Letters to the Editor

Discussion of "Histologic Detection of Fat Emboli"

Dear Sir:

We were quite surprised at reading Davison and Cohle's paper in the *Journal* [*1*], and almost believed we fortunately had received an historic specimen in it. Indeed, the technique of fat detection by osmium tetra oxide has been described by Schultze more than a century ago in 1864 [*2*]. Next, Ciaccio in 1926, Cain in 1950, Lison in 1960, and many others used and described the technique.

We are sure Schultze, Ciaccio, Cain, Lison, and others would be glad to know that the technique is still of use and up to date. However, we wished to give notice of their historical work.

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References

[*1*] Davison, P. R. and Cohle, S. D., "Histologic Detection of Fat Emboli," *Journal of Forensic Sciences*, Vol. 32, No. 5, Sept. 1987. pp. 1426-1430.
[*2*] Schultz, M., in *Sitzber. Nieder. Natur. Heilkunde*, Bonn. 1864.

Authors' Response

Sir:

Had Professor Durigon and colleagues read our paper carefully, they would have found that its purpose was to compare different methods of embedding and staining osmium fixed tissue for optimal microscopic detection of fat emboli.

We do not claim that we are the first or only workers to describe osmium fixation for fat detection. We are aware of the existence of the papers cited by Professor Durigon, as well as other papers not mentioned by him. Had we intended our paper to be a historical review of histologic detection of fat emboli, we would have cited all pertinent references, including the first paper published on the topic.

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Recent Application of DNA Analysis to Issues of Paternity

Dear Sir:

The recent application of deoxyribonucleic acid (DNA) analysis to issues of paternity and individualization of semen and bloodstains has been described as "revolutionary" by many forensic scientists as well as the media. Several companies have played a major role in the development of the DNA technology and in the development of both single and multiple locus probes. A probe is essentially a fragment of DNA consisting of a sequence of nucleotide bases that is complementary to a specific DNA base sequence under analysis. Such probes can recognize and bind to specific regions of DNA on autosomes and on sex chromosomes. Because probes exist to detect regions on sex chromosomes, the sex of the sample donor can be determined.

Many laboratories around the world including the FBI laboratory are currently studying the various systems that have become available to determine their benefits and drawbacks. Regardless of the scientific approach to DNA analysis, several factors must be considered if this technology is to gain wide acceptance and usage. These include personnel, technique, and sample. Properly trained and experienced analysts knowledgeable in the fields of forensic serology and molecular biology are essential. Differences in the quality of the various probes that are available must be studied and a determination made as to which are the best probes to use for a particular sample type. Such probes should also be directed against segments of DNA which are highly polymorphic in the population. Sample type (semen, blood, or other tissue), sample quality (native, denatured or decomposed DNA), and sample size are additional factors that must be taken into account if the analysis is to be successful. Lastly, there must be accuracy in bank pattern data collection and in the statistical analysis that must follow.

An additional factor must also be considered in DNA analysis of samples for paternity determinations. It has been the general practice to exclude a male from paternity if he has been found not to have made a contribution to the genome of the child for any one genetic trait. There are a number of conditions in which a male who is in fact the father of a particular child might, in a DNA analysis procedure, be found not to have provided an expected DNA sequence.

The first possibility arises as a result of the meiotic process. During the early prophase of meiosis in both males and females, when synapsis of homologous chromosomes is evident, an exchange of corresponding segments of nonsister chromatids can occur. This is referred to as a crossover event. The process is normal and relatively frequent. In studies of human spermatocytes, the average number of crossovers seen is about 50, which is slightly more than 2 per synapsed homologous pair of chromosomes [*1*]. A crossover can result in the inheritance in the offspring of a somewhat different DNA sequence than what would have been inherited had no crossover event taken place. If it should happen that a crossover event takes place at a site where restriction enzyme cleavage would normally occur, the analysis of DNA from this individual would provide a banding pattern different from that expected based upon analysis of parental DNA. Thus, regardless of the number of probes used, the alleged father would be excluded from paternity based upon the child's banding pattern.

A second possibility of incorrect exclusion of the biological father in a disputed paternity case can result from a mutation in a spermatogonial cell. If the mutation changes a restriction enzyme cleavage site of one of the father's chromosomes, it would result in his being excluded from paternity. The incidence of such mutations is, as yet, not established, but it does pose a potential problem in these cases.

The last possibility we shall consider is the loss of an entire chromosome in a gamete as a result of nondisjunction during meiosis, thereby producing a nullisomic gamete, and its subsequent complementation in fertilization by a nondisjunction produced disomic gamete. Both members of the chromosome in question would then originate from only one parent.

This situation is referred to as "uniparental disomy." In the first such verified case, which did not involve disputed paternity, the child inherited two identical copies of chromosome 7 from her mother [2]. Based on other studies of aneuploidy in human gametes, it is estimated that such a situation probably occurs in 1/30 000 conceptions.

It should be recognized that the above situations are potential problems not only for cases of disputed paternity, but could also confuse investigations aimed at establishing parenthood where kidnapping or adoption have taken place many years earlier.

Lastly, we would like to point out that the term "allele" has always been taken to mean an alternate form of a gene, a region of DNA responsible for the development of a particular trait. Unfortunately, the same term has become widely used to describe various DNA fragment lengths that result from restriction enzyme activity. Adoption of a more unique and descriptive term would be preferable.

