D. Larry Sparks,[1] *Ph.D.; Peter R. Oeltgen,*[2] *Ph.D.;*
Richard J. Kryscio,[3] *Ph.D.; and*
John C. Hunsaker, III,[4] *J.D., M.D.*

Comparison of Chemical Methods for Determining Postmortem Interval

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ABSTRACT: Accurate determination of postmortem interval (PMI) is a problem for the forensic thanatologist, especially in unwitnessed deaths. A number of objective chemical methods for determining PMI have been developed, the most widely used being accumulation of potassium in the vitreous humor. The authors previously have reported a chemical method for determining PMI from the predictable accumulation or clearance of the dopaminergic metabolite 3-methoxytyramine (3-MT) in the putamen of the brain.

They have extended their previous study to compare directly the accuracy of determining PMI from the level of 3-MT in putamen with the level of potassium in vitreous humor. The data indicate that 3-MT is at least as accurate as, if not more accurate than, potassium accumulation in vitreous humor, although 3-MT levels can be affected by the cause of death and drugs present at the time of death. Nevertheless, determination of both the 3-MT and potassium levels can afford the most accurate method of determining PMI; preliminary nomograms for determining PMI from both variables are presented.

KEYWORDS: pathology and biology, postmortem interval, time of death, 3-methoxytyramine, vitreous humor, postmortem chemistry, postmortem pharmacology, basal ganglia, dopaminergic neurochemistry, thanatology, neuroscience

Accurate determination of the postmortem interval (PMI) is an important medicolegal issue in both civil and criminal law. The issue of PMI can become paramount in unwitnessed deaths, and in such cases, investigative efforts can be effectively directed after the precise PMI is known. Furthermore, the accurate determination of PMI can be a significant factor in legal matters surrounding nonhuman deaths (such as that of game animals).

Subjective assessment of bodily changes along with environmental and associated factors have been long used to derive an educated guess of the PMI. A variety of chemical methods

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[1]Assistant professor of neurology and Sanders-Brown Research Center on Aging, University of Kentucky Medical Center, and chief biochemical consultant, Kentucky State Medical Examiner's Program, Justice Cabinet, Lexington, KY.

[2]Associate professor of pathology, University of Kentucky Medical Center, and head, Chemistry Section, Pathology Service, Lexington Veterans Administration Medical Center, Lexington, KY.

[3]Professor of statistics, University of Kentucky, Lexington, KY.

[4]Assistant professor of pathology, University of Kentucky Medical Center, and associate chief medical examiner, Kentucky State Medical Examiner's Program, Justice Cabinet, Lexington, KY.

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has been employed in past years to determine PMI [*1*]. Of these various means, the most widely used and accepted method is based on the predictable accumulation of potassium (K+) in the vitreous humor of the eye [*2*]. Lack of reliance on that method, however, is exemplified by reports that K+ in the vitreous humor either can predict PMI for only a short period of time (up to approximately 12 h) [*3,4*] or is "insufficiently consistent to be accurate" [*5*].

Recently, we reported a chemical method for determining PMI which is at least as accurate as any other method published [*6*]. Our method is based on the predictable accumulation or "clearance" of 3-methoxytyramine (3-MT) in the dorsal putamen of the brain depending on the cause of death. The putaminal content of 3-MT, predominantly a postmortem metabolite of dopamine, decreases with increasing PMI in cases where the cause of death was a result of significant organic heart disease (OHD), whereas 3-MT accumulated predictably in all other cases (non-OHD) [*6*]. We now report a direct comparison of the two most accurate chemical methods of determining PMI: K+ content in vitreous humor versus the 3-MT content in 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, attending physicians, and various medicolegal investigators. In three cases, the PMI was established on associated factors and reliable details of the decedent's activities (data for these cases were previously reported). In all cases, bodies remained at ambient temperature [range 4 to 29°C (39 to 85°F)] or were stored at 4°C (39°F) within morgue refrigerators for less than 12 h before 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, except when either ethyl alcohol or lidocaine was detected toxicologically. Data for 3-MT and K+ were grouped accordingly for cases in which evidence of premortem drug use/abuse was present or such drugs contributed to the cause of death (for example, barbiturates, stimulants, and opioids). The cause of death in each case was determined by a board-certified forensic pathologist employing standard criteria within the speciality, including correlation of circumstantial and anamnestic information, data from complete necropsy, and toxicologic analysis.

