Letters to the Editor

Importance to Forensic Science of Permanent Curation of Museum Collections of Human Remains

Sir:

In recent years, the science of physical anthropology and the standard museum practice of long-term curation of large collections of human skeletal remains have been directly challenged by contemporary American Indian activists and others who argue for reburial. The issue has emerged as one of the most important, emotional, and dividing problems in the history of American anthropology. Advocates of reburial have succeeded in pressuring many institutions to avoid archeological excavation of human remains or to bury human remains soon after excavation, in many cases with minimal or no study. In recent years, even existing museum collections of excavated human remains have been threatened.

Practioners of forensic anthropology and skeletal biology, as well as anthropologists and other scientists, recognize the importance of long-term curation of human skeletal remains as vital to the well-being of our science. As many have pointed out, the importance of these collections has grown dramatically in recent years with the expanding realization of their potential for the reconstruction of diet, disease, population affinities, and other attributes of populations and individuals. On 9 Feb. 1982 the physical anthropology section of the American Academy of Forensic Sciences, meeting in Orlando, Florida, strongly registered its position on the issue by unanimously passing a resolution in favor of long-term curation. This resolution, authored by the late J. Lawrence Angel of the Smithsonian Institution and Judy Suchey of California State University, Fullerton, California, followed the forensic science laboratory motto "Hic Locus est Ubi Mortui Viventes Docent" (this is the place where the dead teach the living). The resolution emphasized respect for the human remains in museums and laboratories, as well as for the information to be gleaned from them.

Activists from the American Indian community have continued their full-court press with the media, the United States Congress, and state organizations throughout the country, expressing apathy toward and, occasionally, disdain for the scientific perspective. Although the issues and activist positions are extremely complex, an important component is their assertion that nothing of relevance to contemporary American Indian interests results from scientific study of human remains.

As a case in point, I offer the case study, published in the March 1990 issue of the *Journal*, "Positive Identification of American Indian Skeletal Remains from Radiograph Comparison" (Vol. 35, No. 2, March 1990, pp. 466–481). The positive identification of this American Indian homicide victim might not have been possible without my comparative study utilizing our collections of human remains at the Smithsonian Institution. Obviously, this work in forensic science did not bring Leland Ten Fingers back to life, but I know his immediate family greatly appreciated knowing at last what had happened to him and seeing his assailant brought to justice. Or, in other words, yes, there are contemporary American Indians who directly benefit from the curation and study of Indian remains.

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Discussion of "ABH Antigen Typing in Bone Tissue"

Sir:

In Vol. 34, No. 1, of this journal, H. C. Lee and his group describe a combination of two serological methods for a reliable ABH typing of human bone tissue [*1*]. The authors' idea was to bring together the high sensitivity of a semiquantitative absorption-elution (AE) technique with the high specificity of the less sensitive two-dimensional absorption-inhibition (AI) technique. According to Lee and his colleagues the ABH diagnosis can be made, even in specimens more than 6 months old, if both methods yield concordant results.

The results obtained with *fresh* bone material are convincing; however, since the AI technique gave no incorrect results, the value of an additional AE testing is not quite clear from this paper. Concerning the *"older"* specimens, the percentage and the absolute number of concordant results confirming the original ABH type must be small, since the AE technique alone yielded only 20 correct diagnoses out of 41 testings. I wonder whether the concordance of the AE and AI diagnoses can be an infallible basis for establishing an individual's ABH status from older bone material.

To investigate the normal ABH antigen pattern in human bone tissue and the possible effects of aging in a biologically active environment, I performed immunohistochemical studies using an indirect immunoperoxidase technique following acetic acid (10%) decalcification [*2*]. The experiments included aging in tap water (15 and 35°C) and forest soil. The main results were the following:

1. In fresh compact bone tissue, ABH antigens are located on endothelial cell and erythrocyte membranes within the haversian and the Volkmann's canals. In the spongiosa, ABH staining is found on blood vessel endothelia and erythrocytes as well as in erythroblasts and in megakaryocytes of the medullary space.
2. Microbial invasion may lead to an enzymatic destruction of the autochthonous ABH antigens.
3. Some of the microorganisms and macroorganisms invading the specimen exhibit an immunocytochemical staining similar to that of human antigens. The rate of A-positive, B-positive, and A,B-positive microorganism strains is clearly reduced by the use of monoclonal instead of polyclonal antibodies in the first incubation step.
4. The invading organisms follow the blood vessel canals of the compacta and spread diffusely over the medullary spaces of the spongiosa. Eventually, ABH-active microorganisms may completely replace the autochthonous ABH-positive structures.
5. In early stages of contamination, a clear morphologic distinction can be made between autochthonous and microbial antigens (although the characteristic angioarchitecture of soft tissues, if present, furnishes much better morphological criteria).
6. In the final stage, ABH-active organisms as well as autochthonous ABH-positive structures are broken down to amorphous material, sometimes still exhibiting ABH properties. The origin of the ABH-positive material, however, cannot be determined in this stage of decomposition.
7. In 35°C tap water, a complete destruction of autochthonous ABH antigens was found after 1 to 3 weeks; after 6 months of aging, a reliable blood group diagnosis could not be established in our various experiments.

