Int J Leg Med (1993) 106:25–29

International Journal of
Legal Medicine
© Springer-Verlag 1993

# Postmortem activity of lactate and malate dehydrogenase in human liver in relation to time after death

**Tomasz Gos and Stefan Raszeja**

Institute of Forensic Medicine, Medical Academy in Gdańsk, ul. Curie-Skłodowskiej 3a, PL-80-210 Gdańsk, Poland

Received July 20, 1992 / Received in revised form March 26, 1993

**Summary.** Changes in the activity of lactate (EC 1.1.1.27, LDH) and malate (EC 1.1.1.37, MDH) dehydrogenases were measured in tissue extracts of human liver kept at 5 different temperatures until 35 days after death. The investigated activities decreased in proportion to time of storage which enabled an estimation of time after death by statistical analysis of the data.

**Key words:** Time of death – Enzyme activity

**Zusammenfassung.** Teile menschlicher Lebern wurden bis zu 35 Tage nach dem Tod bei −3, 2, 7, 12 und 17°C gelagert. Die in den Extrakten gemessenen Aktivitäten der Lactat- (EC 1.1.1.27, LDH) und Malatdehydrogenase (EC 1.1.1.37, MDH) zeigten eine zeitabhängige Verminderung. Nach statistischer Bearbeitung erlauben die Meßergebnisse eine Schätzung der Todeszeit.

**Schlüsselwörter:** Todeszeit – Enzymaktivität

## Introduction

Attempts to estimate time after death using the measurement of postmortem enzyme activity began in the late 1950s and early 1960s. LDH and aminotransferase activities were investigated in sera from cadavers up to 60 h after death [5, 6, 10] and the data obtained enabled an approximate estimation to be made.

Investigations directly connected with time of death analysis followed and revealed the usefulness of alkaline phosphatase activity measurement in blood and muscle tissue up to 150 h after death [13, 16]. In muscle tissue enzyme activities such as ATP-ase [11, 23], 5-nucleotidase [11], lactate dehydrogenase, α-hydroxybutyric dehydrogenase, asparate aminotransferase [12] and cathepsins [24] were also investigated but the observation time did not exceed a few days after death. The same problem also occurred with further experiments using different enzymes from other organs and body fluids [15, 17]. The only enzyme activities which were measured over longer periods (up to 40 days after death) were muscle protease and creatine kinase [18]. This obvious predominance of studies on the earlier postmortem phase is specific not only for biochemical, but also for other investigations dealing with the problem of time after death [7].

Even in this early period the tendency of enzyme activity values to show significant variation was pointed out which seemed to be discouraging for estimating time after death [1, 22]. On the other hand, very promising biochemical investigations were also published [4, 18].

These problems were crucial for the direction of the present research, which aimed at the elaboration of a simple biochemical method for an estimation of time after death, especially for the later postmortem period, based on the measurement of LDH and MDH activity. These 2 liver enzymes are characterized by an easy extraction from liver tissue, their high activity and simple assay with relatively cheap reagents, which is important for a routine method. In contrast to other enzymes, such as phosphofructokinase [21], the activities of MDH and LDH are independent of developmental changes and hormones as well as dietetic influences.

Various storage temperatures were applied in accordance with earlier reports on the influence of temperature on postmortem enzyme activity [18] and served to approximate to real conditions.

## Material and methods

The investigation was performed on 25 corpses, 4 women and 21 men, aged 6–69 years. Death had occurred suddenly and from different non-toxic causes (17 died from trauma, 5 from heart failure, one each from drowning, hanging and brain aneurysma rupture). The livers showed no macroscopic pathological changes. The time from death until collection of the tissue did not exceed 12 h. A piece of liver (approx. 250 g) was taken, divided into 5 parts and stored at different temperatures (−3, +2, +7, +12, +17°C) in sealed plastic containers. LDH activity was measured every day for the first 14 days and then once every 7 days up to 35 days after death. MDH measurements were performed similarly with the exception that measurements at −3°C were made every seventh day up to 35 days. For each measurement a fragment of liver approx.

