Letters to the Editor

Discussion of "Correlation of Plasma Concentration and Effects of Succinylcholine in Dogs"

Dear Sir:

The report by Drs. Baldwin and Forney [*1*] addresses important but unresolved issues of the pharmacodynamics and pharmacokinetics of succinylcholine. For the following reasons, their report does not alleviate the present dearth of factual information about succinylcholine.

1. The authors omit to specify the method used for the determination of succinylcholine. Both the detection limits and the capability of the analytical method to differentiate between succinyl(di)choline and succinylmonocholine need to be known for proper interpretation of the data. Additionally, neither the number of blood collections nor their timing are reported. Presented are only data (from one dog) for the first 10 min after the administration of succinylcholine. What were the plasma concentrations beyond this period for each of the three doses of succinylcholine?

2. The methods of pharmacokinetic analysis are not described. The results are incomplete and appear incorrect. Only one (the terminal plasma half-life) of the four parameters characterizing the purportedly biexponential decay of succinylcholine in plasma is presented (Table 1).

3. The method used for the compartmental analysis is not reported. Contrary to the authors' statement, the ESTRIP program (Ref *5* in the report) cannot be used for this purpose since it estimates the polyexponential parameters describing the decay of a drug in plasma. The compartment model presented in Fig. 3 is a general one and not one specifically tailored for succinylcholine. The input rate constant, k_o, is irrelevant for the bolus administration of succinylcholine. The use of identical symbols k_{el} for the compartmental elimination rate constant k_{el} in Fig. 3, as well as for the elimination rate constant of the terminal ("beta") half-life in plasma k_{el} in Table 1, is inappropriate since the two are conceptually and numerically different. The authors failed to consider that elimination of succinylcholine can also occur from the peripheral compartment (compartment 2) via enzyme-catalyzed and/or spontaneous hydrolysis of the drug. In addition, based on high hydrophilicity and minimal protein binding of succinylcholine, the estimate for "the total volume of distribution" ($V_{d\ area}$) of 2.184 ± 0.22 L kg^{-1} appears highly exaggerated.

4. The units are frequently incorrectly reported, for example, $V_{d\ area}$ in L instead of L/kg, and the rate constants in min instead of min^{-1}. Microconstants in Table 1 are twice reported to be k_{21}, but k_{12} is not reported. Furthermore, in Table 1 the units for "area under the curve" (AUC) are given as μg min/L. The reported values are incompatible by several orders of magnitude with data presented in Fig. 2 in which the concentrations are expressed in μg/mL. Similarly, the units for clearance of succinylcholine (Cl in Table 1) should have been L min^{-1} kg^{-1} rather than L/min.

5. In Fig. 5, three pairs of experimental points are connected with three straight lines. Since any two points in a coordinate system can always be connected by a straight line, the statement that "This figure suggests that a linear relationship may exist . . ." is fallacious. Even worse is the extrapolation of the lines to obtain A_{min}.

6. In Fig. 9, the two equations do not describe the lines. As a matter of fact, the form of the equations precludes their use to describe lines in a coordinate system with one axis (abscissa) in logarithmic units.

7. The titles of the journals cited in Refs *5* and *10* are incorrect.

8. Table 2 reports succinylcholine concentrations in the muscle. The reader is surprised, since neither the method of sample collection nor the preparation of the samples for analysis are mentioned in the appropriate section of the paper. The results in Table 2 are incomprehensible since the first reference to the Table mentions the plasma concentrations of succinylcholine ("Cps"). The second reference refers, indeed, to the succinylcholine levels in muscle tissue. What are the numbers in the first column? What do "10 Days" or "20 Days" refer to?

9. It is wrong to describe succinylcholine as a "cholinergic antagonist."

These and other deficiencies, too numerous to cite individually, preclude the acceptance of the proferred conclusions.

Vladimir Nigrovic, M.D.
Department of Anesthesiology and Pharmacology
Medical College of Ohio
Toledo, OH 43699

Reference

[*1*] Baldwin, K. A. and Forney, R. Jr., "Correlation of Plasma Concentration and Effects of Succinylcholine in Dogs," *Journal of Forensic Sciences*, Vol. 33, No. 2, March 1988, pp. 470–479.

Author's Response

Sir:

Dr. Nigrovic has mentioned nine problem areas with our paper. The following points correspond to his own:

1. This paper was published back-to-back with another which contained a description of the method of analysis used [*1*]. Measures of precision and sensitivity are as follows: coefficient of variation = 0.99, limit of detection = 10-ng/mL plasma.

Figure 2 depicts a typical plasma concentration versus time plot from one dog at the 1.0-mg/kg dose. Sample times varied as a function of dose owing to the sensitivity limit of the assay. Sample times extended up to 60-min post dose for the 5-mg/kg dose. For lower doses, eight to ten samples were collected as shown in Fig. 2.

2. & 3. The pharmacokinetic parameters were estimated with the use of ESTRIP which solves for areas under the plasma concentration versus time curve and coefficients and exponents for the following general equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

The method of residuals [*2*] (not ESTRIP) was used to estimate volume of distribution and microconstants.

Our k_{el} others may recognize as k_{10}. For multicompartment models, k_{el} does not represent the rate constant of the terminal portion of the disappearance curve as suggested by Dr. Nigrovic. It is, however, used correctly in Table 1. The descriptor of the terminal portion of the disappearance curve is given by the half-life in Table 1.

Any number of models have been used to describe succinylcholine kinetics, including models accounting for elimination from the peripheral compartment as well as a simple one-compartment model. In fact, there is nothing to preclude highly perfused and metabolically active tissue from being conceptualized as part of the central compartment.

We are unaware of any dogma requiring the kinetics of succinylcholine to be analyzed by a particular model.

4. There is are several mistakes in the column headings of Table 1. Column 5 should read k_{12}, the units for Columns 6 and 7 should be litres and for AUC the units should be mg·min/L. The units for Cl are correctly designated as L/min. The units as they appear in the text also are correct. We apologize, though, for the errors in the column headings of Table 1.

5. Dr. Nigrovic takes great exception to the extrapolation of a two-point line. We are mindful of the errors that could be engendered, but two-point extrapolations are not unheard of in pharmacokinetics (for example, Ludden method for estimating V_{max} and km for phenytoin [*3*]). We appreciate the need to refine the estimates of A_{min} with additional data points derived from additional doses of succinylcholine.