Subjects

Data were obtained from 146 subjects whose age ranged from 1 to 84 years at death. The actual 3-MT data, the cause of death and the PMI from 80 of these cases are tabulated in our previous report [*6*]. The cases were divided into groups according to the cause of death: subjects dying as a result of significant organic heart disease ($N = 26$); subjects dying as a result of various forms of mechanical trauma, ethanol abuse, other natural disease, and asphyxia (excluding smokeless carbon monoxide) ($N = 106$); subjects dying as a result of smokeless carbon monoxide poisoning ($N = 8$); and subjects dying as a result of drug intoxication ($N = 6$).

Dissection and Sample Processing

The procedures for the dissection of dorsal putamen have detailed previously [*6*]. The fresh brain was sectioned so that the anterior commissure was apparent on the coronal surface. A core sample of either right or left dorsal putamen (no significant laterality differences

have been found between right and left putamen for any neurochemical detected, including 3-MT; $N = 19$) of approximately 20 mg in weight was sonicated in 6 vols (weight to vol) of 0.1*M* perchloric acid, centrifuged for 10 min (51 000 × *g*), and the supernatent was injected onto the liquid chromatography (LC) column. The time of perchloric acid precipitation was recorded and marked the end point of the determined PMI.

Vitreous humor was collected from both eyes at necropsy as described by Coe [7] using a 25-gage needle and a 10-mL syringe. Vitreous samples were collected in red-top vacutainers and either directly delivered or stored at 4°C (39°F) for no more than 4 h before delivery to the clinical chemistry laboratory for analysis of K+ content using the Beckman Astra 8 (Beckman Instrument, Brea, CA). The time of collection was noted and marked the end point of the determined PMI.

Liquid Chromatography (LC)—Single-point analysis of 3-MT levels in dorsal putamen was performed by the methods of Sparks and Slevin [8], as detailed in our previous article [6] by the senior author (DLS). Briefly, the sample of putaminal extract was analyzed for 3-MT on a fully automated "Waters" LC system (Milipore Waters Corp., Milford, MA) and detected electrochemically (Bioanalytical Systems, West Lafayette, IN). Quantitation of 3-MT was determined by peak height based on external standardization using authentic 3-MT.

ASTRA 8 K+ Analysis—Potassium content in the vitreous humor was determined by the methods of Proda and Simon [9]. A 50-μL sample of vitreous humor was analyzed with an ion-selective electrode having a valinomycin membrane. All vitreous potassium assays were performed in the chemistry section of the Pathology Service Laboratory by American Society of Clinical Pathologists (ASCP) certified medical technologists following standard College of American Pathologists (CAP) quality-assurance guidelines for testing.

STATISTICAL Methods—This is a multivariate calibration problem in which the variables 3-MT and K+ are used to calibrate the variable PMI. Brown [10] discusses the multivariable calibration problem and points out that in this, the random calibration problem, the computations are simple provided the conditional distribution of PMI given 3-MT and K+ is known. For the two large groups of subjects (non-OHD and OHD), scattergrams of 3-MT versus PMI and of K+ versus PMI are each linear. Hence, assuming that the joint distribution of 3-MT, K+, and PMI is that of a trivariate normal variate, the calibration will be based upon the planar regression model in which PMI is regressed on both 3-MT and K+. This permitted the construction of nomograms for determining estimated values of PMI given the values of 3-MT and K+. Estimates of the error in these predictions are obtained by using the usual formulas for constructing 95% prediction limits in multivariate regression [11]. It should be noted that all reported prediction limits are valid near the center of the data (that is, near the mean of the 3-MT and K+ values).