*Correspondence to:* T. Gos

100 mg in weight was obtained from the inner part. The tissue was homogenized in 10 ml 20 m$M$ Tris/HCl buffer pH 7.8 and the homogenate was centrifuged at +4°C and 20000 × g for 30 min. The obtained supernatant was used for activity measurement. Changes in enzyme activities were measured spectrophotometrically on a SPECORD UV-VIS by measuring the decrease in absorbance at 340 n$M$. The final volume of the reaction mixture was 1 ml and the temperature of the assay was 37°C. LDH activity was assayed as follows: to 930 µl of 50 m$M$ Tris/HCl buffer (ph 7.8) was added 20 µl of supernatant and 30 µl of 5 m$M$ NADH solution. The absorbance was measured then 20 µl of freshly prepared 50 m$M$ sodium pyruvate solution was added and the absorbance decrease was assayed. MDH activity was measured in a similar way except that pyruvate was replaced by 50 m$M$ oxaloacetate and kept on ice during the assay. The activity of both enzymes was calculated per gram liver relative to NADH concentrations in the reaction mixture and taking into consideration the molar absorbance of NADH which is 6200.

The protein concentration in the supernatant was estimated by the method of Lowry [14].

The results were analysed statistically using the model of linear regression with the help of a computer programme.

The calculation of the most probable time interval after death and also the upper and the lower limits, which include real death time with known probability, is performed by the following equation:

$$\boxed{\begin{matrix}U\\L\end{matrix}} = \bar{x} + \frac{\beta_1(y_0 - \bar{y})}{\lambda} \mp \frac{t\sigma}{\lambda}\left[c(y_0 - \bar{y})^2 + \lambda\left(\frac{k+1}{k}\right)\right]^{1/2}$$

where the most probable death time is determined by the expression: $\bar{x} + \beta_1(y_0 - \bar{y})/\lambda$ and $y_0$ is the assayed activity value. The coefficients, specific for each of the regression equations are: $\bar{x}$-general mean value of time, $\bar{y}$-general mean value of enzyme activity in appropriate results pool (without excluded cases, see "Results"), $\beta_1$-regression coefficient (from the equation: $y = \beta_0 + \beta_1 x$), $\sigma$-standard deviation of the coefficient $\beta_1$, $t$-Student test value, $k$-number of $x$ and $y$ values appropriate for each regression equation, where $y$ is the activity mean value of statistically analysed cases (without those excluded, see "Results") and $x$ is day of the experiment,

$$c = \frac{1}{\sum(x_i - \bar{x})^2},\ \lambda = \beta_1^2 - ct^2\sigma^2 \text{ (see examples Fig. 3 and 4).}$$

The statistical differentiation of activity decrease of each enzyme dependent on the temperature and, between the two enzymes at each temperature was tested by the variable F, possessing F-Snedecor's distribution.

## Results

Figures 1 and 2 show the mean changes of activity for both enzymes in each of the 25 cases and the investigated temperatures during the total experimental period.

The results of the 5 cases with significantly different activity decreases were excluded from the statistical analysis. The activity assay results taken from 20 cases were used to perform the linear regression and time after death calculations. The difference in activity patterns between the group of 20 and of the 5 cases excluded was particularly obvious with MDH at the temperature +17°C (see Table 1). The excluded cases showed weekly MDH activity decreases lower than 54% (of previous activity before one week) and absolute values ranged from 376 µmol/min × $g^{-1}$ at the beginning and 64 µmol/min × $g^{-1}$ on the 28th day at a temperature of +17°C. MDH assay results obtained at the temperature −3°C were not statistically analysed because no significant activity decrease occured (see Fig. 2).

