Letters to the Editor

Commentary on Morphologic Variations of the External Arcuate Nucleus in Infants Dying of SIDS: A Preliminary Report, *J. Forensic Sci.,* Vol. 39, No. 4, July 1994, pp. 1076–1083.

Dear Sir:

This letter is divided into two sections; the first section specifically comments on the major methodological errors in Dr. Gilson et al.'s paper and the second section addresses the broader problem of the examination of postmortem Sudden Infant Death Syndrome (SIDS) brains.

The findings of this paper are of limited value and will not be discussed because I feel there were major methodological errors that would greatly affect the validity of the results. The errors include a failure to match the cases and controls on pre- and postmortem changes and improper selection of control subjects.

Problems with the methodology used in Dr. Gilson's paper include:

1. The puzzling use of a 15 month old SIDS case. The new definition clearly defined the upper limits for SIDS as 12 months. In addition the closest age matched control was an infant 4 months less developed (an 11 month old). With the rapid neurological changes occurring during the first year of life, the age matching between cases and controls must be fairly narrow to account for the developmental differences.

2. Some of the control cases do not appear ideal for this type of study. Specifically, the infants that died of sepsis, pneumonia, trisomy 18, prematurity and possibly the trauma case. The condition of sepsis brings about several pathogenetic mechanisms that may affect the brain structure. Unless corrected, sepsis will result in multiple organ failure with the lungs being the major shock organ. This in turn will have profound consequences on perfusion of the microvasculature and tissue oxygenation. The resulting tissue hypoxemia will increase in the production of lactic acid. Systemic acidosis usually becomes quite severe in the late stages of septic shock [*1*]. Pneumonia with either a viral or bacterial etiology results in inflammation of the lung with decreased oxygen exchange. Infants with trisomy 18 have severe deformities and mental retardation [*2*] and rarely survive more than a few months [*3*]. Infants defined as premature (a birth weight of 2500 grams or less) have abnormal pulmonary ventilation, incomplete development of capillaries of the lungs and immature alveoli of the lungs [*2*]. Premature infants often do not receive adequate cerebral oxygenation [*2*].

These 4 types of control cases appear unsuited mainly because their pre-mortem condition may result in changes to the cytoarchitecture of the brain by either cerebral hypoxia, as in the cases of sepsis, pneumonia or prematurity, or by abnormal brain development. As for the trauma control, not enough information was provided to make an accurate determination. If the death was instantaneous then it would have made an ideal control; however, if the infant was placed in an ICU on a ventilator for an extended period of time then it would not be acceptable.

3. Some important basic descriptive epidemiological information was lacking from this paper. Very little information, other than the number of SIDS-A and -B (17 and 7 respectively) was provided. An age range and sex breakdown of the two groups would have been useful. This information would have been especially helpful to shed some light on understanding the similarity between SIDS-B and the control group. Were the SIDS-B cases older or predominantly male? Could there be other explanations of the 7 SIDS-B deaths (that is, smothering, medium-chain acyl-Co A dehydrogenase (MCAD) or homicide)?

4. The most serious problem with this study was that no attempt was made to match case and control brains by pre- and postmortem characteristics.

While it is important for any study to control for age, race and sex, studies that utilize post-mortem brain samples must consider and control for the pre- and post-mortem time dependent changes. Pre-mortem factors such as: mode of death (Agonal status (AS)), tissue changes in reaction to chronic hypoxia, ischemia and the postmortem factors: post-mortem interval (PMI), post-mortem stability of the tissue, necrosis, total brain ischemia (TBI), and autolysis must all be taken into account.

PRE-MORTEM Variables: Both Hardy [*4*] and Barton [*5*] showed that biochemical factors and neurochemical measurements are sensitive to the mode of death or agonal state (AS). Brains obtained from patients whose death was fast versus slow showed biochemical and histological differences [*4*]. Brains of patients who died slowly (a long AS) have shown less preservation of structure than those from patients who have died quickly [*4*].

One possible explanation is that patients with long terminal illnesses (long AS) may over time have failing oxygen supplies [*6*]. When the oxygen supply is cut off, the brain obtains energy by metabolizing it's glucose and glycogen reserves. This leads to a production of lactate which causes acidosis, an acid shift to a lower pH [*4*]. Lactic acid has been shown to be neurotoxic in animal models. This lower pH releases certain lysosomal enzymes which cause cell injury and tissue autolysis [*6*]. Therefore, one would expect to observe biochemical and histological differences between brains obtained from fast versus slow death cases [*4*].

