Part 1. Veterinary medicine

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FORENSIC VETERINARY ASSESSMENT OF THE EXPERT INFORMATIVENESS OF BIOTRANSFORMATION PATTERNS OF DOG AND CAT CORPSES IN VARIOUS STATES OF DECOMPOSITION

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Summary. Currently, there is no universal algorithm for determining the time of death of an animal. The purpose of the study was to provide a comprehensive argumentation of the forensic veterinary diagnostic significance of the biotransformation phenomena of 28 dog and cat corpses with justification based on their thorough assessment of expert criteria for the duration of postmortem intervals. The study used special and logical-philosophical methods: physical, observation, cyto/histomorphological, forensic veterinary autopsy, analysis, synthesis, deduction, and induction. Early mortalities: rigor mortis, drying, spots, cooling, and late mortalities: decay, skeletalization, fragmentation, patterns of biotransformation, their time ranges, and morphological characteristics are identified. The criterion informativeness of the 'idiomuscular' and 'pupillary' supravital reactions has been proved. The dynamics of disorganization of venous blood of dog and cat corpses within 48 h after death was determined. The sequence of postmortem succession by the entomofauna is shown. According to the concept of 'evidence-based' veterinary medicine, the key stages of postmortem decomposition of dog and cat corpses at different levels of structural organization are illustrated. Based on the analysis of the results of the empirical study, it is substantiated that in the interval of more than 72 h from the moment of death, the answers to the questions in the expert's opinion, due to the large number of complex processes that occur in the tissues of dog and cat corpses, are often only probable

Keywords: forensic veterinary thanatology, corpse phenomena, postmortem intervals, postmortem decomposition, prescription of death, animals

Introduction. In recent years, forensic veterinary examination has rapidly developed into a distinct area of veterinary practice due to increasing public awareness of animal cruelty and health crimes. Despite the pronounced lag in the development of forensic veterinary medicine compared to forensic medicine, over the past decade forensic veterinary thanatology has been significantly enriched by numerous facts that expand our understanding of the biotransformation of animal carcasses, and in-depth research in this area is catalyzing the accumulation of experience and expansion of the evidence base for determining the cause of death and prescribing its onset (Parry and Stoll, 2020). In the context of animal cruelty issues, Yamada et al. (2023) suggest that a 'virtual' autopsy using radiation research methods should be performed on all sub-expert corpses in criminal cases.

The determination of the time of death of an animal remains a priority task in the forensic veterinary examination of its corpse as questions from pretrial investigation bodies regarding the duration of the postmortem period are found in the vast majority of decisions. In most forensic cases, the approximate time and circumstances of the animal's death are known from the case file, but in some cases, especially when it is not obvious, determining the duration of the post-mortem period has a significant impact on the further course of the investigation and helps to solve crimes against animals that led to their death. Taking into account the different nature and circumstances of the animal's death, as set out in the investigator's decision, the most correct approximation of the duration of the post-mortem interval is one of the most important problems of forensic veterinary medicine.

Many researchers have devoted their work to determining the criterion informativeness of early and late corpse phenomena, structural changes in organs and tissues, biochemistry of animal body fluids, thermometry, supravital reactions, saprotrophic fauna, and animal corpses in various states, including fragmented, skeletalized, and drowned corpses, to establish the duration of the postmortem period. Among them: Sanford (2015) notes the differences in entomological species colonizing human and animal bodies, which can be used to establish postmortem intervals; Brooks (2016) in a systematic review considered the process of biotransformation of animal corpses and methods of its control; Touroo and Fitch (2016) report that for the correct analysis of postmortem phenomena, it is necessary to focus on information and evidence during a thorough examination of the site of the animal corpse; Omond et al. (2017) reports on the prevalence of postmortem necrophagy of dog corpses by their own kind; Listos et al. (2018) evaluated postmortem thermometry of dog corpses; Piegari et al. (2019) studied the animal corpses that died as a result of drowning;

Panasiuk-Flak et al. (2021) determined the diagnostic informativeness of supra-rectal pupillary reactions of dog corpses; Stern and Muralidhar (2022) analyzed the dynamics of electrolyte, creatinine, and urea concentrations in the blood and vitreous of cats, dogs, and horses; Li et al. (2022) determined the entomofauna of dog corpses.

The issue of the expert value of postmortem changes in organs and tissues for forensic veterinary diagnosis of the time and circumstances of animal death has been discussed by many scientists. For example, the dynamics of postmortem decomposition of artificially injured skeletal muscles of dog corpses under the influence of sea water was substantiated (Stacy, Costidis and Keene, 2015); the dynamics of postmortem proteolysis in the pectoral muscle of a female duck was established (Liao et al., 2016); the possibility of estimating the time of death of animals by the development of microflora in the calf muscle of a dog was found (Listos et al., 2017); the metabolic profile of the rat thigh muscle was characterized at different periods after death (Du et al., 2018); the relationship between electrical conductivity and chemical content of the solution for skeletal muscle impregnation in rats to determine the duration of the postmortem period was substantiated (Zheng et al., 2019); the duration of the postmortem interval was estimated by determining the activity of catalase and aminolevulinate dehydratase in the tissues of the liver, kidneys, skeletal muscle and brain of Swiss mice (Paltian et al., 2019); the expression of an autophagy-associated protein in rat muscle tissue after ante- and postmortem injury was recorded (Shi et al., 2020); postmortem protein degradation of skeletal muscle in pig limbs was studied (Geissenberger et al., 2021); the informativeness of postmortem changes in the electrical conductivity of skeletal muscles of *Dicentrarchus labrax* for assessing the duration of the postmortem period was determined (Abbate et al., 2022); the potential use of muscle proteins (desmin and dystrophin) as biomarkers for assessing postmortem intervals in dogs was shown (Piegari et al., 2023). In recent years, the observations of Ukrainian researchers on corpse cooling have gained priority in determining postmortem phenomena, in particular, a correlation between destructive changes in internal organs and the time of death in cats (Serdioucov, Shkundia and Kruchynenko, 2023) and postmortem destructive changes in muscle tissue, in particular, the postmortem dynamics of skeletal muscles according to histomorphological and histochemical parameters of dogs and cats whose death occurred as a result of acute hypoxia in different environmental conditions (Yatsenko and Kazantsev, 2024).

Referring to the achievements of modern science, we can certainly state that the accumulated body of facts has contributed to the solution of some problems. However, it should be emphasized that the issue of finding expert criteria for the duration of the post-mortem interval is still relevant because due to the considerable number of factors that simultaneously affect the animal corpse and determine the development of post-mortem changes (environmental conditions, age and diseases of the animal, etc.), the researchers mentioned above had difficulties in establishing uniform criteria for the time of death.

Despite the numerous experimental studies on the biotransformation of animal corpses reported in scientific journals, modern veterinary medicine has a large amount of incomplete material, the generalization of which can be successfully implemented in the practice of forensic veterinary medicine. The authors of this publication believe that a systematic approach will greatly expand the possibilities for studying the postmortem processes of animal corpses and will allow them to be more thoroughly substantiated from the standpoint of evidence-based veterinary medicine.

The **aim of the study** is therefore to provide a comprehensive forensic veterinary evaluation of biotransformation phenomena in dog and cat corpses and to substantiate expert criteria for determining the duration of postmortem intervals.