The activity of both enzymes from 20 analysed cases revealed statistical differences between LDH and MDH

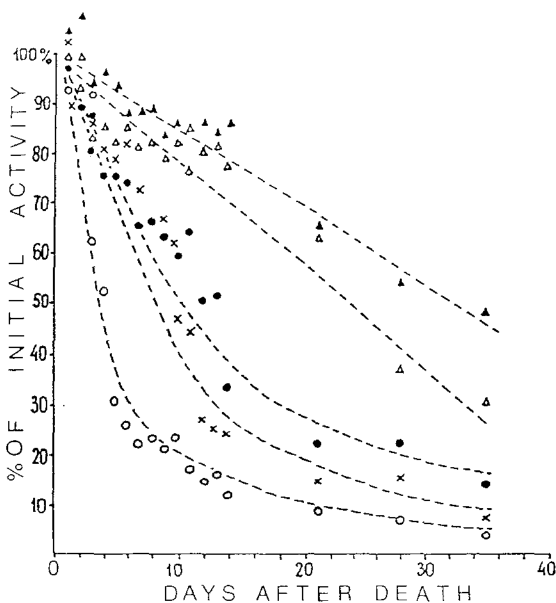


**Fig. 1.** Mean activity values for lactate dehydrogenase expressed in % of initial activity at the temperature: ○ +17°C, × +12°C, ● +7°C, △ +2°C, ▲ −3°C. The initial mean activity value of the 25 investigated cases is given as 100%, with an absolute value of 102 µmol/min × $g^{-1}$, standard deviation 30 µmol/min × $g^{-1}$ (beside the start point representing all the 25 cases other points represent the activity mean value of 8–15 results

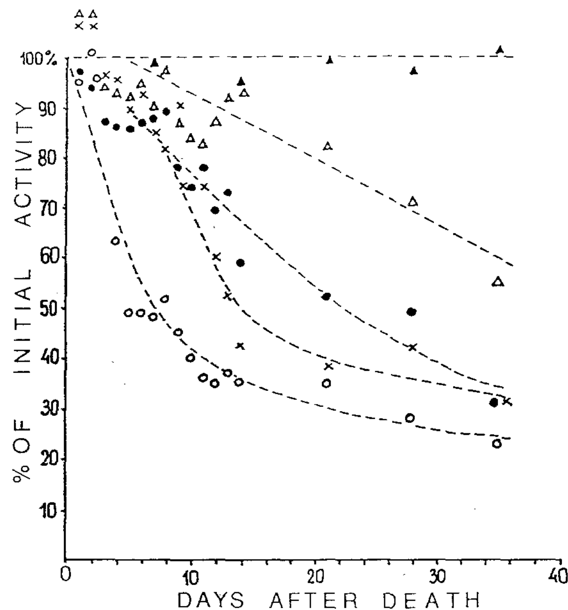


**Fig. 2.** Mean activity values for malate dehydrogenase expressed in % of initial activity at the temperature: ○ +17°C, × +12°C, ● +7°C, △ +2°C, ▲ −3°C. The initial mean activity value of all the 25 investigated cases is given as 100%, with an absolute value of 230 µmol/min × $g^{-1}$, range 124–376 µmol/min × $g^{-1}$, standard deviation 69 µmol/min × $g^{-1}$ (beside the start point representing all the 25 cases other points represent the activity mean value of 8–15 results)

**Table 1.** Activity ranges of LDH and MDH (in μmol/min × $g^{-1}$) during the experiment at +17°C

| Enzyme | Cases group | Initial activity | Activity range on: | | | | |
|---|---|---|---|---|---|---|---|
| | | | 7th day | 14th day | 21st day | 28th day | 35th day |
| MDH | S | 140–335 (20) | 10–120 (6) | 4– 78 (5) | 0– 15 (4) | 0– 13 (4) | 0– 43 (5) |
| | E | 140–376 (5) | 171–337 (3) | 100–280 (3) | 93–214 (4) | 64–188 (4) | 45–150 (4) |
| LDH | S | 72–158 (20) | 0– 25 (6) | 0– 5 (5) | 0– 2 (4) | 0– 2 (4) | 0– 2 (6) |
| | E | 58–177 (5) | 14– 59 (3) | 11– 58 (3) | 5– 32 (4) | 6– 20 (4) | 7– 19 (4) |

*S* – statistically elaborated
*E* – excluded group of cases
In brackets number of results

