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Use of the impedance measurement method to clarify the age of death occurrence

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Impedance measurements of biotissues of model objects — pig carcasses — were carried out at different stages of postmortem period in natural biocenosis and under standardised conditions. The results of the study of impedance dynamics and its dispersion are presented. The existence of a correlation between impedance indices and intervals of postmortem period is established. The possibility of using impedanceometric data to determine the age of death within the framework of reconstruction of stages and conditions of the postmortem period is discussed. The processes occurring in the corpse are analysed.

Keywords: age of death, biological tissues, postmortem changes, impedance, impedance dispersion.

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Introduction

The determination of the prescription of death coming especially in the late post-mortem period is one of the urgent and not fully solved problems of forensic medical examination. A detailed study of the processes occurring in the dead body, reconstruction of the conditions of the post-mortem period and staging of the decomposition process are integral steps in solving this problem.

The modern approach to the consideration of post-mortem changes indicates that the determination of conformance with the pathomorphology of decomposition of cadaver tissues and the underlying abiotic and biotic factors can be a justification for its actual stages. However, the characteristics of the destruction of dead bodies depend on a number of factors acting jointly on the cadaver and, often, catalytically or inhibitingly acting on each other. All these changes, if they can be comprehensively analyzed, play a crucial role in determination of the prescription of death coming. The probability of errors that occur when determining the prescription of death coming only by qualitative assessment of cadaveric phenomena can be reduced by using additional objective methods based on registration of numerical parameters. In this regard, biophysical methods, including the impedance measurement method, are quite promising for solving the problems of forensic medicine.

Measuring the impedance of dead body tissues at different times of the post-mortem period makes it possible to analyze its changes depending on the processes occurring in tissues at the organ and cellular levels. In the applied forensic aspect, this can be the basis for effective expert diagnosis of prescription of death coming, combining the analysis of traditional methods of its determination and the method of impedance measurement, ensuring a more accurate determination of the duration of the post-mortem period as well as in its late phases [1,2].

The impedance measurement method has been widely applied in biology and medicine. An idea of the electrical properties of biological objects is used to interpret the results of measuring the impedance of living tissues. The presence of free ions in cells and intercellular space determines their electrical conductivity. The dielectric properties of biological objects are determined by the presence of membranes, structural components, and polarization phenomena. The impedance dispersion is the variation of the impedance depending on the frequency of the study current which is subject to certain known patterns for the living tissues [3–6].

The impedance of tissues at relatively low frequencies of the study current is determined by the features of their structure, the level of blood supply and the content of conductive fluid in the intercellular spaces, „packing density“ of structural elements in unit volume. It is determined by the polarization of cell membrane phospholipids and dipole polarization of structural formations in cytoplasm at high frequencies (10 kHz−1 MHz) [5]. The impedance dispersion is usually characterized by the dispersion coefficient k_d, which is determined by the ratio of the impedance of biological tissue at a low frequency (100 Hz) to the impedance at a high frequency (1 MHz). The value k_d of a viable tissue is greater than one, and k_d is higher the higher is the metabolic rate [3]. The coefficient of dispersion decreases as the activity of metabolic processes decreases and the processes of destruction develop and it tends to one in case of a dead tissue. The impedance of biological tissues can change significantly under the impact of physiological and pathophysiological factors. This allows using bioimpedance measurement to quantify the state of organs and body systems in various diseases, as well as to detect changes in tissues [7,8].