In view of these data there is no doubt that, in fresh compact or spongiose bone tissue, a reliable ABH diagnosis can be made by immunohistochemistry or by serological techniques; a very high reliability will be achieved by the combination of two different methods, as proposed by Lee and his co-workers. In early stages of microbial contamination, immunohistochemistry seems to be the superior method, since it allows the detection and elimination of microbial artifacts. In late stages, at present, neither ser-

ological methods nor immunohistochemistry is able to discriminate between microbial and human ABH antigens. One perspective for the future is that the rapid development in the field of monoclonal antibodies might lead to the detection of specific mammalian A, B, and H antigenic determinants.

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Author's Response

Sir:

We appreciate the opportunity to respond to the comments by Prof. Pedal concerning our paper "ABH Antigen Typing in Bone Tissue," which appeared in the January 1989 issue of the *Journal.*

There are many different techniques and procedures that can be used in the analysis of most types of forensic science evidence, and the serological typing of human tissues is no exception. A number of ABH typing procedures, as well as numerous technical variations of basic techniques, have been described in this century [*1*]. Each of these procedures has its advantages as well as its drawbacks in the analysis of various kinds of forensic science evidence. The choice of a particular method for practical laboratory work depends on the type of specimen being analyzed, the quantity of the specimen, the availability of necessary reagents and materials, and the skill and training of the analysts. One of our goals in developing the method described in our paper was to make a practical and relatively straightforward procedure available for busy casework laboratories. All forensic serologists are familiar with the basic principles of absorption-elution (AE) and absorption-inhibition (AI) techniques.

We have examined the literature of ABO grouping in bone quite extensively, and we are familiar with the application of immunohistochemical procedures by a number of workers (including Prof. Pedal) to the problem of ABH antigen determination in various human tissues, including bone [*2–9*]. While we have no doubt that the procedures described work well in the hands of the investigators who have reported them, we are less convinced that these procedures would be practical for routine work in busy forensic serology casework laboratories. In addition, it is not clear that a reliable supply of appropriately quality-controlled monoclonal antibodies is available for this testing at the present time.

The results presented in our paper represent our findings on a selected group of bone tissues examined in the initial stages of our current studies. We are currently studying a larger series of bone tissue specimens, and these are being grouped by our procedure not only in their initial condition, but also over a period of months after exposure to a variety of environmental conditions. Grouping tests on the current series of bone specimens in their initial condition, prior to any environmental exposure, show that neither AE nor two-dimensional absorption-inhibition (2D AI) yield uniformly correct results.

In studies on nearly 100 different specimens representing all four ABO blood groups, AE yielded results in about 92% of the samples, while 2D AI yielded results in about 75% of them. Using the combination procedure described in our paper, however, we correctly grouped approximately 70% of the specimens, the remainder giving nonconcordant results. The important point to be made about these results is that using our method, no incorrect results were obtained. We do not yet know what percentage of tested specimens will be amenable to successful grouping by the combination procedure. It is clear that the percentage of specimens that can be correctly grouped decreases with various environmental exposures over various lengths of time. However, our objective is to ensure that those specimens which do give concordant results are giving correct results. This is much more important to us than the percentage of specimens that yield successful results.

As our studies progress, we plan future experiments using monoclonal and polyclonal antisera in both AE and 2D AI tests to ascertain whether there are differences in the results, particularly in those specimens which have clearly "acquired" ABH antigens during exposure to the environment. In addition, we plan experiments involving the titration of eluates obtained from AE tests, as suggested by Prof. Hauser [*10*], to ascertain whether this technique will render the testing more specific.

We are not making the claim, as Prof. Pedal seems to be suggesting, that our procedure yields "infallible" results. The results of our current studies will help us evaluate the percentage of successful groupings that can be obtained from bone tissues exposed to various conditions for various lengths of time. And these results will help us determine whether concordance of AE and 2D AI results is a good criterion for diagnosing ABH antigen types in bone tissues under a variety of exposure conditions. With the relatively fresh and some older specimens we have tested thus far, however, we have observed no false positive results.

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Discussion of "Recent Application of DNA Analysis to Issues of Paternity"

Dear Sir:

We have read with interest the letter of Drs. Koblinsky and Levine in the *Journal* [1] on Recent Applications of DNA Analysis to Issues of Paternity.

These authors mention three genetic reasons why a child might not have inherited the expected deoxyribonucleic acid (DNA) sequence from the father: meiotic crossing-over at the probe restriction site (we wish to add unequal crossing-over, which could also occur, due to the tandem repetitions of the DNA sequences recognized by these probes), mutation, and a recently described case of "uniparental disomy." These are all possible, although extremely rare, events, and it is therefore unlikely that they will happen at any reasonable frequency in the analysis being performed. We feel that an error could be more easily due to a laboratory mistake than to any of the above-mentioned events.

