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Medicolegal relevance of cadaver entomofauna for the determination of the time of death

M.I. Marchenko

pr.Obuchovskoi Oborona 21, 63, 193019 St. Petersburg C-19, St. Petersburg, Russia

1. Present state and trends in development of criteria of determining the time of death in late postmortem period

The optimum criteria for determination time of death in late postmortem period can be developed as a result of comprehensive study of the following processes: first of all there are regular changes occurring directly in the tissues and organs of a cadaver and on the whole completely depending on the specific properties of a particular cadaver. Analysis of over 50 Soviet papers devoted to the study of dynamics of postmortem processes in tissues and organs occurring later than 6 days or more after the moment of death has shown that

1. practically all organs and tissues were studied using biophysical, biochemical, histological, histochemical, neurohistological, spectrographic, spectrophotometric, physical, photometric, chemical, chromatographic, cytological and electronic microscopy methods;
2. studies were carried out mainly on isolated organs;
3. temperature conditions of experiments, when they were accurately specified, did not embrace the complete range of environment temperatures;
4. the majority of studies were done under artificially created conditions, which ensured only the putrefaction process;
5. studies of different tissues and organs by the same method naturally give dissimilar results;
6. the duration of supposed practical application of proposed methods varies considerably depending on the tissues and organs to be studied and on the experimental conditions;
7. studies related to dynamics of investigated processes in dead tissues under varying environmental conditions were not completed, and therefore, the regularities controlling the progress of said processes in time were not determined.

Thus, presently available materials of research works on postmortem changes occurring directly in tissues and organs of a cadaver (as a rule carried out during 3–4 years studies for theses) do not allow reconstruction of an integral picture of cadaver decomposition in its dynamics as consistent with the environmental conditions altered in time. Criteria based on the study of this group of cadaver changes as a rule require initial normal rate of one or other parameter to be known, and therefore, at the present stage normally are within the limits of laboratory studies and hardly suitable for wide practical application.

Secondly, there are processes regularly accompanying postmortem changes in tissues and organs of a cadaver, but developing according to their own specific laws independent of the properties of a particular cadaver. Those are such processes as decay of radioactive isotopes, which entered an organism during its lifetime; cadaver biological decomposition (microorganisms, mold fungi, insects); destruction and revival of plant life beneath and around the cadaver.

Difficulties in practical utilization of the results of studies of the first direction have forced experts in forensic medicine to apply once more to entomology, the fundamentals for applying which for the determination of the time of death were developed by Bergert et al., as early as 19th century. Recently, monographs of applied character were prepared, in UK — Smith [1], in Belgium — Leclercq [2] and in Finland — Nuorteva [3]. This direction is successfully developed in Austria — Reiter et al. [4–7], in Belgium — Leclercq and coworkers [8,9], in USA — Rodriquez and coworkers [10–12], Adkins [13], Catts [14], Greenberg [15], Hall [16], Haskell and Williams [17], Goff [18], Meek [19] and Morris [20], in Finland — Nuorteva [21], in Japan — Nishida and coworkers [22,23], in Italy — Introna et al. [24], in Germany — Benecke [68,69], in Russia (e.g., [60–67]) and in many other countries. Two bibliographic articles on forensic entomology have appeared [25,26].

Analysis of said editions proved forensic entomology to be an independent branch of applied entomology and a promising trend in solving the problem of identifying the time of death.

Scientific base of using entomological data in forensic medicine comprises:

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1. existence of necrobiont insects in nature, which utilize cadaver tissues and pass the major part of their life cycle on cadavers;
2. relative constancy and specificity of cadaver entomofauna in a particular geographical region comprising widely spread predominating species;
3. compliance of species composition of cadaver entomofauna to the degree of its tissues decomposition and to its location;
4. seasonal alterations of predominant necrobiont insect species;
5. beginning of insects' activity in spring and its end in autumn as a result of transition to diapause condition due to changes in temperature and light–time interval, the values thereof being dependent on geographical region and being specific for each species;
6. regulation of number of generations per vegetative period and of species' life cycle duration by strictly definite species-particular thermal parameters;
7. long preservation of insects chitin cuticles in nature.

At the present state of development of forensic entomology, main efforts should be concentrated on the research on flies' biology. Three leads can be distinguished:

1. identification of species, composition of fly association as well as of factors affecting the formation of above composition;
2. study of changes in characteristics of larval growth at different temperatures aimed at working out methods of determining at larvae development periods;
3. determination of thermal values regulating insects development, the obtained data to be further applied for a calculation technique used to determine the time when insects invaded a cadaver in retrospect.

2. Material and methods

2.1. Material and methods of field studies

Studies were carried out in 1971–1983 in the Leningrad region and Kaunas region of Lithuanian SSR on 211 subjects of which 197 had been purposefully delivered to the site of the experiment and 14 found in natural environments. There were 100 cadavers of big animals (mainly dogs), 106 of medium-size ones (cats, rabbits, suckling pigs), five of small animals (mole, pigeon, kittens). A total of 190 cadavers were obtained from the vivarium of the Kirov Military–Medical Academy after clinical studies and seven animals were killed directly before the experiment.

Subjects were placed in 13 biotopes of the above regions, nine of them are the principal ones: mixed forest, mixed underwood, bushes, clearing, hill slope, field, ravine, grass and Sphagnum swamps.

A total of 180 subjects were placed on the soil surface, of which 23 were hung at a height of about 1 m from the ground, seven subjects were buried in the ground to different depths and one subject was placed in unheated service room. Qualitative composition of subjects, their distribution of biotopes and starting date of experiments, as well as distribution of number of observations and experiment duration are given in Tables 1 and 2.

All the studies are subdivided into three groups according to the tasks solved:

Table 1
Distribution of subjects of inquiry according to biotope, year season, cadaver species and period of observations

N	Biotype	Number of cadavers (total)	Number of observations (total)	Animal species				
				Dog	Cat	Rabbit	Suckling pig	Others
1	Mixed forest	12	79	3	1	–	8	–
2	Underwood	11	195	5	2	4	–	–
3	Bushes	11	131	6	3	1	1	–
4	Clearing	15	106	2	3	2	2	6
5	Hill slope	12	287	11	–	–	1	–
6	Field	34	1046	20	5	8	1	–
7	Ravine	18	607	15	–	–	–	3
8	Grass swamp	17	650	10	1	5	–	1
9	Sphagnum swamp	12	355	9	3	–	–	–
10	Other biotopes[a]	11	35	3	5	–	–	3
11	Underwood[b]	23	903	9	5	8	I	–
12	Sphagnum swamp, clearing	27	198	–	12	15	–	–
13	River shore, clearing	6	36	4	2	–	–	–
14	Bushes, clearing	2	36	–	2	–	–	–
Total		211	4664	97	44	43	14	13

[a] Biotypes: town garden, river shore, canyon: 2000 m, room.
[b] Hanged cadavers.

Table 2
Duration of the experiment since the beginning of the experiment

N	Beginning of experiment (month)						Period of experiment of experiment (days)				
	3–4	5	6	7	8	9	Up to 25	Up to 50	Up to 100	Up to 150	Up to 200 and over
1	–	–	–	2	10	–	7	4	1	–	–
2	–	7	1	3	–	–	3	–	4	–	4
3	3	–	–	5	3	–	4	3	1	1	2
4	1	–	–	5	7	–	10	1	3	1	–
5	1	–	–	4	1	6	–	7	4	–	1
6	3	8	4	12	–	7	5	8	6	6	9
7	2	1	10	3	–	2	3	2	–	9	4
8	3	1	10	1	–	2	–	2	2	10	3
9	5	2	4	–	1	1	4	6	1		
10	–	3	2	2	2	2	9	2	–	–	–
11	4	1	13	–	1	4	–	5	1	9	8
12	–	–	27	–	–	–	27	–	–	–	–
13	–	2	–	–	4	–	–	4	–	2	–
14	–	–	2	–	–	–	–	–	–	–	2
Total	22	25	75	37	29	23	68	39	26	44	34

1. Test runs N 1–10 are devoted to the study of cadaver entomofauna and effect of biotope conditions on cadaver decay. They were carried out in the principal biotopes, with a particular test run corresponding to each one.
2. Runs carried out to study the effect of particular cadaver location (hung and buried) on insects, as well as effect of additional agents on cadaver tissues (clothes impregnated with chemicals, effect of flame).

   Run N 11–23 subjects, 903 observations — underwood (hung);

   Run N 12–27 subjects, 198 observations — Sphagnum swamp, clearing (clothing simulation);

   Run N 13–6 subjects, 36 observations — river shore, clearing (buried);

   Run N 14–5 subjects, 58 observations — Sphagnum swamp, clearing bushes (action of flame).
3. Using subjects of 14 principal runs a number of additional (questions were solved, which did not involve any special treatment of cadavers leading to changes in initial conditions of tests. These studies can be subdivided into six subgroups:

   ○ Specificity of tissue decay in cadavers placed under snow in early spring was studied on 22 subjects — 1191 observations;
   ○ Specificity of cadaver tissue decay in autumn season was studied on 21 subjects — 454 observations;
   ○ Temperature conditions of cadaver were studied on 56 subjects — 4472 measurements;
   ○ Role of ants in cadaver decay was studied on eight subjects — 60 observations;
   ○ Changes in cadaver remains and its underlying vegetation were studied on 52 subjects — 1748 observations;
   ○ Study of necrophagus flies phenology on 45 subjects in 1979–1980 in Leningrad region.

