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# The influence of the experimental lactate – acidosis on hemostasis and a structure of endothelium.

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**ABSTRACT**

This work demonstrates experimental data of the influence of different concentrations of lactic acid on the indicators of blood coagulation and vascular-platelet hemostasis. Lactic acid in the experiments in vitro makes a doublephase influence on the process of blood coagulation depending on the dose: small concentrations (2,4-5,9 mmol/l) are shortening, and the big (7,8 - 16,6 mmol/l) are lengthening a fibrin formation. In experiments in vivo the bigger activation of coagulation process is observed at pH change of blood to 7,2-7,1. Hypercoagulation that appears with pH change of blood in acidity is associated with a number of factors: inactivation of antithrombins, a release of procoagulants from erythrocytes and platelets, a spontaneous aggregation and the violation of processes of disaggregation of platelets, the decrease of the electrokinetic potential of platelets and a more rapid polymerization of a fibrin. With the development of acidosis in the blood stream appears the aggregation of platelets and erythrocytes, which at non-compensated lactate-acidosis turns into thrombosis, which is accompanied by disruption of the microcirculation. According to the data of electron microscopy in the endothelial cells appear the signs of mitochondrial dysfunction. Occurs the disintegration of the cytoplasm, the formation of micro-vesicles and micro particles, the disruption of nucleus structure, of mitochondria, appears the rupture of the cytoplasmic membranes of individual cells. The pH change leads to the endothelial cells dysfunction, to the admission into the bloodstream of ogranoids, possessing mostly the pro-coagulant activity.

**Keywords:** lactate acidosis, pH, haemostasis, platelet aggregation, Zeta potential of platelets, fibrinolysis, morphology, endothelial dysfunction.

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## RELEVANCE

**The urgency of the problem**

Violations of acid-base balance are the rule rather than the exception in patients who are in intensive care. Acidotic state complicate the course of many diseases of different genesis, being an essential component of a variety of nosological forms of pathology, including such typical pathological processes as inflammation, fever, shock, DIC etc. [Malyshev V. D., 2005; Alfonso V. et al., 2010]. Products of anaerobic metabolism causing acidosis, pose a real danger to the organism, as they are able not only to destroy the function, but they can also lead to morphological changes in various organs and tissues. The accumulation of lactic sour changes the hemostatic and rheological properties of blood, increases tissue hypoxia and reduces the function of energy formation in the cells due to the uncoupling of glycolysis and the Krebs cycle, reduces the resynthesis of ATP and leads to an increase in entropy in the organism [Tverskoy R. A., 2981; De Backer D, 2003].

A significant role is given to acidosis in the mechanisms of regulation of physiological functions during exercise. In the process of strenuous physical activity there is an increase in the content of acidic products of metabolism that cause shifts in the acid base stats of the blood. The accumulation of acidic products of metabolism are primarily due to the discrepancy between oxygen request and consumption, which leads to an increase of lactate content in blood [D De Backer, 2003]. In these conditions the special importance is the study of the relationship between acidosis and changes in tissue morphology to understand the dynamics of the pathological process in various diseases.

The problem of thrombosis in the modern medicine is one of the most important problems. Currently, there have been numerous studies in which the hemostatic system is no longer studied alone, as a multienzyme cascade of blood flow and its analysis transfers into relationships with other systems of the body [El'chaninov A. P. and others, 2000; Barkagan Z. s, etc., 2005; Bokeria L. A. and others, 2009; Zubairov D. M., et al., 2009; Kuznik B. I., 2010, etc.]. The effect of acidosis on hemostatic parameters was studied by many researchers [Mikhail J., 1999; Zacharias S. R et al., 1999; Roitman E. V., 2000; Alfonsov V. V. et al., 2010 and others]. This has identified main regularities of changes in platelet function, blood coagulation, fibrinolysis, anticoagulant activity, development of disseminated intravascular coagulation. However, until now the mechanisms of pathogenesis and morphogenesis of the syndrome of disseminated coagulation syndrome in the acidosis of varying depth and duration remain largely unexplored.

The aim of the study was to investigate the mechanisms of the development of intravascular coagulation in acute experimental lactate-acidosis.