6. The equations in Fig. 9 represent $y = m \log x + b$. The value for "b" in the equation for the 5-mg/kg dose should be 113.3 instead of 1133. Because the abcissa is a log scale, the values for "b" do not reflect the y intercept shown on the graph since $x = 0.1$ μg/mL at that point.

The data points themselves are derived from the relationships described in Figs. 7 and 2. As derived data all of the points fall on the lines. This is why no standard error bars are used. It might have been less confusing if the points had been omitted with only the lines displayed.

7. The title of the journal cited in Ref *5* should be *Journal of Pharmaceutical Science* and the year 1978. In Ref *10* the journal should be *Anesthesia and Analgesia.*

8. Table 2 presents the results of an experiment performed in an attempt to correlate the recovery from muscle paralysis with tissue concentrations of succinylcholine. Three dogs were anesthesized with sodium pentobarbital and mechanically ventilated. A Grass 48-nerve stimulator was set to deliver train-of-four square pulses as described in the other studies [*1*]. The response of the gastrocnemius muscle to this stimulation was recorded. The dogs were given 1 mg/kg of succinylcholine i.v. in the cephalic vein. Three, 2-g samples of triceps muscle were removed from each animal at three points correlated with the recovery of the gastrocnemius muscle from paralysis. The first point was at the beginning of recovery ("Initial") and at 40 and 70% of recovery, respectively. The muscle sections were isolated before this procedure. Sutures were looped but not tied in preparation so that each could be quickly removed. The samples were kept on ice or refrigerated and analyzed within 48 h of the time of collection. The assay method was the same as in the other experiments [*1*].

The statement "This may also explain why Cps taken at 4 min (Table 2) increased exponentially with increasing doses" should be deleted.

9. Succinylcholine is a depolarizing neuromuscular blocking agent. It has been reported that it stimulates the cardiac vagus and subsequently the sympathetic ganglia [*4*].

Kathleen A. Baldwin, Ph.D.
Sheriff's Crime Laboratory
800 S. Victoria Ave.
Ventura, CA 93009

Robert B. Forney, Jr., Ph.D.
Director of Toxicology
Dept. of Path. Med. College of Ohio
Central Station 10008
Toledo, OH 43699

References

[*1*] Baldwin, K. A. and Forney, R. B., Jr., "The Influence of Storage Temperature and Chemical Preservation on the Stability of Succinylcholine in Canine Tissue," *Journal of Forensic Sciences*, Vol. 33, No. 2, March 1988, pp. 462–469.

[2] Wagner, J. G., *Fundamentals of Clinical Pharmacokinetics*, Drug Intelligence Publications, Inc., Hamilton, IL 62341, 1975, pp. 58–63.
[3] Ludden, T. M., Allen, V. P., Valutsky, W. A., et al., "Individualization of Phenytoin Dosage Regimens," *Clinical Pharmacology and Therapeutics*, Vol. 21, 1977, pp. 287–293.
[4] McEvoy, G. K., McQuarrie, G. M., et al., *Drug Information 84*, American Society of Hospital Pharmacists, Bethesda, MD, 1984, p. 404.

Discussion of "The Shielding Capacity of the Standard Military Flak Jacket Against Ballistic Injury to the Kidney"

Sir:

The paper "The Shielding Capacity of the Standard Military Flak Jacket Against Ballistic Injury to the Kidney" by O'Connell et al. in the March 1988 issue of the *Journal* falls far short of minimum standards for any journal article in that it fails to provide sufficient information to enable another researcher to repeat the experiment. Specifically:

1. No mention is made of which bullet type was used for the reported shots. Those conversant with small arms technology can assume, from the bullet weights given, that the 22 caliber bullet was a lead round-nosed one and that the 45 caliber bullet was of the full-metal-jacketed type; but what were the 38 caliber and the 9 mm bullets? Hollow-point, soft-point, and full-metal-jacketed bullet types are available in the bullet weights given for these calibers. For the M-16 and M-14 rifle calibers, American military ammunition, civilian ammunition of various types, and foreign military ammunition is available in the weights given, yet no particular bullet is specified.
2. No information is given as to the orientation of the bullet's trajectory to the pig's body other than "into" the flank. Did the shot enter from the side or from the back?

We further suggest that in not measuring the velocity of their shots and not describing the deformation and fragmentation of each projectile this paper fails to meet minimum standards for wound ballistics research.

A most striking error is the listing of a 180-grain bullet at 2625-ft/s velocity for the AK-47 rifle. The weight of the Russian military full-metal-jacketed bullet for the AK-47 Assault Rifle is 120 grains and its velocity is about 2340 ft/s; the muzzle energy given for the AK-47 is also overstated by about 90% [*1,2*]. Had the bullet velocities been measured, this inconsistency would have become apparent.

Their results section tells us that all three of the rifle bullets fractured lumbar vertebrae. In our opinion, this unfortunate choice of bullet path negates their results. It is well known that bullets of any type can be expected to behave differently (and more unpredictably) when they strike bone. Which of the damage described was caused by the bullet itself, which by bullet fragments, and which by bone fragments acting as secondary missiles?

Their Fig. 6 shows "Massive hemoperitoneum after assault by 7.62-mm bullet shot from an AK-47." Yes, disrupted blood vessels can bleed—even when injured by the lowest velocity projectiles. *We have also seen the AK-47 cause minimal intraperitoneal damage when it did not hit a large vessel.*

A projectile's shape and construction are at least as critical to the effect it produces as is its velocity. The 12-gauge slug is a blunt projectile constructed of an easily deformed lead; it flattens on the flak jacket, as do other blunt tipped projectiles that fail to perforate it (M. L. Fackler, unpublished data, 1984–1988). The sharp-pointed military rifle bullet will perforate the flak jacket even at extreme ranges where its velocity has fallen to "low-velocity." For example, an M-193 (M-16) bullet at a *measured striking velocity of only 629 ft/s* perforated Kevlar® body armor and continued on to penetrate 14 cm of ordnance gelatin [*3*].