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Anaphylactic Deaths

Dear Sir:

Death from anaphylaxis has long been of interest to forensic pathologists as a cause of death [1], particularly because of implications of iatrogenesis in the case of a reaction to a drug (usually penicillin) or because of jurisdiction in the case of a reaction to a bee sting (hymenoptera venom). Anaphylaxis is not a consequence of natural exposure of an atopic individual to an antigen [2]. Perhaps the allegation of anaphylaxis arises most commonly in the setting of a sudden death of a hospitalized patient, who was administered numerous agents, at least one of which was given shortly before the patient's demise. Nearly 50 cases of fatal reactions to immunotherapy and skin testing have been reported, despite precautions by those attuned to the risk [3]. Most recently, fatal allergic food reactions have been reported [4].

Preformed immunoglobulin E (IgE) antibodies to allergens are thought to mediate anaphylactic reactions. High affinity IgE receptors are found on mast cells and basophils, and low affinity receptors are found on other cell types including eosinophils, macrophages, and platelets. Specifically, histamine release from mast cells and basophils is thought to be the principal basis for systemic anaphylaxis; possible other mediators such as the leukotrienes, platelet activating factor, and so forth may be involved [5].

Death from anaphylaxis is not easy to prove [1,6,7]. Autopsy findings, such as laryngeal edema, pulmonary hyperinflation, and tissue eosinophilia, are subjective and inconstant.

Several groups have suggested that elevated histamine levels may indicate a death as a result of anaphylaxis. Histamine levels have proven useful in live clinical patients, however, measurements must be made on carefully collected samples of plasma. Coagulation (probably through low-level complement activation) or rough handling will cause basophil degranulation which will mask actual in vivo levels (although the number of basophils is quite low, they contain approximately 1.3-pg histamine per cell [*8*]). Hence, postmortem serum levels will yield erratic results. Furthermore, the half-life of histamine is very short, measured in minutes. Menchel, et al. [*7*], of the Suffolk County Medical Examiner's Office, have studied postmortem serum histamine levels in cases of anaphylaxis to penicillin and found that they were not useful in the detection of anaphylaxis. Perhaps, measurement of histamine in a nonclotting bodily fluid, such as vitreous humor, would be useful.

Schwartz has described a protein component of mast cell secretory granules, "tryptase," which appears very promising as a useful marker of anaphylaxis from postmortem human serum [*9*]. Tryptase is found only in mast cells and not in circulating basophils, eosinophils, platelets, or any other cell. It is released under the same stimulus as histamine, particularly during anaphylaxis, and levels correlate with histamine, as both are released together from secretory granules. Its half-life is several fold that of histamine (several hours), and unlike histamine, its concentrations can be measured in serum (rather than plasma). Therefore, tryptase, not histamine, appears to be the key to postmortem determination of anaphylaxis.

Furthermore, we can now test for the putative agent of anaphylaxis in postmortem serum. The radioallergosorbent test, RAST, is a method to detect circulating IgE to a suspect offending allergen. The specific agent must be known and the test will not prove that the particular agent was the cause of an anaphylactic reaction, only that it could have been. In the case of penicillin, RASTs for the major and minor determinants have been well worked out [*7*]. Novel RASTs can be developed for most putative agents (Dr. Yunginger of the Mayo Clinic has graciously done so for me in the past). This testing can even extend to allergies to insects [*10,11*]. In the clinical setting, skin testing (Prausnitz-Kustner) can also be performed to assess allergy to a particular agent.

Laboratory workup should now prove invaluable in cases of death as a result of anaphylaxis.

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can Academy of Forensic Sciences, San Diego, CA, Feb. 1987. (Also, Dr. Menchel, personal communication, 13 April 1988.)

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Discussion of "Statistical Evaluation of Truncated Breath Alcohol Test Measurements"

Dear Sir:

There are two points of technical error in my recent article [1]. Truncating a breath alcohol measurement to two decimal places can yield an error of up to 0.009 g/210 L and not 0.005 g/210 L. On the other hand, rounding a breath alcohol result to two decimal places can yield a result that is 0.005 g/210 L higher and not 0.009 g/210 L higher.

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Reference

[1] Gullberg, R. G., "Statistical Evaluation of Truncated Breath-Alcohol Test Measurements," *Journal of Forensic Sciences*, Vol. 33, No. 2, March 1988, pp. 507-510.

Errata

In the letter to the editor "Anaphylactic deaths" in the Sept. 1988 issue of the *Journal* (Vol. 33, No. 5, pp. 1108-1110), there was an omission. Dr. Emilio B. Gonzalez, Division of Rheumatology/Immunology at the University of Texas Medical Branch at Galveston, was inadvertently omitted as a coauthor with Dr. Victor W. Weedn.

The case report "An Unusual Variant of Blood Group A" in the Nov. 1988 issue of the *Journal* (Vol. 33, No. 6, pp. 1503-1505) by Charles S. Tumosa and Ruby Dowd had the wrong heading on p. 1503. Review of *Violence Prediction: Guidelines for the Forensic Practioner* is incorrect. We are sorry for this inadvertent error.