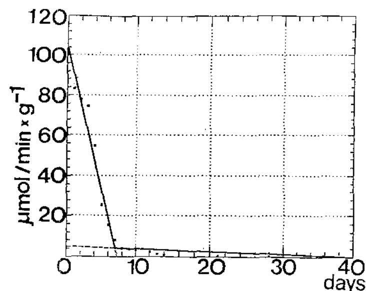


**Fig. 3.** Linear regression of LDH activity *(y)* for the time after death *(x)* at the temperature +17°C.

First time interval (days 0–7, $R = -0.9794$): $y = 104.59–14.06x$; coefficient values: $\bar{x} = 3.5$; $\bar{y} = 55.4$; $\beta_1 = -14.06$; $k = 8$; $c = 0.02359$; $\lambda = 195.40285$; $\sigma = 6.82905$; $t = 1.44$. Activity = 49, death time ≈4 ± 0,7.

Second time interval (days 8–35, $R = -0.7976$): $y = 4.2725–0.14553x$; coefficient values: $\bar{x} = 16.1$; $\bar{y} = 1.9$; $\beta_1 = 0.14553$; $k = 10$; $c = 0.00137$; $\lambda = 0.01606$; $\sigma = 1.04164$; $t = 1.86$. Activity = 1, death time ≈24 ± 17.

Co-ordinates of the intersection point of both linear regression plots: $x \approx 7.2$; $y \approx 3.2$ (for the LDH activity ≥4 the former of the two parameter groups is applied to the death time calculation, for this activity ≤3 the latter)

**Table 2.** Precision of death time estimation

| Temperature | Enzyme | Real time after death (days) | Estimation probability | Span of calculated time interval (days) |
|---|---|---|---|---|
| +17°C | LDH | 0– 7 | 0, 95 | ± 1 |
| | | 8–35 | 0, 95 | ± 18 |
| | MDH | 0– 6 | 0, 95 | ± 2 |
| | | 7–35 | 0, 95 | ± 13 |
| +12°C | LDH | 0–14 | 0, 95 | ± 6 |
| | | 15–35 | 0, 85 | ± 25 |
| | MDH | 0–18 | 0, 95 | ± 3 |
| | | 19–35 | 0, 95 | ± 13 |
| +7°C | LDH | 0–19 | 0, 95 | ± 1 |
| | | 20–35 | 0, 90 | ± 19 |
| | MDH | 0–35 | 0, 95 | ± 14 |
| +2°C | LDH | 0–35 | 0, 95 | ± 11 |
| | MDH | 0–35 | 0, 95 | ± 21 |
| −3°C | LDH | 0–35 | 0, 95 | ± 10 |

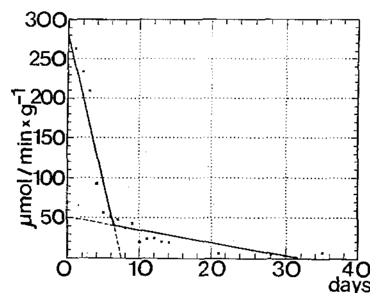


**Fig. 4.** Linear regression of MDH activity *(y)* for the time after death *(x)* at the temperature +17°C.

First time interval (days 0–6, $R = 0.93539$): $y = 278.15–36.666x$ coefficient values: $\bar{x} = 3.5$; $\bar{y} = 149.8$; $\beta_1 = 36.666$; $k = 8$; $c = 0.02359$; $\lambda = 1283.0539$; $\sigma = 35.41594$; $t = 1.44$. Activity = 150, death time ≈3,5 ± 1,5.

Second time interval (days 7–35, $R = 0.82518$): $y = 50, 187–1.6297x$ coefficient values: $\bar{x} = 13.8$; $\bar{y} = 27.8$; $\beta_1 = -1.6297$; $k = 13$; $c = 0.00103$; $\lambda = 2.29039$; $\sigma = 10.48375$; $t = 1.796$. Activity = 25, death time ≈16 ± 13.