The authors add, though, that individual identification errors could ensue "regardless of the number of probes used." We do not think this is a correct statement: the use of different probes, which identify several loci in the same or on different chromosomes, would allow the correct attribution to be made anyway. One new mutation was detected, even with the use of a single minisatellite probe [2], without compromizing the understanding of inheritance. If one uses a number of probes, then the probability of occurrence of two or more of such rare events in the case being studied would be equal to the product of the frequencies of each event taken singly, which makes it practically impossible. For these reasons, we are currently utilizing ten different single-locus variable number of tandem repeat (VNTR) probes [3], which map on various chromosomes, for paternity testing, transplant follow-up, and biological trace determinations.

We agree with the indication that "properly trained and experienced analysts knowledgeable in the fields of forensic serology and molecular biology are essential." We work coordinately as a forensic biology study group which comprises experts from the two areas. We have collected, as suggested, pattern data on a local sample of 100 individuals and performed polymorphic system statistical analysis of the different "alleles" for each (Gasparini et al., manuscript in preparation). We think the word "allele," which Drs. Kobilinsky and Levine suggest should be used for lack of a better term, could be accepted also for DNA polymorphisms, as is widely done already. This is just another example of modern molecular genetics adopting classical genetics terminology, which is not unjustified in this case, as the DNA fragments are inherited as codominant traits according to Mendel's laws. Alleles would then be defined not only as alternate forms of a gene (however *that* is defined!) but also as alternative forms of any identifiable DNA variation in the genome.

We would therefore like to reassure the readers that, perhaps with the added precaution of the usage of several probes, DNA analysis for paternity testing and individualization of biological traces is indeed "revolutionary," as it can identify a sample with a precision and a safety never reached before. If we add the advantages offered by the more recent technique of DNA amplification by polymerase chain reaction [4], to obtain high speed

and low cost responses, to the advantages of using minimal sample size and even degraded DNA for testing, it is easy to predict a brilliant future for nucleic acids in forensic sciences!

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Authors' Response

Dear Sir:

We are grateful to Drs. Pignatti and Marigo for commenting on our recent correspondence to this journal [*1*]. It is very satisfying to realize that our letter has prompted discussions by scientists, first from Canada [*2,3*] and now from Italy. We do feel, however, that some of the points raised require further consideration.

It is certainly true that unequal crossing-over is very frequently the cause of the presence of a unique DNA restriction fragment in a child's genome, and we should have specified this point in our original letter. We are, however, disturbed by the statement, made in reference to the frequencies of meiotic crossing-over, mutation, and uniparental disomy, that "these are all possible, although extremely rare, events, and it is therefore unlikely that they will happen at any reasonable frequency in the analysis being performed." We appreciate that what is "reasonable frequency" and what is "extremely rare" are in part a function of the "eye of the beholder." However, it has been shown that for one hypervariable locus the frequency of unequal crossing-over is, on average, 5% per gamete [*4*]. To this must be added the frequencies of occurrence of unequal crossovers at other loci plus the other perturbing genetic events denoted above. Furthermore, these same factors make it essential that an extensive statistical analysis be performed to verify that the DNA segments probed are in Hardy-Weinberg equilibrium. Only then will the statistical conclusions resulting from the analysis be meaningful.

On the subject of frequency of unequal crossing-over, one should keep in mind that the unique family DNA profiles which form the basis of this powerful test for paternity could only have resulted from a high rate of unequal crossing-over in the past. One must also acknowledge the continuation of the unequal crossing-over phenomenon and its resultant production of other unique DNA fragments which will characterize future

generations of a family line. Unfortunately, whenever it occurs, an unequal crossing-over event will necessarily complicate a paternity determination.

In discussing possible factors affecting paternity determination, one should include "parental imprinting of genes." In mice, some genes inherited from the female have a higher degree of methylation than do the same genes when they are inherited from the male. Thus, if a male mouse inherits such a gene from its mother, the gene will be in a highly methylated form. However, his offspring, both male and female, will have the same gene in a much less methylated form [5]. The importance of this phenomenon for the present discussion rests on the discovery that the action of restriction enzymes can be greatly influenced by the methylation state of DNA. In some cases the methylation of particular bases completely protects the DNA from the action of a restriction enzyme [6]. Thus, in humans the DNA of a father may yield a profile different from that of his child if a particular cleavage site varies in its methylation state, based on parental origin. We do not yet know how frequently this may occur in humans. Therefore, only methylation-insensitive restriction enzymes should be employed for forensic science determinations.