The use of parameters of electrical resistance of tissues of a dead organism for determining the prescription of death coming was most fully studied in the early post-mortem period (up to 72 h after the death) [9,10]. It was found that cells consistently pass the stages from functional activity through stabilization to dystrophy and destruction, which makes it possible to record the residual activity of surviving cells and tissues on the corpse in the early post-mortem period. The authors justified the measurement of the resistance of a corpse using cutaneous electrodes at three frequencies ($R_1\,(1\,\text{kHz})$, $R_2\,(10\,\text{kHz})$ and $R_3\,(200\,\text{kHz})$) and it is proposed to evaluate the dynamics of relative coefficients: R_1/R_3, $(R_1 - R_2)/R_3$ and $(R_1 - R_2)/(R_2 - R_3)$. The difference $(R_1 - R_2)$ corresponds to the resistance of necrobiotically altered cells, the difference $(R_2 - R_3)$ corresponds to the resistance of cells with an unchanged membrane, R_2 characterizes the resistance of all cellular forms and fibrous structures, R_3 characterizes mainly the state of the interstitial fluid. It is noted that autolysis has a wave-like character, when periods of stability are replaced by pronounced necrobiotic processes [9]. A comprehensive assessment of morphological and biophysical changes in tissues and organs confirms the idea that the destruction of complex biological systems occurs as a multi-stage polycausal process accompanied by an increase of the level of entropy of the object, which occurs at different rates at different stages of the post-mortem period.

Studies of the use of the impedance measurement method to determine the prescription of death coming in the late post-mortem period are applied in nature and are aimed at establishing a quantitative relationship between the impedance indicator and the duration of the prescription of death coming [11–14]. It should be noted that all these studies were conducted on fragments of cadaver tissue under certain storage conditions. The papers [11,12] present the results of a study on fragments of 3 cm long Achilles tendon and kidneys (1/4 part) carried out within 56 days after the prescription of death coming. The fragments were stored in leather „envelopes" for preventing their drying. The impedance was measured at five current frequencies in the range from 10 Hz to 100kHz. A wave-shaped variation of the Achilles tendon impedance was found at all current frequencies. Recommendations were developed according to which the measured values of the impedance should be compared with the diagnostic values of the impedance values of the table of diagnostics of the prescription of death coming for kidneys and Achilles tendons.

According to the results of the study on fragments (1.5 cm long) of the median and sciatic nerves of the limbs of human corpses for 8 weeks, separate intervals were identified in the period of the prescription of death coming which can be determined by the absolute values of the impedance measured at frequencies 100 Hz, 1, 10 and 100 kHz, by comparing with the diagnostic parameters of the impedance [13].

The sample was obtained by aspiration of 0.3 ml of the vitreous body for the study of the vitreous body of the eyeball [14]. The impedance was measured at frequencies of 100 Hz (Z_{LF}) and 100kHz (Z_{HF}). The analytical dependences on the period of prescription of death coming (polynomials of the third degree with corresponding coefficients) for two temperature intervals of finding a dead body were obtained for coefficient of dispersion $k_d = Z_{\text{LF}}/Z_{\text{HF}}$. The determined period of prescription of death coming is selected iteratively until the value k_d obtained during measurement matches the value obtained based on the analytical dependence. The authors of paper [11–14] note the impact of the temperature regime on the impedance dynamics.

Therefore, the existence of a certain time dynamics of the impedance and the coefficient of dispersion for dead body tissues was determined, which suggests the prospects of using impedancometric data for determining the prescription of death coming. This approach will be more effective for analyzing the fundamental processes in the decomposing biological tissues. A combination of biochemical processes occurring in a dead body and not observed in a living organism (for example, autolysis), biotic factors (exposure to microorganisms, insects and vertebrates) and abiotic factors determine the rate and intensity of degradation and decomposition of dead body tissues. Currently, there is practically no literature data describing these processes at the cellular level in the post-mortem period, which complicates the interpretation of the impedance data. Therefore, the study of impedance dynamics in the post-mortem period opens up opportunities for the analysis of post-mortem processes in biological tissues.

We would like to note the advantages of the impedance measurement method for forensic medical examination: the possibility of direct recording of objective biophysical parameters of body tissues, simplicity of the technique, high sensitivity, accessibility of research and mobility (the possibility of using portable equipment).

The purpose of the study is to study the possibility of using the impedance measurement method in forensic medical examination to determine the stage of the post-mortem period and define the period of prescription of death coming in the late post-mortem period.

1. Materials and research methods

1.1. Object of the study

The object of the study was model biological objects — complete pig corpses. We believe that it is more expedient to directly record changes of the impedance of various tissues of an integral corpse over a long period from the moment of death, since the duration and characteristics of the decomposition process of an integral dead body or removed tissue fragments differ significantly. Three diagnostic zones were selected for the measurements: the musculoskeletal flap of the shoulder, the cartilaginous part of the third rib and the Achilles tendon. The choice of a musculoskeletal flap is attributable to the accessibility of the zone and the maximum ease of recording of parameters, cartilage and tendons — their low metabolic activity and insignificant impact of individual characteristics of the biological object on these tissues.

