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3-Methoxytyramine in the Putamen as a Gauge of the Postmortem Interval

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ABSTRACT: The armamentarium of the forensic thanatologist includes the examiner's subjective assessment of bodily changes together with comprehensive evaluation of environmental and associated factors to estimate the postmortem interval (PMI). Of the various objective means, postmortem accumulation of potassium in vitreous humor is a widely used gauge. In view of the considerable variability inherent in these techniques, an additional marker with greater accuracy would be a welcome supplement to these indices.

We have developed a method of estimating PMI from the level of 3-methoxytyramine (3-MT) in the dorsal putamen of the brain. The supernatent of a sample of putamen, sonicated in 6 volumes (weight/volume) of 0.1 *M* perchloric acid and centrifuged at 51 000 times gravity for 10 min, was analyzed for 3-MT using high-pressure liquid chromatography with electrochemical detection. The change of 3-MT in the putamen is linearly related to the PMI (range: 5.5 to 60 h), irrespective of patient age (1 to 84 years). This observation is attributable to several factors, including high substrate (dopamine) concentration, preservation of catechol-O-methyl transferase activity, and an inactivation of monoamine oxidase activity, as a result of a decrease in tissue pO_2.

KEYWORDS: pathology and biology, postmortem examinations, time of death, death, postmortem interval, postmortem chemistry, neurochemistry, dopaminergic systems of the central nervous system, thanatology, neuroscience

Medicolegal officials investigating mammalian somatic death have long grappled with two central issues: (1) what is death? and (2) when did death occur? In an era wherein medical science has developed artificial means of continuing respiration and systemic blood circulation, the definition of death has been the subject of ongoing debate and controversy within the United States. No universal criterion has been accepted, either medically or legally, so that currently it is possible to be "dead" in one jurisdiction and "not dead" in another [*1*]. A panoply of considerations, ranging from euthanasia and meaningful life to suitability of a

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potential donor's organs for transplantation, buttresses the conclusion that such issues will not be readily or quickly settled.

In most medicolegal investigative settings, the fact of death is a given by any standard and, for the purpose of this study, is defined as irreversible cessation of cardiorespiratory function. The paramount medicolegal issue becomes determination of the time of death. The question arises most commonly in cases of unwitnessed or unreliably witnessed deaths, but may be of crucial importance even in cases of reliably witnessed "simultaneous" deaths. Accordingly, the accurate determination of the time of death applies not only to civil law, in which specification of an exact time of death is of practical necessity in settling family, social, and business matters, but also to criminal law, where accurate determination of the time of death may either exonerate or inculpate a suspect accused of a particular homicide. Such a determination is an indispensible component of the corpus delecti and may serve as a basis in deciding whether to expand investigative effort in a particular direction.

The testimony of the forensic thanatologist, given the current state of the art based on multiple objective and subjective variables, constitutes merely an "educated guess" expressed as a range of time within which death probably occurred, not as a discrete point in time. Traditionally, the armamentarium of the forensic thanatologist includes the observer's subjective assessment of bodily changes occuring during the early postmortem period, typically observable hours to days after somatic death. Generally accepted postmortem changes during this period include algor mortis, rigor mortis, and livor mortis [*2*]. Other standard physical observations include assessment of corneal cloudiness [*3*] and change in intraocular pressure over time [*2*]. Documentation of the gastric contents contributes to the assessment of the time of death if information about the last known meal of the deceased is available by reliable witnesses. Late postmortem changes after death, typically occurring in a range from days to weeks or even months, occur with the onset of obvious decomposition of the body [*2-4*]. The range of time in which such changes occur is considerably variable, dependent upon such important factors as temperature and climatic conditions [*5*]. In addition, multiple associated factors such as invasion by and characteristics of fauna and flora are also employed to estimate the time of death [*3*].

Various objective means of determining postmortem interval (PMI) have been employed over the past 50 years [*6*]. Almost every body fluid has been tested for enzyme activities and for levels of organic and inorganic constituents, all in an attempt to find changes which would correlate significantly to increasing PMI [*7*]. Of these various objective means, postmortem accumulation of potassium (K+) in vitreous humour is a widely used gauge for assessing the PMI [*7*].