A uniform *worsening of the damage when the flak jacket was used* was shown in extensive studies comparing intraperitoneal and intrathoracic damage from military rifle bullets [*4*]. Shots through flak jackets caused more bullet deformation and fragmentation as well as causing the bullets to yaw at a shallower penetration depth in tissue when compared with identical shots without jackets.

What was the point of these experiments? By showing that the flak jacket does not stop military rifle bullets, do the authors think they have added anything to the literature that was not already well known? In their introductory paragraph, O'Connell et al. state (apparently in attempting to justify the studies) "The lack of research data makes it extremely difficult for military urologists to make specific recommendations concerning the treatment of ballistic injuries to the genitourinary tract." The lack of any "specific recommendations" along these lines in either their discussion or conclusions appears to support our view on the usefulness of their data.

Richard T. Mason, M.D.
Forensic Pathologist
San Jose, CA

Martin L. Fackler, M.D.
Director, Wound Ballistics Laboratory
Letterman Army Institute of Research
Presidio of San Francisco, CA

References

[*1*] Ezell, E. C., *The AK-47 Story*, Stackpole, Harrisburg, PA, 1986.
[*2*] Fackler, M. L., Bellamy, R. F., and Malinowski, J. A., "The Wound Profile: Illustration of the Missile-Tissue Interaction," *Journal of Trauma*, Vol. 28 Supplement, 1988, pp. 21–29.
[*3*] Fackler, M. L. and Bellamy, R. F., "Letter to the editor," *Journal of Trauma*, Vol. 26, 1986, pp. 1157–1159.
[*4*] Fackler, M. L., Breteau, J. P. L., and Sendowski, I., "Protective Vests on the Battlefield. Proceedings of the NATO Research Study Group 11 Workshop," in *Wound-Ballistic Aspects of Personnel Protection*, Ministry of Foreign Affairs, The Hague, Netherlands, in press.

Authors' Response

Sir:

Dr. Mason and Dr. Fackler have brought up several interesting points. We have broken the letter down into seven specific questions and would like to address each question separately:

1. What was the bullet type of the 45 caliber, 38 caliber, and 9 mm bullets? Full metal jacket.
2. What was the bullet type of the M-16 and M-14? Full metal jacket, American military ammunition.
3. Orientation of the bullet into the pig? The projectiles were shot into the side, not the back.
4. Measuring the velocity of the projectiles? The projectile velocity was not measured. It was not part of the experimental design.
5. Weight and muzzle velocity of the AK-47?
   Velocity: 710 m/s or 2340 ft/s,
   Weight: 7.91 grains or 122 g,
   Source: Division of Foreign Weapons Analysis, Aberdeen Proving Grounds, Aberdeen, Maryland.

We apologize for the misinformation printed in our article. We regret any problems this may have caused.

6. Which damage described was caused by the bullet itself (versus bony fragments)? According to the forensic pathologist who performed the autopsy the majority of the damage was caused by the bullet. In fact, very little obvious damage occurred as a result of bony fragments serving as secondary projectiles.

7. What was the point for the experiments? This experiment was performed to evaluate the range of renal injury one can expect from ballistic trauma when the flank is protected by a flak jacket. We deliberately chose a range of weapons systems to evaluate the effects of a range of projectiles and kinetec energy. What might not be apparent to a non-urologist is that it is not uncommon to see renal fractures with significant hemorrhage in cases with little apparent external injury. The purpose was to review the protection offered by the flak jacket.

Kevin J. O'Connell, M.D.
CAPT, MC, USN
Department of the Navy
Naval Hospital
Urology Department
Bethesda, MD 20814-5011

Harold A. Frazier, M.D.
LT, MC, USNR
Department of the Navy
Naval Hospital
Urology Department
Bethesda, MD 20814-5011

Michael A. Clark, Ph.D., M.D.
Medical Science Building
635 Barnhill Dr.
Indianapolis, IN 46223

Discussion of "Validity Testing of Commercial Urine Cocaine Metabolite Assays: Parts I and II"

Sir:

The papers by Cone and Mitchell "Validity Testing of Cocaine Metabolite Assays: Parts I and II" [*1,2*] describe a very well-designed study which provides all the data required for determination of sensitivity and specificity of several commercial assays intended as screens for cocaine use. Unfortunately, in their conclusions, the authors dismiss a very valuable part of their own data. The study was conducted on five male volunteers who had a history of intravenous cocaine abuse. Each volunteer was given a single intravenous dose of 20 mg of *l*-cocaine HCl on the test day. Following collection and freezing, freshly thawed clinical urine specimens from these volunteers were coded and tested in a random fashion. The results of the commercial assays were compared to those by gas chromatography/mass spectrometry (GC/MS). In five specimens from cocaine-dosed volunteers, the Coat-A-Count® and the Double Antibody cocaine metabolite assay gave positive results hours after dosing at a time when the GC/MS procedure was unable to detect cocaine or its metabolite in the urine specimens. The authors called these results "false positives." They are not false positives because the specimens were obtained from volunteers who had been given cocaine intravenously under controlled circumstances and the specimens were coded and randomly tested. A more accurate term would be unconfirmed true positives.

False positives (or Type II errors, beta error, and so forth) are positive test results in patients who are free from the condition under study [*3–5*]. In this case the condition is exposure to cocaine. False positives would be positive test results in volunteers who were

not cocaine abusers and who had not been exposed to cocaine. False negatives (or Type I or alpha errors) would be negative test results in specimens from volunteers who had received cocaine [3–5]. The failure to confirm the immunoassay positives by the GC/MS method might indicate that these were actually false negatives by the particular GC/MS method used in this study.

Because of the greater sensitivity of the radioimmunoassay and its strong cross-reaction with cocaine, as well as benzoylecgonine, norcocaine, and ecgonine [2], the Coat-A-Count radioimmunoassay is able to detect exposure to cocaine longer than any of the other methods tested [1]. According to Cone, for this reason the Coat-A-Count and DPC Double Antibody assays are useful in detecting cocaine in urine at longer time intervals. A more sensitive confirmation method than that used in this study, such as GC/MS with extraction and derivatization for cocaine and other metabolites in addition to benzoylecgonine, should be used to confirm the immunoassay positives. Since Cone et al. [1] report that overall recovery of benzoylecgonine varied from 16.3 to 34.3% of the administered dose, screening tests and confirmation tests which detect cocaine and other metabolites in addition to benzoylecgonine would be more accurate and have longer times of detection.