In POST-MORTEM studies it is crucial to match the PMI of cases and control samples. Such data was not shown or addressed in this study. PMI can be looked at in 2 phases. The first is the delay between the time of death and the autopsy. The second is the time between removal of the brain, dissection and formalin fixation of the sample [*7*].

It is important to accurately determine the PMI so that the exact time of death is known. In most types of deaths this is relatively simple. However, in the case of SIDS, the exact time of death is rarely known. In the majority of SIDS cases one is only given time last seen alive and the time found dead. Body lividity and rectal temperature assist in the determination of approximate time of death, but the former is very general and the latter is rarely used.

One must also consider the behavior of chemicals after death, amino acids concentrations change rapidly, while some enzymes

are relatively stable after death [7]. Chemical concentrations can influence the brain cytoarchitecture after death as in the case of lactic acid.

References

[1] Smith, L. H. and Thier, S. O., *Pathophysiology the Biological Principles of Disease,* W. B. Saunders Co., Philadelphia, 1985.
[2] *Tabers Cyclopedia Medical Dictionary,* C. Thomas, Ed., Edition 17, F. A Davis Co., Philadelphia, 1993.
[3] Price, S. and Wilson, L., *Pathophysiology 4th Edition,* Mosby Year Book, St. Louis, 1992.
[4] Hardy, J. A., Wester, P., and Winblad, B., "The Patients Dying After Long Terminal Phase Have Acidotic Brains; Implications for Biochemical Measurements on Autopsy Tissue," *Neural Transmission,* Vol. 61, 1985, pp. 253–264.
[5] Barton, A. J. L., Pearson, R. C. A., Najlerahim, A., and Harrison, P. J., "Pre- and Postmortem Influences on Brain RNA," *American Journal of Neurochemistry,* Vol. 61, 1993, pp. 1–11.
[6] Karkela, J. T., "Critical Evaluation of Postmortem Changes in Human Autopsy Cisternal Fluid. Enzymes, Electrolytes, Acid-Base Balance, Glucose and Glycolysis, Free Amino Acids and Ammonia. Correlation to Total Brain Ischemia." *Journal of Forensic Science,* Vol. 38, No. 3, 1993, pp. 603–616.
[7] Perry, E. K. and Perry, R. H., "Human Brain Neurochemistry—Some Postmortem Problems," *Life Science,* Vol. 33, 1983, pp. 1733–1743.

Steve A. Koehler, B.S.
Graduate Program of Forensic Epidemiology
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, PA 15261

Author's Response

Dear Sir:

Koehler's letter raises methodological questions about the validity of our findings in the external arcuate nucleus of infants dying of SIDS and controls. While his basic science observations are true, I strongly disagree with his conclusions and will clarify the issues he raises.

First, the 15 month old infant was in fact 1.5 months old. We regret the typographical error.

Second, the bases on which Mr. Koehler suggests we reject certain controls are unnecessarily rigid. While hypoxia and subsequent acidosis may certainly alter morphology, to reject controls simply because there is clinical hypoxia is unreasonable. There are additional microscope markers of cerebral hypoxia (e.g., red neuron degeneration and gliosis) which would clearly indicate specimens unsuitable for evaluation because of hypoxia. These were not present in our cases or controls. Rejecting a trisomy 18 case on the basis of associated mental retardation denies the fact that there are many conditions involving clinical mental retardation for which an anatomic substrate is not demonstrable. Trisomy 18 is one such condition and to our knowledge the only brainstem anomaly described in association with this involved the olives which are not close to our area of study.

Third, additional epidemiological information would certainly be useful in comparing SIDS-B infants with controls and SIDS-A infants. Our preliminary evaluation did not suggest differences in age or sex, but further studies may be helpful. To suggest smothering or homicide as causes of SIDS-B begs the question of SIDS diagnosis. If such conditions were known the infants would not have been signed out as SIDS. This does, however, point up the difficulty in making a diagnosis of exclusion, like SIDS. Tests for inborn errors of metabolism are not routinely performed in most jurisdictions, but may represent an etiology for some "SIDS" deaths.