Materials and methods. The prospective experiment was conducted for two years, from the middle of May 2021 to the end of May 2023, at the municipal veterinary medicine institution in Kharkiv, by observing and analyzing the phenomena of biotransformation of carcasses in the period from the onset of animal death to skeletonization and partial fragmentation of carcasses. The design of the experiment reproduced the meteorological environmental conditions in which animals die under non-obvious circumstances.

The objects of the empirical part of the study were 28 corpses of dogs (n = 16) and cats (n = 12). All corpses were divided into 4 experimental groups (n = 7 per group) according to the criterion of the immediate cause of death of the animal. Each group contained corpses with the following distribution: by taxonomic species (cats, n = 3; dogs, n = 4), sex (females, n = 3; males, n = 4), age (neonatal, n = 2; mature, n = 3; geriatric, n = 2). Group 1 included corpses of animals with sudden death due to cardiac pathology or poisoning; they were stored outdoors at a temperature of +18°C and 59% humidity. Group 2 included the corpses of animals that died from injuries and hyperthermia; they were placed in a tight plastic bag, buried in the ground, and stored at a temperature of +18°C and 64% humidity for 7 days. The death of animals in Group 3 was due to mechanical asphyxiation, in particular, aspiration with liquid during drowning, and the corpses were stored in tap water at a temperature of +2°C and humidity of 73%. Group 4 included the corpses of animals that died of general hypothermia; they were stored in thermal bags at a temperature of –18°C and humidity of 92%. Under the conditions of the experiment, after 7 days of postmortem interval, the corpses of the 2nd, 3rd, and 4th groups were placed in an open space at an average daily temperature of +18°C and relative humidity of 59% and no direct exposure to precipitation for 14 days. Further, the variability of temperature and relative humidity was not

taken into account. After reaching the cadaveric 'plateau', the dynamics of biotransformation and features of succession by necrophilic entomofauna in all groups were documented once a month until the end of the observation period.

The time ranges of supravital reactions and early and late cadaveric changes in the corpses of animals of Group 1 were recorded separately. Supravital reactions were determined in a certain sequence: first, the 'pupillary' reactions to atropine and pilocarpine of the iris muscles were studied, then the 'idiomuscular' reaction of *m. quadriceps femori* to mechanical irritation after impact with an oblong object with a narrow surface. With a syringe with a thin, fixed needle, 0.1 cm^3 of 1% solution of *atropini sulfati* (Darnitsa, Ukraine) was injected into the anterior chamber of the eye of cats every 6 h during the day, followed by 0.1 cm^3 of 1% solution of *pilocarpini hydrochloridi* (Darnitsa, Ukraine), and similar solutions were injected into the eye of dog corpses in the reverse sequence. The height of the muscle roller of the lateral surface of *m. quadriceps femori*, which was formed after a transverse blow with the back of a large sectional knife. Mercury (Medicare, Ukraine) and electronic (Medicare, Ukraine) thermometers with a measuring range of 35.0–42.0°C were used to observe the dynamics of cooling of dog and cat corpses; the sensors were immersed through an artificial hole in the lateral abdominal wall in the projection of the left or right lobe of the liver at an angle of 75° to the segmental plane; the process of cooling the skin surface of animal corpses was visualized using a *ULIRvision TI120* non-contact thermal imaging camera (China).

The onset and development of rigor mortis was determined by touch by the presence of complete contracture of the muscles of the animal carcasses' cervical, thoracic, and pelvic limbs. For the objective examination of the liver, the method of observing the disappearance, pallor, recovery, or absence of a color change of the cadaveric spot when pressing on its visual center and recording the time of color change with a stopwatch was used. For metrological examination of the carcasses, the longitudinal (linea median anterior from the first cervical vertebra to the first caudal vertebra) and circumferential dimensions of the trunk (in the mid-chest and neck region), neck (in the mid-region) and limbs (in the thigh and forearm region) were measured daily for 10 days after the onset of death of the animals using a tape measure (Ukraine).

For the cytomorphological study of the dynamics of destruction of the formative elements and the growth of the number of bacterial colonies in the blood, samples of unclotted blood from the heart chambers of cats and dogs of Group 1 were taken within 48 h after death every 6 h by the vacuum method in *Vacusera* tubes (Ukraine) with a K_3EDTA capillary. Smears were made from it, stained by Romanowsky–Giemza, and, using an optical microscope *Granum R50* (China), blood cytograms were examined at a field of view of ×1000 (Zaporozhan et al., 2002). A forensic veterinary necropsy was performed by the method of partial evisceration. For histomorphological assessment of the severity of liver biotransformation in Group 2 animals, 1 cm^3 samples were used, slides were made, stained with hematoxylin and eosin, and examined at ×400 microscope magnification using the standard method (Horalskyi, Khomych and Kononskyi, 2015). The informative areas were photographed using a digital nozzle *ToupCam UCMOS03100KPA* (China) integrated with the microscope. The obtained information was processed on a personal computer using the software package *'Photo Frame Studio 3.0'*. The definition of 'patterns' in this study refers to any signs of macroscopic decomposition of the animal corpse or elements of microscopic tissue destruction, which were described in the obtained cytograms and histotopograms according to the recommendations of Ressel (2017) and Raskin, Meyer and Boes (2022).

Photogrammetry was carried out using a forensic ruler designed for large-scale photographic recording of objects. The forensic veterinary examination of animal corpses was carried out in four stages: preparatory (preliminary examination), analytical (separate examination), comparative, and synthesizing, which help to trace and evaluate the results obtained when analyzing the expert's opinion (Yatsenko, 2022).

Results and discussion. Our clinical practice has identified a sequential stage that precedes the terminal state of animals: the pre-agonal state, terminal pause, and agony. Our findings indicate that hypoxia is the primary thanatogenetic factor in the onset of terminal states, while circulatory hypoxemia is the initial trigger in the process of thanatogenesis. It is recognized that the causes of terminal circulatory hypoxia can vary. However, refractory disorders of the vital triangle organs (cardiac paralysis, respiratory arrest, and cessation of brain function), which are documented during terminal conditions in animals, should be considered probable signs of death. These conditions directly or indirectly result in clinical death.

We argue that immediately after the onset of biological death, in the early postmortem period, it is advisable to differentiate between the processes inherent in a living animal that cause supravital reactions and those that occur in the postmortem period and cause cadaveric phenomena. In our opinion, the degree of severity of certain supravital reactions and cadaveric phenomena is due to the prevalence of a certain stage of the terminal state.

It is necessary to focus on defining the 'early postmortem period' itself because the literature analysis revealed controversial and sometimes contradictory statements. Therefore, if we consider cadaver autolysis to be intermediate between early and late cadaveric phenomena, then the duration of the early postmortem period should be understood as a period not exceeding 24 h after the onset of death because according to the results of microscopy of parenchymal organs of cat corpses (Kazantsev and Yatsenko, 2021) and dog corpses (Yatsenko and Kazantsev, 2022) proved that the degree

of decomposition after 24 h of the postmortem period does not allow the use of the cytomorphological method to monitor the further biotransformation of the animal corpse at the cellular level. In this regard, the authors of the study evaluated certain cadaveric phenomena and supravital reactions to establish the time range of diagnostic informativeness of the postmortem interval from the moment of biological death of the animal. There is no doubt that damage to corpses, in particular as a result of cannibalism (Fig. 1), and cadaveric changes that are noted by a veterinary specialist by examining the animal corpse at the place of its discovery (Fig. 2) or removal (Fig. 3) the cadaveric bed, during the analysis of the case file, based on the results of a comprehensive examination of the corpse using additional research methods, are the basis for modeling an expert hypothesis about the cause of death and, further, determining the syndromic forensic veterinary diagnosis currently used in expert practice (for example, profuse bleeding, chemical poisoning, pulmonary edema, etc.)

