferritin (particle size 10-12 nM) or colloid lactan (particle size 2 nM). The penetration of these tracers into the intracellular medium gives an idea about the size of pores which have been formed as a result of the damaging action since normally these tracers do not penetrate the cytoplasm.

Many studies have been devoted to the pathological changes of the mitochondrial apparatus of cells. This is due to the fact that mitochondria have been known for a long time, they can even be observed in a light microscope and they are rather resistant to a variety of influences during the technological processing of tissues.

Pathological changes of the mitochondrial apparatus may relate to the entire population of mitochondria in a cell and also to individual changes of separate mitochondria The changes of the population of mitochondria may relate to their number (the density of the chondriom) and localization. Every type of cells is characteristic of its definite number of mitochondria. Thus the number of mitochondria in parietal cells of the stomach which secrete hydrochloric acid, in the cells of the myocardium and in the striated muscular fibers is quite great but it is very low in the lymphocytes. When analyzing the number of mitochondria one should consider the ratio of their number to the overall amount of the cellular substance rather than their absolute number in a cell. A number of physiological conditions rather than pathological influences by themselves act upon the number of mitochondria .

Thus there are 189.10^6 mitochondria in mouse liver cells; following starvation for 24 hours this number reaches 197.10^6 and following 48 hour starvation — 35.10^{10}. More durable starvation results in a decrease in the number of mitochondria in a cell (it is 210.10^6 following 72 hours). There are no doubts that toxic influences upon an organism will result in more pronounced shifts in the number of mitochondrial population.

The alteration of the localization of mitochondria in a cell takes place also in physiological conditions. Thus it was determined by means of microfilming in a phase contrast that the movement of mitochondria is not uniform and has a jerky pattern. This movement, however, is not active but a result of the overall movement of the cell cytoplasm. Toxic actions might paralyze this movement or evoke local concentration in a place which does not correspond to the place of their physiological concentration.

Pathological changes of individual mitochondria relate, in the main, to the density of their matrix, the length of the internal membrane (the forming cryst) and the volume of organelles. Changes in the volume are primarily associated with swelling, a universal response to a toxic influence. The study of live cells by means of microfilming in a phase contrast can show how mitochondria swell completely or only at one end (in the latter case they take shape of a tadpole) and then return to their initial shape.

There are two types of swelling. Anisotic swelling associated with changes in the osmotic concentration of the surrounding medium is passive and at least partially reversible. The swelling of another type is active and associated with

changes in cellular respiration which is connected with enzymatic reactions. This swelling is predetermined by primary functional disorders which produce changes in water metabolism. The latter changes, in turn, depend upon the state of submicroscopic and molecular structures of mitochondria , i.e. the structures where the processes of oxidation phosphorylation and reactions with the participation of ATP take place. The usual consequences of damage to these structures are reduced to the swelling of mitochondria. .

Most likely, swelling is mainly associated with the passage of a liquid from the surrounding medium into the mitochondria. This process is accompanied by an increase in volume, which not infrequently, is quite considerable. Thus the diameter of a mitochondria section in a normal liver is 0.08 μ , and in the liver of a starving rat it is 1.06 μ . In case of ischemia of the myocardium the volume of mitochondria grows from 10 to 60 fold. When swelling occurs the external membrane becomes extended but unlike the internal one, it is not easily ruptured.

One should distinguish the swelling of mitochondria from vacuolization.

Bergman and Knopp (1960) described vacuoli which arise in mitochondria of some cells under different conditions. These vacuoli are of two types: intramitochondrial and adjacent on mitochondrias or their residues.

The swelling of mitochondria involves the external and internal membranes and also the mitochondrial matrix. The external membrane is very elastic. Even in strong swelling of a mitochondria when its volume increases several tens of

3018

times the external membrane is retained and its thickness is not altered. It is not excluded that it possesses a degree of contractility. The properties of the internal membrane differ materially from those of the external membrane. The most characteristic feature of the internal membrane is that it forms numerous folds, crysts. When mitochondria swell the crysts become unfolded and their number decreases. Besides, the inner membrane unlike the external one is rather easily ruptured. The mitochondrial matrix which consists of fine dispersed material of protein origin, in swelling, as a rule, drastically loses its density, is hydrated and in the finally becomes electron-transparent.