Finally, biochemical considerations, durations of terminal illnesses and post-mortem interval are relevant in a study such as ours only in so far as they impact on morphology. All cases would be autopsied on the day of death or the following day as is customary in medical examiner practice. While significant biochemical changes may occur over this time frame, it is not our experience that this is a sufficient time for major morphologic changes. This did not present a problem in the present study. The duration of terminal illness is a potentially important source of microscopic changes, again related primarily to the effects of hypoxia and acidosis. As noted above, histologic markers of these conditions were not present. Determining the "exact" time of death is not the "relatively simple" task which Mr. Koehler seems to imply. Environmental and decedent factors are important and can vary markedly. Frequently an estimation of the time of death can be made and is adequate in virtually all cases. Microscopic changes certainly do not occur with the rapidity which Mr. Koehler seems to suggest.

Thomas P. Gilson, M.D.
Office of Chief Medical Examiner
City of New York
520 First Avenue
New York, NY 10016-6402

IAAI Forensic Science Committee Position on the Use of Accelerant Detection Canines

Sir:

The undersigned members of the International Association of Arson Investigators Forensic Science Committee support the following position statement on the use of accelerant detection canines.

The use of accelerant detection canines, as pioneered by the Connecticut State Police and the Bureau of Alcohol, Tobacco and Firearms, is a major advance in the investigation of fires. This pioneering program has led to other equally competent programs in other states and agencies.

The IAAI Forensic Science Committee endorses the use of accelerant detection canines for the purpose of improving the investigation of fires and particularly the collection of samples for laboratory analysis. The committee agrees that samples collected by a properly trained and maintained canine have a high probability of being confirmed as positive for ignitable liquids.

The Committee recognizes that it is the responsibility of the handler in concert with the laboratory to document the training and performance of the canine. Toward that goal the committee recommends that:

Laboratories conducting fire debris analyses keep records of samples submitted which are noted as having been collected with the aid of accelerant detection canines.

Chemists and handlers should conduct and publish research in peer-reviewed journals, so that additional scientific review can further establish the efficacy of canine ignitable liquid detection.

Canine teams be annually certified by an independent entity or agency.

Teams continually undergo additional discrimination training thus enhancing the canine's proficiency. This is especially neces-

sary if the animals' agreement (confirmation rate) with a laboratory falls below 70%.

In order to reduce concerns about the quality of laboratory testing, canine teams should submit their samples to laboratories utilizing ASTM guidelines for the recovery and identification of ignitable liquids.

Sensitivity

To date, definitive testing has not established a lower detection limit applicable to all accelerant detection canines. Some early testing indicates that the limits of detection for a well trained and maintained canine may be below those of some forensic laboratory methods utilized for recovery and detection of ignitable liquids. This sensitivity may present special challenges to the use and interpretation of canine alerts. Many common products (insecticide solvents, carpet and plywood adhesives, cleaning solvents, etc . . .) produce vapors which may be intrinsic to the fire scene. Extremely low levels of such ignitable liquid vapors are normal to our environment. A canine is not capable of discriminating between these intrinsic vapors and deliberately added ignitable liquid vapors. Neither the canine nor laboratory can indicate when these common products were used as accelerants. Through the use of established threshold limits of detection in their quality control/quality assurance procedures, laboratories attempt to minimize the influence of trace levels of intrinsic materials. This illustrates the need for the investigator to have the source material for any canine alert specifically identified by the laboratory.

Selectivity

Current research does not indicate which individual chemical compounds or classes of chemical compounds are the key "triggers" for canine alerts. Research reveals that most classes of compounds contained in ignitable liquids may be produced from the burning of common synthetic materials. Laboratories which use ASTM guidelines have minimum standards which define those chemical compounds which must be present in order to make a positive determination. The sheer variety of "pyrolytic products" present in fire scenes suggests possible reasons for some unconfirmed indications by canines. The discrimination ability of the canine to distinguish between pyrolysis products and accelerants is remarkable but not infallible. Further publishable research and specific training of the canines to demonstrate selectivity is encouraged.