Results

The relationship between PMI and 3-MT content in the dorsal putamen of subjects ($N = 106$) with negative postmortem toxicologic screens dying as a result of other than organic heart disease (OHD) or smokeless carbon monoxide was positively correlated (Fig. 1). In these cases, the 3-MT levels increased as the PMI increased (Pearson's correlation coefficient, $r = 0.77$; $p < 0.0001$) and the 3-MT level was predictive of the PMI with an accuracy of ±9.9 h.

The relationship between PMI and K+ content in the vitreous humor from 91 of the above 106 subjects was also positively correlated (Pearson's correlation coefficient, $r = 0.87$; $p < 0.0001$). The increase in K+ content was predictive of the PMI with an accuracy of ±10.5 h, but also displayed unequivocal heteroscedasticity (Fig. 2).

As we have previously reported, the relationship between PMI and 3-MT content in dorsal putamen of subjects ($N = 26$) who die as a result of significant OHD is negatively correlated (Pearson's correlation coefficient, $r = -0.78$; $p < 0.0001$). In these subjects, the 3-MT

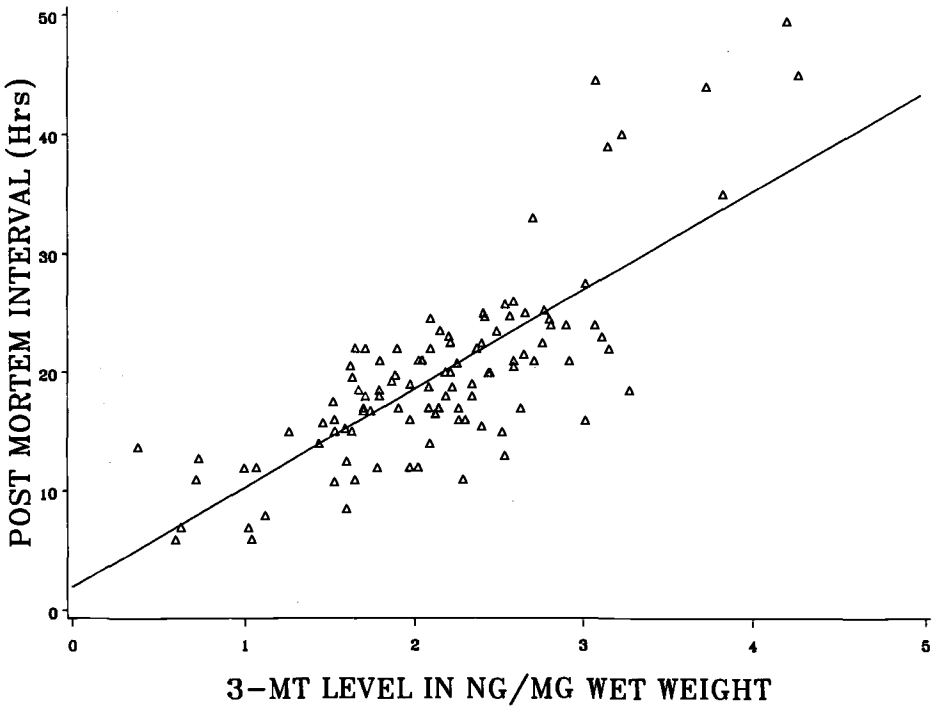

FIG. 1—*3-MT levels in the dorsal putamen from subjects dying from causes other than heart disease (N = 106). Subjects remained at ambient temperature [4 to 29°C (39–85°F)] or were stored at 4°C (39°F) within morgue refrigerators for less than 12 h before tissue collection. Subject 3-MT levels (ng/mg wet weight) are plotted against their respective PMI (hours). Regression analysis of the points creates a line having a correlation coefficient of* r = *0.77 with a 95% prediction error of* ±*9.9 h.*

levels decrease as the PMI increases and is accurate to ±17.6 h for predicting the PMI (Fig. 3).