1.2. Subject of the study

Impedance indicators (impedance modulus $|Z|$ (hereinafter Z) and relative coefficients expressed in terms of absolute values Z at various frequencies of the study current) and their time dependence (dependence on the duration of prescription of death coming) were the subject of the study. Z was measured for each period of prescription of death coming for each diagnostic zone of the biological object at five frequencies of the study current. The impedance was measured using device Keysight U1733C with two needle electrodes made of stainless steel with a diameter of 0.5 mm with a length of the submerged part 5 mm and fixed at a distance of 5 cm. The choice of this device is due to its portability and ease of use. The set of study current frequencies set by the device (100, 120 Hz, 1, 10 and 100 kHz) allows tracking the impedance dispersion.

Both the absolute values of the impedance Z and the relative coefficients expressed in terms of absolute values Z at various frequencies of the study current were used to analyze the time dependence of the impedance parameters. The following coefficients were calculated:

$$k_d = Z_{0.1\,\mathrm{kHz}}/Z_{100\,\mathrm{kHz}}, \quad (1)$$

$$k_1 = (Z_{1\,\mathrm{kHz}} - Z_{10\,\mathrm{kHz}})/(Z_{10\,\mathrm{kHz}} - Z_{100\,\mathrm{kHz}}), \quad (2)$$

$$k_2 = (Z_{10\mathrm{kHz}} - Z_{100\,\mathrm{kHz}})/Z_{100\,\mathrm{kHz}}. \quad (3)$$

The study of the dependence of the impedance and coefficients on the period of prescription of death coming allows tracing the change of the nature of the impedance dispersion, which, in turn, will allow judging the dynamics of post-mortem processes [9].

Preliminarily it was planned to create a database of impedance indicators for various periods of the post-mortem period, which would make it possible to develop recommendations for determining the duration of the prescription of death coming. A number of difficulties arose at the initial stage of the study:

1) the possibility to measure the tissue impedance of only unclaimed human corpses when they are in the forensic medical examination department before burial (which usually takes 2–3 weeks);

2) experimental work could be carried out only under the same type of refrigeration chamber conditions;

3) it turned out to be difficult to standardize the conducted studies according to their conditions (date of death of the object, its age, thickness of subcutaneous fat and some other parameters, frequency of taking readings).

The difficulties of measurements on human corpses resulted in the need to choose a model object. The use of a model object to study the decomposition of a corpse is widely used in forensic medical examination [15–17]. This approach makes it possible to standardize measurements by date of death and by the conditions of the experiment, significantly reducing the variables affecting the studied process. The authors of the article [18] proposed a justification for the choice of a model biological object — pig corpse. The corpses of wild boars aged one to two months weighing up to 10 kg were used for studies.

Z was measured in two series with an interval of 2 years (4 model objects in each series) under standardized conditions (air temperature +4°C, humidity 45%). Measurements were carried out in the period from 0 to 31 days of the prescription of death coming for all diagnostic zones, and from 61 to 80 days of the prescription of death coming only for the musculoskeletal flap, since cartilage and tendon turned out to be unsuitable for taking readings during this period. The readings were taken with a frequency of 1–3 days.

Measurements were performed also in two series (1 and 2) with a break of 20 days (4 biological objects in each series) in terms of 0–28 days for the prescription of death coming for musculoskeletal flap, 0–17 days of the prescription of death coming for cartilage and tendon in the conditions of natural biocenosis (north-west of Russia, the shore of Lake Onega, summer period). The readings were taken once every day. Table 1 shows the parameters of the state of the external environment during measurements.