Given the above, we focus on the early phenomena of biotransformation, in particular, cadaveric rigor mortis, cadaveric desiccation, cadaveric staining, and cadaveric cooling. The phenomenon of postmortem rigor mortis is detailed by the authors of this publication in a prospective study of skeletal muscles of the neck of dog and cat corpses (Yatsenko and Kazantsev, 2024). It is associated with the denaturation of actin and myosin in myofilaments and the subsequent redistribution of calcium ions in them. It was noted that immediately after the death of the animal, a state of atony was recorded in all skeletal muscles, however, in 2–6 h after death, they contracted, became denser, and had passive movements due to postmortem contracture in the joints were not reproduced. The pace and intensity of such phenomena were more pronounced in the corpses of animals of Group 2, because, probably, as a result of damage to the brain stem due to general hyperthermia, rapid processes of postmortem contraction of myofilaments occur. Postmortem contracture of the joints of animal corpses of all groups started in the form of a sequential contraction in the direction from the masticatory muscles, neck, thoracic, and pelvic limbs to the skeletal muscles of the trunk with a peak 12 h after death, then gradually disappeared in a similar sequence and was not observed after 72 h of postmortem interval. It should be emphasized that the least pronounced variant of rigor mortis was recorded in the corpses of animals of Group 1 with a short agonal period and neonatal animals, and the most pronounced — with well-developed skeletal muscle. When moving the cadaver by dragging the thoracic limbs, we found a discrepancy in the degree of rigor mortis compared to the joints of the pelvic limbs, which remained completely immobile.

The early absolute signs of biotransformation of the corpse were confirmed: the phenomena of drying and cornea opacity (Fig. 4) and cadaveric spots (Fig. 5), which occurred immediately after the onset of death.

Undoubtedly, the phenomenon of drying out of the animal corpse is caused by the evaporation of moisture from the surface of the general cover. Signs of cadaveric desiccation were recorded on the cornea, which was not covered by eyelids, in the corpses of animals of all groups except Group 4, indicating that they could not be formed in a humid environment. 6 h after death, the formation of irregular triangular spots of grayish-brown color (Larsche's sign) was observed on the cornea, while the sclera remained shiny.

In fact, the formation of cadaveric spots and hypostases in the internal organs of animals is associated with myocardial paralysis, which results in blood flowing through the vessels to the lowest parts of the body. A certain sequential stage in the development of cadaveric spots has been established. The hypostasis stage began 1–3 h after death and lasts up to 12 h. When pressing on the red center of the spot, the color disappears but is fully restored after 1 second. In animals that were moved or changed position, the spots moved to other parts of the body where they could not form naturally. The stasis stage lasted in the range of 12–24 h after the death of the animal. It is obvious that the plasma of liquid blood from the blood vessels impregnated the adjacent tissues, concentrated over time, and after pressing on the center of the spot, partially disappeared and recovered more slowly within 3 seconds. The stage of imbibition was most pronounced in the period of 24–48 h after death. It was found that when pressing on the center of the spot in the third stage, its color did not change, and the rate of onset of the stage depended on the surrounding temperature in the following way: in the low-temperature regime, the rate of onset of imbibition increased. It is important to emphasize that in the corpses of animals of Group 1, spots of intense blue color with diffuse localization were recorded, but in the corpses of animals of Groups 3 and 4, on the contrary, they were pinkish-red. During the forensic veterinary autopsy of the animals, the formation of cadaveric spots in the viscera was observed as a result of postmortem redistribution of blood to the lowest parts of the viscera, which, in our opinion, is the cadaveric norm, but, taking into account the information in the case file, requires differentiation from pathological conditions.

As for the corpses of animals of Group 3, during prolonged exposure to water, in cases of intentional drowning, fatal injuries, throwing the corpse into the water immediately after death, etc., we noted permanent non-random signs in the form of pink-red cadaveric spots and epidermal maceration at 24 h postmortem. In all corpses of animals that died as a result of drowning, we recorded congestive venous hyperemia and severe pulmonary emphysema, pulmonary vasodilation, rupture of the alveolar walls, alveolar edema, the presence of alveolar transudate, multifocal intra-alveolar hemorrhages, which was confirmed by histomorphological examination.

Figure 1. Fragment of a dog corpse with injuries caused by cannibalism.

Figure 2. Dog corpse at the place of its discovery with postmortem pigmentation.

Figure 3. The corpses of a dog and a cat immersed in tap water containers.

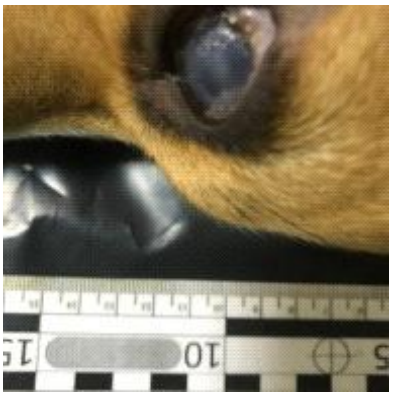


Figure 4. Corneal opacity with Larsche's sign in the medial angle of a dog corpse.

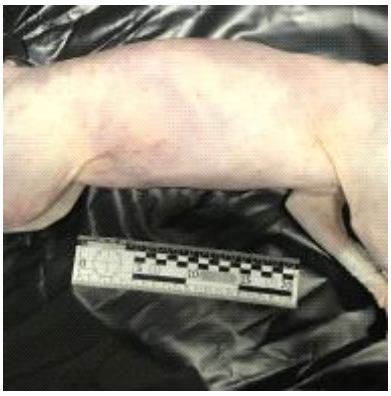


Figure 5. Postmortem spots on a cat corpse 12 h after death.

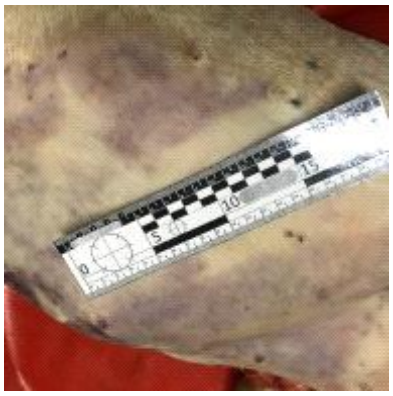


Figure 6. Postmortem stains on a dog corpse 24 h after death.

Regarding the corpses of animals of Group 4, it should be emphasized that freezing of the corpse can occur only if it has been exposed to temperatures below 0°C for a certain period. In this regard, we believe that the freezing of a corpse and the death of an animal from hypothermia are not identical phenomena, and therefore have specific signs. Freezing causes postmortem artifacts in the form of gross soft tissue tears and cracking of the bone sutures of the skull, which must be differentiated from intravital injuries.

Obviously, cadaveric cooling is associated with the inhibition and cessation of heat production and a gradual decrease in body temperature due to the prevailing heat transfer. According to the images of the thermal imaging camera, the cooling of the surface occurs unevenly, both in the corpses of cats (Fig. 7) and dogs (Fig. 8). For example, the highest temperature peaks are recorded in areas with dense soft tissue, in particular, hypodermis and skeletal muscle.

