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Enzyme Alterations in Brain Tissue During the Early Postmortal Interval with Reference to the Histomorphology: Review of the Literature

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Summary. The state of research on enzyme alterations in brain tissue during the early postmortal interval is surveyed with special reference to the histomorphology; the questions currently discussed in the literature are given special consideration. The type of alterations appearing during the postmortal interval and their dependency on the length of the interval are described so that practically applicable conclusions may be drawn. The findings on enzyme alterations presented in the literature (enzymes of the oxidative metabolism, transmitter, enzymes) are compiled in tables.

It could be shown that important structural alterations ascertainable with light microscopy and quantitative alterations in enzyme activity ascertainable with biochemical methods do not usually occur during a 6- to 8-h postmortal interval. Qualitative investigations (i.e., histoenzymatic studies) with longer postmortal intervals and with positive findings are applicable.

Key words: Central nervous system, enzyme activity – Postmortal alterations, enzyme activity in brain

Zusammenfassung. Es wird eine Übersicht zum Stand der Forschung über Enzymveränderungen im Hirngewebe während des frühen postmortalen Intervalls unter Berücksichtigung wesentlicher histomorphologischer Veränderungen gegeben. Dabei wird auf einige, augenblicklich im Schrifttum diskutierte Fragen eingegangen. Die Art der während des postmortalen Intervalls auftretenden Veränderungen sowie ihre Abhängigkeit von der Dauer des Intervalls werden beschrieben, um praktisch verwertbare Schlußfolgerungen zu ermöglichen. Die Befunde über Ezymveränderungen (Enzyme des oxidativen Metabolismus; Transmitter-Enzyme) aus dem Schrifttum werden tabellarisch zusammengestellt.

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Es zeigt sich, daß in der Regel lichtmikroskopisch erfaßbare, wesentliche Strukturveränderungen ebensowenig wie wesentliche quantitative Veränderungen der biochemisch erfaßbaren Enzymaktivität während eines 6–8 h dauernden postmortalen Intervalls zu erwarten sind. Qualitative Untersuchungen im Sinne von histoenzymatischen Untersuchungen sind auch während länger dauernden Zeiträumen post mortem möglich und bei positivem Ausfall verwertbar.

Schlüsselwörter: Hirngewebe, Enzymveränderungen post mortem – Enzymaktivität, im Hirngewebe in der postmortalen Phase

Various supplementary methods of examination including enzyme-histochemical and enzyme-biochemical methods are used to provide additional information for certain diagnostic and forensic questions. A critical consideration of the findings should include an evaluation of artificial and/or postmortal alterations. Since a human cadaver may only be autopsied after a specified postmortal interval has elapsed, such evaluations are unavoidable.

Several studies on the activity of various enzymes in different types of tissue during the postmortal interval have been published (Gössner 1955; King et al. 1959; Van Lancker and Holtzer 1959; Mallach et al. 1965), but only a few are available on the postmortal activity of enzymes in nerve tissue. We studied the literature on the activity of various enzymes in the central nervous system (CNS) during postmortal intervals up to 48 h. Special reference was made to the relationship of histochemical alterations with biochemical alterations. Histomorphologic alterations in brain tissue form the basis for understanding biochemical alterations and, therefore, should be summarized.

The intention of this review was to summarize postmortal brain-enzyme degradation.

Significant Histomorphologic Alterations of Brain Tissue

Few investigations of histomorphologic alterations in nerve tissue during the late postmortal interval have been published (Weimann 1928; Walcher 1928; Orsos 1935). However, histomorphologic alterations in the early postmortal interval, demonstrated by routine staining techniques, were studied extensively.

Histologic investigations during the early postmortal interval show only minor alterations in the white matter, i.e., a slight decrease in stainability and spongious deterioration. These observations correspond with data presented in the few available summarizing descriptions of alterations in the white matter during the postmortal interval (Camerer 1943; Cammermeyer 1972; Oehmichen and Gencic 1980 b).