In all test runs (except run N 13), cadaver inspection was done daily during the first week, and later two to three times a week. Totally 4664 observations were made, from 1 to 68 observations per subject. In the course of observations insects' composition, their life stages, cadaver tissue condition, presence and characters of injuries by vertebrates, changes in underlying vegetation, temperature condition of cadaver and ambient air, air humidity, were registered. Results of observations and measurement were entered into the diary.

2.2. *Material and methods of laboratory studies*

Study of fly development periods was carried out at the laboratory of experimental entomology of the Zoological Institute of the Academy of Sciences of the USSR using special photothermostats featuring automatic control of day length and temperature (temperature deviation $\pm 0.5°C$).

Periods of development of 17 fly species of five families at different constant temperatures were determined experimentally (Table 3). Some of the species were collected from animal cadavers in nature by the author (Tables 3 and 4, N 1, 2, 8–12, 17, 18), while the others were given by laboratory colleagues Vinogradova and Zinovjeva. Live flies of 8–10 species were kept at room temperature in a specially designed photostat, automatically maintaining given day length. Flies lived in compartments of 15 cm × 15 cm × 30 cm size and 30 cm × 30 cm × 15 cm size made of gauze or fine-mesh plastic fabric. They were fed meat, sugar and water. Larvae were fed meat and kept in 0.5–1.0 l jars one third to one half filled with slightly humidified

Table 3
Number and brief specification of laboratory tests carried out on flies

N	Family, species	Total number of tests	Temperature parameters calculated	Conditions of keeping imago	
				Length of day (h)	Temperature (°)
	Calliphoridae				
1	*Calliphora vicina* R.-D.	43	×	20	20–23
2	*Calliphora vomitoria* (L.)	19	×	20	20
3	*Calliphora uralensis* VIII	18		20	20
4	*Lucilia sericata*[a] (Mg.)	1	×	–	–
5	*Lucilia caesar* (L.)	9		19	24
6	*Lucilia hirsutula* (Mg.)	18		10–20	20
7	*Lucilia illustris* (Mg.)	9		19	24
8	*Protophormia terraenovae* (R.-D.)	37	×	20	20–25
9	*Chrysomya albiceps* Wd.	18	×	20	20–25
10	*Phormia regina* (Mg.)	8	×	20	25
	Muscidae				
11	*Muscina assimilis* Flln.	19	×	20	20
12	*Muscina stabulans* Flln.	16	×	19	20
	Sarcophagidae				
13	*Boettcherisca septentrionalis* Rohd.	12	×	20	20–23
14	*Parasarcophaga argyrostoma* (R.-D.)	18		14–20	20–24
15	*Parasarcophaga semenovi*	14		14–19	20–24
16	*Parasarcophaga similis* Meade	12		17–19	20–24
	Piophilidae				
17	*Piophila faveolata* Mg.	19	×	18–19	18–20
	Phoridae				
18	*Phorid* sp.	11		18–19	18–20
Total		300	10	–	–

[a] Calculations cited basing on data by Kozhanchikov (1961).

Table 4
Number of tests under specified temperature and light conditions of keeping preimaginal life stages, day length/temperature

N	12	12–16	12	12–20	20	10–20	16–20	20
	12, 5	15	17	20	23	25	27	30–35
1	8	4	2	11	16	1	1	–
2	2	–	3	5	4	1	4	–
3	–	1	–	4	13	–	–	–
4	–	–	–	–	–	–	–	–
5	–	3	–	4	2	–	–	–
6	–	4	–	9	–	5	–	–
7	–	4	–	2	3	–	–	–
8	3	2	6	9	6	7	4	–
9	5	–	–	4	–	3	3	3
10	–	1	–	2	–	2	2	2
11	–	–	4	8	5	1	1	–
12	3	3	–	4	–	2	4	–
13	–	–	2	5	5	–	–	–
14	–	4	1	5	8	–	–	–
15	–	6	–	4	4	–	–	–
16	–	7	–	2	3	–	–	–
17	6	–	4	4	3	2	–	–
18	1	–	2	2	4	2	–	–
Total	28	39	24	84	76	26	19	4

sawdust. Jar neck were covered with thick cloth or with polyethylene cover having a wire insert and an opening to admit the thermometer. The distribution of species according to the conditions in which they were kept and number of repetition is given in Tables 3 and 4. A total of 300 tests were made at constant environmental laboratory, conditions.

3. Biological decomposition of cadaver

In moderate climatic zone of the USSR decomposition of tissues in a non-buried cadaver is a combined process embracing both destruction by birds and animals and biological decomposition (microbes, mold fungi, insects). Microorganisms and insects take part in decomposition of all the cadavers. Presence of mold fungi and algae is observed after the end of fly larvae nutrition in 30% of cases. This is observed for the cadavers which started to decay in spring — in 55% of cases, in summer — 35% of cases, in autumn —none. The latter effect is accounted for by the fact that fly larvae continue to develop until the very frosts begin and until cadavers are covered with an icy crust, secreting a substance preventing the development of mold fungi [27]. Damage by birds and animals was observed in 26% of cases on the first year of decay and in 20% of the remains on the second year. In spring, 67% of cadavers are damaged, in summer — 17%, in autumn — 26% [28].

Based on predominant and representative for a given time period, activity of one or other necrobiont subgroup, the process of cadaver decomposition is subdivided into five stages[1] and each stage corresponds to a certain degree of cadaver tissue-decay.

The 1st stage — early microbial decomposition follows the autolitic processes and lasts until fly egg-laying and larval emergence. Average duration from May to September is 3 days (1–5 days).

The 2nd stage — active decomposition of cadaver by insects — starts from fly larval emergence, goes on simultaneously with on simultaneously with microbial decay and ends upon the termination of larval development which destroy the major part of cadaver the tissues. Average duration from May to September is 22 days (8–65 days).

The 3rd stage — advanced decomposition of cadaver by insects— starts from pupation of fly larvae and is mainly due to beetle larvae, which destroy almost all the remaining soft tissues. Microbial decay continues and is supplemented by the activity of mold fungi. Duration on varies from 12 to 504 days.

The 4th stage — microbial decomposition of the cadaver — starts from the moment the beetle larvae leave the remains of the cadaver and ends when the skeleton breaks into separate bones. Duration varies from 27 to 976 days.

The 5th stage — disintegration of bony tissues was not completed in the course of the 4-year observation period.

There are no doubts about the effect of meteorological factors on the biological decomposition of the cadaver, however, up to now this problem has not been specially studied. Let us make an attempt to narrow this gap by investigating the cadaver biological decay period CV (Table 5), and the relationship of seven meteorological factors under Leningrad region conditions (Table 6). Correlation analysis between average duration of individual cadaver decay stages and average values (over several years) of meteorological factors for the starting decade of each stage was made (Table 7).

The duration of individual stages of biological decay in the cadaver is considerably affected by meteorological factors. The duration of four stages was found to be inversely proportional to total radiation, air and ground surface temperatures; duration of the 1st, 3rd and 4th stages — to solar

Table 5
Duration of cadaver biological decay stages for Leningrad region

Month	Decade	Mean duration of decay (days)				
		I	II	III	IV	I–III
March	I	57.2				124.7
	II					107.0
	III	47.0				
April	I	35.5				37.0
	III		30.7			
May	I		30.2			
	II		23.0	31.7		
	III	3.0	15.1	34.2		77.6
June	I	3.25	20.3	33.5		77.3
	II	2.1	17.2	70.7		73.5
	III			57.6		
	I–III				93.0	
July	I	3.0	14.0	54.3		
	II	3.0		52.7		35.0
	III	2.6	12.9	17.0		26.5
	I–III				111.5	
August	I	3.0		25.8		
	II	2.5	14.8	14.3		40.0
	III		20.3	6.0		
	I–III				132.0	
September	I			12.0		
	II	2.9	32.4			63.0
	I–III		27.3		598.3	
October	I			426.5		
	II			492.3		
	III			411.5		
November	I–III				887.0	

[1] This division into periods differs from the one earlier proposed [29].