Toxic actions on an organism definitely influences the synthesis of protein. As a rule, this influence is mostly manifest in the action upon the protein synthesizing system of the liver, the organ which is responsible for disintoxication.

The synthesis of protein consists of a number of sequences in the liver; we shall confine ourselves only to a brief enumeration: 1) preparation (activation) of aminoacids, the main blocks for the building of polypeptides; 2) attachment of activated aminoacids to ribonucleoproteids of ribosomes; 3) the action of synthesizing enzymes; 4) the coding of the sequence of protein synthesis from aminoacids thanks to the participation of m-RNA; 5) the arrival of the newly formed proteins into the cytoplasm.

Each of the mentioned processes is subject to disruptions. The latter underly the pathology of ribosomes, our knowledge of these, however, is strictly theoretical. Precise data about ribosomes are scanty and not completely clear.

The cell's protein synthesizing system consists of three elements: monoribosomes, polyribosomes and a rough endoplasmatic network. Each of these elements makes its contribution to protein synthesis. Individual ribosomes synthesize protoproteins or fragments of the protein molecule. A complete molecule of protein can be created only by ribosomes associated by a thread of m'RNA into polysomes or by the same polysomes associated with the membrane of the endoplasmatic network. In the former case the proteins are synthesized for their own intracellular consumption and renovation of the membrane material of the cell proper; in the latter case the proteins are synthesized "for export" which is clearly seen in exocrine cells of the pancreas and in the major cells of the stomach which secrete a large number of protein-enzymes.

The number of monoribosomes in the cell cytoplasm may be large or small. However, it is impossible to say whether it is good or bad judging by their number since an increase in the number of ribosomes may take place owing to the disintegration of polysomes and the rough reticulum. Besides, some of the ribosomes are active, others are inactive. The determination of the number of active and inactive population of monoribosomes requires highly subtle studies making use of labelled aminoacids, precursors of protein synthesis.

The main structural element which ensures protein synthesis is the group of ribosomes associated by the thread of m-RNA, which forms the polyribosome from 5, 6 ribosomes-monomers. An increase in the number of polyribosomes in a cell definitely indicates the mounting of the cell synthetic potential. A decrease, on the contrary, points to the inhibi-

tion of protein synthesis. This system is highly labile and responds consistently to numerous toxic influences and to protein deficiency in the rations by disintegration of polyribosomes and by a decrease of their number in the cell.

The changes in the structure of the rough endoplasmatic network may involve the size of membrane cisterns and the presence or absence of ribosomes attached to the membranes. Most varying influences produce considerable changes in the volume of the cavities in the system of the endoplasmatic network. These changes are of different types.

Some cisterns may turn into large vacuoli, which become more or less deformed owing to the reciprocal pressure. These changes have been described in liver cells in instances of intoxication with carbon tetrachloride. In other instances the tubulae are fragmented and turn into small vesicles; the latter may become broader and turn into large regular vacuoli which, however, are not as large as those which develop as a result of direct swelling without preliminary fragmentation. Individual vacuoli never touch one another, they are always divided by a layer of the hyaloplasm which is from 4 to 5 nM wide. In many damages of the cell large vacuoli arise as a result of edema, swelling of the cisterns of the endoplasmatic network. This reaction passes quite rapidly.

At the same time the mentioned vacuolisation of the endoplasmatic network should not be confused with the widening of the cisterns in the activation of synthetic processes, for instance in plasmatic cells. In the synthesis of protein it accumulates within the cisterns which are filled with fine dispersed material of medium electronic density. As for the

case of edema the content of cisterns of endoplasmatic network is absolutely electrontransparent.

Considerable distinctions in the structure of the endoplasmatic network are determined by the number of ribosomes attached to the membranes of cisterns. Changes in the structure of nutrition, insufficient consumption of protein, starvation and numerous toxic influences are accompanied by a decrease in the number of ribosomes on the membranes and consequently by the inhibition of protein synthesis in the cell.