Vina Spiehler, Ph.D.
Howard Wilson, B.S.
Said El Shami, Ph.D.
Diagnostic Products Corp.
Los Angeles, CA 90045

References

[1] Cone, E. J., Menchen, S. L., Paul, B. D., Mell, L. D., and Mitchell, J., "Validity Testing of Cocaine Metabolite Assays: I. Assay Detection Times, Individual Excretion Patterns, and Kinetics After Cocaine Administration to Humans," *Journal of Forensic Sciences*, Vol. 34, No. 1, Jan. 1989, pp. 15–31.

[2] Cone, E. J. and Mitchell, J., "Validity Testing of Cocaine Metabolite Assays: II. Sensitivity, Specificity, Accuracy, and Confirmation by Gas Chromatography/Mass Spectrometry," *Journal of Forensic Sciences*, Vol. 34, No. 1, Jan. 1989, pp. 32–45.

[3] Galen, R. and Gambino, S., *Beyond Normality: The Predictive Value and Efficiency of Medical Diagnoses*, Wiley Biomedical, New York, 1975.

[4] Swets, J. A., "Measuring the Accuracy of Diagnostic Systems," *Science*, Vol. 240, 1988, pp. 1285–1293.

[5] National Committee for Clinical Laboratory Standards Document GP10, "Assessment of Clinical Sensitivity and Specificity of Laboratory Tests," NCCLS, Vol. 7, No. 6, 1987, pp. 1–174.

Author's Response

Sir:

I am in general agreement with Dr. Spiehler et al.'s comments regarding interpretation of the "false positives" encountered in our evaluation of commercial cocaine metabolite assays. The positive responses obtained with the DPC Coat-A-Count® and Double Antibody cocaine metabolite assays likely were a result of the subject's recent cocaine exposure. Apparently small amounts of cocaine and metabolite are excreted over a period of several days to a week during cocaine withdrawal [1]. The amount of cocaine and metabolite excreted in this late elimination phase is quite small, that is, 5 to 10-ng/mL concentration. However, with the DPC Coat-A-Count and Double Antibody metabolite assays, this is enough cocaine to trigger a positive response in these assays. This is due to the approximate X50 cross-reactivity factor that the antibodies exhibit for cocaine versus benzoylecgonine.

Regarding the issue of how this type of test result should be labeled or defined, I

disagree that the clinical definition cited by Dr. Spiehler et al. is a workable solution. By this definition, a specimen is a true positive if it tests positive and is obtained after cocaine exposure, however, specimens testing negative after cocaine exposure are false negatives regardless of their drug concentration and a false positive cannot be obtained under these conditions. When do specimens collected after exposure become true negatives? on a purely theoretical basis, drug is never completely eliminated after exposure. In light of this difficulty, it seems that we must adhere to our present system of defining positive and negative results in a straightforward manner by use of standardized cutoffs for both initial test and confirmation. We arbitrarily defined "false positive" in the cited study as those specimens testing positive by commercial assay (300-ng/mL cutoff) which had <20-ng/mL benzoylecgonine by GC/MS confirmation. Obviously, it is critically important to define these terms clearly and without ambiguity in forensic science testing.

Edward J. Cone, Ph.D.
Addiction Research Center
National Institute on Drug Abuse
Baltimore, MD 21224

Reference

[1] Cone, E. J. and Weddington, W. W., "Prolonged Occurrence of Cocaine in Human Saliva and Urine After Chronic Use," *Journal of Analytical Toxicology*, 1989, in press.

Discussion of "A Case of Panhypogammaglobulinemia Masquerading as Child Abuse" with Regard to the Involution of the Thymus

Dear Sir:

Arthur R. Copeland's article [*1*] demonstrates the importance of immunological examination in a case suspected child abuse or neglect. While the mechanism of injuries and the circumstance of abused children have been mentioned in most papers and reviews, very few reports refers to the immunological state [*1*] or involution of the thymus [*1,2*].

I investigated 44 autopsy cases of child abuse/neglect in the Department of Legal Medicine, Kobe University School of Medicine, and the Medical Examiner's Office of Hyogo Prefecture during the period 1967 to 1989. Most of them, not only physically abused children but also neglected cases, showed a markedly involuted thymus. Furthermore, the involution of the thymus correlated to the degree and the period of child abuse/neglect [*3*]. It is well known that the thymus is the most sensitive organ to stress since Selye's stress theory [*4*]. I would like to propose here that the involution of the thymus is one of the indices of child abuse/neglect and that early involution in childhood may be related to an immunological insufficiency.

In the report of Dr. Copeland, a 15-month-old boy also revealed a markedly involved thymus and relatively low levels of gammaglobulin and complements. But, his cause of death was thought to be sepsis from the infection of noma in the face and pneumonia. If the generalized infectious disease was related to immunodeficiency, still more to atrophy of the thymus, his cause of death might more possibly have been due to child abuse, that is, maltreatment of wounds and refusal of medical care, rather than a result of genetic origin.

Several other cases of child abuse with severe polymicrobial infection have been reported [*5–7*]. Though these reports are not concerned with thymic function, abused children may have an easily infectious state.

In the court of justice, it is a major problem whether the injuries or sick conditions of children are due to any other intention or not. The judgment based on only parental assertion and police investigation is so critical. Therefore, forensic pathologists should

evaluate the stress on children correctly. The implication shows the importance of immunological examination in abused children and of thymic function. I would like to emphasize again that the involution of the thymus is one of the indices of child abuse/neglect. The further investigation of immunological function in abused children is needed.