Table 6
Decade average values (data of several years) of meteorological factors for Leningrad region

Month	Decade	Decade average value of meteorological factors						
		Period of solar radiation (h)	Total radiation (kcal/cm^2)	Air temperature (°C)	Soil surface temperature (°C)	Relative air humidity (%)	Precipitation quantity	Precipitations (day)
March	I	2.8	1.134	−6.4	−6.3	80.7	11.0	6.2
	II	4.1	1.651	−4.4	−6.1	77.7	10.0	6.5
	III	5.0	2.086	−1.9	−2.5	76.3	11.0	6.1
April	I	4.6	2.196	0.6	1.0	74.9	12.0	6.0
	III	6.7	3.122	5.4	6.7	68.9	13.0	5.3
May	I	8.1	3.794	7.7	10.1	65.3	16.0	4.4
	II	8.4	4.158	9.5	13.4	64.1	16.0	5.0
	III	8.9	4.429	11.7	15.0	61.9	16.0	4.2
June	I	9.3	4.623	13.5	18.6	64.4	21.0	3.5
	II	8.7	4.558	14.8	19.2	65.8	22.0	4.4
	III	8.4	4.376	16.2	20.2	69.3	22.0	5.6
	I–III	254.1	13.450	14.8	19.3	66.3	65.0	13.6
July	I	8.2	4.254	17.4	20.3	72.6	21.0	5.4
	II	8.1	4.201	18.0	21.0	71.3	22.0	4.6
	III	8.2	4.074	18.0	21.0	72.1	22.0	5.3
	I–III	265.0	13.490	17.8	20.8	71.4	65.0	15.3
August	I	7.5	3.544	17.4	20.0	74.6	28.0	5.3
	II	7.1	3.196	16.4	18.2	76.8	28.0	5.0
	III	5.7	2.561	14.5	16.2	78.2	28.0	6.5
	I–III	209.5	9.950	16.0	18.1	76.3	84.0	16.8
September	I	5.4	2.245	12.8	14.3	79.8	22.0	5.7
	II	4.2	1.790	10.8	11.6	81.3	22.0	6.5
	III	3.5	1.384	8.8	8.5	81.4	22.0	6.0
	I–III	131.3	5.590	11.1	11.5	80.2	66.0	18.3
October	I	2.5	0.946	6.2	6.5	82.8	19.0	6.6
	II	1.9	0.754	4.8	4.7	83.4	19.0	6.5
	III	1.5	0.506	2.9	2.4	85.7	18.0	7.9
November	I–III	220.0	0.670	−0.5	−1.0	87.1	48.0	23.9

radiation period; of the 1st stage also to the amount of precipitation. Duration of the 1st, 3rd and 4th stages and 3rd and 4th were found to be directly proportional to the number of days with precipitation and to relative air humidity, respectively. No pronounced effect of relative air humidity, number of days with precipitation and amount of precipitation on the duration of the 2nd stage was observed. This is connected with the ability of fly larvae to maintain the humidity of their life environment at a certain level through parental nutrition [30].

The average period of cadaver skeletization varies from 205 to 829 days (54–1074 days) depending on the month when decay started. In 65% of cases, skeletization occurs the same year as a cadaver arrived at the place where it was detected in March–June. Extensive variability in the duration of individual decomposition stages of cadavers even under identical conditions makes it impossible to use the average data on their duration in order to determine the time of death [28,31].

Effect of cadaver location (biotope) on the duration of decay stages is the conditioned by the possibility and the degree of the meteorological factors effect on cadaver tissues and feeding necrobionts. In a moderate climatic zone, primary importance is attributed to the conditions of cadaver-environmental heat exchange, particularly to the absorption of part of the solar radiation by the cadaver. It should be noted in this connection that assuming that cadaver temperature is equal to the environment at temperature would be a wrong thing to do, especially if the cadaver is exposed to direct solar radiation or heat source effect. The duration of the decay of cadaver tissue different biotopes at different seasons of the year is determined by the character of radiation regime. In spring and autumn, decay progresses more quickly at the slopes oriented perpendicular to sunbeams, and more slowly at horizontal areas of the country.

Hanged cadavers decompose slower than ones lying on the surface of the ground, because of higher convective heat

Table 7
Duration of cadaver biological decay stages CV, decade average values (data of several years) of meteorological factors (Leningrad) relationship[a]

Meteorological factors	Exponent	Cadaver biological decay stage				
		I	II	III	IV	I–III
Period of solar radiation	*r*	−0.708	−0.565	−0.874	−0.978	−0.617
	R	−0.067	−0.149	−0.013	−0.274	−0.483
	P	=0.01	>0.05	<0.01	<0.01	<0.05
Total radiation	*r*	−0.695	−0.589	−0.830	−0.967	−0.610
	R	−0.037	−0.091	−0.007	−0.014	−0.127
	P	<0.02	<0.05	<0.01	<0.01	<0.05
Air temperature	*r*	−0.683	−0.857	−0.844	−0.938	−0.905
	R	−0.326	−0.484	−0.031	−0.019	−0.262
	P	<0.01	<0.01	<0.01	<0.02	<0.01
Soil surface temperature	*r*	−0.864	−0.931	−0.881	−0.969	−0.862
	R	−0.381	−0.632	−0.031	−0.024	−0.292
	P	<0.01	<0.01	<0.01	<0.01	<0.01
Relative air humidity	*r*	0.435	0.207	0.669	0.911	0.219
	R	0.117	0.205	0.025	0.020	0.047
	P	>0.1	>0.1	<0.01	<0.05	>0.1
Precipitations quantity	*r*	−0.751	−0.388	−0.417	−0.711	−0.818
	R	−0.197	−0.256	−0.009	−0.025	−0.155
	P	<0.01	<0.1	>0.1	>0.1	<0.01
Precipitations number of days	*r*	0.759	0.305	−0.674	0.934	0.355
	R	0.032	0.039	0.004	0.010	0.011
	P	<0.01	>0.1	<0.01	=0.02	>0.1

[a] Statistical data.

transfer and mummification of the surface layers of the tissues. When a cadaver has been in a natural environment since March, the average duration of the 1st stage is 63 days, 2nd stage — 46 days, 3rd stage — 311 days, since May, average duration of the 1st stage is 2 days, 2nd stage — 40 days, 3rd stage — 75 days. Further at the 3rd and 4th stages, depending on the time when the cadaver got into the natural environment, the tissues decomposition is abruptly decelerated, and mummified remains preserve the form of the body in a hanged position for up to 3 years.

The influence of cadaver mass on its decay period depends on meteorological factors involving changes in the tissue condition by making them more or less suitable for necrobiont feeding. Relevance of each of above factors varies depending on month and biotope.

Clothes on a cadaver do not delay the invasion of insects though the decay period will be somewhat longer. If clothes are stained with combustibles, lubricants and paintwork materials, the time before insects invade the cadaver will be approximately doubled, and in 47% of cases decomposition is perceptibly postponed (Table 8). Meteorological factors such as solar radiation, precipitation, etc. produce a considerable effect on the character and durability of ‘‘dirt’’ influence. Rain and higher air humidity contribute to the transfer of chemicals into cadaver tissues thus increasing their repellent effect. Solar radiation and increased air temperature tend to evaporate and dry out substances on clothes, thus preventing the transfer of chemicals into tissues, which results in accelerated insect invasion [32].

The leading part in biological decay of a cadaver is played by necrobiont insects. Based on taxonomic and ecological features the cadaver entomofauna can be divided into two groups:[2] (1) cadavericole entomofauna properly. It comprises necrobiont insects, for which cadaver is a permanent environment for life and development. According to trophic specialization they are subdivided into necrophages and entomophages, (2) arbitrary entomofauna of cadaver. It comprise the species for which the cadaver is not a constant scene of life and development. This group is subdivided into polyphages, entomophages and necroentomophages.

Properly cadavericole entomofauna is the most important component of biological decay of a cadaver. The leading position is held by representatives of Diptera families: Calliphoridae, Muscidae, Sarcophagidae, Helemizidae, Phoridae and Piophilidae. The second order of importance is Coleptera (with horny fore wings), families: Silphidae, Dermestidae, Histeridae and Staphylinidae [34].

[2] Classification of Marchenko [33] is outdated.

Table 8
Time of insects invasion and character of soft tissues decay in cadaver, clothes stained with various contaminants being simulated [32][a]

Type of dirt on clothes	N	Time when insects started to invade cadaver (day)		Decomposition of cadaver soft tissues		
		Insolated event	Mass event	Complete	Partial	Absent
Gasoline	1	5	8	+		×
	2	5	9		+	×
	3	–	4	× +		
Kerosene	1	2	5		+	×
	2	5	8			× +
	3	–	4	× +		
Diesel fuel	1	2	8	+	×	
	2	5	9	+	×	
	3	5	7	+	×	
Nitrocellulose enamel	1	2	9			× +
	2	2	8			× +
	3	4	7		× +	
Lubricant	1	2	5	× +		
	2	2	5			× +
	3	4	5	+	×	
Lacquer S-4	1	2	8			× +
	2	8	11		+	×
	3	6	7	× +		
Oil plant	1	2	5	× +		
	2	–	5		+	×
	3	4	6	× +		
Remains of burnt clothes	1	–	5	× +		
	2	5	8		× +	
	3	4	6	× +		
Clean clothes, reference	1	2	5		× +	
	2	2	5		+	×
	3	–	4	+		×
No clothes reference	1	–	5	× +		
	2	2	5		× +	
	3	–	4	× +		

[a] (×): Cadaver tissues condition by the moment when clothes were taken off on the 14th day; (+): cadaver tissues condition by the 23rd day.