The nerve cell alterations are striking when compared with the relatively minor alterations of the white matter, oligodendrocytes, and astrocytes: shrinkage not directly related to the length of the postmortal interval and increasing autolysis as the postmortal interval progresses. Nerve-cell alterations during the postmortal interval have been described extensively in the literature. One

important reason for these investigations may well be the fact that some postmortal neuronal changes resemble intravital hypoxic neuronal alterations (Spielmeyer 1922). Subsequent problems in interpretation and differentiation of nerve cell alterations have provided the basis for extensive discussions (Camerer 1943; Scholz 1943; Lindenberg 1956; Becker 1961; Becker and Barron 1961; Petersohn 1962; Cammermeyer 1973, 1975).

The presence of hyperchromatic cells ("dark neurons") was particularly mentioned in these discussions. It has been established that this type of nerve-cell alteration is due to a postmortal mechanical lesion. The number of cells appearing in the brain tissue depend on the method by which the brain is removed and the method of postmortal treatment, but not on the length of the postmortal interval (Cammermeyer 1960, 1961, 1975, 1978a; Friede 1963). The development of hyperchromatic cells is thought to be due to dehydration. According to the investigations conducted by Friede (1963), this dehydration probably results from decreased osmolarity in the cytoplasm. The affinity of these nerve cells for plasma proteins would also tend to indicate altered permeability (Sasaki and Schneider 1976; Oehmichen and Gencic 1980a; Oehmichen et al. 1979). Even though extensive literature is available, clear-cut criteria for differentiating postmortal alterations from intravital hypoxic nerve cell damage (i.e., Spielmeyer's nerve cell damage) have not been established.

Autolytic deterioration is the nerve cell alteration characteristic for the postmortal interval. The observations indicated that the autolytic process is first discernible as a swelling of the nucleus and cytoplasm together with increasing chromatolysis and liquefaction of the cytoplasm that may or may not involve the nucleus. Some authors reported that, using light microscopy, they observed the first alterations 30 min (Koenig and Koenig 1952), 40 min (Becker and Barron 1961), 60 min (Oehmichen and Gencic 1980), and 2–3 h (Petersohn 1962) post mortem. Submicroscopical alterations, particularly swelling of the cytoplasm, were reported in the nerve cells 15–20 min post mortem (Karlsson and Schultz 1966; David et al. 1971).

The fact that the cells swell also tends to indicate a change in intracellular permeability. According to David et al. (1971) this change in permeability damages the mitochondria. Extensive cristolysis changes the mitochondria into what appears to be empty bubbles. The increasing chromatolysis is an expression of beginning RNA synthesis, separation of the ribosomes and polysomes from the surface of the membrane, and peripheral displacement of the endoplasmic reticulum.

The author's investigations with rats (Oehmichen and Gencic 1980b) also showed that the rate of the autolytic process in the neurons will differ, depending on the localization. Structurally, intact nerve cells were found 48 h post mortem in the hippocampus major, while the neurons of the formatio reticularis, e.g., showed signs of autolysis within a few hours post mortem. The authors therefore suspect a biocline process similar to that described by Voigt for diseases and degenerative processes of the nervous system. Cammermeyer (1978b), on the other hand, assumed that the various rates of autolysis might possibly be attributable to the different times at which the fixation solution immersed the brain tissue.

Table 1. Summary of histochemical, biochemical, and cytophotometric investigations of the activity of brain enzymes mainly involved in oxidative metabolism during the postmortal interval. The numbers listed in the second column bearing the heading "Methods of quantification" refer to the following investigators who are presented in alphabetical order: 1. Anderson 1965; 2. Anderson and Christoff 1964; 3. Becker 1961; Becker and Barron 1961; 4. Chason et al. 1963; 5. Fahn and Côté 1976; 6. Lazerus et al. 1962; 7. Mann et al. 1978; 8. Naidoo and Pratt 1951; 9. Oehmichen and Gencic 1980b; 10. Robins et al. 1958; 11. Smith et al. 1957; 12. Tyrer et al. 1971