In these same OHD subjects ($N = 26$), as in the non-OHD subjects, there was a positive (Pearson's correlation coefficient, $r = 0.90$; $p < 0.0001$) relationship between the level of K+ in the vitreous humor and the PMI (Fig. 4). The predictive accuracy of K+ in these cases was ±12.3 h.

Because of the inverse relationship between PMI and 3-MT, the two basic groups were treated separately in our analysis. Age of each subject at death was not correlated with PMI in either group and was not considered further in the analysis (simple correlations as well as partial correlations between PMI and age given 3-MT and K+ were not significant).

Abnormally high levels of 3-MT (for the PMI) were found in the putamen in cases where amphetamines, cocaine, caffeine, or opioids were detected in the blood or urine (data not shown). On the other hand, abnormally low levels of 3-MT were present if barbiturates were detected or if the subject died as a result of smokeless carbon monoxide intoxication (data not shown).

Paired analysis of the 3-MT and K+ content at a specific PMI led to the construction of nomograms for the two basic groups of subjects (OHD and non-OHD). The regression of PMI on 3-MT and K+ simultaneously provided a better prediction of PMI than the separate regression of PMI on 3-MT or PMI on K+ within each group of subjects. In these regressions only 91 of the 106 subjects in the non-OHD group were used since 15 of these subjects had missing K+ values.

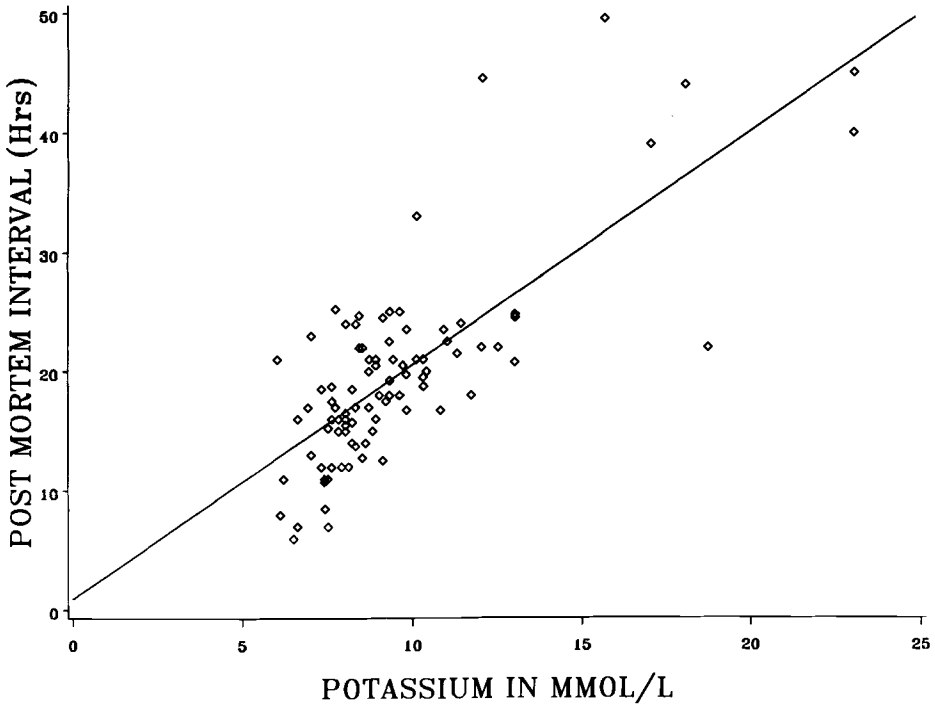

FIG. 2—*Potassium (K+) content in the vitreous humor of subjects dying from causes other than heart disease (*N = *91). Subjects remained at ambient temperature [4 to 29°C (39–85°F)] or were stored at 4°C (39°F) within morgue refrigerators for less than 12 h prior to tissue collection. Subject K+ levels (mmol/L) are plotted against their respective PMI (hours). Regression analysis of the points creates a line having a correlation coefficient of* r = *0.87 with a 95% prediction error of* ±*10.5 h.*