The process of cadavericole fauna formation in a man's living quarters is characterized by a certain specificity. Initially this is connected with the steady microclimate maintained in the living quarters, favoring round-the-year insects activity. Secondly, this depends on whether necrophage insects did have or had no access to the room with the cadaver, on the one hand, and depends on whether there existed in the room already a complex of obligate synanthropic species of insects capable of round-the-year development. Thirdly, species composition of such a complex varies depending on the intrusion of new insect species, brought in with food stuffs, or whether disinsectization measure were being taken. Insect species composition can be dissimilar even in the rooms of the same house [35].

In 12% of cases, partial mummification of cadavers was observed. The average periods of cadaver parts mummification as a function of biotope and month, when the cadaver is in natural conditions, are presented in Table 9. An insignificant formation of ''grave wax'' (in 1–1.5 years in average) was noted in 4% of cases, more frequently on ''autumnal'' cadavers.

A cadaver and products of its decomposition produce both mechanical and chemical effects on vegetation on the underlying ground. Mechanical effect, apart from broken plants, is manifested as the color of plant being altered from green to different shades of yellow, which is connected with a change of a plants respiratory pigments. As can be seen from Table 10, in spring, color changes twice as slow than in

Table 9
Average periods of mummification of individual parts of cadaver in relation to month and biotope in USSR north-west climate conditions (days)

Biotope	Month			
	4	5	6	9
Field	66.0	–	15.7	30.0
Swamp	47.5	35.0	23.6	–
Ravine	36.0	–	20.5	–
Underwood	–	–	25.0	–
Average for four biotopes	49.8	35.0	21.2	30.0

summer or autumn, Chemical effect is mainly connected with the period of fly larval development on a cadaver, because their parental digestion leads to an abrupt increase liquid fraction of decomposition products, and the rate of chemical reactions increases due to metabolic heat release (temperature of larval mass is 45–49°C). On the average chemical burning out of plants occurs by the 7th day from the beginning of larval development. Dynamics of vegetation revival on the cadaver bed is presented in Table 11. In the 2nd year a vegetation billow is formed around the cadaver bed, which looks distinctly unlike the surrounding plants. A sort of bed decamouflage occurs. Revival of vegetation the cadaver bed properly starts in the 2nd or 3rd year and ends only in the 4th year [36].

The above data may be of importance for the determination of the season, when the cadaver arrived at the place, where it was discovered.

4. Biology of flies and their significance in forensic medicine

At the present state of development of forensic entomology the main efforts are made to study the biology of flies. The reason for this consists in the fact that flies are the first to invade a cadaver, and this is done quickly and simultaneously, so that their species composition accurately corresponds to the degree of cadaver tissue decomposition and alters in a regular pattern during the decomposition process [37]. Flies are predominant in quantity and consume the major part of the tissues.

Three directions of study on necrobiont flies species can be distinguished.

1. Identification of the species composition of the necrobiont fly community and of factors affecting the formation of said composition.

Table 10
Changes of vegetation condition in cadaver bed area; average periods, days

Time of the year	Number of cadaver	Period of green color fading to yellow	Complete destruction of plants in cadaver bad area	
			After flies appeared on cadaver	After fly larvae emerged
Spring	10	14.4	28.6	23.1
Summer	9	8.4	10.9	8.0
Autumn	17	8.7	23.8	19.0

Table 11
Periods of cadaver bed vegetation restitution

Character of revived vegetation	Number of studies cadaver beds	Periods of vegetation revival, years			
		1	2	3	4
Grass around cadaver bed	36	9	26	33[a]	No observation
Formation of vegetation billow around cadaver bed	36	4	15	21	No observation
Grass in cadaver bed area	36				
Complete restitution		0	2	6	12
Partial revival		0	22	30	24
No revival		36	2	0	0
Moss in cadaver bed area	6				
Complete restitution		0	0	0	4
Partial revival		0	3	5	2
No revival		6	3	I	0

[a] In three cases, no grass spreading around the cadaver bed was observed.

This direction is being successfully developed by Porta [38], Fuller [39], Bornemissza [40], Reed [41], Payne and Crossley [42], Nuorteva [3,21,43], Hanski [44], Hanski and Kuusela [45], Leclercq [2], Leclercq and Brahy [8], Putman [46], Kuusela and Nanski [47], Vernon [48], Smith [1], Ozerov [49]. Ozerov has discovered, while studying nercobiont flies in the woods of the southern part of Far East of the USSR: (i) the necrobiont flies community comprises 128 species of 16 families and is divided into obligate necrobionts (26 species of five families) and facultative necrobionts (102 species of 16 families); (ii) the structure of community depends on the weight and type of the fly cadaver, the degree of its decomposition, season of the year, biotope condition, climatic factors; (iii) the variety of species reduces as the cadaver weight decreases; (iv) periods of egg-laying in the cadaver (larvae emergence) are connected with certain stages of its tissue decay and were identified for 72 species; (v) flies of anthropogenetic biocenoses lay eggs into the cadaver (or larvae emerge) for a limited period of time and only once; (vi) the wider the variety of species composition collected from a cadaver, the higher is the probability of the obtained data to be efficiently used for their expertise.

The above findings completely comply with the results of studies of cadaver entomofauna in the northwest region of the USSR. Study on the variation of larvae growth parameters at different temperatures (length of the body, weight) aimed to develop a method for determining the period of their development.

The second direction is developed in Japan (Nishida) and in Austria (Reiter). It is based o registering the length and weight of fly larvae as they grow. So, Reiter [6] gives data on the length variation of *Calliphora vicina* R.-D. larvae at the 6.5–30°C range of temperatures. Based on these data, he proposed an isomegalodiagram for practical application. Studies, carried out by Nishida et al. [23] in Japan on seven fly species at different temperatures, resulted in a statistically true dependence of length and weight of larvae CV development period, which permitted the compilation of diagnostic Tables. Independently of each other, scientists have pointed out that the *increase* of length and weight of larvae changes to their *decrease* from the moment when feeding is over, prior pupation. Development at temperatures higher than the optimum ones leads to an increased duration of development, delayed pupation or larval death. In the study of flies development on dead bodies, a process of larvae mass self-heating was observed owing to metabolic heat release and results in an up to 50% shortening of the development in a certain period of pre-imago stages, compared to the time on the environmental temperature data [50–53]. The method proposed by Reiter and Nishida attracts by its simplicity, but requires a thorough practical testing especially in connection with the above observations. It is effective only till the pupation moment, which is a significant disadvantage. From the above paper by Nishida et al. [23] one can utilize data on development periods of preimaginal stages and how they correspond to each other on a cadaver.

2. Determination of thermal parameters values regulating insects development, to be further used in calculation technique for retrospective determination of the time of insects invasion into cadaver.

The third direction has been successfully developed by Nuorteva [3], Leclercq [2], Smith [1] and Introna et al. [24]. The direction is being developed in the USSR and comprises a calculation technique for retrospective determination of the time when insects started to develop on a cadaver, the said technique being based on thermal parameters regulating the insect's development [29,50–52,54–56].

The early summer activity and reproduction of flies is predominantly regulated by temperature. In moderate climatic zone fly reproduction starts in spring when 24 h

Table 12
Thermal parameters (°C), regulating necrobiont flies development

N	Fly species	Lover development threshold	Heat constant of species	Sum of effective temperatures of develop from egg to puparium
1	*Calliphora vicina*	2.0	388.0	191.0
2	*Calliphora vomitoria*	3.0	472.0	213.0
3	*Protophormia terraenovae*	7.8	251.0	191.0
4	*Lucilia sericata*[a]	9.0	207.0	–
5	*Chrysomya albiceps*	10.2	186.0	123.0
6	*Phormia regina*	11.4	148.0	101.0
7	*Muscina stabulans*	7.2	269.0	139.0
8	*Muscina assimilis*	7.9	240.0	102.0
9	*Boettcherisca septenrionalis*	7.8	279.0	117.0
10	*Piophila foveolata*	6.4	434.0	278.0

[a] According to Kozhanchikov (1961).

temperature is stabilized at 10–15°C level and ends in autumn, when temperature drops below 10–13°C [57]. Thermal parameters regulating the development of nine fly species are given in Table 12, the meaning of those is considered in Section 5 [52]. For the species cited in Table 12 and for *Lucilia sericata* Mg. there were calculated development periods from egg to puparium and to imago emergence and hourly development indices at temperatures from 11 to 30°C, which are presented in Table 13. Egg development period for *C. vicina* R.-D., according to Reiter [6], is 2.83 days at 11°C, 1.21 days at 15°C, 0.95 days at 19°C and 0.86 days at 22.5°C. In the article by Marchenko [34], data on development periods at constant temperatures of another eight fly species are given.