Enzymes investigated	Methods of quantification			Species	Type of nervous tissue	Storage temperature (°C)	Maximum postmortal interval without alterations with special regard to the investigated interval
	Histochemical	Biochemical	Cytophotometrical				
Acid phosphatase	1			Rat		Room temperature	6 h stable; within 48 h intracellular alterations
	2			Rat		Room temperature	48 h (7 days investigated)
		2		Rat		Room temperature	Early increase in unbound activity, and decrease in bound activity
	3			Rat		37	10 min; progressive swelling, clumping and reduction of activity
	6			Rabbit	Cerebellum	Room temperature	24 h (24 h investigated)
	8	8		Rat	Endbrain	4/16–18	48 h (48 h investigated)
	9			Rat		22	24 h (48 h investigated)
	11	11		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
Alkaline phosphatase	8	8		Rat	Endbrain	4/16–18	48 h (48 h investigated)
	9			Rat		22	48 h (48 h investigated)
	11	11		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
Alpha-naphthyl acetate esterase	9			Rat		22	24 h (48 h investigated)
Naphthol AS-D chloroacetate esterase	9			Rat		22	32 h (48 h investigated)

Adenosine triphosphatase	6			Rabbit	Cerebellum	Room temperature	24 h (24 h investigated)
	8	8		Rat	Endbrain	4/16–18	24/48 h (48 h investigated)
	9			Rat		22	24 h (48 h investigated)
	10	10		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
Na-K-ATPase		5		Rat		Room temperature	14 h (14 h investigated)
Mg-ATPase		5		Rat		Room temperature	14 h (14 h investigated)
Uridine diphosphatase	9			Rat		22	40 h (48 h investigated)
Adenosine-5-phosphatase	8	8		Rat	Endbrain	4/16–18	24 h (48 h investigated)
Diphosphopyridine nucleotide tetrazolium reductase	3			Rat		37	30–45 min; swelling, clumping and reduction of activity
	4			Rat		25	6 h (6 h investigated)
			7	Man	Cerebellum	Room temperature	16 h (16 h investigated)
	9			Rat		22	48 h (48 h investigated)
			12	Rabbit		37/Room temperature	24 h (24 h investigated)
Triphosphopyridine nucleotide tetrazolium reductase	3			Rat		37	30–45 min; swelling, clumping and reduction of activity
			7	Man	Cerebellum	Room temperature	16 h (16 h investigated)
	9			Rat		22	48 h (48 h investigated)
Malate dehydrogenase			7	Man	Cerebellum	Room temperature	16 h (16 h investigated)
	8	8		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
	11	11		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
– DPN linked	4			Rat		25	6 h (6 h investigated)
Lactate dehydrogenase	6			Rabbit	Cerebellum	Room temperature	24 h (24 h investigated)
		5		Rat		Room temperature	14 h—less than 15% decrease in activity
		7		Man	Cerebellum	Room temperature	Activity increase during the first hour, decreases between 10–25 h, then is stable
		11		Rabbit	Cerebellum	25	6 h (6 h investigated)
	12		12	Rabbit		37/Room temperature	24 h—stable at 22°C, less stable at 37°C

Table 1 (continued)

Enzymes investigated	Methods of quantification			Species	Type of nervous tissue	Storage temperature (°C)	Maximum postmortal interval without alterations with special regard to the investigated interval
	Histochemical	Biochemical	Cytophotometrical				
Glutamate dehydrogenase	9			Rat		22	48 h (48 h investigated)
		10		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
		11		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
	12		12	Rabbit		37/Room temperature	Significantly reduced activity at 22°C within 24 h
Alpha-glycerolphosphate dehydrogenase	4			Rat		25	6 h (6 h investigated)
	9			Rat		22	48 h (48 h investigated)
Isocitrate dehydrogenase	4			Rat		25	6 h (6 h investigated)
Beta-hydroxy butyrate dehydrogenase	4			Rat		25	6 h (6 h investigated)
Cytochromeoxidase			7	Man	Cerebellum	Room temperature	16 h (16 h investigated)
Glucose-6-phosphate dehydrogenase	6			Rabbit	Cerebellum	Room temperature	24 h (24 h investigated)
		7		Man	Cerebellum	Room temperature	Activity increases during the first 25 h but subsequently decreases
		3		Rabbit	Cerebellum	Room temperature	6 h (6 h investigated)
			12	Rabbit		37/Room temperature	Activity significantly reduced within 24 h at 22°C
Succinic dehydrogenase	4			Rat		25	6 h (6 h investigated)
			7	Man	Cerebellum	Room temperature	16 h (16 h investigated)
	9			Rat		22	48 h (48 h investigated)
			12	Rabbit		37/Room temperature	24 h (24 h investigated)
Phosphofructokinase		7		Man	Cerebellum	Room temperature	40 h—minimal activity; 145 h investigated

Detailed data concerning the beginning and extent of the autolysis of nerve cells will therefore also depend on the localization of the cells.