Co-ordinates of the intersection point of both linear regression plots: $x \approx 6,5$; $y \approx 39,6$ (for the MDH activity ≥40 the former of the two parameter groups is applied to the death time calculation, for this activity ≤39 the latter)

at all of investigated temperatures and a dependence on the temperature. LDH at the temperature +7°C and both enzymes at temperatures +12 and +17°C revealed different dispersion of activity values dependent on the time after death. In these cases the total 35-day experimental period was subdivided into 2 intervals with pooled results, described by 2 different linear regression plots. This enabled a higher precision of death time estimation to be obtained (see examples, Figs. 3 and 4). The ordinate of intersection point of two linear regression plots gives the border value determining which regression equation should be applied for the time estimation in an actual case. If the given activity is higher than this ordinate, the former equation, describing the pooled results nearest to the actual time of death is used. If the activity is lower than this ordinate, the latter equation is applied (see examples Figs. 3 and 4).

The remaining pooled results were calculated by only one linear regression equation.

The correlation coefficient R ranged between −0.98129 and −0.76364.

The highest precision of death time estimation (greater than ten hours) was achieved for LDH up to 7 days after

death at +17°C and up to 19 days at +7°C. Table 2 gives an overview of the achieved precision of the death time estimation (the limits of the calculated time intervals are rounded up).

Examples of the applied mathematical formulae, and the linear regression plots are presented in Figs. 3 and 4 together with the appropriate regression equations and coefficient values needed for the calculation of time after death (accordingly to the equation in "Materials and methods").

**Discussion**

MDH activity was more stable than LDH at the investigated temperatures. The difference between these 2 enzymes seems to correspond with earlier reports about the protective influence of mitochondrial membranes on postmortem enzyme activity [20], since LDH is exclusively located in the cytoplasmic fraction but MDH is present in the cytoplasmic and mitochondrial fractions.

During the total experimental period no changes in soluble protein concentration were observed, so that the enzyme activity decrease calculated on the weight of a tissue corresponds to that calculated on the weight of protein.

The decrease in assayed activity for both enzymes showed good correlation with the actual time after death, which proved that the choice of enzymes and the organ for the purpose of death time estimation was correct.

The best mathematical expression of LDH and MDH activity plots were linear regression equations. Contrary to other methods elaborated for the late postmortem phase, death time estimation using LDH and MDH activities assay enables not only the determination of the most probable time of death, but also the time interval, which includes with a high probability the real time of death. An exclusion of cases with abnormal activity plots was the most important factor for the precision of time analysis and enabled the dispersion of the results observed in earlier reports to be reduced. A subdivision of the pooled results from one temperature into two groups, characterized by different statistical parameters was also important. Both operations enabled the time after death to be estimated in a period as long as 19 days with the precision obtained by earlier enzyme activity investigations only for short postmortem intervals.

The enzyme activity decrease is not comparable to the results of LDH investigations in animal brains [20, 21] and human muscles, which did not reveal activity decrease up to 110 h after death, but a decrease of soluble protein was observed [13]. Additionally, no difference between MDH and LDH was found, which is contrary to the results obtained at each of experimental temperatures.

Contrary to other investigations on enzyme activity, this method enabled an estimation of time after death. Cathepsins are an example of such an activity, revealing increases at 13–18 and 25–36 h after death (for heart and skeletal muscle respectively), and then a decrease [24], which is disadvantageous for the death time estimation.

Results different from those discussed above were obtained by Enticknap, who followed changes in LDH in blood serum from the brachial vein. He found a linear increase of activity up to 60 h post mortem [6]. Such an increase could be due to a release of enzyme into body fluids from necrotic tissues after death. The decrease in enzyme activity due to postmortem changes proceeds simultaneously. So the activity assayed in blood is determined by these 2 processes. Thus one could suppose, that it is more variable than the activity measured in a tissue, reflecting only the changes in enzyme activity. In this way, an enzyme activity assay in a tissue seems to be more reliable in death time estimation.