## MATERIALS AND METHODS

To study the role of metabolic factors in the mechanisms of blood coagulation and vascular-platelet hemostasis were conducted in vitro studies. Were studied 20 dogs weighing from 15 to 25 kg, they were used as donors. Were studied the effects of various concentrations of lactic acid prepared in normal saline. Were studied blood coagulation, fibrinolysis, platelet hemostasis. In the experiments we used different concentrations of lactate (2.5 - 17 mmol/l). Blood samples were taken in silicon tubes containing 3.8% sodium citrate, so that the final ratio of citrate and blood was 1 to 9. To obtain plasma, the blood was centrifuged for 10 minutes at 1500 rpm, and the plasma rich in platelets for the study of Zeta potential and aggregation - at 1,000 rpm. A determination of the electrokinetic mobility of the platelets was carried out in the chamber of H. A. Abramson (1928) in modification of Alfonsov V. V. (1977).

Indicators of acid base status (ABS) were determined by the micromethod of Astrup "Micro-Astrup". In experiments in vivo lactate-acidosis was made by the intake of a 3% solution of lactic acid in isotonic NaCl solution into the femoral vein with use of hexenal anesthesia. Various pH change in acidity was reached by a metered drip adding of lactate from 20 to 38 drops per min with the pH control until the pH level reaches of 7.25-6.5 and up to 30-180 min. For the determination of hemostatic parameters, the blood samples were taken before and after the adding of lactate from the femoral artery. To characterize the system of hemostasis were determined: time of recalcification, prothrombin time, thrombin time, fibrinogen, SFMC, platelet count, aggregation and ξ-potential of platelets. In the study of the morphology of the myocardium and endothelium was used light and electron microscopy.

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Electron microscopy of pieces of the bodies was carried out at pH 7.4 (control), and 7.2 and 7.0 (experiment) in 15-30 minutes after the beginning of acidosis. In work with experimental animals were followed the requirements outlined in the "Methodological recommendations for conducting biomedical research using animals", 1985 year. The statistical analysis was performed using the software package Microsoft Excel 2007 to the Windows 7 operating system. The significance of differences of indicators in groups were evaluated according to the value of Student's t-test.

## THE RESULTS OF THE STUDY AND THEIR DISCUSSION

The results of these studies indicate that lactic acid in vitro has a marked effect on the blood clotting system. The pH change in acidity in both plasma and a whole blood leads to biphasic changes in coagulation. As acidification of plasma from 7.4 to 7.22 – 7,10 the rate of formation of fibrin clot increases, a further change of the medium reaction in acidity leads to the slowing of blood clotting, and at pH 6.0 clot is not formed.

The activity of factors included in the prothrombin complex, is the most resistant to pH change in acidity, the VII factor exhibits an optimal activity at a pH of 7.40 – of 7.22, a further acidification of the medium leads to its rapid inactivation. Thrombin time in the presence of different concentrations of lactic acid also undergoes two phase changes. In small doses (up to 5.95 mmol/l) lactate shortens the time of the transition of fibrinogen to fibrin by binding of natural anticoagulants, and in high doses (16.6 mmol/l) – blocks the formation of fibrin because of the impaired polymerization of the fibrin – monomer (table. 1).

**Table 1. The effect of different concentrations of lactic acid on hemostasis in vitro (M±M).**

| Studied parameters n=15 | Before the injection | On the background of injection | After injection | |
|---|---|---|---|---|
| | | | After 10 minutes | After 60 minutes |
| pH of sample | 7,35±0,043 | 7,07±0,02*** | 7,23±0,03*** | 7,29 ± 0,02 |
| Time of recalcification (sec) | 121 ± 5,2 | 65±4,1*** | 82±7,0** | 120 ± 6,0 |
| Factor V ( sec ) | 19 ± 0,85 | 20 ± 0,9 | 22 ± 1,2 | 23 ± 2,0 |
| Factor VII ( sec ) | 57 ± 2,6 | 54 ± 3,0 | 65±3,0* | 68±3,5* |
| Factor VIII ( sec ) | 17 ± 1,5 | 11±1,5* | 16 ± 2,0 | 17 ± 2,1 |
| Factor X ( sec ) | 22 ± 1,8 | 22 ±1,6 | 22 ± 2,0 | 23 ±1,8 |
| Prothrombin time (sec) | 15 ± 0,85 | 14 ± 0,76 | 14 ± 0,95 | 14 ± 1,3 |
| Thrombin time (sec) | 36 ± 1,5 | 39±0,9* | 43±0,1*** | 45±0,7*** |
| Fibrinogen (mg%) | 395 ± 17,8 | 346±18* | 364 ± 26,7 | 370 ± 28,9 |
| Fibrinogen B | – | +++ | +++ | +++ |
| Euglobulin fibrinolysis (min) | 49 ± 2,2 | 51 ± 3,4 | 54 ± 3,1 | 58±3,1* |
| Lactate (mmol/l) | 0,70 ± 0,034 | 1,49±0,11*** | 1,02±0,08* | 0,74 ±0,07* |
| Alkaline reserve (MEq/l) | 110 ± 4,2 | 90 ±5,0* | 95 ± 5,5 | 105 ± 2,1 |
| Oxygen (vol%) | 17 ± 1,0 | 14±1,2* | 14 ± 1,2 | 16 ± 1,1 |
| The Zeta potential of platelets | 14,958 + | 13,940±0,16* p<0,05 + | 14,825±0,13* p<0,05 | 14,950 |