Tatsushige Fukunaga, M.D.
Assistant Professor
Department of Legal Medicine
Shiga University of Medical Science
Seta-Tsukinowa-cho
Ohtsu 520-21, Japan

References

[1] Copeland, A. R., "A Case of Panhypogammaglobulinemia Masquerading as Child Abuse," *Journal of Forensic Sciences,* Vol. 33, No. 6, Nov. 1988, pp. 1493–1496.
[2] Morrow, P. L., "Caffeine Toxicity: A Case of Child Abuse by Drug Ingestion," *Journal of Forensic Sciences,* Vol. 32, No. 6, Nov. 1987, pp. 1801–1805.
[3] Fukunaga, T., Ueno, Y., Imabayashi, T., and Nakagawa, K., "Thymus Gland in Battered Child Syndrome," *Japanese Journal of Legal Medicine,* Vol. 43, Suppl., May 1989, p. 160.
[4] Selye, H., *Stress,* Acta Inc. Medical Publishers, Montreal, 1950, pp. 452–488.
[5] Koch, C. and Høiby, N., "Severe Child Abuse Presenting as Polymicrobial Bacteremia," *Acta Paediatrica Scandinavica,* Vol. 77, No. 6, 1988, pp. 940–943.
[6] Halsey, N. A., Frentz, J. M., Tucker, T. W., Sproles, T., Redding, J., and Daum, R. S., "Recurrent Nosocomial Polymicrobial Sepsis Secondary to Child Abuse," *The Lancet,* Vol. II, Sept. 1983, pp. 558–560.
[7] Zohal, Y., Avidan, G., Shvili, Y., and Laurian, N., "Otolaryngologic Cases of Munchausen's Syndrome," *Laryngoscope,* Vol. 97, Feb. 1987, pp. 201–203.

Author's Response

Dear Sir:

I read with interest the letter to the editor by Dr. Fukunaga of Japan. I am not familiar with his research article published in the *Japanese Journal of Legal Medicine.* However, a study of the thymus and immunology of the neonate is important. My own practical experience with child abuse cases and with forensic pediatric pathology is that the thymus does NOT involute as much as in a genetic disorder. I would encourage response from the rest of the readership on this point. I would also recommend a controlled study in which the thymus' weight, thymus weight/kilogram body weight, and thymus weight/body mass index is studied in both acute *and* chronic states of child abuse, traumatic accidental deaths (for example, traffic, drowning, fires, and so forth), Sudden Infant Death Syndrome (SIDS) deaths, and in other childhood illnesses of nontraumatic but stressful scenarios (for example, congenital heart disease, sickle cell disease, and so forth).

As far as the use of the thymus as a "marker" for abuse/neglect, I believe that Dr. Fukanaga has committed one of the most serious "cardinal sins" of forensic pathology expounded by the late Dr. Alan Moritz—namely, relying on anatomic findings without adequate scene investigation. Here in Miami, one works with a medical examiner system in which autopsies and scene visitations are performed by the physician. One does not work here under a coroner's system with certification of death by individuals remote from the scenario, nor does one operate here a legal medicine department with information supplied solely by referral. Here, the doctor goes to the scene and does the autopsy. Both facets of a case must correlate before the certification of death.

In this specific case, such scene visitation and investigation of the clinical records revealed a child who was repeatedly taken to the hospital by the mother for medical

care, with a height and weight growth curve BELOW the third percentile, with no fractures either recent or old, with manifestations of sepsis, with evidence of past sepsis in lungs and in the hip joint, and with abnormalities in the circulating immune system. Additionally, since the time of the autopsy, (2 October 1987) there have been no further inquiries made to this office by officials investigating this family.

Accordingly, I recommend to all who read this letter to *CAVEAT SCRUTATOR* (that is, let the examiner beware). Do not rely solely on anatomic findings. Please perform autopsies *and* scene visitations.

Arthur R. Copeland, M.D., Ph.D.
Associate Medical Examiner
Number One Bob Hope Rd.
Miami, FL 33136

Discussion of "Clay Mineralogical Analysis for Forensic Science Investigation"

Sir:

The recent trend towards using the clay-size fraction of soils as a means of increasing their forensic science applicability is an unfortunate example of placing before the forensic science community a promising application of an area of science without establishing a protocol that is both scientifically unflawed and practical in the justice system.

In two papers [*1,2*], Marumo et al. present several cogent arguments for the use of the clay-sized fraction in the characterization of soils involved in criminal investigations. They point out among other things that clay mineralogists and soils scientists have shown that the clay minerals present in soils are dependent upon not only parent materials but more importantly on the local chemical weathering factors which in turn are dependent upon the local climate and topography [*3–5*].

Over the past 30 years a standard protocol for the general identification of clay minerals has evolved and been adopted by workers in this highly specialized field [*6*]. This method as it stands is not practical as a standard procedure in most forensic science laboratories because it involves the use of an X-ray diffractometer scanning over the 3 to 35° range of two-theta (equipment which is too limited as a general applications technique to be cost-effective in many laboratories). Scans are made upon randomly oriented samples as well as samples that are aligned parallel to the basal spacing. It also involves a time-consuming set of sample treatments more or less as given by the Marumo group, and it requires, as they point out, a quantity of <0.005-mm size fraction that is greater than that often encountered in questioned soils samples.

The alternative protocol they propose uses a much smaller range of observed two-theta values, covering only the first order of basal reflections of oriented samples. Their procedure also selects a coarser upper limit of grain size [*1*]. These changes of protocol, however, restrict the determination of clay mineral types to a much fewer number while they leave indeterminate the positive identification of many others and create confusion between coarser-sized detrital phyllosilicate minerals and the clay minerals. This last point is not insignificant if the investigator is interested in the source of the sample.

The fruits of this oversimplification are seen in their second paper. They comment: "A very strange thing is observed in the examination of the soil samples from the alluvial plain. Whereas the XRD peak of Kn (0.7-nm basal spacing) is very strong, its absorption bands in IR spectra are weak." The standard references for the identification of clay minerals by infrared (IR) methods, Farmer [*7*] and van der Marel and Beutelspacher [*8*], do not describe this effect, nor have I seen it myself or found it referred to in the literature. Their explanation of the effect as a dehydroxylated kaolinite which occurs in a fluvial/alluvial environment is not reasonable in light of present understanding of the chemistry

of clay minerals. However, Farmer [7] reports a spectrum obtained by Velde on a synthetic 0.7-nm chlorite that lacks hydroxyl bands.

It should be pointed out that, by the Marumo protocol, which does not permit examination of the (060) reflections, kaolinite cannot be distinguished from 0.7-nm chlorite (so-called "septechlorite"). The 0.7-nm phase the Marumo group report in their alluvial samples cannot be identified definitively without doing a standard analysis, but it is probably a 0.7-nm chlorite with a minor amount of kaolinite admixed.