Numerous studies further investigating the relationship between PMI and vitreous humour K+ established that K+ accumulates with increasing PMI [*8-14*], but the accuracy is quite variable, with some investigators concluding a limitation of ±5 h [*8*], while others determine that the levels were "insufficiently consistent to be accurate" [*9*]. More recent studies indicate that the K+ method may show linear increase for a brief period of only 12 h, with continually increasing standard error associated with longer PMI [*10,11*].

In view of the considerable variability inherent in these subjective and objective techniques, an additional marker with greater accuracy would be a welcome supplement to these indices. We report a method of estimating PMI from the level of 3-methoxytyramine (3-MT) in the dorsal putamen.

Materials and Methods

General

Putaminal samples were harvested from coroner's cases for which the time of death had been determined from eyewitness accounts, including both interested and disinterested by-

TABLE 1—*Age, cause of death (COD), postmortem interval (PMI) and 3-MT level in dorsal putamen from each case.*

Case	Age, years	COD	PMI, h	3-MT, ng/mg wet weight
1	23	GSW	20	2.45
2	64	trauma	18.75	2.22
3	39	GSW	12	1.97
4	64	nat	11	1.65
5	33	crush	16	1.53
6	32	GSW-H	20	2.21
7	61	GSW	23	3.11
8	59	trauma-car acc.	18.5	3.27
9	49	crush	17	1.90
10	32	GSW	26	2.59
11	49	GSW	19	2.34
12	71	peritoneal hem.	6	1.04
13	24	GSW	22.5	2.21
14	63	crush	22.5	2.76
15	80	Tx-aorta	21	2.71
16	34	GSW	35	3.82
17	37	crush	22	2.09
18	6	Tx-brainstem	16.75	1.70
19	4	Tx-brainstem	18.75	2.08
20	35	nat	20.75	2.25
21	31	strangulation	15	1.53
22	18	strangulation	24	2.81
23	20	crush	20	2.44
24	22	GSW-H	21	2.59
25	38	drown	17	1.70
26	17	GSW	16	1.97
27	75	GSW	22	1.71
28	20	skull-Fx	7	0.63
29	9	Tx-brainstem	19	1.97
30	32	skull-Fx	12	2.02
31	65	pulmonary emb.	45*	4.26
32	27	multiple-Fx	15	1.00
33	31	GSW	21	2.02
34	44	ETOH	18	1.79
35	32	GSW	16	2.30
36	42	crush	24	2.90
37	30	GSW	12	1.07
38	20	nat	25	2.41
39	32	GSW	14	1.44
40	12	trauma-car acc.	24	3.07
41	36	GSW	23.5	2.15
42	25	cerebellar hem.	21	1.79
43	19	GSW	19.25	1.86
44	59	Tx-aorta	6	0.60
45	80	Tx-juglar	21	2.04
46	7	GSW	19.5	1.63
47	31	GSW	10.75	1.53
48	41	GSW	12	1.78
49	20	GSW	15	1.63
50	49	smoke	14	2.09
51	1	smoke	16.75	1.74
52	39	GSW-H	15	1.26
53	27	GSW-H	8.5	1.60
54	44	GSW-H	17	2.63
55	30	GSW-H	18	2.34
56	54	GSW-H	18	1.71
57	63	GSW-H	11	2.29
58	18	GSW-H	18	2.18

TABLE 1—*Continued.*

Case	Age, years	COD	PMI, h	3-MT, ng/mg wet weight
59	33	fatty liver	22	2.37
60	84	GSW-H	18.5	1.79
61	28	ETOH-trauma	15.75	1.46
62	39	GSW	17	2.14
63	44	OHD	15	1.97
64	30	OHD	21	3.17
65	38	OHD	12	2.28
66	21	OHD	17.5	2.09
67	39	OHD	17.5	2.69
68	67	OHD	6.5	2.82
69	45	OHD	20	2.56
70	46	OHD	8	3.29
71	51	OHD	7	2.80
72	62	OHD	60*	0.61
73	76	OHD	48*	1.57
74	37	OHD	26	1.94
75	51	OHD	15.5	2.56
76	70	OHD	12	2.34
77	50	OHD	5.5	3.26
78	65	OHD	25.5	2.18
79	34	OHD	6	2.67
80	66	OHD	14	2.18