Although our discussion has been geared to paternity determination, forensic science must also deal with problems involving determination of maternity. These problems arise in cases of abduction and of accidental or deliberate switching of babies in hospitals. Very briefly, we would like to point out that the analysis of DNA found in mitochondria (mtDNA) has proven to be an extremely efficient test for maternity. Unlike chromosomes of the nucleus, which are inherited equally from both parents, mitochondria are inherited exclusively from one's mother. Spermatozoa do not transfer mitochondria to the egg during fertilization. Therefore, DNA profiles of mtDNA are strictly maternal in their inheritance. It has been demonstrated that a comparison of the mtDNA profiles of a child, its mother (or any maternal biological relative), and unrelated females can unequivocally resolve cases of disputed maternity [7].

We must confess our continued frustration with the use of the term "allele" to describe a fragment of DNA delineated by adjacent palindromes, which act as cutting sites for particular bacterial restriction enzymes. This use of the term destroys the concept of an allele as an alternate form of a gene which functions either in transcription or as a regulator of transcription. Since a restriction enzyme DNA fragment is not a gene, to have an "allele" without a "gene" makes no sense and, in this case, even continued usage will not make it correct.

We therefore propose that a new term be used to describe a DNA fragment whose length is determined by adjacent palindromes. We propose to call such a DNA fragment a "palon." (Readers will recognize the similarity to the gene-oriented terms cistron, muton, and recon, which are now part of our vocabulary.) The term palon would, in no way, imply anything about the function of such a DNA fragment. Changes in palon length could occur as a result of: (1) unequal crossing-over during meiosis, (2) mutation of a nucleotide base, (3) methylation of a critical nucleotide base within the restriction site, as found in parental imprinting, or (4) the formation of a chromosomal aberration (deletion, inversion, etc.) that involves the palindrome sequence. We would urge serious consideration of the proposed new term "palon" to describe DNA fragments delineated by adjacent palindromes and revealed through the use of bacterial restriction enzymes.

In closing, we want to assure Drs. Pignatti and Marigo, as well as the readers of this journal, that we are not suggesting that DNA profiles are inadequate or unsuitable for paternity determination. On the contrary, our discussion of the problems involved with their use is designed to take into account and dispose of these complicating genetic phenomena. Also, we feel strongly that the use of multiple probes for paternity determination is essential to avoid confusion in the interpretation of banding patterns. If this is done, any ambiguous situations can be clarified and resolved in a truly scientific manner,

thereby assuring all concerned that the cause of justice has indeed been served. The need for such an approach is required not only in cases of paternity determination, but also in cases of disputed parentage where kidnapping had occurred many years earlier and in cases where an individual, many years after an abduction, claims to have been the kidnapped child.

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Discussion of "Heroin, Morphine, and Hydromorphone Determination in Postmortem Material by High Performance Liquid Chromatography"

Dear Sir:

We feel bound to comment on an article by W. R. Sawyer, G. A. W. Waterhouse, D. J. Doedens, and R. B. Forney, appearing in the September 1988 issue of the *Journal* [1], which describes a simple method for the high-performance liquid chromatography (HPLC) determination of morphine in biological matrices, using electrochemical (EC) (amperometric) detection (HPLC/EC).

The authors of the article achieved morphine determination by using a fairly low electrode potential (+0.5 V versus Ag/AgCl), in comparison with the majority of other researchers, who adopted voltages in the range +0.6 to +1.1 V [2].

This approach of Sawyer et al. is in agreement with our own experience.

In a recent paper on the HPLC/EC determination of morphine in biological samples [3], we reported the use of even lower potentials (we chose +0.35 V versus Ag/AgCl,

with a basic eluent at pH 9.5) in order to obtain a quite stable baseline, especially at the lowest detector ranges (greatest sensitivities), and to "tune out" a number of compounds present in complex biological matrices, which could interfere at higher voltages.

In short, this approach seems promising in the development of rugged methods to be used with "dirty" samples or in heavy routine work.

On the other hand, we disagree on another important point of the same article, on which we hope that more information will be given by the authors. It was surprisingly reported that the described HPLC/EC method allowed the direct determination of heroin itself, in the same run as morphine. According to W. R. Sawyer and his co-workers, diacetylmorphine would elute in a well-resolved peak after morphine and hydromorphone but before nalorphine, and could be detected at the same electrode potential as morphine.

Although the oxidation reaction of opiates is not yet fully understood, it mainly depends on the phenolic group, which activates the aromatic ring, leading to the initiation of the reaction [*4*]. In the heroin molecule, this hydroxyl is esterified, and therefore it seems unlikely that it would be available for electrochemical oxidation to the same degree as in morphine.

While it has been published that in some cases (e.g., 8-acetylpentazocine) its acyl substitution does not prevent amperometric detection, it has been found that the reaction requires substantially higher potentials than those needed to oxidize morphine and its derivatives with a free phenolic group [*4*]. Moreover, other authors, either in voltametric [*5*] or in HPLC/EC [*6*] studies, under conditions roughly comparable to those adopted by W. R. Sawyer and his co-workers, reported that heroin is not amenable to electrolysis and therefore cannot be detected amperometrically.