I suggest that this protocol not be accepted by the forensic science community.

John P. Wehrenberg, Ph.D.
Department of Geology
University of Montana
Missoula, MT 59812

References

[1] Marumo, Y., Nagatsuka, S., and Oba, Y., "Clay Mineralogical Analysis using the <0.05-mm Fraction for Forensic Science Investigation—Its Application to Volcanic Ash Soils and Yellow-Brown Forest Soils," *Journal of Forensic Sciences*, Vol. 31, No. 1, Jan. 1986, pp. 92–105.

[2] Marumo, Y., Nagatsuka, S., and Oba, Y., "Rapid Clay Mineralogical Analysis for Forensic Science Investigation—Clay Mineralogy over the Short Distances," *Journal of Forensic Sciences*, Vol. 33, No. 6, Nov. 1988, pp. 1360–1368.

[3] Bates, T. F., "Halloysite and Gibbsite Formation in Hawaii," in *Clays and Clay Minerals: Proceedings of the Nineth National Conference*, Monograph No. 11, Earth Science Series, Pergamon, New York, 1962, pp. 307–314.

[4] Barshad, I., "The Effect of a Variation in Precipitation on the Nature of Clay Mineral Formation in Soils from Acid and Basic Igneous Rocks," in *Proceedings of the International Clay Conference, 1966, Jerusalem, Vol. 1*, L. Heller and A. Weiss, Eds., Israel Prog. Sci. Transl., Jerusalem, 1966, pp. 167–173.

[5] April, R., Hluchy, M. M., and Newton, R. M., "The Nature of Vermiculite in Adirondack Soils and Till," *Clays and Clay Mineralogy*, Vol. 34, No. 5, Oct. 1986, pp. 549–556.

[6] Brindley, G. W. and Brown, G., Eds., *Crystal Structures of Clay Minerals and Their X-ray Identification*, Monograph No. 5, Mineralogical Society, London, 1980, 495 pp.

[7] Farmer, V. C., "The Layer Silicates," in *The Infrared Spectra of Minerals*, Monograph No. 4, V. C. Farmer, Ed., Mineralogical Society, London, 1974, pp. 331–364.

[8] van der Marel, H. W. and Beutelspracher, H., *Atlas of Infrared Spectroscopy of Clay Minerals and Their Admixtures*, Elsevier, Amsterdam, 1976, 396 pp.

Author's Response

Dear Sir:

I appreciate the editor allowing me the opportunity to respond, and I would like to make comments on the letter of Dr. Wehrenberg about the method which we have reported [1,2]. If he concluded "not accepted by the geological community" I should agree with him. But I cannot agree with his statement "not accepted by the forensic science community". Certainly, it is ideal to apply the established method completely in clay mineralogy, but in the case of forensic soil examination such as a comparison between soil samples often the sample amount obtained is very small. Thus, with this restrictive condition, a forensic scientist is required to make an examination and attempt to identify minerals as much as possible. The method that we reported is one of the suggestions applicable to the case of a small amount of sample. This method, naturally, should be applied accompanied by the examination of primary minerals including phyllosilicate. It does not prevent further additional examination or using the standard protocol as Dr. Wehrenberg described. A forensic scientist who performs soil examination ought to have enough knowledge about mineralogical identification, and the most effective method for the samples should be decided by the individual forensic scientist based on various

conditions such as the amount of samples, local geology, soil type distributed, and so forth.

I have never completely identified the 0.7-mm spacing mineral in the alluvial soil samples [2] as dehydrated kaolinite, but it is natural to be classified into kaolin minerals (not kaolinite) because it has not been reported that septechlorite is widely distributed in alluvial soils, nor that it is formed pedogenetically, at least in Japan. This problem should be delved into through the detailed investigation of this mineral, as I wrote that it is necessary to conduct a more detailed study.

Y. Marumo
National Research Institute
of Police Science
6 Sanban-cho, Chiyoda-ku
Tokyo, 102, Japan

References

[1] Marumo, Y., Nagatsuka, S., and Oba, Y., "Clay Mineralogical Analysis Using the 0.05-mm Fraction for Forensic Science Investigation—Its Application to Volcanic Ash Soils and Yellow-Brown Forest Soils," *Journal of Forensic Sciences,* Vol. 31, No. 1, Jan. 1986, pp. 92–105.

[2] Marumo, Y., Nagatsuka, S., and Oba, Y., "Rapid Clay Mineralogical Analysis for Forensic Science Investigation—Clay Mineralogy Over the Short Distances," *Journal of Forensic Sciences,* Vol. 33, No. 6, Nov. 1988, pp. 1360–1368.

Report of 1988 Ad Hoc Committee on Forensic GC/MS: Recommended Guidelines for Forensic GC/MS Procedures in Toxicology Laboratories Associated with Offices of Medical Examiners and/or Coroners

Dear Sir:

The ad hoc Committee on Forensic GC/MS was charged with the responsibility of drafting "Recommended Guidelines for Forensic GC/MS Procedures in Toxicology Laboratories Associated with Offices of Medical Examiners and/or Coroners," and such guidelines were drafted and forwarded to Toxicology Section members of the American Academy of Forensic Sciences (AAFS) on 27 Dec. 1988. It was voted upon and officially accepted by the Toxicology Section at the business meeting of the 41st Annual Meeting of AAFS in Las Vegas, Nevada on 15 Feb. 1989. The Committee appreciates Mrs. Ruth Foltz for editing this committee report.

These guidelines are designed for laboratories engaged in detection and quantitation of drugs in specimens of interest to medical examiners and coroners. They refer only to gas chromatographic/mass spectrometric (GC/MS) analysis and do not address other aspects of operations in a forensic toxicology laboratory, such as chain-of-custody documents, quality-assurance programs, choice of specimens, preparation of procedure manuals, extraction methods, or proficiency testing. The members of the GC/MS committee feel that these factors are beyond their assignment, recognizing that high standards with respect to all of the subjects mentioned are crucial for validation of any analytical result obtained by GC/MS for forensic science purposes.