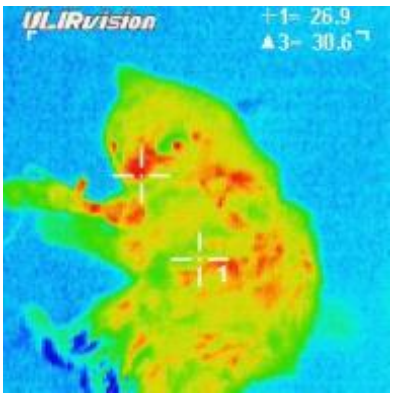


Figure 7. Thermography of the surface of a cat corpse with a thermal imaging camera.

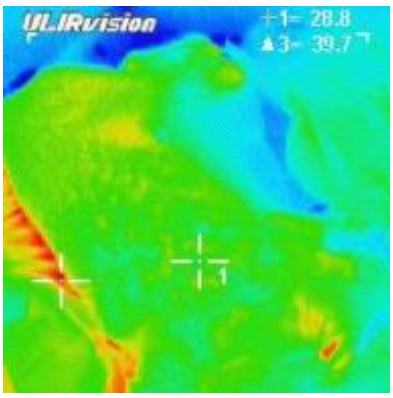


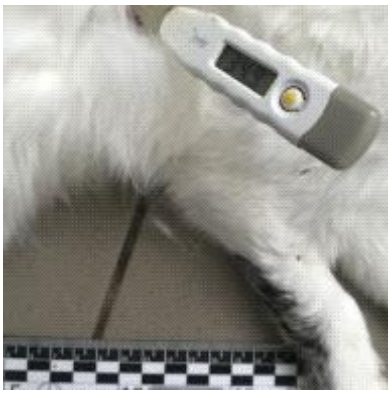
Figure 8. Thermography of the surface of a dog corpse with a thermal imaging camera.

During the terminal pause, a significant increase in temperature up to 40.0°C was recorded in the animals.

According to our data, the liver temperature of the corpses of animals in Group 1 and the environment finally equalized after 27 h postmortem. The results of hepatic thermography show that the temperature of the corpse decreased steadily by 3.0°C in the first hour after the death of the animal. At 2–3 h after death, the decrease in temperature level was about 1.0°C, in the range of 3–18 h — from 0.5°C to 1.0°C, then in the following hours — decreased by 0.1°C every hour until the end of the observation period. The liver temperature of the cat carcasses was measured with electronic (Fig. 9a) and mercury (Fig. 9b) thermometers. The liver temperature of the dog carcass was measured with a mercury thermometer (Fig. 10). The temperatures were not significantly different, but were higher than on the skin surface.

It has been shown that after the transverse impact of the *quadriceps femori* of animal corpses with the back of a large sectional knife in the early postmortem period, an 'idiomuscular' roll was formed (Fig. 11). In the first 2 h it was pronouncedly high, appeared and disappeared quickly, in the period of 2–6 h it was low, appeared and disappeared more slowly than in the previous period, and 6–8 h after the onset of the animal's death it appeared only in the form of a focal thickening at the site of mechanical irritation.

As for the reaction of the pupil to the injection of pilocarpine (Fig. 12) into the anterior chamber of the eye in the form of constriction and its reverse dilation after atropine (Fig. 13), it should be noted that such reflexes persisted for 24 h after death, but the duration of the reactions slowed down every 6 h.



a

b

Figure 9. Hepatic thermometry of a cat corpse: a — electronic thermometer; b — mercury thermometer.

Figure 10. Hepatic thermometry of a dog corpse with a mercury thermometer.

Figure 11. 'Idiomuscular' reaction of *m. quadriceps femori* of a dog corpse after mechanical stimulation.

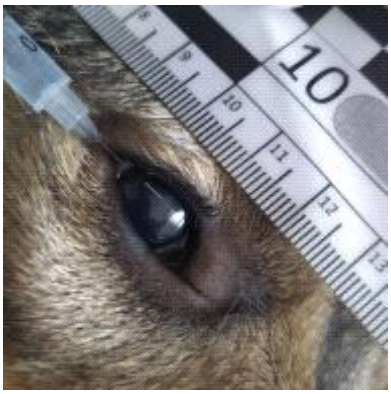


Figure 12. Pupillary reaction of a dog corpse after pharmacological stimulation with pilocarpine hydrochloride.

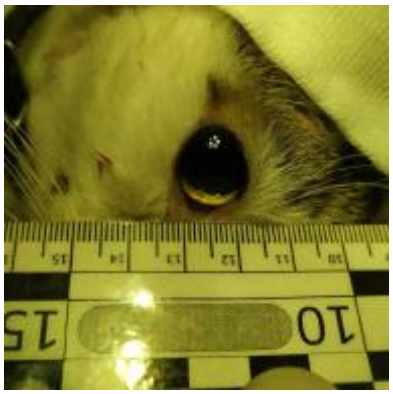


Figure 13. 'Pupillary' reaction of a cat corpse after pharmacological stimulation with atropine sulfate.

As for the cytomorphologic examination of the blood of cats of Group 1, in 6 h after their death, the formative elements look morphologically typical for the taxonomic species of animals. No structural changes were visualized in erythrocytes and segmented neutrophils (Fig. 14).

12 h after the onset of death, the erythrocyte membrane remains unchanged. However, changes are observed in the structure of leukocytes. Thus, some of them show changes in the shape of the nuclei in the form of karyopyknosis and condensation of nuclear chromatin (Fig. 15).

18 h after the death of the cats, the nuclear membrane of the erythrocytes remained unchanged. The number of altered leukocytes visually increased compared to the previous observation time (Fig. 16).

Twenty-four hours after the onset of death, all identified leukocytes were morphologically altered. The destruction of the leukocyte nucleus in the form of karyolysis enhanced cytoplasmic degranulation. The tinctorial properties inherent in the granules also change, which looks like an atypical staining according to the standard method. The nuclear membrane of erythrocytes remains unchanged (Fig. 17).

In the postmortem interval of 24–30 h after the death of cats, there are pronounced processes of destruction of blood cells, which break down into fragments, and debris is formed, which becomes a nutrient medium for the colonization of saprotrophic bacteria (Fig. 18).

36 h after the death of cats, the number of cellular debris visually increases, and rod-shaped bacteria form colonies (Fig. 19).

Blood cytograms of cat corpses obtained in the interval of 42–48 h after death do not visually differ from each other (Figs 20, 21) and are represented by patterns of residual decomposition of formative elements and colonies of polymorphic bacteria.

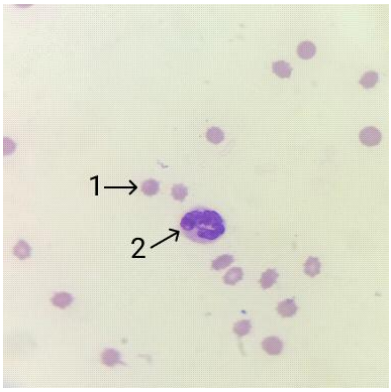


Figure 14. Blood cytogram of a dead cat 6 h after death; ×1000, Romanovski–Giemsa staining. 1 — erythrocyte without noticeable changes in shape; 2 — segmented neutrophil.