Utility

The ultimate objective of the use of accelerant detection canines must be to select and secure fire scene samples which have a high probability of laboratory confirmation. Certain cautions or restrictions are in order whenever an alert or indication is not confirmed by the laboratory. Any alert or indication not confirmed by laboratory analysis must be considered a false positive or unconfirmed indication for the purposes of origin and cause determination. The accelerant detection canine is a tool used to assist the fire investigator in the collection of samples for forensic laboratory analysis. **If the forensic laboratory examination of the sample is negative for the presence of identifiable ignitable liquids, any positive indication by the canine for that sample *must* be deemed as *not relevant.***

It must also be remembered that destructive fires may be set without the use of ignitable liquids. Materials used to enhance fire spread such as waxed paper, crumpled newspaper, or styrofoam packing are easily ignited to produce intense fires. Such materials do not leave significant complex chemical traces which are detectable by canines or laboratories. In the technical sense these materials may be termed "accelerants." A negative indication by either a canine or a laboratory thus cannot be used to infer that a fire was *not* incendiary. Conversely, a positive canine indication or laboratory finding does not in itself establish that the fire was deliberately set. Investigators must utilize all the origin and cause facts available (burn patterns, laboratory analyses, canine indications, and background investigations) in order to support the charge of arson.

It is the position of the Forensic Science Committee that the trier of fact should have an understanding of the limitations of any investigative tool. The chemical processes which canines use in alerting to ignitable liquids are not fully understood. As a result, unconfirmed indications are possible. Any alert or non-alert is data which may or may not be utilized by the investigator in the overall fire investigation. The trier of fact should have all relevant investigative data available, not just what is beneficial to one party. Giving testimony which states or infers that unconfirmed indications are sufficient in themselves to establish the presence of an ignitable liquid or "accelerant" does not present the trier of fact with accurate data within the scope of scientific certainty. Testimony regarding unconfirmed indications should be restricted to issues of probable cause and should not play a part in the actual trial.

It is the position of the Forensic Science Committee that misuse of accelerant detection canines, particularly in the presentation of "evidence" of unconfirmed positives, will be detrimental to the proper utilization of this important tool.

Summary

It is the position of the Forensic Science Committee that the proper role of the canine is to assist the fire investigator in the selection of samples for subsequent laboratory analysis and to provide a preliminary indication of the presence of an ignitable liquid. The use of a canine alert to the possible presence of an ignitable liquid is only one of the many factors used in a fire investigator's preliminary origin and cause determination and just like laboratory findings, can *never* be the sole basis for that determination.

The Committee wishes to thank Mr. Richard A. Strobel of the Bureau of Alcohol Tobacco and Firearms for his substantial contributions to the development of this position paper.

IAAI Forensic Science Committee
Carl E. Chasteen, Chair
Florida Div. of State Fire Marshal
Fire and Arson Laboratory
Rt. 1 Box 3252 Hwy 90W
Havana, FL 32333
John D. DeHaan, Co-Chair
California Criminalistics Institute
California Department of Justice
4949 Broadway, Room A104
Sacramento, CA 95820
Michael K. Higgins, Co-Chair
K-Chem Laboratories
46 Clayton Street
Boston, MA 02122

Andrew T. Armstrong, Ph.D.
Armstrong Forensic Laboratory, Inc.
330 Loch'N Green Trail
Arlington, TX
John C. Colver
Colver Technical Consultants
0855 Mountain Laurel Drive
Aspen, CO 81611
Richard L. P. Custer
Custer Powell, Inc.
P.O. Box 741
Wrentham, MA 02093
Barker W. Davie
Barker and Herbert Laborator
207 Main Street
P.O. Box 438
New Haven, IN 46774
Claude J. Fortin
Laboratorie de Police Scientific
1701 Rue Parthenais
5 Etage
Montreal, Quebec H2K 387
CANADA
Mary Lou Fultz, Ph.D.
Atlanta Forensic Laboratory
ATF Suite 410
2600 Century Pkwy, NE
Atlanta, GA 30345
Richard W. Henderson, Ph.D.
Southeastern Research Laboratories, Inc.
P.O. Box 15010
Quinby, SC 29501
John J. Lentini
Applied Technical Services, Inc.
1190 Atlanta Industrial Dr.
Marietta, GA 30066
Murray I. Nystrom
McLarens (Forensic) Pty. Lt.
27 Black Street, P.O. Box 1039
Milton, Queensland 4064
AUSTRALIA
Gordon R. Orr
Canadian Standards Association
178 Rexdale Blvd.
Rexdale, Ontario M9W 1R3
CANADA

DNA Versus Fingerprints

Dear Sir:

Forensic DNA testing is frequently compared to fingerprinting, that is, just as each person's fingerprints are thought to be unique, so is their DNA (unless they are an identical twin). Unfortunately, this is where the analogy usually ends. I think it worthwhile to carry this analogy even further.