The data for two series of measurements of Z obtained under appropriate conditions are in good agreement with each other, therefore, a sample of 8 objects was used to analyze the data under specific conditions. The numerical characteristics of a sample of 8 biological objects are the median values of the impedance value Z and the corresponding coefficients, the values of the lower and upper quartiles. The differences between the quantitative parameters corresponding to different periods of prescription of death coming were evaluated using the nonparametric Wilcoxon criterion for dependent samples [19]. Statistical processing of the results of the study was carried out using Statistica 8.0 program.

2. Results and discussion

The discussion of the results was focused on two objectives: the use of impedance indicators, firstly, to clarify the interval in the timing of prescription of death coming and, secondly, to analyze the processes occurring in tissues of a dead body.

An identical dependence of Z on the time (time of the prescription of death coming) is observed for all frequen-

Table 1. Parameters of temperature and humidity of the environment during biological observations and impedance measurement in conditions of natural biocenosis

Study series No.	Daily average temperature (t,°C)		Air humidity (φ, %)	
	t_{min}	t_{max}	φ_{min}	φ_{max}
1	13.7	22.6	61.6	86.1
2	12.6	25.1	51.6	84.2

cies of the study current which characteristic of a specific diagnostic zone and specified measurement conditions.

2.1. Standardized conditions

The nature of the impedance dynamics and relative coefficients was evaluated for all diagnostic zones. The following was found:

a) the variation of Z over time with a general downward trend has a wave-shaped character most pronounced for cartilage tissue with the prescription of death coming from 0 to 31 days for all diagnostic zones at all current frequencies (Fig. 1, line *1*). The value of Z practically did not change in the period from 61 to 80 days for the musculoskeletal flap, and its value was on average 4 times lower compared to the initial period;

b) the dependences of the coefficient value k_1 on time are identical for all diagnostic zones. Line *1* on Fig. 2 corresponds to the dependence of k_1 on the duration of the prescription of death coming for the musculoskeletal flap;

c) the dependences of the coefficient value k_2 on time are identical for all diagnostic zones (Fig. 2, line *2* — for the musculoskeletal flap);

d) intervals in the timing of the prescription of death coming are conditionally defined according to the analysis of time dependencies Z, k_1 and k_2: interval I — from 0 to 9 days; interval II — from 10 to 21 days, interval III — from 22 to 31 days, interval IV — from 61 to 80 days (only for the musculoskeletal flap). Z in case of a wave-shaped variation of Z has close (statistically indistinguishable) values for different periods of the prescription of death coming which belong to the intervals I and III. At the same time, the coefficient k_1 has significantly different values in the intervals I and III (Table 2). It should be emphasized that the median values of k_1 in the interval I are less than one, and they exceed this value in the interval III, which indicates a change of the nature of the dispersion Z;

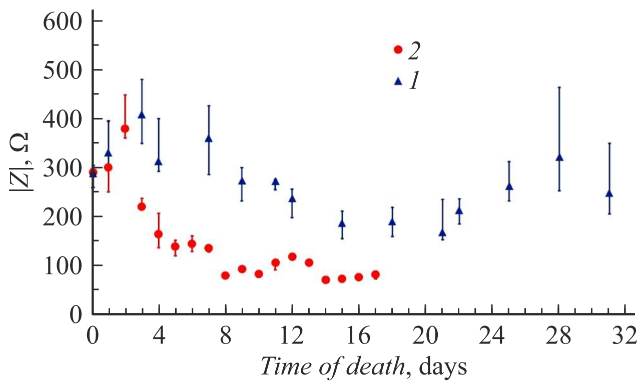

Figure 1. Dependence of the impedance modulus $|Z|$ of cartilage tissue on time (time of the prescription of death coming) at frequency 1 kHz under standardized conditions (*1*) and conditions of natural biocenosis (*2*). The points on the graphs are the median values of Z. The edges of the vertical segments are the boundaries of the lower and upper quartiles.