Note: * p <0.05, ** - p <0.01, * * * p <0.001 – the difference is significant between control and experience.

Prior to the injection of lactic acid the Zeta potential of platelets obtained from blood of the femoral artery was 14,958 mV. Due to the injection of lactate the electrokinetic charge of the platelets fell to 13,940 mV or by 6.9% (P <. 0,05). At the same time in the systemic circulation was observed an increase in the concentration of lactic acid from 11 to 26 volume percent (P < 0,002), the decrease in pH from 7.30 to 7.07 (P < 0.001), in alkaline reserve from 110 to 90 m-equiv./l (P < 0.05) and oxygen levels from 17 to 14 volume percent (P<0.05). On the 10-th minute, after the injection of the acid the metabolic parameters partially recovered, however, there has been an increase in Zeta potential of platelets. The data show (table 1)

that metabolic shifts, that occur in the body in response to the appearance in the bloodstream of lactate, are accompanied by a decrease in electrokinetic potential of platelets.

The decrease of charge of platelets creates favorable conditions for their mutual bonding and the disruption of blood flow through the microcirculatory channel. Acidosis is likely to decrease not only the electrokinetic potential of blood cells but also the vascular wall, which must be accompanied by the adhesion of platelets to the endothelial surface. In this regard, one can observe two simultaneous processes: on the one hand, - the disruption of the microcirculation by the platelet aggregates, and with another hand – a reduction of platelets number due to their adhesion to the modified inner surface of the large vessels.

**Table 2. The effect of intravenous injection of 4% solution of lactic acid on coagulation, fibrinolysis and some physico-chemical parameters of blood (M±M)**

| Studied parameters n=15 | Control | The concentration of lactate in plasma in mmol/l | | | | |
|---|---|---|---|---|---|---|
| | | 2,5 | 4,0 | 5,95 | 7,77 | 16,6 |
| Time of recalzification | 130,0± 3,5 | 127±3,2 | 120±3,6* | 124 ± 4,8 | 154 ± 10,0* | No clot |
| Prothrombin time | 14,±0,9 | 14±0,95 | 14± 0,90 | 15±1,05 | 17± 1,5 | 28 ±5,0* |
| Factor VII | 30,0± 1,5 | 30 ± 1,8 | 30 ±1,9 | 37 ±2,5* | 48 ±3,1** | No clot |
| Thrombin time | 19±1,2 | 15±0,8** | 13±0,72*** | 13±0,82*** | 14 ±1,0** | 30 ±3,1* |
| Thrombin time of heparinized plasma | 98 ±16 | 26 ±16** | 18 ±12*** | 15±13*** | 15±13** | 28±22* |
| Fibrinolysis | 38±1,8 | 41 ± 2,0 | 43 ±2,0* | 47 ±1,8** | 52±2,1*** | 78±4,5*** |
| pH | 7,55 ± 0,05 | 7,35 ± 0,08** | 7,22 ± 0,10** | 7,10 ± 0,15** | 6,85 ± 0,20** | 6,0 ± 0,31*** |

Note: * p <0.05, ** - p <0.01, * * * p <0.001 – the difference is significant between control and the experiment.