The death time was estimated by Enticknap [6] exact to more than 10 h. Similar precision of death time estimation was achieved by LDH activity assays in isolated liver at +17°C up to the 7th day and at +7°C up to the 19th day after death. This was an advantage in comparison with the method cited above as well as that of Mallach and Laudahn [16], which dealt only with short postmortem periods. They followed acid and alkaline phosphatase activities and their substrate and product concentrations in human serum during a time interval 21–140 h after death when corpses were kept in cold storage. The achieved precision of time estimation was comparable with that of Enticknap [6].

Although other enzyme activities were assayed over longer periods and the results obtained seemed to be hopeful for death time calculations, the authors did not perform any further elaboration [18]. For instance muscle protease and creatine kinase activities were followed in skeletal muscle of rats up to 40 days after death, where the corpses were stored at room temperature and +4°C [18]. The former enzyme activity increased at both temperatures, when the latter constantly decreased, reached zero value after 12 days at room temperature and half of the initial value after 35 days at +4°C. Those results of creatine kinase activity assay are similar to these of human liver LDH, which revealed about 15% of initial activity after 12 days at +17°C and about 30% after 35 days at +2°C (corresponding MDH values were 35% and 60%). Although the standard deviation and the logarithm of the ratio of protease/creatine kinase activities seemed to be promising for death time estimation, the continuation was not reported.

The concentration of δ-aminovaleric acid as a marker of postmortem proteolyse was followed by Bonte and Theusner [2] in different tissues and body fluids in man and animals up to 45 days after death. A significant dispersion of results probably made the authors give up the elaboration of death time equation, so with the help of their method one can determine only one limit of the interval including death time. Electrofocusing of protein fractions appearing with proteolysis [19] has a similar value for the death time estimation. Contrary to these qualitative rather than quantitative methods, Daldrup [4] elaborated the mathematical equation based on the concentration of 3 amino acids from a human putrefying brain: α-aminobutiric (ABU), γ-aminobutiric (GABA) and glutaminic (GLU). These showed increasing values in a period 4–20 days after death at temperatures rang-

ing between 15 and 25°C with values of the coefficient: $\ln \{([ABU] + [GABA]) \times [GLU]^{-1}\} + 1$. Thus this equation enabled the most probable death time to be estimated, but it does not allow a calculation of the limits of a time interval, which include with known probability the real death time. The determination of such an interval is often more useful in forensic practice.

The investigations of Daldrup revealed the role of GABA as a putrefaction marker [3] and linked its concentration increase with the activity of anaerobic bacteria. The present MDH and LDH investigations were performed on isolated fragments of the liver, i.e. in aerobic conditions. The fragments were obtained within a relatively short time period after death (up to 12 h). In relation to earlier reports [8] no growth of anaerobic bacteria was assumed. Additionally, the temperature range was not appropriate for that growth of these microbes [3]. The facts stated above suggested that no influence of anaerobic bacteria on the assayed enzyme activities was found under the described conditions. However it would be interesting to know whether these bacteria influence MDH and LDH activity in the liver of putrefied cadavers ("in situ") in comparison to isolated fragments of the liver. The comparison of enzyme activity assayed in the experiment with the "in situ" activity gives a comparison of death time calculated from enzyme activity with the real one. Such investigations, performed on the large number of corpses are absolutely necessary before the method can be applied in the practice. The first results are encouraging in the matter of the death time estimation [9]. They also suggest the cause of death has a greater influence on the activity change pattern than the storage conditions (i.e. liver "in situ" or isolated fragments).

In summary, the data presented above indicate that MDH and LDH activity assays in human liver could be valuable for the estimation of time after death over longer time periods. They also suggest, that for this purpose enzyme activity investigations have not yet been entirely exploited.