Cause-of-death abbreviations:

GSW	gunshot wound to trunk or neck.
GSW-H	gunshot would to head.
ETOH	ethanol intoxication.
hem.	hemorrhage.
Tx	Transection.
Fx	fracture(s).
emb.	embolism.
smoke	smoke inhalation.
OHD	organic heart disease.
nat	natural.

standers, attending physicians, and various medicolegal investigators. In three cases, denoted with an asterisk (*) in Table 1, involving individuals who were found dead and for whom eyewitness testimony was not directly available, the time of death was established within several hours to a high degree of confidence on the basis of associated factors and reliable information concerning the decedent's activities. In all cases, regardless of time of year, the bodies remained at ambient temperature or were stored at 4°C within morgue refrigerators for 12 h or less until the time of tissue collection, which occurred within 4 h after commencement of the necropsy. Samples were analyzed solely from decedents whose drug history and toxicologic screens of postmortem heart blood and urine were negative. Cases were not excluded from this study, however, when either ethyl alcohol or lidocaine was detected toxicologically. The cause of death in each case was determined by a board-certified forensic pathologist employing standard criteria within the specialty, including correlation of circumstantial and anamnestic information, data from complete necropsy, and toxicologic analysis.

Dissection and Sample Processing

As part of the complete autopsy procedure, brains were removed in accordance with traditional autopsy routine and examined in the unfixed state. Following removal of the brain-

stem and cerebellum, the cerebral hemispheres were cut in coronal fashion, each section measuring approximately 15 mm in thickness. One of the coronal incisions was made immediately anterior to the optic chiasm to expose the caudate, putamen, and anterior commisure on the posterior face of the section (see Fig. 1). Twenty-milligram core cubes of either right or left dorsal putamen were collected from areas routinely examined or stored per standard autopsy protocol. In cases of unilateral putaminal trauma, samples of the intact contralateral putamen were extracted for analysis. Within 60 min of harvest, the core samples were sonicated in 120 mL of 0.1*M* perchloric acid (PCA), centrifuged for 10 min (51 000 $\times g$), and injected onto a liquid chromatography (LC) column. The time of PCA precipitation was recorded and marked the end point of the determined PMI.

Liquid Chromatography (LC)—Single-point analysis of 3-MT levels in dorsal putamen was done by the method of Sparks and Slevin [*15*]. The LC solvent delivery system used in the studies consisted of a Model 6000 A high-pressure liquid chromatography (HPLC) pump in series with a Model 710B automatic injector (Waters Associates, Milford, MA). A Waters steel-jacketed 10-μm Bondapak C-18 column protected with a guard column packed with 50-μm Corasil C-18 was used to separate compounds of interest. An LC-4A electrochemical detector (Bioanalytic Systems, Lafayette, IN) using a glassy carbon electrode (detector setting: +0.85 V; sensitivity 0.5 nA by 1.0 V) was connected to a Waters 730 data module for signal detection and quantitation. Chromatographic runs were initiated from a Waters 720 system controller. The column was maintained at ambient temperature (25°C) and the flow rate of mobile phase was 1.1 mL/min.

Mobile phase was prepared using distilled, deionized water (960 mL) containing 6 g/L of sodium acetate (Fisher Chemical Co.), 6.0 mL/L of concentrated glacial acetic acid, and 15 mg/L of ethylenediaminetetracetate (EDTA) (Sigma Chemical Co.) and 125 mg/L sodium octyl sulfonic acid (Eastman Kodak Co.). This solution was degassed under vacuum after which 40 mL/L of HPLC-grade degassed methanol was added and the pH adjusted to 4.30 with glacial acetic acid. External standards, all obtained from the Sigma Chemical Co., were dissolved in 0.1*M* (PCA). Quantitation of these standards was determined internally by the data module using the "Waters" peak height program.