The intravenous injection of 3% solution of lactate leads to a significant acidosis (pH of 7.10), the fall in alkali reserve and oxygen levels in the blood. In the presence of changes in the internal environment of the body there is a sharp activation of the processes of coagulation – time of recalcification is shortened almost by half. The prothrombin time of normal plasmas and plasmas with a low content of V and VII factors is almost constant, the activity of antihemophilic globulin increases. Acidosis is accompanied by a steady fall in the level of fibrinogen. At the same time, lysis of euglobulin during the experience is slowing. The decrease in the concentration of fibrinogen and the appearance of fibrinogen B in the blood makes us assume that the injection of large doses of lactate is accompanied by intravascular coagulation of blood (table. 2)

In the next series of experiments we studied platelet aggregation at various pH change (7,50; 7,4; 7,34; 7,2; 7,18; 6,92; 6,8; 6,50; 6,11). Analysis of the curves of aggregation in the control observations shows that the platelets without the addition of an aggregating agent do not stick together. Aggregation curves that are recorded at pH of 7.4 and 7.5, describe the normal response of platelets to ADP dose of 0.0005 mc/ml of plasma.

With pH decreasing to 7.34 in some cases appeared a spontaneous platelet aggregation, which was according on average to 2.5°, and the time of the beginning of aggregation after application of ADP does not increase significantly. The angle of aggregation decreased to 59,0°. The process was more stretched, it lengthened to 369 sec. However, due to the large variability of the results, and these changes were not reliable. The amplitude of aggregation of platelets was decreased; the amount of aggregates remained virtually unchanged. A change of the medium reaction in acidity also leads to a decrease in the intensity of disaggregation. More significant changes in aggregation were observed when the pH of the samples was equal to 7,2. At this hydrogen ion concentration in all experiments was discovered a spontaneous platelet aggregation. The application of the aggregating agent into the cell of the calorimeter was increasing the

sticking of the blood platelets, however, the average value of the angle of the aggregation fell to 42°, the aggregation process was endless and after 900 sec. the plasma density has reached 33.5%.

The value of aggregates at pH of 7.13 remained the same as in the control, disaggregation was absent in all experiments.

A further acidification of the environment has led to more significant changes of aggregation, and its main feature was the increase in the angle of spontaneous aggregation in the presence of lactate and blocking specific actions of ADF. At pH of 6.92 the angle spontaneous aggregation of platelets increased to 11.3° ($p < 0,2$), at 6.50 to 16° ($p < 0.05$), at 6,11 to 21.8° ($p < 0.05$). The slope of the aggregation under the influence of ADP in the sample with pH of 6.92 was slightly increased, and at pH 6.5 and 6.11 the applying of the ADP has not led to a further change in the angle of aggregation. "Spontaneous" sticking of the blood platelets in the acidic environment was always irreversible, the amplitude of aggregation was increasing with time.

Thus, the acidosis induced by the intravenous injection of lactic acid, leads to biphasic changes of hemostasis. At pH 7,2 – 7,1 develops a hypercoagulation, shortens the time of coagulation of blood, decreases the Zeta potential and the number of platelets, occurs spontaneous platelet aggregation, reduces anticoagulant activity of blood. The change of pH 6.8 is accompanied by the consumption coagulopathy; fibrinogen level decreases. The products of degradation of fibrinogen and fibrin appear in the blood flow. At pH of 6.5 in most of the experiments the blood does not coagulate, in connection with developing a fibrinogenemia. The change of pH to 7.2 leads to disseminate intravascular coagulation, then hypercoagulation, with a further decrease of pH changes with hypocoagulation, which is accompanied by a decrease in fibrinogen levels and the number of blood plates in the blood stream.

All this creates favorable conditions for the occurrence of hemorrhagic phenomena and the development of disseminated intravascular coagulation. Platelets at a shift to the acidity or acidosis contribute to the increase of hypercoagulation properties of the blood and gain the ability to spontaneous aggregation. It is interesting to trace changes in electrokinetic potential of blood platelets in acidosis caused by intravenous injection of lactic acid.

At the same time, the morphological studies indicate the violation of the structural integrity of the endothelial cells of the vascular wall, of the extracellular matrix and parenchymal organs. The various components of cells and tissues can get into the bloodstream to influence clotting and vascular-platelet hemostasis.