Nomograms were constructed for determining an estimate of PMI given the values of 3-MT and K+ for each group of subjects; these appear as text Figs. 5 and 6. The estimated values produced by these nomograms are accurate (with 95% confidence) to within ±11.3 h in the OHD group and ±8.0 h in the non-OHD group. These accuracy figures refer to predictions near the mean values of 3-MT and K+. Prediction should not be made outside the range of the data; ranges for 3-MT is 0.61 to 3.30 and K+ is 4.7 to 26 in the OHD group and ranges for 3-MT is 0.38 to 4.26 and K+ is 6 to 23 in the non-OHD group.

Discussion

We have extended our previous finding that 3-MT could be used as a chemical marker for the determination of PMI, and we have directly compared this method to the most widely accepted chemical method, namely, accumulation of K+ in the vitreous humor. Since our initial report [6], the number of non-OHD subjects with both a known PMI and a negative toxicologic screens has nearly doubled. In these cases, the 3-MT content in the dorsal putamen increased in a highly predictable manner as the PMI increased. Furthermore, the nearly twofold increase in cases yielded no significant alteration in the correlation coefficient, and there was only a slight decrease in the accuracy of determining the PMI compared to our previous report ($N = 62$ versus $N = 106$; $r = 0.83$ versus $r = 0.77$; ±7.5 h versus

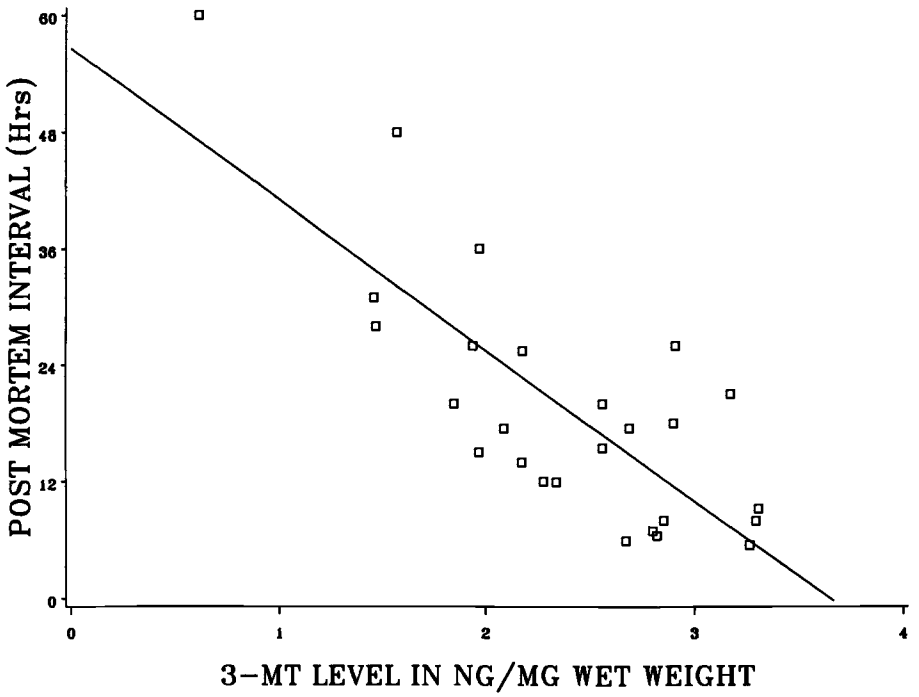

FIG. 3—*3-MT levels in dorsal putamen of subjects dying from organic heart disease (*N = *26). Subjects remained at ambient temperature [4 to 29°C (39–85°F)] or were stored at 4°C (39°F) within morgue refrigerators for less than 12 h before tissue collection. Subject 3-MT levels (ng/mg wet weight) are plotted against their respective PMI (hours). Regression analysis of the 3-MT points creates a line having a correlation coefficient of* r = −*0.78 with a 95% prediction error of* ±*17.6 h.*

±9.9 h). Therefore, the observation that 3-MT can be used as a gauge of PMI is still viable and is slightly more accurate than K+ accumulation in the same cases.