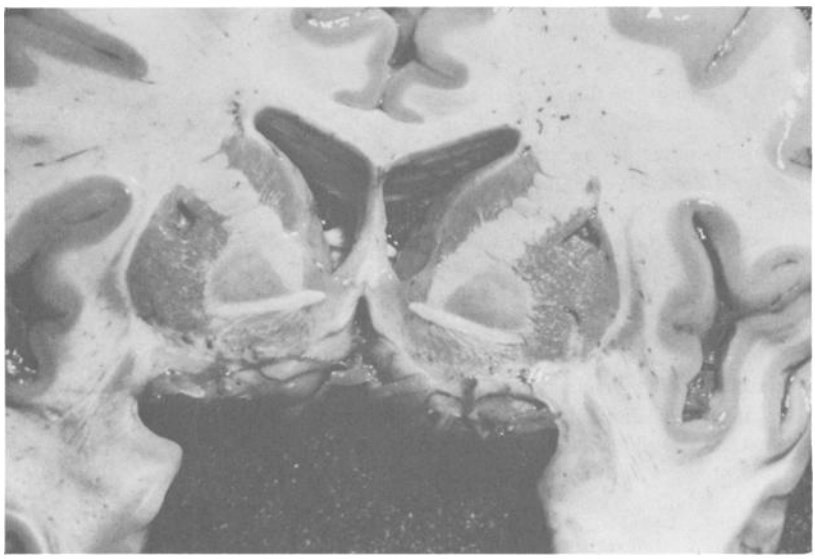

FIG. 1—*Representative coronal section of unfixed human brain at the level of the anterior commissure. Approximately 20-mg core samples of both right and left dorsal putamen have been removed.*

Verification of authentic 3-MT was accomplished by differential chromatography, and run-to-run variation in the peak height of a known amount of 3-MT within a day never exceeded 3%. We have previously reported a daily decrease in sensitivity [*15*], but the system was recalibrated each day in this study.

Results

All data, including the age of the decedent, cause of death, PMI (time between death and PCA precipitation of tissue in hours), and 3-MT level (ng/mg wet weight putamen) are given in Table 1. The relationship between PMI and 3-MT level in dorsal putamen is linear and either negatively (Fig. 2) or positively (Fig. 3) correlated depending on the cause of death. Decedents who die as a result of organic heart disease (OHD) show a negative correlation ($r = -0.83$), creating a line with the equation: PMI (hours) = 62.7 − 18.4 3-MT (ng/mg). All other cases reported here show a positive correlation ($r = 0.83$) between PMI and 3-MT level, creating a line with the equation: PMI (hours) = 2.22 + 7.66 3-MT (ng/mg). The 95% confidence [*16*] of predicting PMI from experimentally determined levels of 3-MT is ±18.2 h for OHD and ±7.5 h for all others. No correlation exists between PMI and any biogenic amine other than 3-MT found in dorsal putamen. Furthermore, patient age does not appear to play a role in the relationship between PMI and 3-MT.

Discussion

3-MT is synthesized from dopamine (DA) by the enzyme catechol-O-methyl transferase (COMT) [*17,18*]; this is a minor pathway for DA catabolism antemortem. Under in vivo conditions, DA is first deaminated by monoamine oxidase (MAO), which has an absolute requirement for molecular oxygen. In the putamen, the aldehyde intermediate is rapidly dehydrogenated to dihydroxyphenylacetic acid (DOPAC), which is then converted to homovanillic acid (HVA) by COMT [*17*]. In vivo, a small percentage of DA is first metabolized by COMT to 3-MT, which is then deaminated by MAO and oxidized to give HVA [*18*] (Fig.