In the study of *Protophormia terraenovae* R.-D. development on dead bodies, a process of larvae mass self-heating was observed owing to metabolic heat release. While studying cadaver decomposition by insects under natural conditions of the north-west European part of the USSR in 1971, thermometry of 17 cadavers (256 measurements) confirmed that temperature increases to 45°C in cadaver tissues [50]. It was established that this fact is connected with the development of flies the larvae mass. This fact is already described in the literature [41,58]. For all the big animals (six subjects) and for a portion of medium-size ones (five subjects) temperature increase to 18–45°C was registered inside the cadavers in the mass of fly larvae, the air temperature being 4–15°C. The measurements were made at 9–11 h per mp when any heating due to direct solar radiation was excepted. Temperature range at the cadavers surface was 4–13°C.

In 1980, the ascertained fact was made a subject of special study on 39 dog cadavers. A total of 4216 measurements of their temperature five times per day (7.00, 11.00, 14.00, 19.00 and 22.00 h) were made, air temperature was registered at the same time. In Table 14, the results of measurements on one subject are presented as examples. According to the observation of temperature conditions of 21 cadavers during the spring–summer season maximum temperature in the mass of developing *P. terraenovae* larvae was 30–49°C.

In spring, self-heating of feeding larvae mass was distinguished by a great stability in cadavers located on the soil surface (variation factor of maximum temperatures $CV = 6.0\%$) if compared to hanged cadavers ($CV = 16.4\%$) for which heat exchange was intensified due to turbulence effects. This is reflected by statistically true difference ($P = 0.999$) of average maximum temperature: for cadavers on the ground they are 44.4°C, for hanged ones — 36°C (Table 15, rows 1 and 2).

Summer group of four cadavers lying on the soil surface was characterized by average maximum temperature 45.5°C ($CV = 5.5\%$). No statistically true difference between maximum temperatures of spring and summer cadavers was found ($P = 0.788$), which is due to predominance of the same of species larvae, namely *P. terraenovae*. Maximum temperature level attained 2 or 3 days after larvae emergence, it keeps up for 6–20 days and then decreases in 2–4 days. The average time period during which temperature in the larvae mass was maintained at the highest level varied from 18.2 days (in spring) to 12.0 days (in summer) for the cadavers on the soil surface and 12.7 days (in spring) for hanged cadavers, manifesting great instability in all the above cases (CV — 20.3, 11.7 and 21.2%, respectively).

Of 18 cadavers observed in the autumn, self-heating effect was registered on 16 cadavers lying on the ground; this process was not noticed for two hanged cadavers. At that time mainly *Calliphora vomitoria* and *C. vicina* developed on cadavers; average maximum temperature equal to 29.6°C was lower than in the case of *P. terraenovae* larvae during spring–summer period. Truth of difference between autumn group and spring–summer groups is 1.0. Instability of the autumn self-heating process found to be the highest — $CV = 23\%$. This is accounted for by peculiarities of fly species also by the fact that in spring there is a constant increase of solar radiation intensity and air temperature, while in the autumn they decrease. The time of expected fly emergence was calculated for 45 dead bodies based on air temperature data and then compared to actual values. In all cases, flies started to emerge before the calculated time. In 21 of the 26 cases under study the maximum peak of flies emergence also came earlier than expected. The greatest acceleration of development is observed in open land, the lowest one in closed, shadowy places and especially on hanged cadavers due to higher heat transfer. The intensity of heat release depends on the season of the year, biotope and conditions of cadaver heat exchange with the environment. In nature, the warming up of the mass of feeding larvae helps to maintain a favorable microclimate for their development and supports their survival during temporary periods when the environment at temperature drops to 0°C, it reduces by up to 50% the development period of part of the preimaginal stages compared to the period predicted bas on environmental temperature only.

The regularities of the process of warming up, taking place in the mass of feeding fly larvae, were established for seven fly species, the families of Calliphoridae, Muscidae, Sarcophagidae and Piophilidae. For *P. terraenovae*, a larval environment temperature value of 10°C was determined, which is the starting point of the food substrate heating process. In the study of *Chrysomya albiceps* and *P. terraenovae* development in Laboratory conditions, the sums of effective temperatures which larvae could have by the moment of pupation were calculated. When the calculation was made based on ambient air temperature, the sum of effective temperatures for said species amounted to 87 and 81°C, respectively. The same parameter was determined based on larval mass temperatures of 112 and 178°C, respectively. The latter values are closed to the sum of effective temperatures needed for development from egg to pupation. So *Chrysomya albiceps* requires 123°C (we have 112°C), and *P. terraenovae* requires 191°C (we have 178°C). Consequently, practical application of the method for retrospectively determining the time of

Table 13
Development periods (days) and hourly indices of fly development from egg to puparium and from egg to imago emergence

Development temperature (°C)	Development period from egg to		Index $\times 10^{-4}$	Development period from egg to		Index $\times 10^{-4}$
	Puparium	Imago		Puparium	Imago	
	Protophormia terraenovae			*Calliphora vicina*		
11	50.0	78.4	5.312	21.2	43.1	9.664
12	38.1	59.8	6.972	19.1	38.8	10.738
13	30.8	48.3	8.632	17.4	35.3	11.812
14	25.8	40.5	10.293	15.9	32.3	12.886
15	22.2	34.9	11.952	14.7	29.8	13.960
16	19.5	30.6	13.616	13.6	27.7	15.034
17	17.4	27.3	15.273	12.7	25.9	16.108
18	15.7	24.6	16.937	11.9	24.3	17.182
19	14.3	22.4	18.952	11.2	22.9	18.256
20	13.1	20.6	20.256	10.6	21.6	19.329
21	12.1	19.0	21.918	10.0	20.4	20.403
22	11.3	17.7	23.580	9.6	19.4	21.477
23	10.5	16.5	25.237	9.1	18.5	22.551
24	9.9	15.5	26.899	8.7	17.6	23.625
25	9.3	14.6	28.588	8.3	16.9	24.699
26	8.8	13.8	30.215	7.9	16.2	25.773
27	8.3	13.1	31.879	7.6	15.5	26.847
28	7.9	12.4	33.548	7.3	14.9	27.920
29	7.5	11.8	35.192	Larvae death		
30	7.2	11.3	36.873			
	Chrvsomva albices			*Phormia regina*		
11	Larvae death			Lower development threshold		
12	Larvae death			168.3	246.6	1.689
13	43.9	66.3	6.289	63.1	92.5	4.504
14	32.4	48.8	8.535	38.8	57.0	7.319
15	25.6	38.6	10.781	28.0	41.1	10.135
16	21.2	32.0	13.027	22.0	32.2	12.950
17	18.1	27.3	15.273	18.0	26.4	15.785
18	15.8	23.8	17.520	15.3	22.4	18.581
19	14.0	21.1	19.766	13.3	19.5	22.804
20	12.6	18.9	22.012	11.7	17.2	24.211
21	11.4	17.2	24.258	10.5	15.4	27.027
22	10.4	15.7	26.504	9.5	13.9	29.842
23	9.6	14.5	28.750	8.7	12.7	32.657
24	8.9	13.4	30.997	8.0	11.7	35.472
25	8.3	12.5	33.243	7.4	10.9	38.288
26	7.8	11.7	35.489	6.9	10.1	41.103
27	7.3	11.0	37.735	6.5	9.5	43.918
28	6.9	10.4	39.981	6.1	8.9	46.734
29	6.5	9.8	42.227	5.7	8.4	49.549
30	6.2	9.4	44.474	5.4	7.9	52.364
	Calliphora vomitoria			*Lucilia sericata*		
11	26.6	59.0	7.062	103.5	4.025	
12	23.7	52.4	7.944	69.0	6.038	
13	21.3	47.2	8.826	51.8	8.051	
14	19.4	42.9	9.710	41.4	10.064	
15	17.7	39.3	10.593	34.5	12.077	
16	16.4	36.3	11.475	29.6	14.090	
17	15.2	33.7	12.358	25.9	16.103	
18	14.2	31.5	13.241	23.0	18.11£	
19	13.3	29.5	14.124	20.7	20.128	
20	12.5	27.8	15.007	18.8	22.141	
21	11.8	26.2	15.889	17.3	24.154	
22	11.2	24.8	16.772	15.9	26.167	

Table 13 (*Continued*)