The first nerve cells completely altered by autolysis were, however, usually observed after 6–8 h, using light microscopy. Many such altered nerve cells could be observed approximately 24 h post mortem. Apparently, the number of altered nerve cells increases in direct relationship to the length of the postmortal interval.

Enzyme Alteration in Brain Tissue

Detailed histochemical investigations concerning substrates found during the postmortal interval (Friede and Van Houton 1961; Tewari and Bourne 1963; Fishman et al. 1977; Mann et al. 1978) and investigations concerning changes in the affinity of brain tissue for metalic silver (Dixon 1964) are not available. No general investigations have been published on alterations occurring under various postmortal conditions which are only suggested and therefore often lead to surprising findings (Petersohn 1962). Changes in enzyme activity, however, have been studied extensively. Only one relevant study on the peripheral nervous system has apparently been published (Pribor 1952; autonomic ganglion cell of cat and dog; alkaline and acid phosphatase activity progressively decreased within 30 h after death).

Feigin et al. (1950) as well as Leduc and Dempsey (1950) first established the correlation between decreased enzyme activity demonstrated by histochemical methods and the diffusion of enzymes into neighboring tissue. Systematic investigations of alterations in enzyme activity during the early postmortal interval are compiled in Tables 1 and 2. Table 1 shows all those enzymes studied in the literature that were involved in oxidative metabolism; Table 2, the transmitter enzymes. Histochemical, biochemical, and cytomorphometric findings are taken into consideration.

Although considerable differences in enzyme activity have been described, histochemical investigations showed an *appreciable* decline in the activity of only a few enzymes during the early postmortal interval (e.g., phosphofructokinase). Other enzymes were surprisingly stable. Nearly all enzymes showed unaltered activity during the course of 6–8 h. Slight variations in the findings reported by the individual authors may basically be attributed to the method; no two authors treated tissue specimens prior to and during the demonstration of the enzymes with the same method. For example, Lacerus et al. (1962) pointed out the influence of various procedures prior to fixation. They observed important alterations in enzyme activity only after the brain tissue had been stored at 20° C for 4 h and the temperature then lowered to 4° C for the rest of the storage period.

Using biochemical and cytophotometric methods, a decrease in activity was determined for almost all enzymes during the various postmortal intervals. The decrease in enzyme activity and the diffusion of enzymes is due to alterations resulting from proteolysis, inactivation by inhibitors, or a combination of both (Mann et al. 1978). Using biochemical and cytophotometric methods, some authors were able to establish a temporary increase in activity for individual enzymes. This information, however, has not been included in the tables. The

Table 2. Biochemical (and 2 cytochemical) studies of the activity of various transmitter enzymes in the brain. The numbers listed in the second column refer to publications of the following authors who are presented in alphabetical order: 1. Bird and Iversen 1974; 2. Black and Geen 1975; 3. Bowen et al. 1976; 4. Fahn and Côté 1976; 5. Grote et al. 1974; 6. Mahoney et al. 1971; 7. McGeer and McGeer 1976; 8. Oehmichen and Gencic 1980b; 9. Puymirat et al. 1979; 10. Robins et al. 1967; 11. Silbergeld et al. 1971; 12. Tyrer et al. 1971; 13. Vogel et al. 1969; 14. Wise and Stein 1975; 15. Wyatt et al 1975