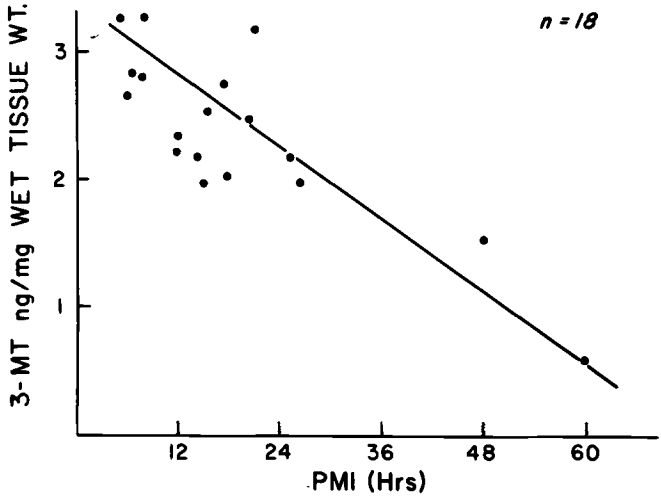

FIG. 2—*3-MT levels in dorsal putamen from patients dying from organic heart disease. Patient 3-MT levels (ng/mg wet weight) in dorsal putamen are plotted against their respective PMI (hours). Regression analysis of the points creates a line with the equation: PMI = 62.7 − 18.4 (3-MT). The correlation coefficient is* r = *−0.83 with a 95% confidence of ±18.2 h.*

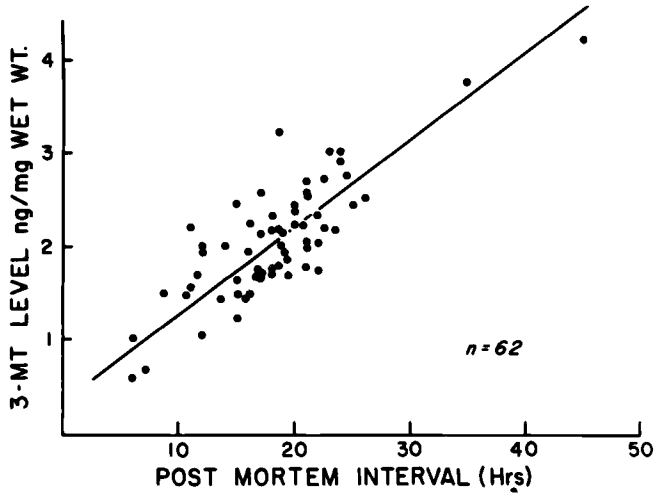

FIG. 3—*3-MT levels in dorsal putamen from patients dying from causes other than heart disease. Patient 3-MT levels (ng/mg wet weight) in dorsal putamen are plotted against their respective PMI (hours). Regression analysis of the points creates a line with the equation: PMI = 2.22 + 7.66 (3-MT). The correlation coefficient is* r = *0.83 with a 95% confidence of* ±*7.5 h.*

4). The postmortem fate of central nervous system dopamine has been investigated to a limited degree in rats [*17-20*]. Carlsson et al. [*19*] consistently demonstrated 3-MT in rat brain postmortem and also induced an accelerated and elevated postmortem accumulation of 3-MT in rat striatum (analogous structure to human putamen) by giving the animals the MAO-inhibitor, pargyline, before death.

Further human investigation by Carlsson and Winblad [*21*] showed no positive correlation between increasing 3-MT levels in putamen and increasing PMI. These authors based their conclusions on data obtained from patients dying from both cardiovascular and malignant disease. Our data, if combined, would yield a similar conclusion.

We attribute the *accumulation* of 3-MT in postmortem putamen (Fig. 3) to three factors. First is the preservation of COMT. COMT has been shown to be contained in both nerve cells [*22*] and supporting tissue (glia [*23*]) as a soluble [*24*] temperature-dependent enzyme [*20*] which is extremely stable [*25*]. This stability is demonstrable in rats sacrificed by microwave irradiation, where the brain temperature is elevated to 86°C in 1.8 s. Under these conditions, the activity of enzymes of dopamine metabolism (that is, tyrosine hydroxylase), acetylcholine metabolism (that is, choline acetyltransferase), basic metabolic systems (that is, phosphodiesterase), and oxidative systems (that is, MAO) are virtually eliminated, while 18% of the total COMT activity found in the striatum from animals sacrificed by decapitation is retained [*25,26*]. Secondly, the continued accumulation of putaminal 3-MT is predicated on the availability of a large precursor (DA) pool, and, thirdly, to a decrease in pO_2 (tissue partial pressure of oxygen) to low levels. This third factor, caused by cardiorespiratory failure, precludes oxidative deamination of both DA and 3-MT by the strictly O_2-requiring MAO.