Development temperature (°C)	Development period from egg to		Index ×10^{-4}	Development period from egg to		Index ×10^{-4}
	Puparium	Imago		Puparium	Imago	
23	10.6	23.6	17.655	14.8	28.180	
24	10.1	22.5	18.538	13.8	30.193	
25	9.7	21.5	19420	12.9	32.206	
26	9.3	20.5	20.303	12.2	34.219	
27	Pupariae death			11.5	36.231	
28				10.9	38.244	
29				10.4	40.257	
30				9.9	42.270	
	Muscina assimilis			*Muscina stabulans*		
11	32.9	77.4	5.381	Larvae death		
12	24.9	58.3	7.118	Larvae death		
13	20.0	47.1	8.854	24.0	46.4	8.983
14	16.7	39.3	10.590	20.4	39.5	10.532
15	14.4	33.8	12.326	17.8	34.5	12.081
16	12.6	29.6	14.062	15.8	30.6	13.630
17	11.2	26.4	15.798	14.2	27.4	15.179
18	10.1	23.8	17.534	12.9	24.9	16.728
19	9.2	21.6	19.270	11.8	22.8	18.277
20	8.4	19.8	21.006	10.4	21.0	19.841
21	7.8	18.3	22.743	10.1	19.5	21.375
22	7.2	17.0	24.479	9.4	18.2	22.924
23	6.7	15.9	26.215	8.8	17.0	24.473
24	6.3	14.9	27.951	8.3	16.0	26.022
25	6.0	14.0	29.687	7.8	15.I	27.571
26	5.6	13.3	31.423	7.4	14.3	29.120
27	5.3	12.6	33.159	7.2	13.6	30.669
28	5.1	11.9	34.895	6.7	12.9	32.218
29	4.8	11.4	36.631	6.3	12.3	33.767
30	4.6	10.9	38.368	6.1	11.8	35.315
	Boettcherisca septentrionalis			*Piophila foveolata*		
11	36.6	103.3	4.032	Diapause		
12	27.9	75.4	5.525			
13	22.5	59.4	7.019			
14	18.9	49.0	8.512			
15	16.3	41.6	10.016			
16	14.3	36.2	11.499			
17	12.7	32.0	12.992	Diapause		
18	11.5	28.8	14.482	24.0	37.4	11.136
19	10.4	26.1	15.979	22.1	34.4	12.096
20	9.6	23.8	17.473	20.4	31.9	13.056
21	8.9	22.0	18.966	19.0	29.7	14.016
22	8.2	20.4	20.459	17.8	27.8	14.976
23	7.7	19.0	21.953	16.8	26.1	15.937
24	7.2	17.8	23.446	15.8	24.7	16.897
25	6.8	16.7	24.940	15.4	23.4	17.818
26	6.4	15.8	26.433	14.2	22.1	18.817
27	6.1	14.9	27.927	13.5	21.1	19.777
28	5.8	14.2	29.420	12.9	20.1	20.737
29	5.5	13.5	30.913	12.3	19.2	21.697
30	5.3	12.9	32.407	11.8	18.4	22.657

insects settling in a cadaver based on air temperature alone may result in certain errors.

Experiments carried cut on *C. albiceps*, *P. terraenovae* and *Musca domestica* L. have shown a direct connection between the rate of temperature increase in the mass of feeding larvae and their quantity, age and conditions of heat exchange the environment. It was also confirmed that larvae development is accelerated due to metabolic heat release

Table 14
Temperature characteristic of fly larvae development on cadaver (biotope-hill slope)

Data	Air temperature/cadaver temperature (hour when temperature was measured)				
	7 a.m.	11 a.m.	2 p.m.	7 p.m.	10 p.m.
May					
3	0.5/6.0	–	–	3.0/7.0	–
4	5.5/40	11.5/4.5	11.0/9.0	10.0/10.5	4.5/9.0
5	4.5/7.0	8.5/9.5	9.5/8.0	9.0/9.5	7.0/10.0
6	5.5/5.0	11.5/6.0	10.0/11.5	8.0/12.5	3.5/7.0
7	8.0/9.5	–	17.0/11.5	–	12.0/10.0
8	15.0/11.0	–	11.0/14.0	–	10.5/16.0
9	11.0/13.5	18.0/16.0	17.0/19.0	14.0/19.0	10.0/13.5
10	8.0/12.0	12.0/12.0	12.0/15.0	9.0/13.0	6.5/9.5
11	8.5/6.5	9.0/7.0	9.0/18.0	8.0/17.0	5.0/15.5
12	4.0/17.0	9.0/19.0	11.0/21.0	8.5/17.0	3.5/10.0
13	2.0/7.0	7.5/11.5	7.5/21.5	7.0/14.0	5.0/18.0
14	9.0/31.0	14.0/39.0	14.5/46.5	8.5/44.0	–
15	7.0/31.5	13.0/45.0	15.0/48.0	I5./43.5	–
16	9.0/38.0	7.5/40.0	10.0/45.5	9.5/44.0	8.0/44.5
17	–	15.5/40.0	–	–	–
18	8.5/37.5	14.0/41.0	16.0/43.5	12.0/40.0	7.5/39.5
19	10.0/30.0	13.5/36.0	17.0/41.5	14.5/39.0	8.0/38.0
20	–	15.5/40.0	–	–	–
21	–	3.0/25.0	2.0/19.0	2.0/11.0	0/8.0
22	1.0/6.0	3.0/6.0	4.0/7.0	4.0/7.0	2.0/7.0
23	2.0/6.0	11.0/11.0	15.0/16.0	10.0/10.0	7.5/12.0
24	7.0/10.0	12.0/12.0	11.0/13.5	13.0/14.5	8.0/9.0

[52,53,59,60]. The presented data on necrobiont flies biology will be useful to make correct evaluation of their development in each particular case.

5. Method of retrospective determination of the starting moment of insects development on a cadaver

Prior to discussing the method, definitions and explanation the meaning of individual thermal parameters regulating insects development periods will be given. Lower development threshold is the lowermost limit value of environment temperature, above which a progressive development of species becomes possible. The threshold value is specific for each species. At temperatures below this threshold value cold torpidity is observed until certain limit, then insects die.

Table 15
Characteristics of average maximum temperatures stability in the mass of fly larvae living on cadaver as a function of year season and cadaver location[a]

N	n	$\overline{X}$	σ	CV
1	12	44.4	2.67	6.0
2	4	36.0	5.89	16.4
3	4	45.5	2.52	5.5
4	16	29.6	6.8.2	23.0

[a] N 1: spring, cadavers are on soil surface, N 2: spring, hanged cadavers, N 3: summer, cadavers on soil surface, N 4: autumn, cadavers on soil surface.

Effective temperature is the temperature value which directly involves progressive development of a species. At each specific time interval for a given species it is the difference between the environmental temperature value and the lower threshold value of this particular species. As a rule, average 24 h effective temperature is used.

Heat constant of species is the sum of effective temperatures, a species needs to develop from the egg to the emergence of adult insect (imago). The value of heat constant of species, as well as lower threshold of development is specific for each species and is not changed depending on the geographical region. Heat constant of species governs the development period under specific environmental conditions and area of this species. The sum of effective temperatures necessary for the development from egg to pupa (puparium) is a constant value for each species, exactly like the heat constant.

Development index is that part of development, which a species undergoes per unit of time (hour, day) at a given temperature. Development from egg to imago is conventionally assumed to be a unit. Development index is determined for a specific temperature value by dividing the unit into development period at a given temperature value, which remained constant during the total development period from

egg to imago. Thus, the development index is a specific value for each species, varying with temperature. When development indices are applied, calculations of effective temperatures can be avoided when determining species development period under specific conditions.

The parameters listed above allowed development of a method of retrospective determination of the moment/when insects started to develop on a cadaver, which is based on said development indices and effective temperatures, and to adopt it for practical application in forensic medicine. The essence of the method consists in breeding preimaginal insects stages found on a cadaver until imago emergence in laboratory. If "grown up" insects emerged from pupae are found on the cadaver, one can directly start from point 3 of the calculations (see below). The same is done when pupae (puparia) of those species are found, for which the life cycle is well studied. Having determined experimentally the part of development in the laboratory, the part of development on the cadaver before the insects were collected from it is interpolated. Basing on temperatures observed in nature during the days preceding the moment of cadaver discovery the probable date of a given species invasion into the cadaver is determined by fairly simple calculations.

Initial data to make calculations using thermal parameters for *C. vicina* R.-D., *P. terraenovae* R.-D. and *Muscina assimilis* Flln. are given in Fig. 1. In the same figure an example of calculations using effective temperatures is presented. Below the Procedure of calculations with development the index is given.