The above described developments do not take place in an isolated manner but, as a rule, occur in a complex. The fragmentation of cisterns of the endoplasmatic network in case of toxic influence, as a rule, is parallelled by vacuolisation and dissociation of ribosomes. In as much as the synthesis of a protein molecule is implemented by a group of ribosomes attached to the membrane and since the cistern proper performs in this case the function of a support and also of accumulation and distribution of the synthesized product one can understand that the decrease in the number of ribosomes per unit of length of the membrane and the fragmentation of cisterns which leads to a partial dissociation of the synthetic ribosomal group serve as the morphological substrate of the decrease in the production of protein by the cell.

The Golgi apparatus or the intracellular reticulum is functionally connected with the endoplasmatic network. It consists of three components: a packet of double membranes which form the dictyosome; an inconsistent number of small vesicles scattered in the cytoplasm and which are formed of dictyosomes; and of considerably larger vacuoli.

The application of aminoacids labelled by tritium and radioactive carbon made it possible to demonstrate that the material synthesized in the endoplasmatic network by means of vesicles which bud off from it is transferred into the zone of the Golgi apparatus. The synthesized molecules become more complex within the Golgi apparatus; for instance, there takes place the joining of the hydrocarbon part of the molecules of glycoproteids. In other words, the function of the reticulum is not confined to packing the synthesized material. It is much more intricate. A number of complex processes takes place in the Golgi apparatus: the formation of lysosomes, the production of globules of mucus etc. The extent of the development of the Golgi apparatus in every cell depends most likely upon its metabolic activity. The changes in the Golgi apparatus in pathology may be of different orientation. At times it manifests itself in the disappearance of the structures of intracellular reticulum. More often than not these changes are expressed by the intensified formation of a multitude of small vesicles which develop from the edges of the plates of dictyosomes. This creates an impression that there is an intensification of the normal process in this case.

The cellular orgenelle which is called a lysosome became known only in 1955 thanks to the studies of De Duve. Having been first described by biochemists it occupied a highly important place in physiology and pathology. The processes of proteolysis which occur in the cell or which begin in the cell are associated with lysosomes.

Two categories of structures which possess the properties of lysosomes are distinguished. Some are, so to say,

"professional" lysosomes. Neutrophilic granules of polymorphonuclear leukocytes may serve as an example; their main peculiarity is that they contain the potentially proteolytic enzymes which demonstrate their activity following various influences (activation of lysosomes). Those are primary lysosomes. Other lysosomal structures, secondary lysosomes, are the consequence of the combination of primary lysosomes with other proteins or structures which had not initially possessed a proteolytic activity. They may arise as a consequence of phagocytosis (phagosomes) or as a result of local degeneration of the cytoplasm (cytolysosomes, cytolysomes). They may be very varied in normal and specifically in pathological conditions.

Proteolytic enzymes in primary lysosomes are in a latent state. The realization of their proteolytic activity is possible but after the rupture of the membrane which surrounds the lysosome or after it becomes permeable for enzymes. Normally this membrane is a barrier for enzymes. Proteolysis becomes active only when the membrane is labilized. Proteolysis may cover the entire cell in which case the latter is subject to autolysis, i.e. it is subject to the extending proteolysis which leads to the death of the cell and its disintegration. Under definite conditions the proteolysis of the cytoplasm is localized only in some sites of the cell. The site which is in the state of proteolysis is surrounded by a membrane and is sequestered in the form of cytolysosome or autophagic vacuoli. Thanks to this the spread of proteolysis is discontinued and there develop the secondary lysosome. The latter may exist as an inclusion to which the cell is

tolerant. However, the lysosome of this type possesses a latent proteolytic activity. This activity may demonstrate itself, specifically during the destruction of the membrane. The final stage of litic processes within the secondary lysosome consists in the inactivation of enzymes and the formation of residual bodies. As a rule, residual bodies are represented by myeloid lamellas which form peculiar concentric figures with lipofuscin.

At present more than 50 enzymes in the lysosomes have been identified. In the main they are hydrolases. However, one among them — esterase — is an acid phosphotase which serves as a lysosomal enzyme marker for histochemical identification of these organelles.