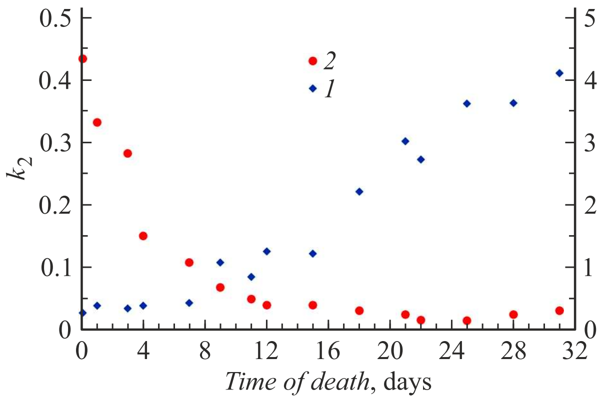

Figure 2. Dependence of coefficients k_1 (*1*) and k_2 (*2*) on time (time of the prescription of death coming) for musculoskeletal flap in standardized conditions.

e) the coefficient of dispersion k_d for all diagnostic zones remained greater than one during the study period ($k_d \approx 1.5$). An increase of k_d to 2.5 was found for the musculoskeletal flap in the period from 75 to 80 days.

Therefore, a comprehensive quantitative assessment of Z, k_1 and k_2 under standardized conditions for the studied biological objects allows distinguishing the time intervals I and III in the timing of prescription of death coming.

Is it possible to interpret the results obtained from the point of view of describing the processes occurring in tissues with the determination of their phasing? The presence of dispersion Z at all studied periods of the prescription of death coming may indicate the course of certain processes in a dead body. The termination of the functioning of the membranes in the absence of other processes should result in the complete disappearance of

Table 2. Coefficient values k_1 in the prescription of death coming times corresponding to time intervals from 0 to 9 days (interval I) and from 22 to 31 days (interval III) for studied diagnostic zones

Interval of the prescription of death coming	Median k_1 [lower quartile; upper quartile]		
	Cartilage	Musculoskeletal flap	Tendon
I	0.69 [0.58; 0.80]	0.58 [0.37; 0.78]	0.95 [0.82; 1.15]
III	3.71 [3.25; 4.76]	3.71 [2.11; 4.23]	3.44 [1.67; 4.78]

the dispersion Z [3,5]. Experimentally established presence of dispersion Z and a change of its nature depending on the period of the prescription of death coming in the late post-mortem period in standardized conditions can be caused by a destruction of the membrane structure itself along with the cessation of membrane functioning. This occurs as a result of autolysis under the impact of its own enzymes and the developing process of putrefaction (an increase of the mass of microorganisms that produce sets of enzymes that are activated at different stages of putrification). The processes of autolysis and putrefaction can have different velocities and intensities at different time periods, which is determined, among other things, by the volume of biomass of microorganisms producing enzymes, which, in turn, depends on the ambient temperature. The most comfortable temperature for the development of putrefactive microflora ranges from +25 to +35°C, and this process slows down at temperatures below +6°C [20]. The wave-shaped impedance dynamics observed by us under standardized conditions seems to be associated with periods of some activation and attenuation of autolysis and putrefaction processes.

According to Ref. [5], the dispersion Z of living tissues in the frequency range from 10 kHz to 1 MHz should be determined by the polarization of phospholipids of cell membranes and dipole polarization of structural formations in the cytoplasm. So, the difference of values Z is used for frequencies 10 and 100kHz when calculating the coefficients k_1 and k_2 (formulas (2) and (3)). The almost unchanged values of k_1 and k_2 in the interval III (Fig. 2) may indicate the absence of polarization processes due to the cessation of functioning of cell membranes and organoids along with the course of other processes, since a change of Z is observed. The change of the value of k_1 in the interval II and the change of the value of k_2 in the interval I (Fig. 2) may be attributable to a change of the ratio of cells with damaged (their increase) and preserved (their decrease) membranes.

Thus, the presence of time dynamics for Z, k_d, k_1 and k_2 under standardized conditions indicates the existence of a certain stage of the destruction of cells and tissues of a dead body, characterized by periods of activation and attenuation the processes of their autolysis and putrefaction. Additional studies and their analysis (namely, determining the ratio of the active and capacitive components of the impedance, establishing the frequency dependence of the phase shift) will allow for a more detailed interpretation of the results.

2.2. Conditions of natural biocenosis

Observations of biological changes in cadaver tissues of model biological objects and measurements of the impedance of Z diagnostic zones were carried out.