1. There is determined the part of development undergone by insect in laboratory ($Y_{\text{lab.}}$).
   1.1. at constant temperature in laboratory: $Y_{\text{lab.}} = it$, where i is the development index and t the development time, hours, days. $Y_{\text{lab.1}} = 0.062916 \times 8.5 = 0.534786$, where 0.062916 is 24 h development index for *M. assimilis* at 23°C; $Y_{\text{lab.2}} = 0.0605688 \times 8.9 = 0.83906232$ where 0.0605688 is 24 h development index for *P. terraenovae* at 23°C; $Y_{\text{lab.3}} = 0.05412249.4 = 0.50875056$, where 0.0541224 is 24 h development index for *C. vicina* at 23°C;
   1.2. at varying temperature in laboratory calculations are made in the same way, as in point 3: $Y_{\text{lab.}} = \sum_{j=1}^{n} ij$ where j is time (hours, days).
2. There is determined the part of development which took place on the cadaver ($Y_{\text{ext.}}$)

$$Y_{\text{ext.}} = 1 - Y_{\text{lab.}}$$

$$Y_{\text{ext.1}} = 1 - 0.534786 = 0.465214;$$

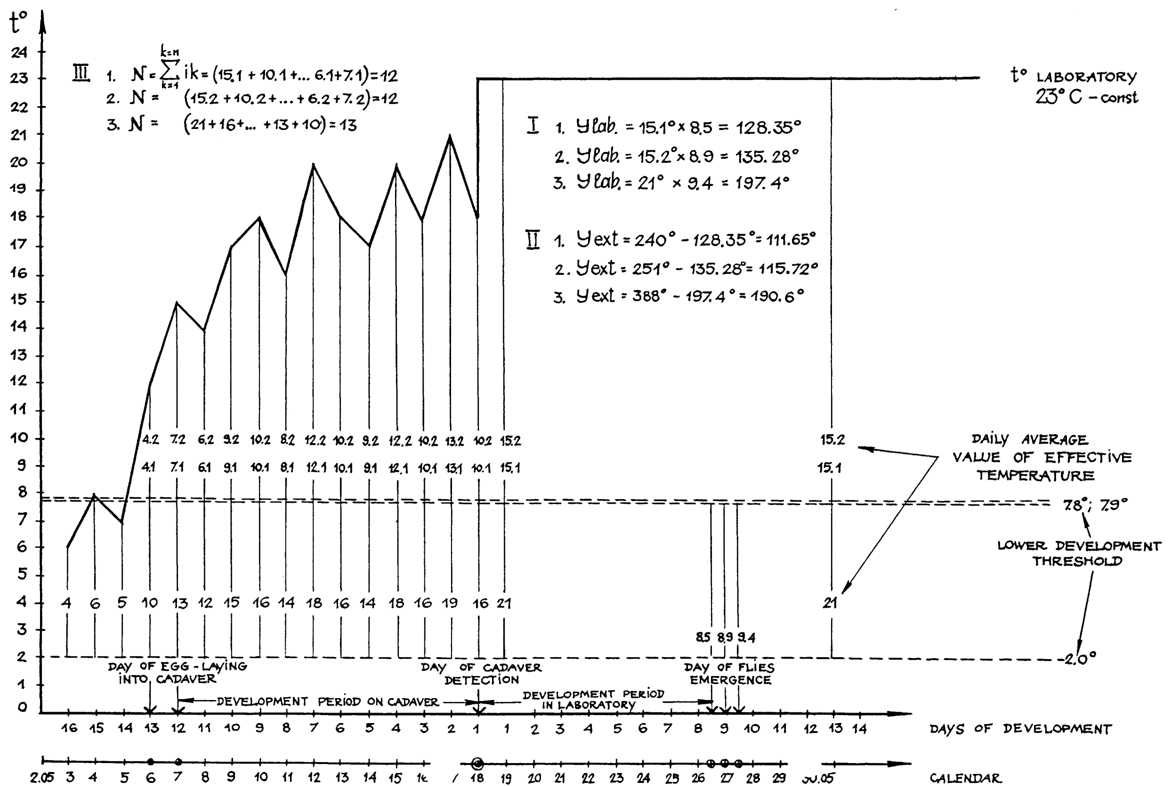

Fig. 1. Methods of retrospective determination of the date, when insects started to develop on a cadaver (examples: (1) *M. assimilis*; (2) *P. terraenovae*; (3) *C. vicina*).

$Y_{\text{ext.2}} = 1 - 0.53906232 = 0.4609377;$

$Y_{\text{ext.3}} = 1 - 0.50875056 = 0.4912494.$

3. The duration of insect development on the cadaver is determined (N)

$$N = \sum_{K=1}^{K=n} iK = (i_1 + i_2 + i_3 + \ldots + i_n),$$

where K is the number of sum members corresponding to the time of development of the cadaver; $(i_1 + i_2 + i_3 + \ldots + i_n)$ the respective values of 24 h temperatures of development index.

$$\begin{aligned} N_1 = (&0.0420816 + 0.0379152 + 0.0420816 \\ &+ 0.0504144 + 0.0379152 + 0.0420816 \\ &+ 0.0504144 + 0.0337488 + 0.0295824 \\ &+ 0.0379152 + 0.025416 + 0.0295824 \\ &+ 0.17832 = 0.476232) = 13\,\text{days}; \end{aligned}$$

$$\begin{aligned} N_2 = (&0.0406488 + 0.0366552 + 0.0406488 \\ &+ 0.0486144 + 0.0366552 + 0.0406488 \\ &+ 0.0486144 + 0.0326784 + 0.0286848 \\ &+ 0.0366552 + 0.0247032 + 0.0286848 \\ &+ 0.0167328 = 0.4606248) = 13\,\text{days}; \end{aligned}$$

$$\begin{aligned} N_3 = (&0.0412368 + 0.0386592 + 0.0412368 \\ &+ 0.0463896 + 0.0386592 + 0.0412368 \\ &+ 0.0463896 + 0.0360816 + 0.033504 \\ &+ 0.0386592 + 0.0309264 + 0.033504 \\ &+ 0.0257712 = 0.4922544) = 13\,\text{days}. \end{aligned}$$

4. Calendar date when insects started to develop on a cadaver is determined.

In our example, it is 6–7 May.

The given example of two alternative calculations proves that the method itself is sufficiently simple and does not require the special training of an medicolegal expert, whereas the determination of insect species is a fairly complicated task calling for serious taxonomic training and practical skills.

The proposed method was experimentally checked through solving "blind" problems based the temperature parameters of fly development. First, nine problems to determine probable emergence data were solved for *P. terraenovae* and *C. vicina*, egg-laying dates, periods of retention in each temperature condition during the development being known. As clear from the calculation results presented in Table 16, in five cases, the date of summer was determined precisely, in other cases deviation amounted to ±2 days.

Statistical evaluation of the difference between actual period of fly development and theoretically calculated one was made through the application of χ^2, correspondence criterion. In nine tests, $\chi^2 = 0.31$ ($P = 1.0$). It means that there is observed practically complete compliance between the actual data on development duration and their theoretical prediction.

Later on, the task became more complicated. The experimenter received, from laboratory personnel, data on the starting date and mass ending of the flies emergence, and also data on dates and temperature conditions and their variation, which changed at random, simulating conditions of the natural environment. The aim was to determine the egg-laying. Altogether 35 problems were solved for three fly species: *C. albiceps*, *P. terraenovae*, *C. vicina* (Calliphoridae).

Results obtained from solving the problems were statistically assessed using χ^2 criterion. Accuracy evaluation of

Table 16
Results of testing prediction accuracy for dates flies emergence for Calliphoridae

N	Actual data					Calculated data	
	Number of changes of temperature condition (number of rearrangements)	Temperature at the start of development (°C)	Date of eggs laying (start of development)	Date of flies emergence	Actual development period (days)	Date of flies emergence	Deviation of calculated data from actual data (days)
Protophormia terraenovae							
1	4	14.5	12.03	10.04	30	10.04	0
2	4	14.5	12.03	11.04	31	10.04	1
3	4	14.5	12.03	12.04	32	10.04	2
4	4	20.0	16.03	17.04	33	17.04	0
5	4	20.0	21.04	27–28.05	37–38	25.05	−2
6	4	20.0	23.04	27–28.05	35–36	27.05	0
7	4	20.0	28.04	2.06	36	31.05	−2
Calliphora vicina							
8	2	20.0	28.03	14.04	18	14.04	0
9	3	20.0	28.03	17–18.04	2.1–22	18.04	0

Table 17
Evaluation of accuracy of method for retrospective determination of starting date of Calliphoridae development under laboratory conditions using squared criterion

N	Flies species	Calculations made basing on start/mass emergence	Number of calculations (n)	Correspondence criterion (χ^2)	Probability of the given value (P_{χ^2})
1	*Crysomya albiceps*	Start	8	1.47	0.99
		Mass	6	0.1	1.0
2	*Protophormia terraenovae*	Start	6	1.18	0.95
		Mass	9	0.95	1.0
3	*Calliphora vicina*	Start	10	2.10	0.99
		Mass	17	1.88	1.0
4	Total for three species	Start	24	4.75	1.0
		Mass	32	2.93	1.0

the method for retrospective determination of the starting date of three fly species development is given in Table 17.

Correctness of the proposed method is fully confirmed for all the three species ($P = 1.0$). Slightly lower probability of χ^2 criterion ($P = 0.95$ was obtained for *P. terranovae* when calculations were made based on the beginning of emergence. On the whole for the three species χ^2 criterion probability is 1.0 if calculated bas on the beginning of emergence. To avoid any possible inaccuracies, practical calculations should be made for all the species found on a cadaver.