Enzymes investigated	Investigators	Species	Type of nervous tissue	Storage temperature (°C)	Postmortal period investigated (h)	Remarks
Tyrosine hydroxylase	2	Rat		Room temperature	20	50% decrease after 5 h
	4	Rat		Room temperature	14	64% decrease after 14 h
	5	Rabbit		Room temperature	24	21% decrease after 2 h
	7	Rat		20	8	62% decrease after 8 h
		Rat		4	8	50% decrease after 8 h
		Human		(?)	10	52% decrease after 10 h
	9	Rat		20	48	40–50% decrease after 48 h
		Rat		4	48	Stable
	11	Rat		24	12	95% decrease after 12 h
		Rat		4	12	80% decrease after 12 h
	13	Rat		4/10	16	Stable
DOPA decarboxylase	2	Rat		Room temperature	20	Stable
	4	Rat		Room temperature	14	Less than 15% decrease after 14 h
	7	Rat		20	8	58% decrease after 8 h
		Rat		4	8	(?) decrease after 8 h
		Human		(?)	10	No significant decrease
	10	Rabbit		24	14	Stable 8 h; 11% decrease after 24 h
Dopamin-β-hydroxylase	2	Rat		Room temperature	20	Stable
	5	Rat		Room temperature	24	Stable
	11	Rat	Adrenal gland	24	12	No significant decrease
		Rat	Adrenal gland	4	12	Stable
	14	Animal (?)		Room temperature	24	23% decrease after 24 h
	15	Rat		Room temperature	6	15% decrease after 6 h

Glutamic acid decarboxylase	1	Mouse		Room temperature	24	50% decrease after 24 h
		Human		4	48	Stable
	3	Human	Frontal lobe + caudate nucl.	Room temperature	27	20% decrease after 27 h
		Human		37°C, mean dissipation rate of 1°C/h	27	20% decrease after 27 h
		Rat		37°C for 4 h, and 4°C for 18 h	22	Stable
	4	Rat		Room temperature	14	Less than 15% decrease after 14 h
	7	Rat		4	8	23% decrease after 8 h
	9	Rat		20	8	64% decrease after 8 h
		Rat		20	48	40–50% decrease after 48 h
		Rat		4	48	Stable
Choline acetyltransferase	1	Mouse		Room temperature	24	50% decrease after 24 h
	3	Human	Frontal lobe + caudate nucl.	Room temperature	27	24% decrease after 27 h
		Human		37°C, mean dissipation rate of 1°C/h	27	33% decrease after 27 h
		Rat		37°C for 4 h, and 4°C for 18 h	22	22% decrease after 22 h
	4	Rat		Room temperature	14	60% decrease after 14 h
	6	Rat		Room temperature for 3 h, and 4°C for 16 h	19	Stable
	7	Rat		20	8	Stable
	9	Rat		20	48	40–50% decrease after 48 h
		Rat		4	48	Stable
		Human		(?)	10	35% decrease after 10 h
Monamine oxidase (MAO)	4	Rat		Room temperature	14	78% decrease after 14 h
	5	Rabbit		Room temperature	24	Stable
	12	Rabbit		22/37	24	*Cytochemically* stable at 22°C; decrease at 37°C
	13	Rat		4/10	16	Stable

Table 2 (continued)

Enzymes investigated	Investigators	Species	Type of nervous tissue	Storage temperature (°C)	Postmortal period investigated (h)	Remarks
Acetylcholinesterase	4	Rat		Room temperature	14	58% decrease after 14 h
	7	Rat		20	8	No significant decrease
	8	Rat		22	48	*Cytochemically,* decrease after 32 h
Catechol-0-methyltransferase	5	Rabbit		Room temperature	24	Stable
	13	Rat		4/10	16	Stable
Aromatic amino acid decarboxylase	3	Human	Caudate nucl.	Room temperature	27	40% decrease after 27 h
		Human	Caudate nucl.	37°C, mean dissipation rate of 1°C/h	27	52% decrease after 27 h
		Rat		37°C for 4 h, and 4°C for 18 h	22	More than 30% decrease after 6 h
Phenylethanolamine-N-methyl transferase	11	Rat	Adrenal gland	24	12	40% decrease after 12 h
		Rat	Adrenal gland	4	12	20% decrease after 12 h

increase may well be due to the release of normally inactive lysosomal enzymes during the postmortal interval. Dvořák (1967) suggested that the increase in acid phosphatase is due to the autolysis of acid-phosphatase-positive structures; these structures resemble Golgi bodies in regard to form and localization. Apparently, the direct concentration in the Golgi zone is produced by adsorption on enzymes exuded from the lysosomes, by submicroscopical structures of the Golgi bodies, or by autolytic activation of acid phosphatase.