In non-OHD subjects, the accumulation of K+ in the vitreous humor is accurate to ±10.5 h for determining the PMI. On the other hand, the increased scatter of data points with increased PMI (statistically significant) severely devalues the usefulness of this method. Our data agree with previous reports that K+ in vitreous humor can accurately predict PMI for only a narrow window of time [*3,4*].

In a limited sampling of OHD subjects we found that, as in non-OHD subjects, the K+ in vitreous humor increases with increasing PMI. Interestingly, the accuracy of predicting the PMI in OHD subjects was not as good as in non-OHD subjects. Furthermore, K+ accumulation in OHD subjects was more accurate than putaminal 3-MT clearance from the same cases.

We have previously discussed the biosynthesis of 3-MT [*6*] and concluded that there is limited production *intra vitam,* but the postmortem production of 3-MT may be a major outlet of dopamine metabolism in the putamen. We further proposed hypothetically that the inverse nature of the 3-MT/PMI relationship in OHD subjects maybe related to ante- or perimortem release or both of dopamine into extraneuronal spaces with subsequent mass conversion to 3-MT postmortem. Our current studies of the toxicologic effects on the 3-MT/PMI relationship would tend to support this hypothesis.

A wide variety of stimulants including amphetamines and cocaine is known to cause the release of dopamine in the basal ganglia of animals [*12*]. We have found in each non-OHD