Even more surprising is the authors' observation that 6-monoacetylmorphine could not be detected by the proposed HPLC/EC method. This compound has a free phenolic group like morphine and theoretically should have a similar oxidative electrochemical behavior. Moreover, given its intermediate polarity, it should elute between morphine and heroin in reversed-phase chromatography [*7,8*].

It is difficult to explain these apparent discrepancies, and we wonder if, perhaps, the chromatographic peak identified as heroin should not in reality to be attributed to 6-monoacetylmorphine, which could have been present in the injected heroin as a partial hydrolysis product [*9*].

In this case, of course, the identity confirmation of the HPLC "heroin peak" carried out by the authors, using off-column *in vitro* hydrolysis (to morphine) and reinjection, could have resulted in a false positive.

Much more difficult to explain is the gas chromatography/mass spectrometry (GC/MS) confirmation of the "heroin peak" collected after HPLC separation. We tentatively suggest that, because of a postulated incomplete chromatographic resolution of the 6-monoacetylmorphine peak (believed to be heroin) from heroin (inapparent), the peak collection could have included both compounds, leading to the GC/MS identification of heroin in the eluate.

In conclusion, we think that the analytical approach to heroin HPLC/EC determination proposed by W. R. Sawyer and his co-workers is questionable and should at least be corroborated by additional studies on the electrochemical behavior of heroin, as an attempt to describe and, possibly, to explain those phenomena on which the analytical method is based.

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Authors' Response

Dear Sir:

We agree that the structure of heroin makes electrochemical detection curious and would welcome further studies of the phenomenon. Other reports that support the concept that heroin should not be detectable may have used electrochemical conditions different from ours.

No data on the electrochemical or chromatographic behavior of 6-monoacetylmorphine was presented, but we concur that it would probably elute between heroin and morphine. We have shifted the elution order of morphine, heroin, nalorphine, to morphine, nalorphine, heroin by adjusting the mobile phase pH from 7.3 to 4.2. Inasmuch as we attempted unsuccessfully to resolve 6-monoacetylmorphine over a full 0.3 to 1.0-V potential range with a mobile phase of various pHs, we were unable to include this compound in our assay. The monoacetylmorphine used was not standard reference material but was synthesized by a modification of the method of Fehn & Megges [1].

The gas chromatography/mass spectrometry (GC/MS) analysis was performed on an unhydrolyzed high-performance liquid chromatography (HPLC) eluate that corresponded to the retention time of heroin. This fraction was unlikely to be contaminated with 6-monoacetylmorphine, given the separation between closely related opiates and derivatives that has been achieved with our system.

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Drugs and Transportation Safety

Sir:

Widespread random testing of transportation workers has been proposed as a cost-effective way of improving the safety, health, performance, and productivity of workers, particularly those performing safety-related tasks. One of the reasons given in support of random testing is that there is evidence that drug impairment plays a major role in many transportation accidents. Within days of a major transportation accident, the finding of drugs in an involved worker is announced to the public, implying that drug impairment was responsible. After investigation which may take a year or more, the probable cause or causes are established but rarely receive the publicity of the early drug announcement. Evaluations of the findings in these accidents reveal that rarely is there scientific evidence that the employees were impaired by drugs other than alcohol or that random urine drug testing would have prevented the accident. Millions of dollars have been expended on urine drug testing by government and industry, yet no evidence can be found that improvement in safety, health, or performance has resulted. Blood, breath, and urine alcohol concentrations have been linked scientifically to alcohol impairment, which has been related to transportation accidents. Drug testing when there is probable cause might provide some useful information.

Positive drug findings in apparently unimpaired workers tend to support the contention that drugs, as they are generally used by workers, are not a serious and widespread problem, whereas a similar finding in impaired workers could support the need for much more extensive and expensive testing of these few individuals. For testing to be fully effective, every worker would have to be tested daily for every drug that might impair performance, the results would have to be available before he started work, and he would have to be under constant surveillance while at work to make sure that he did not use a drug while working. Our lives would be greatly altered if we had to wait for the results of such tests before trusting workers with safety-related positions—if we were able to get the vast amounts of money and resources that would be required by such testing programs. Random testing of a population where the vast majority is free of the few drugs for which testing is done once a year or once a month is not cost-effective. Workers might stop using drugs included in the analyses and use more alcohol or use more impairing drugs which are not included in the analyses.

An early mention of marijuana involvement was made in 1977 when a Chicago Transit Authority train rear-ended a stopped train on the Loop [1]. Four cars fell to the street, killing 11 and injuring 268 people. Four marijuana cigarettes allegedly were found in a handbag belonging to the motorman of the moving train.

> Urine specimens from the motorman were tested in two laboratories. One laboratory reported traces of "THC" in a urine specimen. The second laboratory failed to detect anything unusual by its first test. A second test, performed after the laboratory's instruments were recalibrated, detected the presence of "THC." Neither laboratory could determine how long before the accident the marijuana had been ingested.
>
> The extent that the motorman may have been under the influence of marijuana at the time of the accident could not be determined by the tests. Because of the nature of THC and its chemical behavior in a human body, tests could conclude only that the operator had ingested marijuana at some time before the accident. There is no scale available to relate levels of THC or its effect on the behavior of individuals.