The important question here is how to explain the relative stability of the activity observed in the histochemical investigations, particularly the activity of those enzymes involved in oxidative metabolism, even though assays of oxygen uptake (Mann et al. 1978) indicate that the efficiency of mitochondria as respiratory units declines rapidly after death. Mann et al. (1978) suggested a plausible explanation: Electron-microscopical investigations during the early phase of anoxic cell alterations showed that the mitochondria in the neurons swelled. Although the double outer membranes of the mitochondria were intact, the internal cristae were progressively disrupted (Van Nimwegen and Scheldon 1966; Brown and Brierley 1971). It is highly probable that the limiting membrane of the mitochondria protects the enzyme from immediate deterioration as a result of the penetration of lysosomal enzymes. The function, however, is cancelled by the destruction of the internal cristae. This protection is not provided for those enzymes which are freely available in the cytoplasm and not bound to the organelles (e.g., phosphofructokinase).

Another somewhat simpler explanation (Oehmichen and Gencic 1980 b) is that biochemical investigations always involve an entire brain tissue specimen. In accordance with an "all or nothing law," the enzyme activity in some non-autolytic cells is virtually unaltered and no activity at all is present in other cells during the last stage of autolysis. Quantitatively altered activity may be established when the brain tissue as a whole is examined, but not when individual cells are examined with histochemical methods. In this case, only the enzyme-positive cells and not enzyme-negative cells are recorded. A change in enzyme activity due exclusively to enzyme diffusion, as described by Feigin et al. (1950) as well as Leduc and Dempsen (1950), does not clarify the distinct decrease in biochemically determined enzyme activity. In such cases, virtually unaltered enzyme activity must be demonstrated in the tissue homogenate.

Only one research team has investigated the intracellular distribution and localization of enzymes during the postmortal interval with special reference to the relationship between enzyme localization and submicroscopical cell structures (Becker 1961; Becker and Barron 1961): localization of acid phosphatase in the lysosomes and of nucleotidases in the mitochondria. Within 10 min both lysosomes and mitochondria begin to swell. Progressive clumping of the organelles in the cell and a reduction in the number of organelles were observed during the postmortal interval.

Conclusion

Apart from a few exceptions, structural alterations demonstrable with light microscopy and histochemical methods are virtually unchanged and not yet

significantly influenced by autolytic processes within 6–8 h post mortem. Even biochemical quantitative methods reveal no significant changes during this period. The findings are based in part on investigations with animal cadavers since no human material is obtainable for these postmortal intervals. Individual comparative studies using human tissue have shown that the findings may be almost unreserved applied to human tissue.

Interpretation problems arise when enzyme activity is demonstrated to obtain information about intravital or postmortal (autolytic) intracellular processes. At present, it is impossible to differentiate between the two processes with any degree of reliability. Although differences between intravital and postmortal alterations are demonstrable with routine staining techniques, the possibility of an identical course cannot definitely be excluded. The autolytic process per structural unit should especially be considered when enzymes are demonstrated. A possible change in enzymatic activity per structural unit can only then be understood if the degree of autolysis is also considered.

The following conclusion is possible if questions concerning the intravital and postmortal event can be excluded: Distinct structural and quantitative enzyme alterations are observed after a postmortal interval of approximately 6–8 h. Studies of intracellular structures and quantitative enzyme content may therefore be carried out only in tissue frozen within this postmortal period (Anderson 1965; McKeown 1977, 1979). Qualitative investigations, particularly histoenzymatic studies, however, are still possible with tissue removed as late as 48 h post mortem. In such cases, positive findings are applicable, but negative findings cannot be evaluated and considered.

In summary, the cadaver should therefore be refrigerated as quickly as possible after death if questions of this nature are to be studied. The brain tissue to be examined should be quick-frozen in small blocks and stored at −70° C (Puymirat et al. 1979). Biochemical and light-microscopical examinations are usually possible within a postmortal interval of 6–8 h. Autolytic process must be taken into consideration when morphologic and biochemical examinations are carried out on material obtained after longer postmortal intervals.

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