The following was found in biophysical studies:

a) dependence of Z on the time of the prescription of death coming is identical for all frequencies of the current of the study of all diagnostic zones of model biological objects: an initial interval of 0−4 days can be distinguished, where Z gives a „surge" with further by its uneven decrease (Fig. 1, line *2*) [21];

b) the nature of the change of the coefficients k_1, k_2 and k_d is qualitatively similar to their change in standardized conditions, but the time intervals are shortened. So, the initial interval for k_1, when its value is less than (or of the order of) one, was reduced to 4 days compared to the interval I (from 0 to 9 days) under standardized conditions;

c) a jump in its magnitude is observed for cartilage and musculoskeletal tissue, against the background of a decrease of k_2 on the 11th day of the prescription of death coming (Fig. 3). Statistically significant differences in the values of k_2 were found on 10 and 11 days prescription of death coming and on 11 and 12 days. These results were obtained in both series of measurements (Table. 1);

d) the coefficient of dispersion k_d had a value greater than one (about 1.5) throughout the entire study period. An increase of the value of k_d is observed For the musculoskeletal flap (Fig. 4), like in standardized conditions, only at an earlier date — from 17 days of the prescription of death coming.

Biological observations made it possible to establish that the time sequence of biological changes for the series of studies 1 and 2 coincides with the timing of prescription of death coming, despite some differences in the indicators of the main abiotic environmental factors at the time of observation (Table 1). Two stages of decomposition of soft tissues of corpses of model biological objects have been traced, which can be conditionally designated as „mainly microbiological", and then „microbial-zoological" disposal. Mainly the microbial destruction corresponds to the interval of 0−4 days according to Ref. [22] and the conducted observations. The second stage begins with the appearance of necrophilous insects [23]. This stage begins with 4–5 days of the prescription of death coming period in our observations. It should be noted that the initial interval of change of Z(„surge“) under conditions of natural biocenosis in terms of prescription of death coming clearly corresponds to the first stage of biological decomposition (microbial destruction) of the model biological object.

A comparative analysis of the dynamics of the same type of impedance indicators under different conditions allows making an assumption about the occurrence or absence of any processes. The discussed studies require the consideration of the microbiological and entomological components of destruction in conditions of natural biocenosis, whereas only the microbiological component can be taken into account in standardized conditions. The increase of the coefficient of dispersion k_d (Fig. 4), observed both in standardized and natural conditions, but occurring at different times, may be associated with different velocities and intensity of microbiological processes and autolysis.

A sharp change of the coefficient k_2 on the 11th day of the prescription of death coming and an increase in the spread of its values (Fig. 3) compared with the practical absence of a spread of values k_2 in the adjacent periods allows considering this jump as a reason for discussion. According to biological observations, it was during this period that the mass movement of necrophilous fly larvae directly

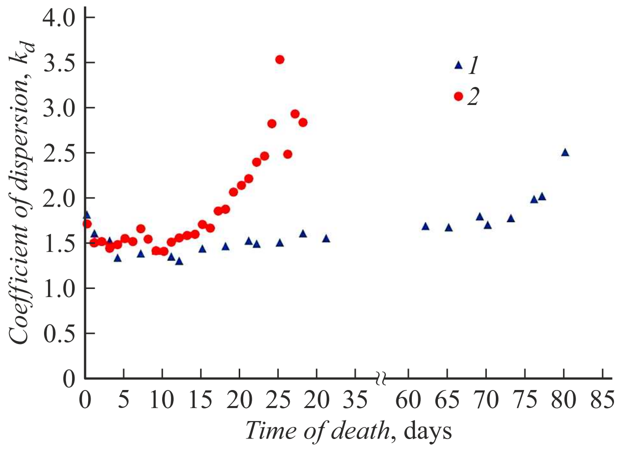

Figure 4. Dependence of the coefficient of dispersion k_d on time of the prescription of death coming for musculoskeletal tissue under standardized conditions (*1*) and conditions of natural biocenosis (*2*).

from the corpse into the soil for further pupation occurred, which led to a change in the biomass of necrobionts and the decomposition of dead organic matter. This may indicate the cessation and completion of entomological processes of destruction and the prevalence of microbiological processes in the future. Thus, it is possible to use changes of the impedance parameters under conditions of natural biocenosis to record and study entomological processes in the post-mortem period.