The lysosomal apparatus becomes particularly complex in the system of specilized phagocytes represented by cellular elements of two types: micro- and macrophages. The lysosomal apparatus of microphages has a perfect and intricately organized mechanism for extracellular functioning of lysosomal hydrolases. It has been possible to demonstrate that lysosomes located in the peripheral layers of the cytoplasm are evacuated from the cells by detaching parts of the cytoplasm. Participating in this process is the actomyosin fibrillary system. The second type of phagocytes (macrophages) in its conventional, inactive state, has a comparatively small number of lysosomes. However, in case of activation of these cells, they acquire the ability to form large numbers of lysosomes through intensive activity of their protein synthesizing apparatus.

Besides the digestive and protective functions lysosomes

are used also to implement other functions. For instance, the cells of the epithelium of some liver tubulae possess well-expressed phagocytic activity and accordingly a rather large number of lysosomes. The latter participate in the intracellular digestion of organic formations in phagosomes if the activity of the filtration apparatus of the nephron is disrupted and if large molecules or their complexes enter the primary urine.

The universality of the spread of lysosomes is determined by their role during autophagia — intracellular digestion of individual organelles and sites of cell cytoplasm and also, most likely, of molecules and supramolecular complexes which have lost their importance either owing to ageing or being used as material for maintaining the life of the cell under favourable extreme conditions.

The autophagic activity of lysosomes essentially is an apparatus of physiological regeneration of individual cellular structures. However, just as in case of heterophagia with its primary trophic function, autophagia in specialized cells may have a different nature performing some other specific functions.

For instance, quite important is the so-called reconstructive function of lysosomes. It is associated with the ability of multicellular organisms to maintain, under the conditions of starvation, the viability of cells thanks to endogenous nutrition, i.e. digestion with the help of lysosomes of some of cytoplasmatic structures and consuming the low molecular compounds for the needs of energy metabolism. The specific autophagia is common in glandular cells whenever

it is necessary to utilize the surplus of the secretion.

All this proves that lysosomes are not merely vesicles with enzymes which are used from time to time in case of hetero- and autophagia. In keeping with present-day notions lysosomes are a morphologically developed old and intensively evolutionized catabolic system of the cell. It is under regular control of integrative cellular mechanisms and, in its turn, most likely can influence these mechanisms up to altering the orientation of cell differentiation as it is seen, for instance, when a surplus of vitamin A acts upon the rudiments of a multilayered cornea epithelium. Vitamin A selectively acts upon the membranes of lysosomes predetermining the emergence of enzymes into the hyaloplasm. The latter, quite possibly enter the nuclear apparatus or influence the genetic apparatus of cells indirectly by acting through cytoplasmatic proteins as a result of which we observe a change in the direction of the cell differentiation in the epithelium and instead of corneal cells goblet and whip cells emerge.

An action similar to that of vitamin A on the membranes of lysosomes is observed during the action of sporofusarin. During electron microscopic investigation of rat liver following intraperitoneal injection of crystalline sporofusarin at a dose of 75 mg per a kg of body mass we found large clarification foci in the cytoplasm of hepatocytes at times taking up to half the cell. Along the periphery of these foci there is a large number of lysosomes and autophagic vacuoli surrounded by an elementary membrane. The vacuoli quite frequently include mitochondria with an altered structure, sites of cytoplasm and lysosomes, the membrane of which is

partially destroyed. A picture of this type attests to active processes and to the participation of lysosomes in local lysis of the cytoplasm.

Biochemical studies of the same material correlate well with the results of morphological investigation of liver. The findings included a sharp disruption of the stability of membranes of liver lysosomes, the emergence of lysosomal enzymes into the cytoplasm and an increase of their non-sedimented activity.

This, however, is but one example of the morpho-functional approach to a specific experimental task.

All above described structural-functional reactions of intracellular organelles have been demonstrated with integrated application of electron mocroscopy and cellular structural biochemistry. At present this integration is conventionally known as functional morphology. The main peculiarity of the morphofunctional approach to the study of a cell and its responses to pathologic influences is the desire to comprehend the structural foundation of biochemical processes which determine the given function, i.e. to relate these processes to specific cellular structures.