The probable cause of the accident was the failure of the motorman to exercise due care in meeting his responsibilities. No mention was made of drug involvement. The questionable urinary cannabinoid identifications did not prevent the media from exploiting the marijuana issue in this tragedy.

Aviation

In 1981, the U.S. Navy drug testing program was hastily expanded after the crash landing of an aircraft on the U.S.S. *Nimitz* killed 14 servicemen and injured 48 [*2*]. Cannabinoids were found in the urines of 6 of the crewmen. The answer a clinical chemist gave to the question of when the servicemen had used marijuana based on the levels of cannabinoids in their urines was, "You can bracket it roughly between six hours and ten days." The carrier had been at sea for 11 days. During the congressional hearings [*2*], these statements were made: "A comment relative to impairment cannot be made since there is no present scientific tool to correlate levels of impairment and cannabinoid level." "There is some indication that some antihistamines may have been present" (in the aircraft crew). The Secretary of the Navy concluded, "The data I have provided confirms my belief that drug use or abuse did not contribute to this tragic crash. The only thing which the data does show is that six members of the deck crew had used marijuana in some amount and at some time. It does not establish in any way that any of these men were in the least impaired in the performance of their duty." The hastily expanded drug testing program was for one drug, marijuana, not for antihistamines or any other drug.

In 1985 the Federal Railroad Administration (FRA) prepared rules and regulations titled "Control Alcohol and Drug Use in Railroad Operations" [*3*]. "Accidents/Incidents 1975–1984 with Alcohol or Drugs Directly Affecting Cause" were reported. In Tables 1 through 4 of that report, 67 accidents are listed. Alcohol was found or alleged to be a factor in all but 4 accidents. Marijuana was mentioned in 4 accidents, together with alcohol in 2 and barbiturates and imipramine in 1 each. The imipramine report resulted from a positive and unconfirmed colorimetric test performed on urine from an embalmed body. The test was performed in the Toxicology Laboratory of the North Carolina Office of the Chief Medical Examiner in 1976. The circumstances of the accident did not warrant further testing of the embalmed specimens.

The National Transportation Safety Board (NTSB) reported that in a fatal airplane landing crash [*4*] the pilot's urine contained 197 μg/L of THC-COOH, and alcohol swabs were "positive for marijuana." It was concluded that "tests for marijuana indicated that the captain had used marijuana in the past 24 hours." Expert testimony stated "that values above 100 μg/L found in urine of casual and chronic users of marijuana is indicative of use within the previous 24 to 48 hours." The report continues, "The Safety Board was unable to find comprehensive data which correlates postmortem toxicological blood/urine analyses findings with operator behavioral degradation from marijuana use." Although there was no report that marijuana was found in the blood or urine of the dead copilot, alcohol swabs were "positive for marijuana." It was concluded that "the copilot had used or been exposed to marijuana in the past 24 hours." Where is the scientific foundation for the statement that "swabs of the mucous membrane can be tested for marijuana by the Duquenois test or other similar colorimetric tests" and for the interpretation of the time of exposure?

Railways

The National Transportation Safety Board reported on a fatal train accident [*5*] which was cited in the media as being related to drug use. The engineer's blood, which was obtained 4 h after the crash, contained 1 μg/L of THC and 79 μg/L of THC-COOH.

The rear brakeman's blood, which was obtained 4 h after the crash, contained 3 μg/L of THC-COOH. It was concluded by the NTSB that an enzyme multiple immunoassay technique (EMIT®) positive result, which was unconfirmable by thin-layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS) was a "false positive." The NTSB stated, "Even if the engineer was a heavy user and had used marijuana more than 4 to 6 hours before being tested, the presence of THC in his blood indicates that he was still under the influence of marijuana at the time of the accident and therefore his failure to respond to the signals may have been the result of his use of marijuana." There is other information in this report that is more enlightening. "The train's rapid acceleration going downhill alerted the conductor (in the caboose), and he called the engineer on the radio. The call awoke the engineer, who saw the speed indicator registering a speed of 60 to 62 mph." "The sound of the initial air brake awoke the head brakeman." The engineer and head brakeman had had about 6 h sleep in the prior 48 h.