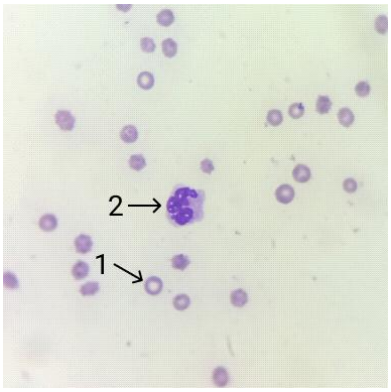


Figure 15. Blood cytogram of a dead cat 12 h after death; ×1000, Romanovski–Giemsa staining. 1 — erythrocyte without noticeable changes in shape; 2 — leukocyte with an altered nucleus.

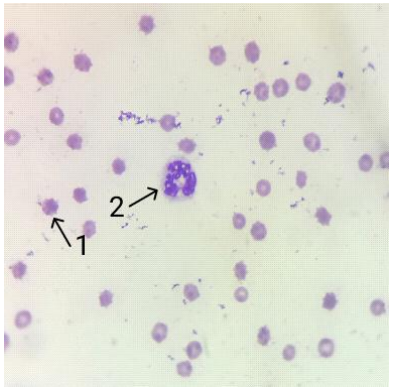


Figure 16. Blood cytogram of a cat corpse 18 h after death; ×1000, Romanovski–Giemsa staining. 1 — erythrocyte without noticeable changes in shape; 2 — leukocyte with changes in the nucleus.

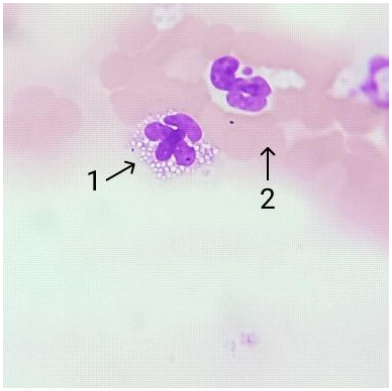


Figure 17. Blood cytogram of a dead cat 24 h after death; ×1000, Romanovski–Giemsa staining. 1 — a leukocyte with changes in the tintorial properties of cytoplasmic granules; 2 — accumulation of red blood cells without noticeable changes.

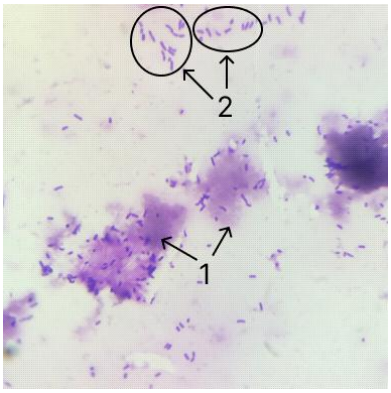


Figure 18. Blood cytogram of a cat corpse 30 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

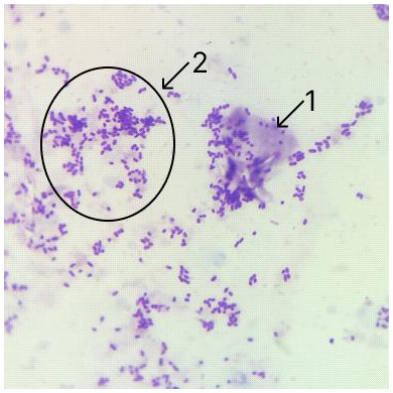


Figure 19. Blood cytogram of a cat corpse 36 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

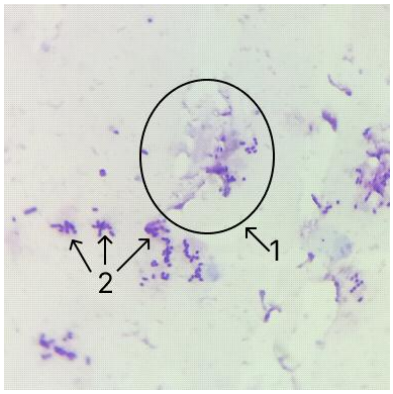


Figure 20. Blood cytogram of a dead cat 42 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of polymorphic bacteria.

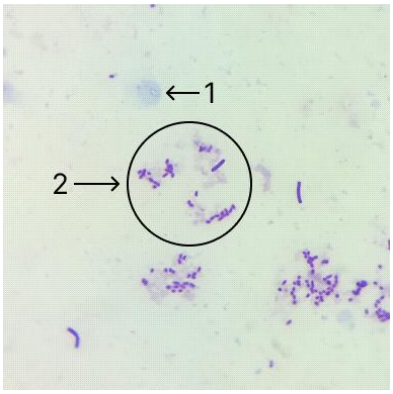


Figure 21. Blood cytogram of a cat corpse 48 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of polymorphic bacteria.

On blood cytograms of corpses of dogs of Group 1 in 6 h after the onset of death, the blood cells look morphologically typical. There are no visual changes in thrombocytes, segmented neutrophils, and monocytes (Fig. 22). 12 h after the onset of death, structural changes in white blood cells are visualized. Thus, karyopyknosis and nuclear chromatin dyscomplexity are observed in almost a quarter of the studied rod-shaped neutrophils. The nuclear membrane of erythrocytes remains without noticeable structural changes (Fig. 23).

The nuclear membrane of erythrocytes of dog corpses 18 h after death remains unchanged. However, the cytograms of white blood of dog corpses, compared to the previous period, indicate a rapid increase in the number of leukocytes with signs of nuclear destruction in the form of karyolysis (Fig. 24).

The study of the red blood picture 24 h after the onset of death of dogs showed no noticeable structural changes in erythrocytes. However, compared to the previous observation interval, all leukocytes were found to have a certain structural disorganization. Thus, some of the identified leukocytes contained nuclei in a state of rhexis. The nuclei of other white blood cells were in a state of total fragmentation (Fig. 25).

In 30 h after the death of dogs, there are pronounced processes of destruction of blood cells, they break down into fragments, forming debris, which becomes a nutrient medium for colonization by saprotrophic bacteria (Fig. 26).

In the postmortem interval of 30–36 h after the death of dogs, the number of cellular debris visually increases, and rod-shaped bacteria form colonies (Fig. 27). The blood cytograms of dog corpses obtained in the time interval of 42–48 h from the moment of death do not visually differ from each other (Figs 28, 29) and are represented by patterns of residual decomposition of cellular elements and colonies of rod-shaped and coccal forms of bacteria.

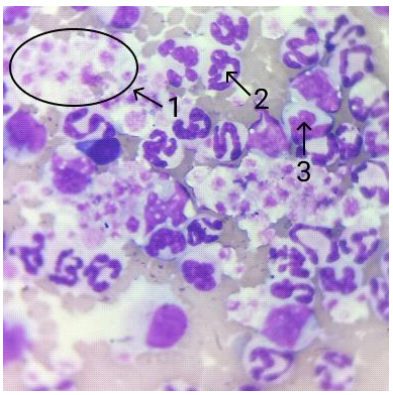


Figure 22. Blood cytogram of a dog corpse 6 h after death; ×1000, Romanovski–Giemsa staining. 1 — the shape of thrombocytes in clusters is not changed; 2 — segmented neutrophil without noticeable morphological changes; 3 — monocyte with preserved shape.

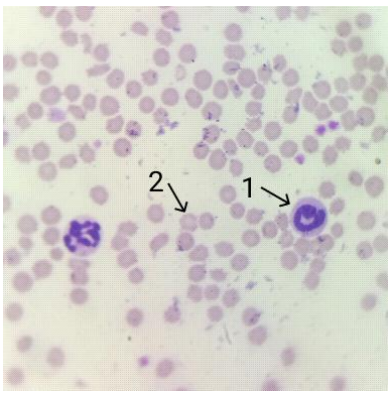


Figure 23. Blood cytogram of a dog corpse 12 h after death; ×1000, Romanovski–Giemsa staining. 1 — a rod-shaped neutrophil without noticeable changes in shape; 2 — a morphologically unchanged erythrocyte.