According to the electron microscopy during the first 15-30 minutes of acidosis at the blood pH of 7,2 is changing a configuration of the cell surface of endothelial cells of the capillaries of the myocardium. Increases the number of micropinocytosis vesicles that come off and fall into the lumen of the capillaries (1). For the first is changing the structure of mitochondria. In some endothelial cells the mitochondria are ovoid in shape with dense matrix and a little bit dilated cristae, the mitochondrial matrix is enlightened sharply, and cristae are shortened or completely reduced. In the nuclei of endothelial cells changes the structure of karyoplasm: its granular components accumulate mainly near the inner leaf of the nuclear envelope, the perinuclear space expands. Part of the endothelial cells is in a state of swelling: their outer contours are strongly smoothed, and plasma membrane in separate sections shows some signs of degradation. A further change of pH to 7.0 with duration of 30 min results in degradation of cytoplasmic membrane of endothelial cells and the release of organelles into the lumen of the capillaries, which have apparently procoagulant activity, is also observed the detachment from the basement membrane, and later – desquamation of endotheliocytes (photo 2).

In the nuclei of endothelial cells changes the structure of karyoplasm: its granular components accumulate mainly near the inner leaf of the nuclear envelope, the perinuclear space expands. In some endothelial cells mitochondria are ovoid in shape with dense matrix and few cristae extended. Cisterns and tubules of sarcoplasmic reticulum and lamellar complex are expanded. Part of endothelial cells look like swollen: the outer contours are strongly smoothed, and plasma membrane in separate sections shows some signs of degradation. The cytoplasm of endotheliocytes is bright and substantially free of microvesicles. The mitochondrial matrix is sharply enlightened, and cristae are shortened or completely reduced. In granular cytoplasmic reticulum and in lamellar complex is observed significant expansion cisterns, fragmenting to large

vacuoles. The number of ribosomes is dramatically reduced. In many endotheliocytes the cell surface suffers from the significant ultrastructural changes. These changes are usually presented by the local destruction of the plasma membrane and by the release of organelles into the lumen of the capillaries. Is observed a gap between some endothelial cells from the basement membrane, and later – a desquamation of endotheliocytes (photo 1-4).

Endothelium for a long time was considered as non-wettable semipermeable membrane separating the blood stream from the underlying tissues. In fact, the endothelium is a dynamic organ involved in a wide range of processes of hemostasis, which includes maintaining the liquid state of the blood, control of vascular tone, the transfer of nutrients between the blood and underlying tissues. Vascular endothelium maintains the liquid state of aggregation of blood by reducing blood clotting, inhibition of platelet aggregation. Endothelial cells secrete mediators that regulate hemodynamics, and the enzymes involved in the processes of blood coagulation. For example, nitric oxide that causes vasodilation and inhibits adhesion of leukocytes, adhesion and aggregation of platelets and induces their disaggregation, suppresses migration and proliferation of smooth muscle cells. In addition, activated endothelial cells can release into the extracellular space fragments of their plasma membrane (micro - and nanovesicles), which retain procoagulant phenotype of the original cells, and thus create a mechanism of disseminated coagulation process. We also observed a deformation of the cell membrane of endothelial cells and the formation of microvesicles in acidosis. Endoteliocytes microvesicles in some diseases reflect a state of endothelial dysfunction [Zubairov D. M., et al., 2009].

## CONCLUSION

Hypercoagulation occurs in the acidic change of pH, due to several factors: the inactivation of antithrombin, increased activity of plasma factors, a more rapid polymerization of fibrin, spontaneous aggregation, a violation of the processes of disaggregation and a decrease in electrokinetic potential of the platelets and also due to the endothelial dysfunction that occurs in acidosis.

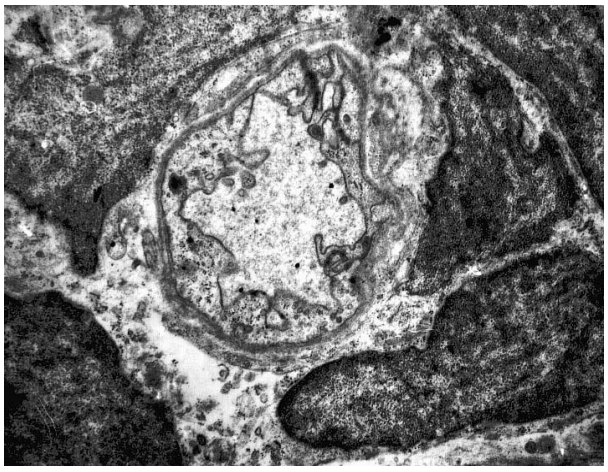

**Photo 1. The myocardium of the cat. Capillaries surrounded by pericytes in metabolic acidosis (blood pH of 7.2, exposure 15 min). The endothelial cells form capillary outgrowths that break off and fall into the lumen of the capillary.**
**Pericapillary swelling. Magnification - 7 000. Electron micrograph.**