## References


1. Balasooriya BAW, Hill CAS, Williams AR (1984) The biochemical changes in pericardial fluid after death. An investigation of the relationship between time since death and the rise or fall in electrolyte and enzyme concentrations and their possible usefulness in determining the time of death. Forensic Sci Int 26:93–102
2. Bonte W, Theusner J (1979) Nachweis und thanatologische Bedeutung von Delta-Aminovaleriansäure. Z Rechtsmed 83: 139–146
3. Daldrup T (1983) Die Aminosäure des Leichengehirns und ihre Bedeutung für Todesursachen- und Leichenaltersbestimmungen. Habilitationsschrift, Universität Düsseldorf
4. Daldrup T (1983) Praktische Erfahrungen mit der Leichenalterbestimmung durch Auswertung bakterieller Stoffwechselprodukte. Z Rechtsmed 90:19–25
5. Dotzauer G (1962) Enzymatische Aktivitäts-Untersuchungen im Leichenserum. Beitr Gerichtl Med 22:78–85
6. Enticknap JB (1960) Biochemical changes in cadaver sera in fatal acute heart attacks. J Forensic Med 7:136–146
7. Forster B (1986) Praxis der Rechtsmedizin für Mediziner und Juristen. Georg Thieme, Stuttgart New York, pp 11–45
8. Fukita K (1967) Bacteriological studies on postmortem changes. Jpn J Leg Med 21:49–74
9. Gos T (1992) Die ersten praktischen Erfahrungen mit eigener Todeszeitbestimmungsmethode. Zentralbl Rechtsmed 38:11
10. Hall WEB (1958) The medico-legal application of the serum transaminase test. J Forensic Sci 3:117–122
11. Jakliński A, Bryc R (1974) Zachowanie aktywności adenozynotrójfosfatazy i 5-nukleotydazy w mięśniach szkieletowych szczurów we wczesnym okresie pośmiertnym. Arch Med Sąd i Krym 24:275–278
12. Laarse A van der, Dijkshoorn NJ, Hollaar L, Caspers T (1980) The (iso)enzyme activities of lactate dehydrogenase, α-hydroxybutyrate dehydrogenase, creatine kinase and asparate aminotransferase in human myocardial biopsies and autopsies. Clin Chim Acta 104:381–391
13. Laudahn G, Mallach HJ (1965) Untersuchungen zur postmortalen Aktivität von Hauptkettenenzymen in menschlicher Skelettmuskulatur. Klin Wochenschr 43:959–960
14. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurment with the Folin phenol reagent. J Biol Chem 193:265–275
15. Lythogoe AS (1980) The activity of lactate dehydrogenase in cadaver sera: a comparison of different sampling sites. Med Sci Law 20:48–55
16. Mallach HJ, Laudahn G (1964) Vergleichende Untersuchungen mit enzymatischen Methoden an Vital- und Leichenblut im Hinblick auf die Todeszeit. Klin Wochenschr 42:693–699
17. Mann DMA, Barton CM, Davies JS (1978) Post mortem changes in human central nervous tissue and the effects on quantitation of nucleic acids and enzymes. Histochem J 10: 127–135
18. Mayer M, Neufeld B (1980) Post mortem changes in skeletal muscle protease and creatine phosphokinase activity – a possible marker for determination of time of death. Forensic Sci Int 15:197–203
19. Mittmeyer HJ, Strebel KH (1980) Experimentelle Untersuchungen zur forensischen Liegezeitbestimmung durch Elektrofokussierung von löslichen Muskelproteinen. Z Rechtsmed 85:235–240
20. Oemichen M (1980) Enzyme alterations in brain tissue during the early postmortal interval with reference to the literature. Z Rechtsmed 85:81–95
21. Pla A (1986) Postmortem activity of the key enzymes of glycolysis in rat brain regions in relation to time after death. Z Rechtsmed 97:49–59
22. Tsunenari S, Lythogoe AS, Gee DJ (1981) The activity of the rate-limiting glycolytic enzymes in the erythrocyte with relation to the time after death. J Forensic Sci 21:333–336
23. Vandor E, Jozsa L (1978) Postmortale Veränderungen in den myofibrillaren Adenosintriphosphatasenaktivität der Skelettmuskulatur. Z Rechtsmed 80:265–272
24. Zharov VV, Melnikova GM (1989) Aktiwnost katiepsinow w miokardie i skielietnych myszcach kak priznak dawnosti nastuplienija smierti. Sud Med Expert 32:38–40