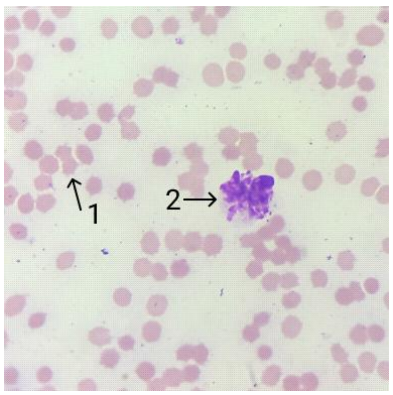


Figure 24. Blood cytogram of a dog corpse 18 h after death; ×1000, Romanovski-Gimza staining. 1 — erythrocyte without noticeable structural changes; 2 — leukocyte with changes in the nucleus.

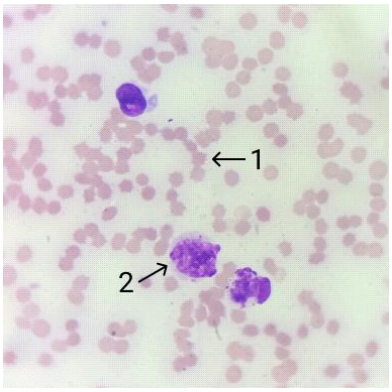


Figure 25: Blood cytogram of a dog corpse 24 h after death; ×1000, Romanovski–Giemsa staining. 1 — morphologically unchanged erythrocyte; 2 — leukocyte with changes in the nucleus.

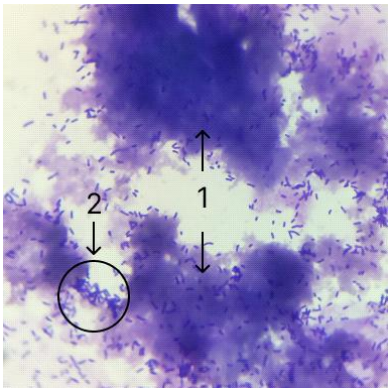


Figure 26. Blood cytogram of a dog corpse 30 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

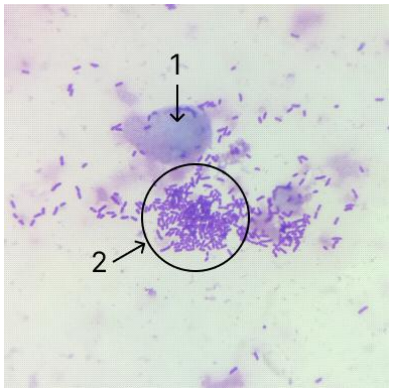


Figure 27. Blood cytogram of a dog corpse 36 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

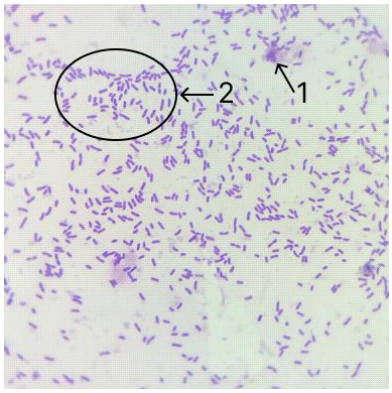


Figure 28. Blood cytogram of a dog corpse 40 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

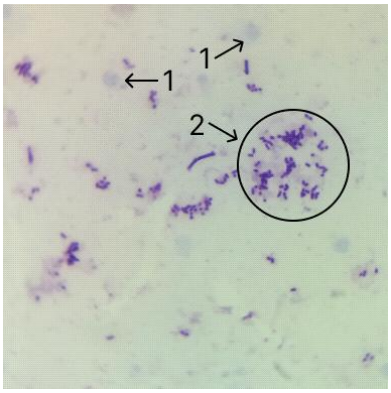


Figure 29. Blood cytogram of a dog corpse 48 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of polymorphic bacteria.

It has been established that cadaveric autolysis begins within a day after the onset of animal death and is further observed simultaneously with late cadaveric changes, and therefore probably occupies an intermediate position between early and late postmortem phenomena, catalyzing the development of the latter. Postmortem tissue autolysis, for example, in the pancreas, causes its enzymatic melting, which macroscopically looks like pancreatic necrosis, autolysis of the convoluted tubule epithelium — like necronephrosis, and myocardial autolysis — like intramural infarct.

It has been empirically proven that late cadaveric phenomena include those that lead to pronounced changes in appearance, in particular, a characteristic feature of late biotransformation is a change in the color of the animal's corpse. The skin acquires a dark, 'metallic' luster. Such cadaveric pigmentation begins with the transformation of primary cadaveric spots, at an average daily temperature above 18.0°C and after 48–72 h at a temperature of 10.0°C, and then, in the abdomen, in places where the intestines come into contact with the abdominal wall, a 'putrid venous network' of dirty green color is formed over time. The skin, due to the uneven drying of its parts due to the presence of a hair coat, becomes dense to the touch and looks like parchment.

Let's highlight the late postmortem phenomena: putrefaction, skeletalization, and fragmentation. It is known that putrefaction is the process of decomposition of protein substances in the corpse under the influence of the vital activity of various microorganisms. Along with the color change, the corpse's volume increased due to the formation and distribution of gases in certain body cavities, confirmed by the relevant metric studies of the dog (Fig. 30) and cat corpses (Fig. 31).

Microscopically, putrefaction of animal corpses is characterized by disorganization of the parenchyma, destruction of stromal elements, formation of cavities of different diameters filled with gases, focal accumulation of bacterial colonies, which are visualized around the vessels during light microscopy, confirming the vascular mechanism of microflora dissemination.

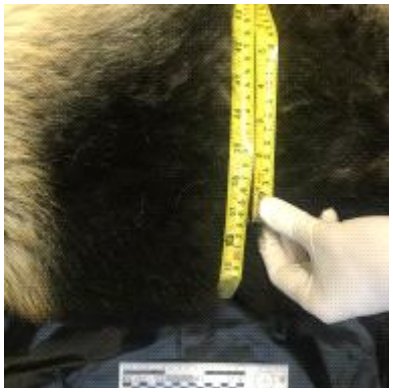


Figure 30. Girth measurement of the chest of a dog corpse.

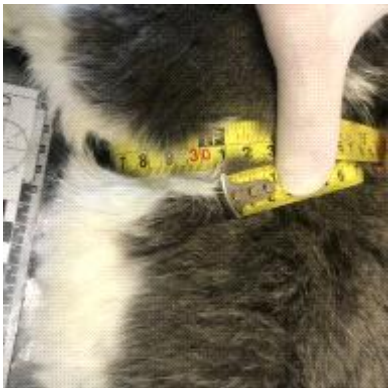


Figure 31. Girth measurement of the chest of a cat corpse.

The most representative phenomena of putrefactive biotransformation were found in the corpses of animals of Group 2. After digging up the animal corpses from the soil a week after the onset of death (Fig. 32), a pungent putrefactive odor was immediately felt, which was released through natural openings.