The result obtained using the proposed method corresponds to the maximum possible development time of species under specific conditions. The development can be accelerated by metabolic heat release in the course of fly larval development. The time when insects started to develop on a cadaver does not always coincide with the time of death, just as the place where a cadaver was discovered is not always the place of death.

6. Methods of making entomological and botanic studies while examining the place of cadaver detection

Successful application of entomological data in the investigation practice depends on the correct collection of insects on the place, where cadaver was found, and their timely delivery alive for entomological study. Only a qualified expert can properly and reliably collect cadaver fauna at the discovery place and to register proper parameters influencing the vital activities of insects. To accomplish this task an investigator may cooperate with an entomologist from local sanitary and epidemiological inspection (in USSR) or with a medicolegal expert, participating in the examination of the cadaver and the place, where it was found.

To collect and deliver insect to the laboratory one should prepare beforehand a set of tools (pincers, spoons, spatulas, compass, thermometer or better thermograph) and containers, designed to transport alive insects. Containers should meet stringent requirements. These should be glass or plastic jars (cases) sealed with perforated lids or lids having fine-mesh metallic wires, or covered with dense cloth, which can be secured to the neck by rubber rings. It is strictly forbidden to employ polyethylene bags or rubber, instead of cloth. A jar will be filled half-height with sawdust or sand.

In the course of cadaver examination, it is necessary to collect as many species of insects and other living organisms as possible from different spots, noting their precise location relative to cadaver the remains and not only the area should be taken into consideration, but also the depth, from which they were taken.

Special attention should be paid to collecting flies stages, as it is the earliest, most numerous and well-studied component of cadaver entomofauna. Flies are caught by a conventional chemical test tube, a sitting insect is covered with it. Several flies can be put into the same test tube with cotton wool interlayers there-between. Young flies (Phorate adult) just emerged from puparia are necessarily collected separately. They differ from the ‘‘grown-up’’ ones by the folded or yet not completely unfolded, wings or, if the wings are already unfolded, by a whitish shade reluctance and fly. Huge armies of colored flies on cadaver remains and adjacent objects point to recent mass emergence of flies from puparia; some 20–30 of such flies should be caught. Young flies are to be placed separately from the mature ones and a label accurately specifying the date and, hour of collection is to be provided. Laid eggs will be transported separately in jars on a piece of nutrient substrate or wet soil, to prevent them from quickly drying out.

Flies larvae will be taken from different spots on the cadaver, since they tend to be grouped species to species, and all age groups will be collected which can be visually distinguished by their length and thickness of body. Eggs and larvae are delivered to the laboratory alive, so that flies can be raised there from. This helps to identify species and is an obligatory component of calculation techniques discussed earlier. In parallel, some larvae are preserved in 70° ethyl alcohol; formaline is undesirable.

Puparia (pupae), just as their chitin cuticles are taken from different parts over after fly emergence and also from the ground under the cadaver (pieces of furniture, slits in the floor, etc.), since larvae of the majority of fly species pupate in the soil with the exception of *P. terraenovae* R.-D. and small species of Phoridae and Piophilidae families; and are grouped according to size and resemblance in appearance. They are transported in jars on slightly wet sawdust or sand. In the laboratory flies will emerge the puparia. Puparia should be placed are per tube as often different species may be very similar superficially.

It is important to fix the interrelation between different life stages of flies on the cadaver, because this may prove to be helpful in determining the multiplicity of flies invasion, which is especially essential when a suspicion exists of a cadaver being moved.

Each sample of fly life-stages should amount to at least 30–50 items from different spots on the cadaver itself, cadaver bed and bed soil at 25–30 cm depth, in buildings-samples from pieces of furniture and slits in the floor will be taken.

Beetles are collected using pincers, then killed by ether and put into test tubes apart from other insects. If beetles or their larvae are not killed, then each specimen should be put into separate jar or test tube with air-permeable cork plug (beetles gnaw through the cloth and cork eventually). When samples are taken from a mummified cadaver, especially when Dermestidae are present or their larvae (covered with thick bristles (hairs)), one should remember that they are perniciously affected by rotten meat and moisture. Nutrient substrate should be dried. In the dust around a mummified cadaver large amounts of very small insects (1–1.5 mm) can be found, therefore, samples should be also taken, closed tightly and delivered to entomologists for study.

Beetle pupae found in the ground under a cadaver at depths of 340 cm are distributed between the jars according to places where they were taken and powdered with soil. A portion of larvae and pupae specimens is fixed in alcohol. In case it is impossible to collect insects from soil during examination, three to four soil samples sized $0.3\,\text{m} \times 0.3\,\text{m} \times 0.4\,\text{m}$ should be taken for further treatment in the laboratory.

It is necessary to preserve some of the collected insects in order to carry out studies in case insects taken alive should die in the course of transportation or wrong handling in the laboratory. Jars containing live insects are not to be exposed to direct sunbeams or left near sources of heat.

During the examination the interrelation between insect location and clothes position, their dirty patches and any damage, zones of cadaver tissues injuries should be registered. If individual parts of the cadaver show some kind of preservation condition, factors entailing this type of preservation should be determined at the cadaver site, since they may affect vital activity of insects.

When inspecting a cadaver site in a natural environment it is practical along with insect collection to pay attention to nearby vegetation at its condition. The protocol of inspection of the incident site should contain the distinctions of "cadaver bed" vegetation from that around it (species composition, color, density, shoots richness), presence of area with dead plants and its relation to cadaver projection and to released cadaver decay products and fly larvae location, revival vegetation on cadaver bed. Samples of plants and their remains should be fixed between sturdy paper sheets or flooded with 96° ethyl alcohol. Branches and grass used to hide the cadaver should be collected as well.

Attention should be paid to the character of any cadaver damage by rodents or birds, or whether it was dragged asunder by bigger animals. Examination of the incident place is supplemented by a more detailed study of the characteristics of the locality, which includes: description of vegetable and animals environment, soil, relief watercourse, living and service houses; evaluation of light conditions, especially illumination with direct sunbeams, and light variation during the day, humidity, regime of temperatures, particularly in the layer near the ground, cadaver tissues temperature (fly larvae mass), soil water level and its probable variation, possibility of the cadaver location being flooded with rain or melted snow, probable snow-retention time.

If a room is the place of the incident, it is necessary to ensure at least 24 h fixation of air temperature by means of thermograph, condition of windows and air vents (open–closed) should be noted, presence of ventilation openings, smoke stacks, refuse chutes and also the distance between

Table 18
Intervals of necrobiont flies development periods versus environment temperature relationships (days)

Development temperature (°C)	Development period from egg to puparium	Development period from egg to fly emergence
11	21.2–50.0	43.1–103.5
12	19.1–38.1	38.8–69.0
13	17.4–30.8	35.3–51.8
14	15.9–25.8	32.3–42.9
15	14.7–22.2	29.8–39.3
16	13.6–19.5	27.7–36.3
17	12.7–17.4	25.9–33.7
18	11.9–15.7	23.0–31.5
19	11.2–14.3	20.7–29.5
20	10.6–13.1	18.8–27.8
21	10.0–12.1	17.3–26.2
22	9.6–11.3	15.9–24.8
23	9.1–10.6	14.8–23.6
24	8.7–10.1	13.8–22.5
25	8.3–9.7	12.9–21.5
26	7.9–9.3	12.2–20.5
27	7.6–8.3	11.5–15.5
28	7.3–7.9	10.9–14.9
29	7.5	10.4–11.8
30	7.2	9.9–11.3

the cadaver and heat sources. While working at the place where the cadaver was found, it is desirable to find explanations for all the distinctions between the development of cadaver fauna and that, which would be expected based on the characteristics of locality, meteorological conditions, season of the year and state of cadaver tissues.

Decision to seek entomological expertise should be taken right at the incident place and collected material should be sent to entomologists by special delivery. Any delay in delivery can make entomological study a useless task. A formal warrant approving such expertise may be sent later. Further on, an entomologist should receive: a warrant, a copy of the protocol of incident place inspection, a copy of incident place layout, photographs, a copy of medicolegal-autopsy in the morgue (not a medicolegal conclusive statement), hourly meteorological reports for 1–2 months preceding the date of cadaver discovery (temperature, humidity, precipitations, time of sunrise and sunset), temperature measurements in the building.

To plan investigation activities before the results of entomological expertise are obtained, various average periods of fly development on a cadaver can be used (Table 18).

7. Conclusion

Using the results of entomological expertise one should not forget, that the time when insects started to develop on a cadaver does not necessarily coincide with the moment of death, and the place, where the cadaver was found may not be the one of death. It is inadmissible to make any conclusions about the time of death based on the degree of cadaver tissues decomposition or cadaver skeletization. Entomological studies are the base for solving the following problems:

1. to determine the season when a cadaver the arrived at the place where it was found;
2. to identify the time of death or the time when a cadaver arrived to the place where it was discovered;
3. to establish the fact of cadaver being moved;
4. to identify the initial location of a cadaver or the place where it was hidden (by means of investigational simulation experiment).

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