The determination of a relation between the impedance data (Z, coefficients) and the time of the prescription of death coming implies the formulation of clear recommendations and/or tables, graphs, equations, etc. in an applied plan, which can be used by a forensic medical expert to determine the prescription of death coming. Objectively, a comprehensive approach to the determination of the prescription of death coming is needed. It is very difficult to carry out such a procedure „manually“ due to the many factors that should be taken into account. The creation of a software module for determining prescription of death coming is considered by the authors as the most promising option at the current stage of development of IT technologies. Further research is aimed both at expanding the database of impedance indicators (in terms of the number of biological objects studied and the timing of the prescription of death coming), which will improve the accuracy of the estimates obtained, and at finding and developing approaches to creating a software module for determining the prescription of death coming.

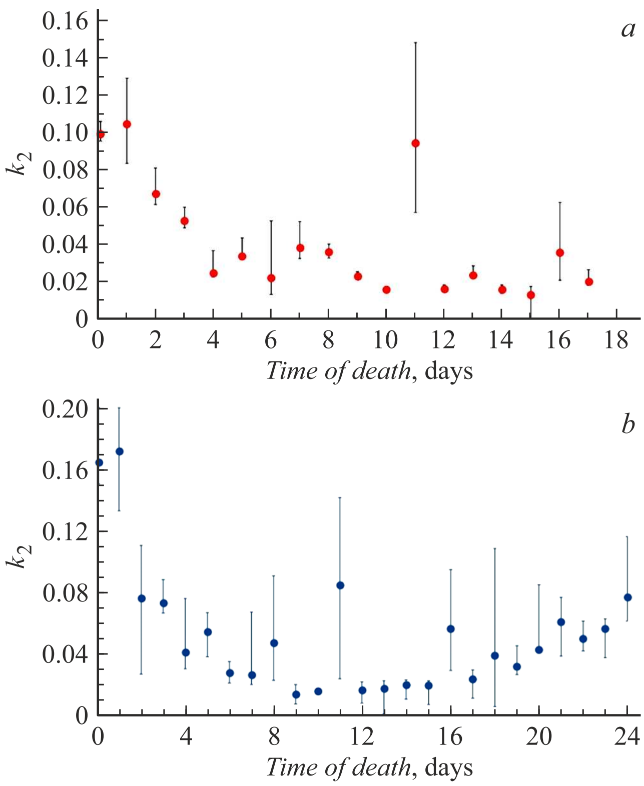

Figure 3. Dependence of the coefficient k_2 on time (time of the prescription of death coming) for cartilage tissue (*a*) and musculoskeletal flap (*b*) in the conditions of natural biocenosis.

Conclusion

The presence of impedance dispersion for all observed periods of the prescription of death coming reflects the course of destructive processes in a dead body. A change of the nature of the impedance dispersion at different intervals of the prescription of death coming can be caused by both a change in stages in the decomposition process and a change in the speed and intensity of their passage. The change of the conditions of a dead body affects the impedance parameters.

Understanding the causes and essence of the processes occurring in the body post-mortem makes it possible to assess and justify the nature of changes in any quantifiable tissue characteristics, including impedance indicators, as well as predict the prospects of their use for specific practical purposes, namely, to diagnose the prescription of death. It is also important to find a convenient and rational form of presenting recommendations on the use of impedance indicators to determine the duration of the prescription of death coming.

The impedance-metric approach to determining the duration of the post-mortem interval should be used in combination with traditional methods of forensic medical examination and based on the analysis of fundamental processes occurring in dead biological tissues and determined by the biochemical state of the object (autolysis), taking into account the microbiological and entomological components of destruction and abiotic factors.

Compliance with ethical standards

All experiments were carried out in compliance with relevant ethical standards and legal documents (conclusion of the Committee on Medical Ethics under the Ministry of Health and Social Development of the Republic of Karelia and Petrozavodsk State University No.43 dated May 21, 2019).

Conflict of interest

The authors state that they have no conflict of interest.

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