Except in unusual circumstances, the finding at a crime scene of a fingerprint that is a match for a suspect only shows that the individual was present at some time in the past. It is usually not possible to date a fingerprint, or even from its quality determine if it was recently deposited. The failure to find fingerprints at a crime scene that match a suspect is not considered exculpatory, since there may be many reasons why recoverable and identifiable fingerprints were not deposited. It is not at all unusual to recover fingerprints that are of identification quality, and yet do not match the record prints of any of the suspects, victims, family members, or of investigators who were at the scene. This too is usually not exculpatory. Because of the extreme variation in the persistence of fingerprints, these prints may have been deposited at any time in the past by visitors, laborers, etc.

Would the analogy hold if in the preceding paragraph we were to substitute "DNA" for "fingerprints" or "a fingerprint" and instead of just the crime scene, consider all sources of physical evidence? Fingerprints may be left due to innocent, casual contact; the same is not normally true for DNA if we limit the possible sources to blood, and vaginal and seminal fluids. But DNA could potentially be extracted from head hairs, or from nasal secretions on discarded tissues, or saliva residues found on sources such as cigarette butts and chewing gum. Visitors might leave blood traces due to nosebleeds or picking at scabs, and workmen might suffer minor injuries while using tools. Traces of seminal fluid could arise from acts of masturbation. But can there be an innocent explanation in sexual assault cases if the DNA extracted from seminal fluids recovered from deep vaginal swabs from the victim doesn't match the DNA profile of the suspect? What if the suspect wore a condom? The seminal fluid recovered may be from previous consensual sex with someone else. A deceased victim can't tell you this, but even living victims may choose to remain silent. Victims may be in relationships varying anywhere from long-term affairs, one night stands, or even those of a homosexual nature. If these relationships are divulged great harm may be done to the lives and reputations of the victims, their lovers, and their families.

If blood or other secretions were recovered in sufficient quantity, an estimate of the age of the stain might be possible, but I doubt that this is feasible when only traces are recovered which must then be amplified by the polymerase chain reaction (PCR).

As with fingerprints, there may be many reasons why no DNA-containing traces that could have originated from the suspect were recovered. The suspect may not have bled. If the crime was a sexual assault, the suspect may have worn a condom (victims are not always aware of this). Overcome by feelings of revulsion and shame, the victim may have showered, douched, and gargled.

In recent years several cases have come to light in which fingerprints allegedly recovered from the crime scene were falsely fabricated. Could this occur with DNA profiling evidence? Most of the fingerprint fabrication cases involved law enforcement personnel. Usually they honestly believed that the suspect was guilty, but wanted the fingerprint evidence in order to make the case "airtight." Obtaining blood and saliva samples for comparison standards, portions could be diluted with normal saline or water and deposited on evidence garments or swabs. Chain of custody procedures might prevent this if the medical personnel obtaining the standard samples made sure that they were in sealed containers before turning them over to investigators, and forensic laboratory technicians receipting for the evidence noted whether the seals were intact. But what about the fabrication of DNA evidence by others? Scott Turow in his fictional mystery, *Presumed Innocent,* had the hero's wife recover his seminal fluid from her diaphragm and use it to frame him for sexual assault and murder. In real life, a more likely scenario could occur if an estranged wife were embroiled in a bitter child custody suit. She would obtain a diaphragm (not having used one previously), and suggest an attempt at reconciliation. After a romantic candle light dinner they would retire to the bedroom. She would go into the bathroom to put on a sexy negligee,

and (unbeknownst to him) insert her new diaphragm. Of course the reconciliation would not work out. After their little girl had returned from a weekend visit with her father, the wife would accuse him of child sexual abuse. The forensic laboratory would then find his seminal fluid on the toddler's panties.

Yes, the above analogy between fingerprints and DNA profiling is simplistic and speculative. The point I am making is that we must insure, on a case-by-case, item-by-item basis, that DNA profiling evidence is trustworthy. Guilt or innocence must be based on the totality of the evidence, not decided in knee-jerk fashion on DNA profiling alone.

Robert D. Blackledge
Senior Chemist
NCISRFL-San Diego
Box 368220
3475 Senn Rd Ste 3
San Diego, CA 92136-5054
(619) 556-4979 FAX: (619) 556-5072