The final objective given this approach is identical to the objective pursued by molecular biology and cellular structural biochemistry. However, the methods used by those sciences to resolve common objectives are fundamentally different. While an indispensable condition for molecular biology and structural biology is cell destruction and the isolation of the studied structure in a more or less pure fraction, the prerequisite in cytological studies is, on the contrary, the

preservation of the wholesomeness of the cell. In this case it is necessary to strive to minimize external interference and to study the structural-biochemical organization of some or other components precisely within the confines of an intact cellular system.

We have already stated that the most widespread, method which has become classical in structural-biochemical studies is the method of electron microscopy with its different modifications. These modifications are predetermined both by differing approaches to the analysis of the studied structures and by the peculiarities of the preparation of cells for ultrastructural studies. The high resolution of conventional transmission microscopes make it possible to analyze not only all organoids of the nuclear and cytoplasmatic apparatus but also some structures in the supramolecular level of organization, for instance, the suporting and contractile microfibrils, microtubulae, some multienzyme complexes. The present study of cells in the systemic and subsystemic levels of their organization increasingly and successfully involves the method of high-voltage electron microscopy. Thanks to its much greater compared to the convevtional electron microscope energy of the penetrating beam of electron this method makes it possible to study "thick" sections and even whole flattened cells which allows, for instance, analysis of the complex system of intracellular organelles as a whole.

The method of scanning electron microscopy which allows for a three-dimensional study of the surface of an object becomes important in the study of the function of the surface apparatus of the cell, the interrelationship of indivi-

dual subsystems of the surface apparatus of the nucleus and for a number of other problems. The method of freeze-etching holds a special and fundamentally important place in cytological studies of the morphobiochemical orientation. It is the most sparing method of preparing biological objects for ultrastructural analysis, i.e. it causes the least changes in cellular structures compared to their native state. The substance of the method is that the object is placed in liquid propane cooled by liquid nitrogen at a temperature of -185-196°C which instantaneously discontinues all metabolic processes. Then etchings are made in the frozen object using special installations at a high vacuum. Replications are taken from the surface of the etching by putting upon them the finest coat of platinum or silver. The metal replication taken from the surface of the etching is subsequently investigated in an electron microscope. The advantage of this method is that the face of the etching usually passes along the hydrophobic phase of the membrane and this makes it possible to study on the etchings the quantity, size and the nature of distribution of integral proteins of the membrane, i.e. the directly internal morphobiochemical organization of the membranes. This method produced highly valuable results in investigating different types of membrane structurss and special formations, for instance certain types of cellular contacts. Up to now, however, the cryomethods have not been used for the study of the action of mycotoxins, though they could produce valuable information on the influence of their subtoxic doses on the membrane formation of cells.

Cytochemical methods play an exceptionally important

part for the fundamental task of structural-biochemical aspect of cytological investigations -- the elucidation of the functional importance of structures by analyzing their biochemical organization. They are being presently refined both from the point of view of precise qualitative identification of chemical compounds in the studied structures and in the sense of their quantitative evaluation. Making use of special instruments which allow for quantitative cytospectrophotometry it is possible to identify the content of the given substance, for instance, RNA and DNA not only in the cell as a whole but also on the level of nuclear and cytoplasmatic structures.

Also possible at present is a very accurate qualitative analysis of individual proteins of cellular structures in the framework of an intact cellular system. This analysis is carried out by means of immunocytochemical methods. The substance of this method is that a concrete protein serves as an antigen to which an organism of some mammals produces antigens. The latter are combined with a fluorescent stain (for light microscopy) or with an electron dense marker of the type of colloid gold, ferritin or peroxidase (for electron microscopy). Specifically marked antibodies are bonded strictly selectively with the structures containing the studied protein. Thus this method was instrumental in identifying the localization of the main and auxiliary contractile proteins of the actin-myosin system in the submembrane fibrillary apparatus of non-muscular cells. The same method was successfully applied to prove the validity of the liquid-mosaic model of membrane organization.