Urine and blood specimens were obtained from the engineer and brakeman of three standing Conrail locomotives which were rear-ended by an Amtrak passenger train which had been traveling at 128 mph (206 km/h) and exceeded the speed limit of 105 mph (169 km/h) by 23 mph (37 km/h) [*6*]. Specimens were taken 5 and 8 h, respectively, after the crash. The Conrail engineer's serum was reported to contain no THC and 42 μg/L of THC-COOH, and his urine contained 70 or 182 μg/L of THC-COOH. The brakeman's serum was reported to contain no THC and 13 or 23 μg/L of THC-COOH, and his urine contained 80 or 144 μg/L of THC-COOH. Two laboratories reported the different results. Urine and blood specimens taken from the crew of the passenger train four days after the crash were of no value in assessing their condition at the time of the crash. Little can be said about the cannabinoid concentrations in the specimens obtained from the locomotive crew, assuming that the results were correct, other than they had probably used marijuana at some prior time, that the concentrations at the time of the crash could not be estimated, and that the possibility of marijuana-related deterioration of performance could not be determined. The time between the crash and the collection of specimens, about 5 and 8 h, could allow about 0.07 and 0.12% of alcohol, respectively, to disappear. There is no evidence that these crewmen had alcohol in their blood at the time of the crash. Attempts may be made to interpret the findings in this crash using the preliminary reports of Yesavage for "hangover" effects of marijuana for 24 h [*7*] and of alcohol for 14 h [*8*]. Automatic train controls, which the Conrail locomotives were supposed to have had, other properly functioning safety devices, and proper scheduling would have prevented this horrible crash.

Public Law 100-342, the Rail Safety Improvement Act of 1988, was approved on 22 June 1988. If implemented and enforced, many of the above safety problems would be solved. Some of the requirements of this law follow: The motor vehicle driving record of locomotive operators will be checked. For trains on the Northeast corridor, baggage rack restraints, seat back guards, seat lock devices, automatic train stop, and other safety devices are to be installed.

The Federal Railroad Administration (FRA) issued summary reports of postaccident tests for the period from 10 Feb. 1986 through 31 Dec. 1987 [*9*]. Specimens from 1508 trainmen in 349 events were analyzed. The presence of drugs was reported in specimens obtained from 16 engineers: alcohol was found in 3; cocaine in 5; butalbital, a barbiturate, in 1; and cannabinoids in 7. The 16 engineers represented about 1% of the trainmen tested, and less than 5% of the number of events. A careful evaluation of accident responsibility in each incidence would have been helpful in evaluating the meaning of this summary. An assessment of whether the presence of drugs in the trainmen resulted in impairment which could have contributed to the accident would be helpful also. The effects of marijuana and other drugs on the performance of those responsible for the

safety of railroads have not been tested in controlled experiments while these individuals are performing tasks which could be related to their jobs. The tasks are well learned and with proper signals, maintenance, and scheduling there should be little need for complex judgement decisions, short reaction times, and critical tracking ability.

Postaccident drug tests performed in the Oklahoma City laboratory used by the Federal Aviation Administration (FAA) and FRA on specimens from airplane crews for over 20 years and on specimens from train crews for about 1.5 years prior to January 1987 could have been seriously flawed because of incompetency and falsified results [*10*]. Doubt concerning the value of testing for drugs other than alcohol and on the availability of "forensic" quality work was mentioned in the *Federal Register* in 1985 [*3*].

The results of one year of drug testing under the FRA Mandatory Post-Accident Testing Program have been reported recently [*11*]. "During the year beginning 4/1/87, specimens were collected for 183 qualifying events from 743 individuals, including 40 fatalities." It was reported that drugs were found in 48 employees. It was not possible from the abstract or the presentation to establish how many drugs, whether the compounds were metabolites or drugs, or what concentrations were found in the 741 blood specimens. Before the proposed random urine drug testing is initiated, which is estimated to cost $2 to $22 million a year [*12*], such data ought to be made available so that they can be evaluated and interpreted independently. Since the reported testing was incidental testing, it is likely that random testing will result in less than 6% positives and not be helpful in establishing the role of drugs in railroad operation. No data have been found that indicate that there are significant drug problems on the railroads.

An air taxi freight airplane crashed into a mountainous wooded area killing the sole occupant, the pilot, who had over 6000 h of flight time [*13*]. His driver's license had been revoked continuously since 1980 for 20 traffic violation convictions, including 7 for driving while intoxicated (DWI). His latest revocation, which was for 5 years as a habitual traffic offender, started in June 1985. Following his seventh DWI arrest, a county attorney filed a formal complaint with the Federal Aviation Administration (FAA) [*13*]. "The attorney was concerned about the obvious conflict between the pilot's driving history and his professional flight duties." At the time of the crash, the pilot had not slept in 21 h. He had been flying for 3 h, primarily at night, using instrument procedures, and at 11 000 ft (3353 m) without oxygen. His blood alcohol concentration at the time of his death was 0.158%, and he had a heavy smoking habit. The FAA has proposed random urine drug testing of an estimated 511 628 commercial aviation employees at a cost of $4.4 to $60 million a year [*14*].