After the corpses were excavated a week later, macroscopic examination revealed localized 'saponification' in the head area of the cat (Fig. 33) and dog (Fig. 34) and dirty green in the abdomen, apparently as a result of the formation of sulfhemoglobin in it; then a putrid venous network is formed, which may be caused by the contamination of blood vessels by bacteria from the liquid fraction of blood; cadaveric emphysema, which occurs as a result of the release of gases by anaerobes with the formation of blisters in the hypodermis and internal organs, which is most pronounced in the liver.

The external examination of the dog's corpse revealed its unnatural position (Fig. 35), and an X-ray examination of the abdominal organs revealed the presence of a foreign body with a 'metallic' density (Fig. 36). The forensic veterinary autopsy illustrated the total blood imbibition of soft tissues and internal organs, and due to explosive gas production by anaerobes, the intestinal loops were visually distended (Fig. 37). As a result of putrefactive emphysema, gases accumulated in the subcutaneous fatty tissue and swelled it. It was noted that simultaneously with the bloating of the corpse, the epidermis of the skin was raised by gases, blisters filled with dirty bloody liquid were formed.

An X-ray examination of the cat's corpse revealed fragmentary pulmonary atelectasis and accumulation of free fluid (Fig. 38), which was further confirmed by a forensic veterinary autopsy (Fig. 39).

It is shown that the methods for estimating the postmortem interval based on histological changes in the animal corpse demonstrate the possibility of their application in the late postmortem interval, when most of the currently used methods cannot be informative. We have noted the possibility of determining such changes 72 h after the onset of death. At the same time, we emphasize that autolysis and progression of biotransformation of cadaveric material cause uninformative histomorphological examination in late postmortem intervals. However, despite the impossibility of using this type of research in every forensic case, in early cadaveric changes, such additional studies contribute to the identification of microscopic lesions in the absence of visible macroscopic patterns and narrow the range of differential diagnoses.

Figure 32. A container with sand in which animal corpses were buried.

Figure 33. A fragment of a cat's corpse after being dug up a week later.

Figure 34. A fragment of a dog's corpse after being dug up a week later.

Figure 35. Appearance of a dog corpse after excavation 7 days after death.

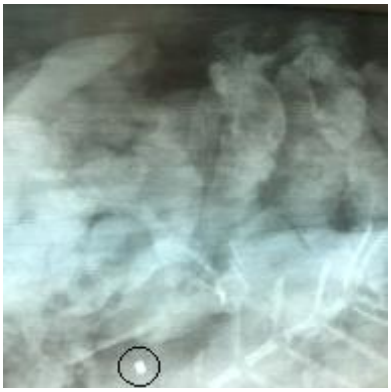


Figure 36. Overview X-ray in LL projection of the abdominal organs of a dog corpse excavated from the ground 7 days after death (a foreign object with a 'metallic' density is outlined).

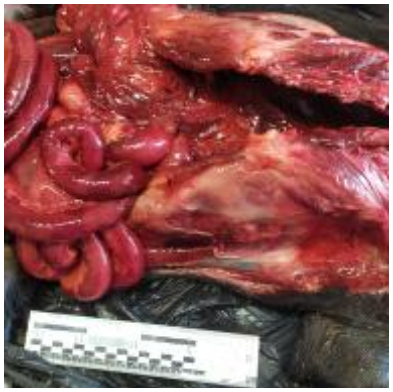


Figure 37. Internal examination of a dog corpse excavated from the soil 7 days after death.

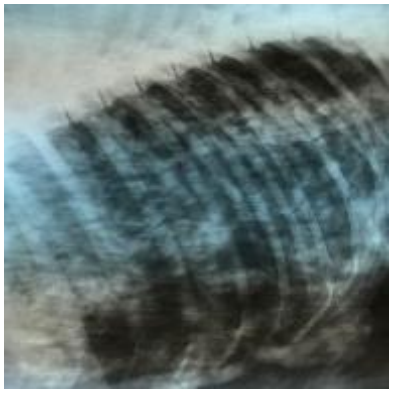


Figure 38. Overview X-ray in RL projection of the chest organs of a cat corpse excavated from the ground 7 days after death.

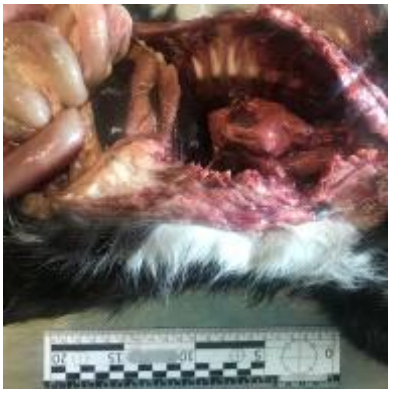
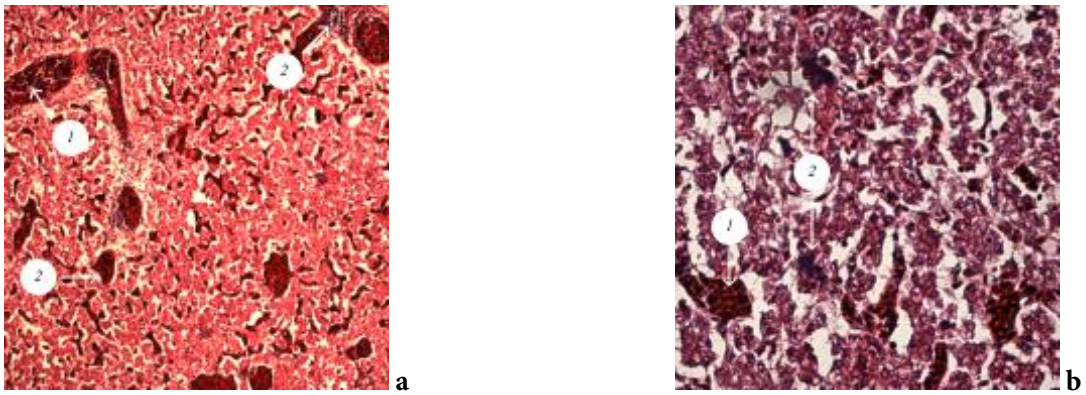


Figure 39. Internal examination of a cat corpse excavated from the soil 7 days after death.

Thus, to establish the presence and assessment of pathological changes in organs and tissues caused by violent acts and/or diseases, to determine the vitality and prescription of bodily injury, and to resolve other issues related to the determination of the microscopic structure of animal organs and tissues, we consider it necessary to conduct additional cytological/histological studies using special staining that is consistent with the principles of evidence-based veterinary medicine. It is advisable to carry them out taking into account the goal set during the forensic veterinary examination of a corpse or its fragments, and the results must be taken into account when drawing up an expert opinion, which traces the logical connection between the analytical and synthesizing parts.

We suggest that the text describing the pathological changes should be placed in the research part of the examination, the detected microscopic changes should be described in the state language, systematized into a forensic veterinary histological diagnosis according to the pathogenetic principle using the syntax of pathomorphological nomenclature, and histotopograms illustrating the conclusion should be attached in the form of photo tables. For example: 'Examination of liver histopreparations of a cat and dog corpse was performed in light transmitted under a Granum R50 microscope with an image magnification of ×400. Using a ToupCam UCMOS03100KPA digital camera installed on the microscope and the Photo Frame Studio 3.0 software, 2 histotopograms (2 samples, 2 sections) were made, reproductions of which with appropriate markings and explanatory captions are shown in the photo table (Fig. 40). Forensic-veterinary histologic diagnosis: (a) smoothed histoarchitectonics of the liver, (b) hydropic dystrophy of hepatocytes, (c) capillary hyperemia, (d) bacterial colonies'. Late changes in the corpse actually appear immediately after death, but they progress more slowly, appear later and lead to the destruction of the corpse.