Broad prospects are opened up by the method of auto-

radiography with the application of radiactive precursors of micromolecule synthesis labelled by artificial isotopes (^{3}H, ^{14}C, ^{35} etc.) the action of which upon an organism is still to be investigated. This method makes it possible not only to determine the site of the synthesis of some or other macromolecules but to trace specific pathways of the intracellular transport of compounds, offers a relative quantitative appraisal of the intensity of the synthesis and the rate of movement of macromolecules in cellular structures. In this way, specifically, it was demonstrated for the first time how RNA moves from the nucleus to the cytoplasm of cells, the localization of the synthesis was traced in detail as well as the intracellular transport of the secretion in secretory cells and many other processes. It is understandable that if the method makes it possible to study a process it is applicable also to the examination of the process changes after the influence of a variety of actions, for instance when mycotoxins are introduced into an organism.

Essentially the autoradiographic method is one of the most typical methods characteristic of the structural-biochemical orientation of studies in as much as it allows a direct investigation into the processes of metabolism in intracellular structures in a wholesome, intact (just as in biochemical studies) cell.

The synthesis of molecular-biological and structural-biochemical methods is most indicative for the elaboration of many important problems of general biology. This type of an interaction is carried out either by undertaking integrated studies with the application both of biochemical and of

cytological methods by biochemists and cytologists or by applying specific integrated methods bordering on biochemical and cytological analysis of cellular structures. These methods, however, are complex and are beyond the framework of conventional morphological methods.

At present in connection with the appearance of instruments for semiautomatic morphometric analysis, visual study of ultrastructures is accompanied by a sterometric treatment of results of electron microscopic examination. The quantitative expression of the results (instead of qualitative) has essentially strengthened the positions of morphological methods and offered the possibility to correlate with greater confidence the structure and the functions of cells.

The necessity of assessing the intensity of energy processes in a cell has given rise to a large number of studies covering stereological analysis of mitochondrial apparatus. As a rule, the measured parameter is the area under mitochondria in the cell as well as their number; the extent of swelling of mitochondria is assessed, the surface area of the inner mitochondrial membrane where, in the main, the energy processes take place, and also the coefficient of crystae fragmentation. All these parameters taken together make it possible to evaluate the energy potential of the cell and, what is particularly important, its changes not only on their pathological influences but also in case of physiological deviations, i.e. then when the visual (qualitative) study does not produce results.

Unlike the case with the mitochondrial apparatus only a few papers are devoted to the analysis of the structures be-

longing to the protein synthesizing system. We have already stated that the protein synthesizing system consists of three independent and at the same time interrelated compartments: isolated monoribosomes, ribosomes which are combined into polysomes and ribosomes which are associated with the membranes of the endoplasmatic network. In as much as the three parts of the protein synthesizing system are well seen in the investigation of the cell in an electron microscope we have the possibility of measuring stereometrically their quantitative characteristics. As a rule, the investigators measure the area of the surface of membranes of the endoplasmatic network in the cell, the coefficient of fragmentation of the EPS cisterns, the degree of granulation of membranes, i.e. the number of ribosomes per unit of membrane length. Besides, they measure the number of monoribosomes and polyribosomes per unit of section area, ordinarily per 1μ .

All measured parameters give a rather adequate characteristics of the protein synthesizing system of the cell. To obtain an integrated evaluation one may make use of the total coefficient of activity which takes into consideration not only the state of each of the system components but also the percentage contribution into the overall pool of protein synthesis.

Stereological analysis can also cover other intracellular components: Golgi's apparatus, primary and secondary lysosomes, secretory granules. A complete stereological analysis of the cell gives exhaustive characteristics of the functional state of organelles which together with biochemical studies provides an insight into the energy and the synthetic and catabolic processes.

x x x

The analysis of morphological methods shows their possibilities and the ways of application in the study of the influence of various mycotoxins upon organism's organs and tissues. At the same time it should be mentioned that at present only routine morphological methods are being used in mycotoxicology though they do not provide a complete scope of information. The main morphofunctional approaches including electron microscopic histochemistry are still beyond the field of vision of investigators who are engaged in the study of mycotoxins.

Зак. 3018 ПИК ВИНИТИ