In the cases of organic heart disease-related death, we speculate that there is an antemortem stress-related dumping of DA into extraneuronal spaces (stress-related release of DA is well documented in rats [*27*]), which is converted to 3-MT postmortem in the extraneuronal space. We are unsure of how 3-MT is cleared from the putamen of OHD patients, but diffusion or further enzymatic modification of 3-MT extraneuronally are strong possibilities.

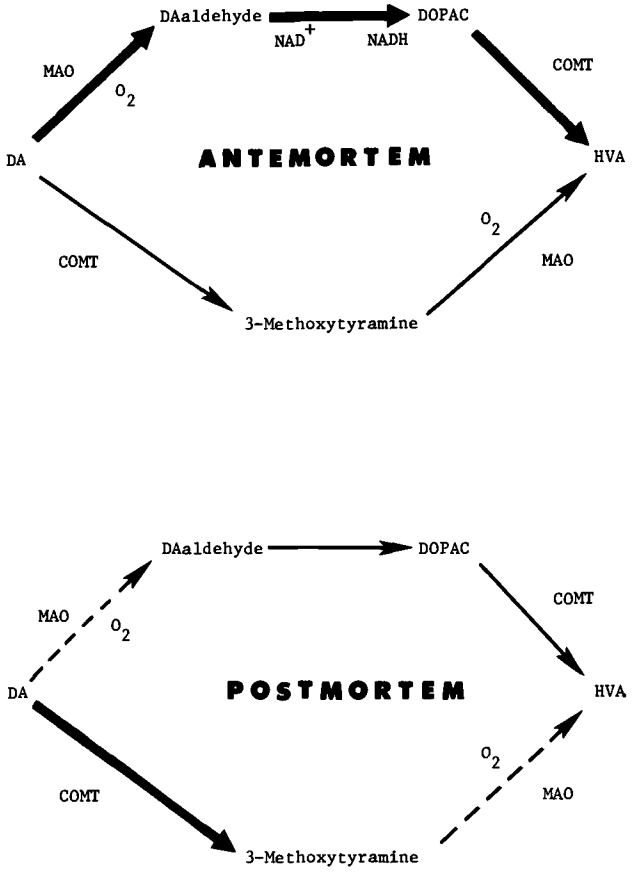

FIG. 4—*Antemortem and postmortem fate of putaminal dopamine.*

Other OHD-related postmortem biochemical alterations have recently been reported, suggesting that several metabolic systems change in a manner unique to this pathologic entity. These OHD-related changes include increased urinary catechols [*28*], increased fibrinogen degradation products [*29*], elevated lactate dehydrogenase [*30*] in blood, decreased lactate dehydrogenase in cardiac muscle [*30*], and decreased serotonin binding in frontal cortex [*31*].

Conclusion

We conclude that 3-MT levels in dorsal putamen show linear change as the postmortem interval increases. With the exception of causes of death related to various forms of organic heart disease, the linear change represents a steady rise with a quite good coefficient of correlation, probably higher than any chemical method proposed to date. In cases of death as a result of organic heart disease, a negative correlation obtains and may be occasioned by unique dopaminergic changes specific to stress induced by that complex of pathological cardiac conditions. Additional research with rigid observation and control of the plethora of variables affecting postmortem chemical reactions is mandatory. To date, determination of 3-MT levels is at least as accurate as any other chemical method utilized in making an "edu-

cated guess" about the postmortem interval. With increased experience and refinement, it may, in the future, be used on a routine basis to determine PMI with sufficient accuracy to be material and relevant in a court of law.

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