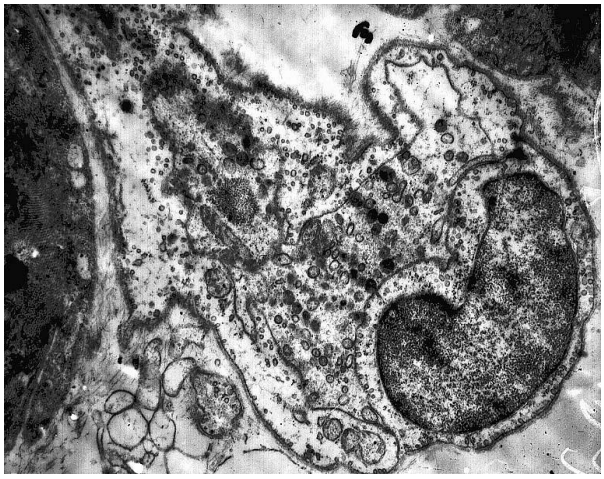

**Photo 2. Heart of a cat with metabolic acidosis (blood pH of 7.0, exposure 30 min). Cytoplasmic membrane of endotheliocyte is destroyed, the organelles of cells entered the lumen of the capillary and the interstitial space. Magnification - 20 000. Electron micrograph.**

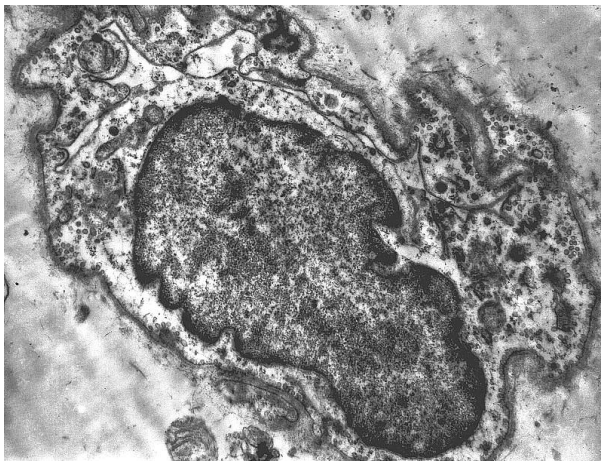

**Photo 3. Heart of a cat with metabolic acidosis (blood pH of 7.2, 30 minutes). Capillary. The nuclear envelope is blurred, there is marginalia of chromatin, cytoplasmic membrane is slightly deformed, it forms protuberances facing into the lumen of the capillary. Individual mitochondria are located in the extracellular space. Pericapillary swelling. Electron microscopy.**

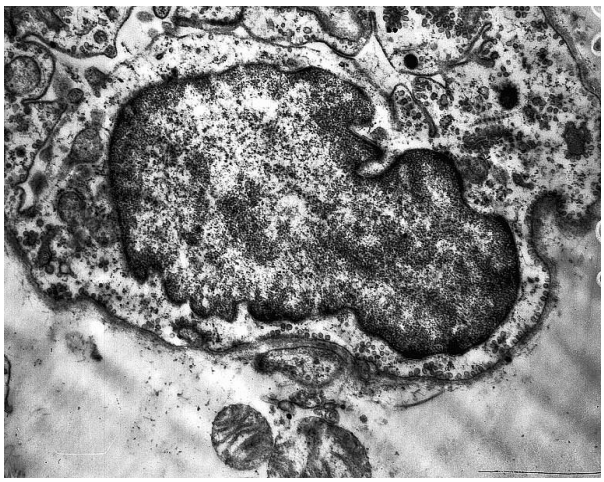

**Photo 4. The myocardium of cat with metabolic acidosis (blood pH of 7.0, exposure 30 min). The cytoplasmic cell membranes are destroyed; the organelles are located in the extracellular space. Magnification - 25 000. Electron micrograph.**

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