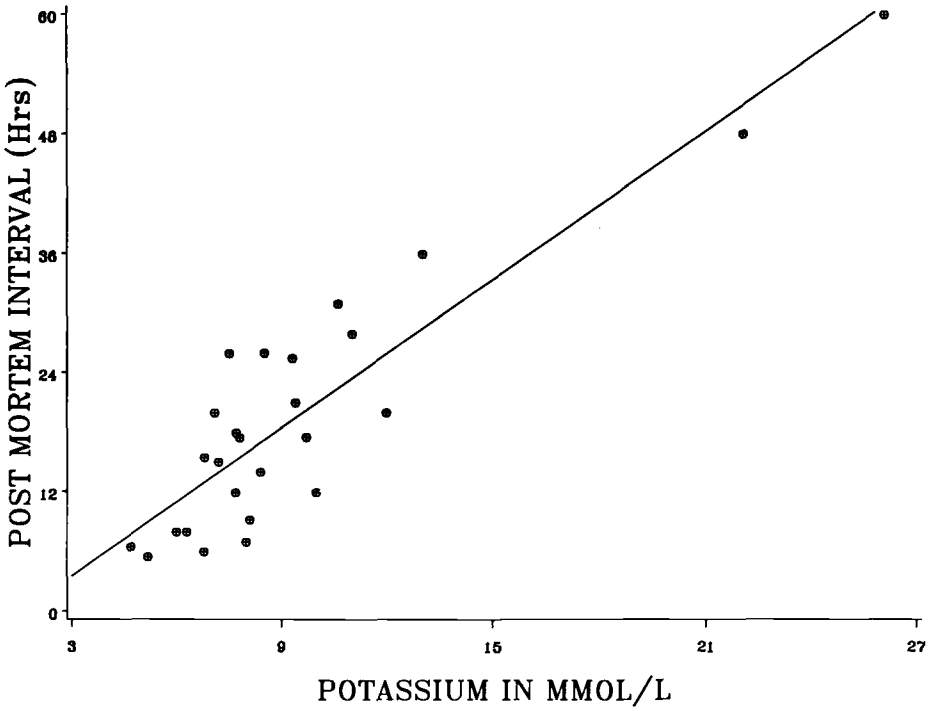

FIG. 4—*K+ content in vitreous humor of subjects dying from organic heart disease (*N = *26). Subjects remained at ambient temperature [4 to 29°C (39–85°F)] or were stored at 4°C (39°F) within morgue refrigerators for less than 12 h before tissue collection. Subject K+ content (mmol/L) is plotted against their respective PMI (hours). Regression analysis of the K+ points creates a line having a correlation coefficient of* r = *0.90 with a 95% prediction error of* ±*12.3 h.*

case where amphetamine, caffeine, or cocaine was detected toxicologically that the 3-MT content in the putamen was significantly higher (up to fivefold) than the PMI would have predicted.

Interestingly, in cases involving the presence of opioids, the 3-MT content was also higher than would have been predicted by the PMI. Many opioids are mixed-function drugs: at a high dose they are sedative, but at low doses they can be stimulative. Behavioral and biochemical studies have indicated that the stimulatory actions of opioids may be mediated by the enhanced release of dopamine (DA) where the opiate acts at the level of the dopaminergic presynapse [*13*]. Thus, the increased 3-MT level could be explained as an indirect effect of opioids on the antemortem "release state" of DA.

Logically, but speculatively, if the "release state" were decreased, the 3-MT levels would be decreased in relation to the PMI. Our data tend to support this concept. Barbiturates are known to cause decreased neurotransmitter release [*14*]. We have found low levels of 3-MT for the PMI in cases where barbiturates were present.

A decrease in the release of DA could account for the reduced levels of 3-MT in subjects dying from smokeless carbon monoxide, but this also is speculative. We are unsure why, on the one hand, 3-MT content follows the normal non-OHD pattern in cases of fire-related asphyxia where carbon monoxide is detected in the blood and why, on the other hand, the 3-MT is significantly reduced (vis-a-vis the PMI) when the cause of death is attributable to carbon monoxide absent other products of combustion.

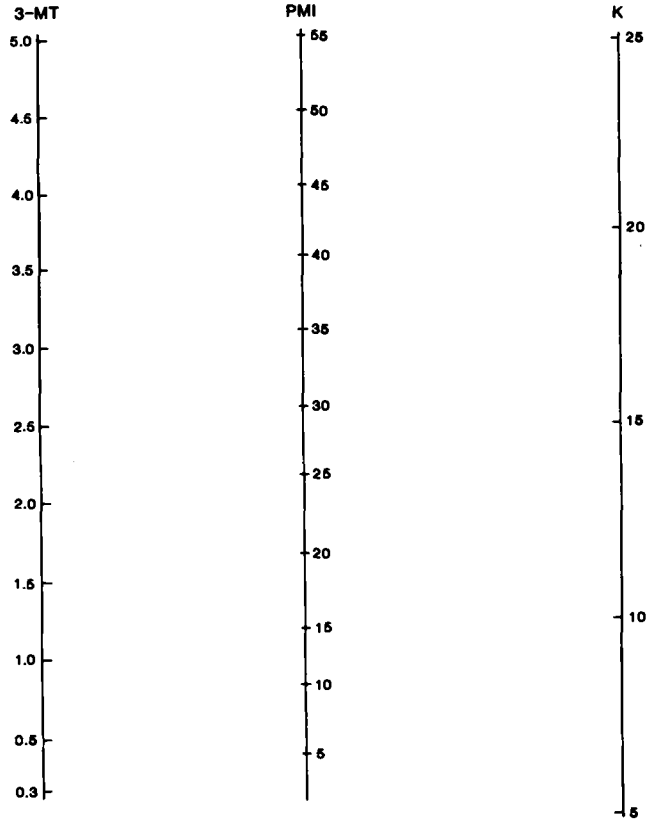

FIG. 5—*Nomogram for determining PMI from both 3-MT levels and K+ content in non-OHD subjects. Subjects remained at ambient temperature [4 to 29°C (39–85°F)] or were stored at 4°C (39°F) within morgue refrigerators for less than 12 h before tissue collection. The nomogram is based on 91 matched determinations having a correlation coefficient of* r = *0.74 with a 95% prediction error of* ±*8.0 h.*