It was found that after 7 days the corpses of animals putrefy — a kind of rotting in conditions of sufficient air and moderate humidity. The process of decomposition is more intensive than ordinary decomposition, with more complete oxidation, and is accompanied by the formation of a relatively small amount of gases with an unpleasant odor.



a b

Figure 40. Histotopogram of the liver 72 h after animal death: a — cat corpse, b — dog corpse; ×400, hematoxylin and eosin staining; 1 — hyperemia of hepatic hemocapillaries, 2 — bacterial colonies.

In our opinion, the general drying of the corpse is an important prerequisite for its 'conservation', which depends on a set of certain natural conditions, in particular, humidity, temperature, mineral composition of the soil, etc.

Corpse decomposition processes are related to the vital activity of bacteria, fungi, plants, and animals, particularly insects, and can be caused by insects, rodents, and predators.

Seasonal dynamics of carcass decomposition under the influence of entomofauna were observed. In the second half of May, the carcasses were massively colonized by blue flesh flies, and in June by green flies. The predominant localization of larvae in the oral cavity is the mucous membrane of the tongue (Fig. 41a) and the inner surface of the cheeks. At the same time, the mucosa becomes darker due to desiccation (Fig. 41b), and the number of larvae gradually decreases. It was found that dipterans were the first to inhabit the carcass, and as the number of their larvae increased, predatory species of beetles that destroy fly larvae appeared. Larvae of beetles and adult beetles appeared on the corpse and accumulated mainly in its location. Representatives of Hymenoptera — ants were found on the corpses from the first days and throughout the observation period. The first egg-laying flies on such carcasses appeared later. On the carcasses of the animals of Group 1, egg-laying flies appeared on the 4th day of observation. However, in the summer, dipteran oviposition was much more frequent.

As for dog carcasses, the predominant localization of blue and green fly larvae is the mucous membrane of the tongue (Fig. 43a) and the inner surface of the cheeks. At the same time, the mucous membrane also gradually changes color to darker due to drying (Fig. 43b), and the number of larvae gradually decreases.

The succession of necrophilous insects had similarities and significant differences. The common feature was that two-winged flesh flies were the first to discover the carcasses. Obligate species of necrophagous beetles — the notched-wing scavenger and the red-breasted carrion beetle — were observed at similar times. The differences found in the keratophagous group under normal decomposition conditions appeared on the corpses on days 10–14 of the observation (Figs 42, 44).

By studying the intervals of decomposition of dog and cat corpses in different states, no differences in the timing of the onset of biotransformation phenomena were found. Thus, the process of biotransformation of the animal corpse occurs through a period of microbial decomposition, which occurs from the moment of death to the development of putrefactive emphysema (from 1–2 days to 1–5 weeks): (a) 'fresh' corpse — up to 2 h; (b) early cadaveric changes (up to 2–3 days); (c) early putrefactive changes — the appearance of cadaveric green and putrefactive venous network; (d) putrefactive gigantism of the corpse. Then comes the period of active destruction of the corpse by insects (from 15–20 days to 2 months): (a) early destruction of soft tissues, mainly due to fly larvae; (b) late destruction of soft tissues, during the activity of necrophage beetle larvae (developmental duration — 30–45 days) and predator beetles (developmental duration 45–65 days), which ends with almost complete destruction of soft tissues. At the end of this interval, which we propose to consider the 'plateau' period, all corpses acquired the same appearance.

It has been empirically proven that incomplete skeletonization of a corpse (Fig. 45) lasts until the end of the warm season and can continue into the next year in winter. As for the complete skeletonization of the corpse, it has been shown that it can last for years and end in fragmentation and complete destruction of the organic and mineral bone base (Fig. 46).

We believe that the forensic veterinary examination of a skeletal corpse under certain conditions allows us to determine the possible cause of death in case of severe bone trauma, the approximate time of death. At the same time, it is necessary to clearly differentiate the signs of biotransformation from bodily injuries that occurred during the movement and transportation of the corpse to the autopsy hall of a specialized expert institution, fragmentation of the body caused by accidents during transportation trauma, dismemberment of the corpse by predators, etc.


a


b

Figure 41. A fragment of a cat's corpse on the 4th day after death: a — drying of the mucous membrane of the tongue; b — pigmentation of the scalp.

Figure 42. The corpse of a cat 10 days after death.


a


b

Figure 43. Fragment of a dog corpse on the 4th day after death: a — drying of the tongue mucosa; b — pigmentation of the scalp.

Figure 44. Dog corpse 10 days after death.

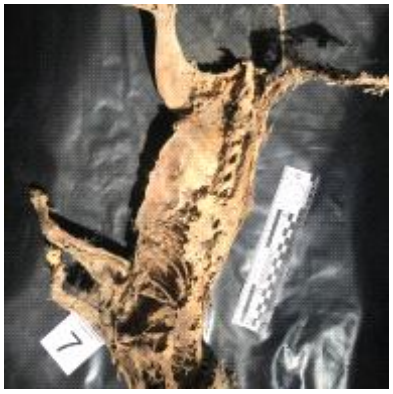


Figure 45. Skeletonization and fragmentation of a cat corpse 6 months after death.

Figure 46. Skeletonization and fragmentation of cat and dog corpses 12 months after death.

Conclusions. Morphological signs of early phenomena of biotransformation of animal corpses were detected. Postmortem joint contracture started in the direction of contraction from the masticatory muscles to the skeletal muscles of the trunk with a peak 12 h after death, then gradually disappeared in a similar sequence and did not recover after 72 h of postmortem interval. 6 h after the death of the animals, the formation of irregular triangular spots of grayish-brown color (Larsche's sign) was observed on the cornea. A certain sequential stage in the development of cadaveric spots was established: hypostasis, stasis, imbibition, and their time ranges were identified. The cooling of the corpse took place in the form of a gradual decrease of the temperature in the first hour after death by 3.0°C, then, 2–3 h after death, the temperature level decreased by about 1.0°C, in the range of 3–18 h — from 0.5°C to 1.0°C, and in the following hours — by 0.1°C every hour

until the end of the observation period. The 'idiomuscular' roll was pronouncedly high in the first 2 h, appeared and disappeared quickly, in the period of 2–6 h it was low, appeared and disappeared more slowly, and in 6–8 h it appeared only in the form of focal thickening at the site of mechanical irritation. Pupillary reflexes were preserved for 24 h after death, but the duration of reactions slowed down every 6 h. The dynamics of putrefactive disorganization of venous blood of dog and cat corpses within 48 h after death was determined.

Cadaveric autolysis begins within a day after the onset of animal death and is observed simultaneously with late cadaveric changes. It has been shown that the decomposition of animal corpses is macroscopically characterized by specific pigmentation, blood imbibition of soft tissues, explosive gas formation of internal organs, and putrefactive emphysema. Microscopic patterns of disorganization of the liver of animal corpses are characterized by the destruction of the parenchyma, hydropic degeneration, and bacterial dissemination. The sequence of cadaveric succession by entomofauna is shown: blue flies, green flies, ants, and necrophagous beetles. Incomplete skeletonization of animal carcasses lasts until the end of the warm season, followed by complete skeletonization, which ends with fragmentation and destruction of bones.

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