The committee's purpose is to offer laboratories some guidance and ideas for establishing a high standard of practice in routine GC/MS analysis. Developing guidelines for such a diversity of intoxicants and biological samples as are met with in forensic science laboratories is a difficult task. Many of our recommendations are general ones that may be incorporated into any laboratory's procedures, but there will always be exceptional circumstances that challenge the analytical process and cannot be anticipated by any given set of guidelines. In those cases, the toxicologist, with the assistance of the mass spec-

troscopist, must conduct the analysis on the basis of sound scientific principles that will stand the scrutiny of challenge in court. Adhering to the guidelines proposed in this document, as much as reasonably possible, will offer a sound foundation for meeting such challenges to the laboratory's analytical procedure. Documentation of all steps taken in these special cases is critically important for potential litigation. Moreover, the circumstances may well arise again; even if that happens only rarely, having the procedure written down will make subsequent analyses easier and will add to the credibility of the result.

I. Operation and Maintenance of GC/MS Instruments

(*Note:* In the following statements, "daily" means any day GC/MS analysis is being performed. "Ion ratio" is defined as the ratio of peak area or peak height between two ions formed from the same molecule.)

Recommended activities, all of which should be carefully documented:

I-1. Change the septum daily. However, the required frequency will be influenced by the number of injections and the type of septum; each laboratory should specify, on the basis of its own equipment and analytical schedule, how often a septum should be changed.

I-2. Calibrate (autotune) the mass spectrometer daily with perfluorotributylamine or other suitable tuning compound, or at least confirm and document that the instrument is properly tuned. Each laboratory should specify how often calibration will be done, and observe this regimen faithfully.

I-2.1. Record the mass spectrum and the ion ratios of the tuning compound and the appropriate instrument settings (that is, the daily autotune report) in a log book. (Each laboratory should maintain written criteria for acceptable tuning of each of its instruments; these criteria may vary when a laboratory contains mass spectrometers from different manufacturers.)

I-2.2. Record any corrective action if the mass spectrometer is out of calibration.

I-3. In addition to calibration with perfluorotributylamine, a laboratory should determine a performance standard for each of the GC/MS instruments it operates and specify its choice of standard(s) in writing. The standard compound(s) should be injected daily to evaluate the overall performance of an instrument, and the procedure for preparation of the performance standard should be documented. Performance standards prepared in the laboratory with the compound(s) of greatest interest are often the most useful; for example, a laboratory that tests frequently for phencyclidine may choose a known concentration of phencyclidine in a suitable solvent, such as ethyl acetate. Alternatively, one can inject a known concentration of whatever analyte is to be measured when a "target compound" analysis is being undertaken on a given day. The important objective is to verify that the current performance level of the GC/MS system is adequate for the analytical task at hand.

I-3.1. Maintain records relating to the performance standard runs. Each laboratory's SOP manual should specify the nature of these records and the criteria for acceptability of the performance data. The performance standard should be evaluated in terms of retention time, ion ratios, peak area, and peak width.

I-4. Record periodic maintenance of each GC/MS instrument. The following are examples of maintenance activities that should be documented:

(a) cleaning or changing or both the injector liner, injector nut, O-ring seal, and any other injector parts;
(b) installing a new GC column;
(c) altering the GC column (removing a section of a capillary column, for example);
(d) changing the ion volume, the filament, and so forth;

(e) cleaning the ion source;
(f) changing pump oil; and
(g) changing the carrier gas cylinder.

(After a service call or other repair, keep records to show that the GC/MS is back in service. A satisfactory calibration with perfluorotributylamine or a satisfactory injection of an appropriate performance standard or both would provide adequate evidence.)

I-5. Maintain general records for each instrument:

(a) serial number, make, and model of the GC/MS and
(b) date(s) of initial installation and any major update of the instrument.

II. Standards and Controls for GC/MS Analysis

II-1. *Calibration standards* consist of one or more samples containing known concentrations of analyte which are analyzed for the purpose of establishing a calibration curve. *Controls* are samples of known concentration that are used during the analysis of unknowns to validate the calibration curve. Ideally, the calibration standards and controls should be prepared in the same biological matrix as that of the specimens to be analyzed. If that is not feasible, the matrices should be as similar as possible.

II-2. Guidelines for documentation of standard solutions:

II-2.1. Record the sources of all standards and keep this information on file.

II-2.2. Establish a written procedure for preparation of stock and working standard solutions.

II-2.3. Record, in a logbook, the preparation and expiration dates of stock solutions and their assigned lot numbers. The preparer's initials are usually enough for such records; however, in a large laboratory a quality-control person or supervisor should also sign these documents.

II-2.4. Establish a procedure to certify the accuracy of new solutions. For example, a stock standard solution may be analyzed by GC/MS against the current standard or a reference standard; the results must agree within a certain acceptable range specified by the laboratory (for example, $\pm 10\%$ of target value) to certify the new solution for use. For qualitative standards, the purity of the material must be established.

II-2.5. Include the following information on the labels of stock and working standards:

(a) name of standard,
(b) concentration,
(c) solvent,
(d) preparation date,
(e) expiration date,
(f) initials of preparer, and
(g) lot number.

II-2.6. Store stock and working standards in a *limited-access area and under refrigeration*, unless stability at other temperatures has been documented.

II-3. Guidelines for documentation of quality control samples.

II-3.1. Record the source of the quality control sample. Control samples and calibration standards should be obtained from separate sources or at least prepared separately.

II-3.2. Establish written procedures for preparation of quality control samples and for verification of the accuracy of controls purchased from outside sources and

control samples prepared in-house. For example, analyze the controls by GC/MS against current standards; the results should agree within a predetermined acceptable range (for example, ±20% of target values) to certify a new control sample for use.

II-4. The number of calibration standards and controls to be analyzed within a batch depends on the quantitative accuracy and dynamic range required. At a minimum, one should run a standard or control containing the analyte at a concentration *below* the lowest reported specimen concentration to show that the instrument's performance was sufficient at the time of analysis to permit acceptable quantitative accuracy at the lowest concentration encountered in the specimens. A preferred procedure is to include, within a batch, standards and controls at concentrations that encompass (both above and below) the entire range of reported specimen concentrations.

III. Guidelines for the Analytical Procedure

III-1. Specify the GC/MS analytical conditions to be used for each drug assay in a manual or on file with the instrument.