These data indicate that the thanatologist must be prudent and cautiously cognizant of the cause of death (COD) when applying this method for forensic science purposes. We have grouped a number of CODs because they seem to fall into a general pattern, while other CODs apparently fall into a different pattern. Any subtle but predictable changes in the 3-MT/PMI relationship because of COD, once determined, could further enhance the sophistication of this aspect of postmortem forensic chemistry. More importantly, for the human neuroscientist, these data point out the profound effect COD could have on neurochemical data gathered in the study of disease-related dysfunction (for example, Parkinson's disease, Alzheimer's disease) where inappropriate controls are used (OHD).

Conclusions

Our data indicate that under the appropriate circumstances, 3-MT is as accurate as any other chemical determinant of PMI. It is apparent from these data, except in certain cases toxicologically positive for several specific classes of drugs, that both 3-MT and K+ can be predictive of PMI and that simultaneous determination of both parameters can yield a

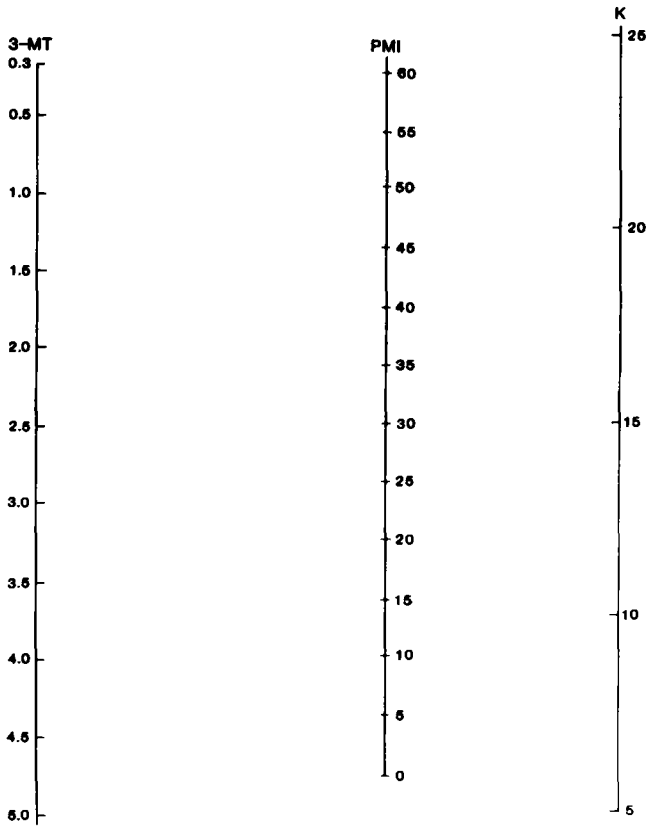

FIG. 6—*Nomogram for determining PMI from both 3-MT levels and K+ content in OHD subjects. Subjects remained at ambient temperature [4 to 29°C (39–85°F)] or were stored at 4°C (39°F) within morgue refrigerators for less than 12 h before tissue collection. The nomogram is based on 26 matched determinations having a correlation coefficient of* r = *0.85 with a 95% prediction error of* ±*11.3 h.*

highly respectable estimation of the time of an unwitnessed death (±8.0 h for non-OHD, ±11.3 h for OHD).

Acknowledgments

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Address requests for reprints or additional information to
D. L. Sparks
203 Sanders-Brown Bldg.
Lexington, KY 40536-0230