Trucks

Alcohol and other drug use determined by analyses of blood and urine specimens of 317 truck drivers has been reported [*15*]. The specimens were analyzed for more than 80 commonly used drugs, including alcohol. No alcohol was found in 305 (99.3%) of the 307 drivers tested and non-impairing concentrations of 0.01 and 0.02% were found in 2 blood specimens. The drugs which were not found are impressive, and no alcohol or other drug was found in 71% of the drivers. No mention was made that any driver was impaired. Evidence of prior marijuana use was found in 47 (15%) of the drivers but only 3 had cannabinoid concentrations in their bloods which might indicate recent use. About 10% of the drivers had stimulants in their blood and 70% said they drank coffee. Evaluation of the report indicates that the results do not support random or without-cause urine drug testing as a cost-effective or efficient way of improving the health, safety, and performance of truck drivers or that any of the drivers should have been prevented from completing their driving. It has been estimated [*16*] that the costs of the proposed drug testing program for truck drivers alone will cost $0.4 to $2 billion a year, and that the

benefits will equal or exceed the costs. It is stated that the benefit estimates are based on a June 1984 Department of Health and Social Security (DHSS) survey which estimated that the costs to society of drug abuse are $66 billion annually. An analysis of the report does not support a fraction of this estimate [*17*].

Frequency

The number of employees who would have drug-positive urines has been greatly exaggerated [*18*].

> Of the 11,233 Army civilian urinalysis drug tests conducted through January 31, 1988, 74 or 0.66 percent were positive for drug metabolites. Of these 63 were for marijuana, 8 were for cocaine and 3 were for both. The court noted that no evidence suggested that any of those persons testing positive used drugs while on duty.

In 1987, only 40 or 0.58% of the urines of 6950 Federal employees who worked in "sensitive positions" tested positive for drugs [*19*]. A 1985 survey reported that about 9% of the military said they had used drugs in the last 30 days [*20*]. Another 1985 report revealed that the urines of 6.2% of U.S. Navy recruits and 3.2% of U.S. Marine Corps recruits tested positive for cannabinoids. The results were 1.22% for the Navy and 1.36% for the Marines in service schools. Testing those in service schools did not deter cocaine use, since cocaine-positive urines increased 55% in the Navy and 27% in the Marines from 1985 to 1988 [*21*]. If the survey is any indication of those using drugs (9%), then the testing is only discovering about 20% of those who say they used drugs.

Effects

The effects of smoking 2% THC cigarettes by 7 professional and 3 private pilots have been evaluated using a flight simulator [*22*]. Individual performances varied considerably from pilot to pilot and from variable to variable. The results indicated that smoking marijuana caused significant deterioration in simulated instrument flying ability for at least 30 min in experienced pilots. The effect probably lasted 2 h and disappeared in 4 h. A preliminary report stated that there were performance decrements on a flight simulator by 10 private pilots 24 h after each had smoked an entire marijuana cigarette which contained 19 mg of THC [*7*], but the urine and blood specimens were not tested for cannabinoids, alcohol, or any other drugs, and the study lacked adequate controls. Another preliminary report dealt with a similar study of 10 private pilots after they had drunk enough alcohol for their blood concentrations to reach 100 mg/dL (0.10%). Significant decrements in performance were still evident 14 h later when their bloods should have contained no alcohol [*8*]. It might be concluded from these two reports that a pilot whose blood contained no alcohol and no THC but did contain THC-COOH could have had a performance decrement because the blood alcohol concentration could have been 100 mg/dL about 14 h earlier or marijuana might have been smoked 24 h before the time of the blood test.

Four studies, one simulator and three on-road studies, looked at extended effects of marijuana and alcohol. Two of the four studies reported that alcohol affects driving for extended time periods, 3 and 4 h [*23*]. None of the studies revealed any effects of marijuana after the initial test performed shortly after smoking. In another study subjects smoked up to 200 mg/kg (14 mg of THC) and drank enough alcohol to produce blood concentrations of 0.08% [*24*]. Although the plasma concentration of THC was 5 μg/L and of THC-COOH was 30 μg/L, on the morning after, the only effect was that the alcohol group slowed their speed. No effect was measurable in the laboratory 2 to 4 h after the use of strong marijuana cigarettes (4% THC) and marijuana had little effect

on tasks for which the subjects were well trained, but it had a greater effect on the learning of new tasks.[1] Other findings [25] suggest that marijuana smoking (2.9% THC) can produce residual (hangover) effects the day after (9 h). The precise nature and extent of those effects as well as their practical implications remain to be determined. The THC plasma concentrations the morning after were 3 μg/L for the placebo and 5 μg/L for the 2.9% THC cigarette smokers.

The National Master Freight Agreement Drug Testing Program is an example of the confused efforts at trying to interpret the meaning of marijuana concentrations [26]. An employee can be discharged if:

> (*a*) the blood/serum contains at least 2 and up to 5 nanograms THC/mL and at least 10 nanograms THC metabolites/mL; (*b*) the blood/serum contains at least 5 or more nanograms THC/mL, regardless of the THC metabolite concentration; (*c*) the blood/serum contains 20 or more nanograms THC metabolites/mL regardless of THC concentration.

Also, an employee is subject to discharge for other Schedule I drugs. "Where other Schedule I drugs in blood are detected, the laboratory is to report a positive test based on a forensically acceptable positive quantum of proof." How do the results of such testing determine those who are under the influence of drugs?

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