III-2. Establish a protocol for the sequence of injections. A suggested sequence follows:

(a) a performance standard containing the compound(s) being analyzed on that day;
(b) calibration standards, if quantitative determinations are to be made;
(c) a negative control (blank); and
(d) specimens and controls.

III-3. It is critically important to be able to provide documentation that the GC/MS evidence for a drug in a specimen was not a result of "carryover" from the sample injected before it. One way of documenting the absence of carryover is to run a "blank" or a solvent injection before injecting each specimen. The GC/MS data for the injections of blanks or solvent must be retained with the data for each specimen.

III-4. Mode of mass analysis: acquisition of full spectra or selected ion monitoring.

III-4.1. Qualitative analysis: whether it is better to scan repetitively the mass spectrometer over the full mass range, or to monitor only selected ions, continues to be debated. Both modes of operation have their strengths and limitations, so it is unwise to adhere rigidly to only one mode of operation. A good quality full-scan mass spectrum generally provides the best qualitative identification; however, the SIM mode generally is more sensitive and less affected by potential interferences from coeluting compounds. The specificity (certainty of identification) of a SIM assay depends on many factors including: the number of ions monitored, the uniqueness of the monitored ions, the selectivity of the extraction procedure, the type of derivative, the efficiency of the chromatographic separation, and the selectivity of the method of ionization. A well-designed SIM assay can provide a very reliable method of identification. However, it may be difficult to evaluate the reliability of a SIM assay without personal experience with the method or access to data from the analysis of a substantial number of specimens. With either mode of mass analysis, the analyte's retention time (or better, its retention time relative to a reference standard) should agree well with the analyte's expected retention time.

III-4.2. Quantitative analysis: The accuracy of quantitative measurements performed by GC/MS is highly dependent on the intensity of the analyte's ion current relative to the background ion current ("noise") intensity. Acceptable quantitative measurements can be obtained from reconstructed ion chromatograms, or total ion current chromatograms obtained under full-scan data acquisition, if a relatively

high concentration of analyte is present in the specimen. However, when analyte concentrations are in the low nanogram/millilitre range, it is generally necessary to use selected ion monitoring to obtain sufficient ion current intensity for accurate quantitation.

IV. Criteria for Identification of Drugs in Biological Specimens

Each laboratory should establish criteria, and describe them in its SOP manual, for what constitutes conclusive identification of a drug. It is difficult to make a comprehensive statement that would cover all types of compounds and specimens. Ultimately the interpretation of all GC/MS data available should rest with the professional judgment of an experienced mass spectroscopist.

V. Quantitation

V-1. For any quantitative analysis, the linear range of each commonly analyzed compound should be established and maintained on file in each laboratory. There is no need to reestablish linearity OVER THE ENTIRE RANGE with each batch of samples so long as calibration standard(s) or control(s) or both are run with each batch. For those compounds analyzed only infrequently, a new calibration curve should be prepared on the day of analysis.

V-2. Some laboratories may choose to prepare a calibration curve by injection of calibration standards on a particular day.

V-2.1. One or more calibration standards are usually required. However, the number will depend on the compound being tested and on the individual laboratory's criteria. Acceptable results from testing the quality control samples against the calibration curve should be specified by each laboratory.

V-2.2. Each laboratory should specify in its SOP manual a practical, acceptable range for ion ratios.

V-3. Some laboratories may choose to use a historical calibration curve. In that event, acceptable results for at least one quality control sample analyzed on that day against the historical curve should be within a predesignated range of the target value. More than one control is desirable. The results(s) of the control measurement(s) should be within $\pm 20\%$ of target value(s).

V-4. A negative control (blank) should be included in every batch to demonstrate the absence of interference from deuterated internal standards or other compounds remaining from the extraction and derivatization procedures.

V-5. If a need to retest the specimen should arise, the retest result may be quantitatively different from the original as a result of normal fluctuation in instrument performance or degradation of the analyte or both. Few data are available on the stability of compounds in various matrices. Given that degradation is a very real possibility, it is sufficient to demonstrate that the compound is still present at or above the limit of detection of the analytical system. The detection limit must be well documented. Sometimes it will be necessary to use a larger specimen aliquot or a larger injection volume or both.

VI. Information Constituting a Complete GC/MS Report

VI-1. A GC/MS report sheet with the following data:

(a) toxicology case number or medical examiner's case number or both;
(b) type of specimen;

(c) qualitative result;
(d) quantitative result, when applicable;
(e) storage location of the standard file;
(f) initials and date on each data sheet;
(g) batch number (optional); and
(h) reviewer's initials or signature and date of review.

VI-2. An extraction sheet for the specimen with the following:

(a) toxicology case number or medical examiner's case number or both;
(b) specimen type and amount;
(c) internal standard and amount, when applicable;
(d) date and initials of analyst;
(e) batch number (optional); and
(f) extraction procedure accompanied by a brief description and literature references (a copy of the entire protocol should be on file or in the manual).

VI-3. A hard copy of the GC/MS data.
VI-3.1. For SIM mode:

(a) header of the file with specimen identification;
(b) mass chromatograms of the appropriate ions;
(c) peak areas or heights of the appropriate ions, with retention times;
(d) ion ratio(s), and the acceptable range, included here or on a separate form for each batch of samples; and
(e) qualitative and quantitative results.

VI-3.2. For SCAN mode (specimen and standard):

(a) header of the file with specimen identification;
(b) mass spectrum of the peak of interest, with retention time;
(c) computer library search result (optional);
(d) mass chromatograms of characteristic ions (optional); and
(e) total ion chromatograms (optional).

Note: the above data should be saved also for the standards and controls. It is also desirable to record a list of samples in the batch, including standards and controls, and the order in which they were analyzed.

VII. Review Procedure

VII-1. Ideally, the analyst and at least one supervisor should review a GC/MS result before the chief toxicologist/director reviews it. Whether this is possible will depend on the number of people in the laboratory and their experience levels.
VII-2. Each reviewer should date and initial the review.
VII-3. Some laboratories may prefer signatures to initials. Initials as well as signatures should be on file with the laboratory.

Chairman

Nancy B. Wu Chen, Ph.D.
Assistant Chief Toxicologist
Office of the Medical Examiner
County of Cook
Chicago, IL 60612