Forensic Science

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This article marks the first appearance of a survey of Forensic Science in Applications Reviews. Given the tremendous diversity of materials received by forensic science facilities and the varied assortment of analytical techniques applied to their characterization, there has, and will remain, serious interest on the part of forensic scientists in the subject content of most reviews appearing within this Journal. It is the aim of this article to augment these reviews with a concise survey of articles appearing in publications that primarily appeal to forensic practitioners. To accomplish this objective, we have focused our attention on the following journals: *Journal of Forensic Sciences*, *Journal of the Forensic Science Society*, *Forensic Science International*, *Journal of the Canadian Society of Forensic Science*, *Arson Analysis Newsletter*, and *Analytical Toxicology*, as well as *Chemical Abstracts Selects*: *Forensic Chemistry*. Our survey encompasses the period from October 1980 through October 1982. Because of normal delays in the abstraction of journal articles by *Chemical Abstracts*, some work covering this period will inadvertently be omitted. Hopefully, these references will be included in the next biennial review.

The format selected for presentation of this survey divides coverage into three distinct areas: Drugs and Poisons, Forensic Biochemistry, and Trace Evidence. Within the scope of each of these areas, articles have been selected to describe current forensic science practices in analytical chemistry and to outline relevant forensic science research interests. To keep our discussion concise and meaningful, we have limited our survey to drugs regulated under the United States Controlled Substances Act, ethanol, and common poisons. Furthermore, to eliminate unnecessary duplication of effort, citations of articles appearing in *Clinical Chemistry*, the *Journal of Pharmaceutical Sciences*, and other pharmaceutical journals have been avoided. We believe that ample coverage of these journals is provided within the Pharmaceuticals and Clinical Chemistry sections of this Journal, and recommend that interested readers consult these sections in order to obtain a complete survey of the drug-abuse subject.

Finally, we wish to express our appreciation to the editors of this Volume for including a literature survey of the forensic sciences within its contents. We strongly believe that its presence brings with it recognition of the unique contributions being made by forensic scientists to analytical chemistry and offers opportunities for cross-fertilization of ideas and concepts between forensic scientists and their colleagues in the analytical chemistry community. We look forward to the continued presence of this survey in future editions of Application Reviews and would welcome comments on its style and content from interested readers.

DRUGS AND POISONS

Ethanol and Volatiles. Ethanol determination is by far the most common and one of the most important areas of modern forensic toxicology. The determination of blood ethanol by sampling of the headspace gas and quantifying by gas chromatography (GC) is the most common way of determining blood ethanol today. The evaluation of ethanol analysis on brain and liver by headspace GC has been reported by Bastos et al. (*1*). The determination of blood ethanol was accomplished by headspace GC analysis on two columns of differing polarity by Schmidt et al. (*2*, *3*). Acetaldehyde accumulation during the headspace GC determination of ethanol has been studied by Chiarotti et al. (*4*). Frey et al. (*5*) have determined blood and serum ethanol in 20-μL samples with an alcohol dehydrogenase micromethod. Blood alcohol determinations with automated systems have also been reported (*6*, *7*). A study of the effects of storage on ethanol concentrations in bone marrow has been reported (*8*). Blood ethanol values have been compared to other body fluids (*9*, *10*) and the effects of oral contraceptive steroids on the rate of postabsorptive phase decline of blood ethanol concentration in adult women have been reported (*11*). In addition, Dittman et al. (*12*) determined blood alcohol concentrations in order to monitor the absorption and diffusion–equilibrium phases of alcohol in humans. Zumwalt et al. (*13*) have evaluated the ethanol concentration in decomposed bodies. They found that the ethanol concentrations of the vitreous humor was helpful in establishing whether any ethanol detected in blood or putrefied fluid samples was endogenous or exogenous. Bilzer et al. (*14*) developed an apparatus to obtain a breath volume of 20 mL which could be transferred to a gas chromatograph for breath alcohol measurements. Breath alcohol measurements have been performed also by infrared (IR) absorption (*15*). The effect of inhalation of gasoline fumes on breath ethanol testing by IR absorption has been examined (*16*). The rate of breath ethanol decrease over a period of time has been studied by the Alcotest IR Breath Analyzer (*17*).

Wells (*18*) has studied the temperature effects of simulator solutions on the expected readings of the breathalyzer. Wilkinson et al. (*19*) have compared three methods of analyzing trapped ethanol from the breath and have studied the trapping, storing, and subsequent analysis of ethanol in in vitro samples. Dubowski and Essary (*20*) have applied an automated GC headspace method to the measurement of alcohol in trapped whole breath. Ethanol congeners have also been determined in alcoholic products and biological fluids (*21*, *22*).

The detection and identification of volatile organic compounds are also an important part of forensic toxicology. Hensman et al. (*23*) have published an annotated bibliography of recent literature on solvent and aerosol abuse. Ramsay and Flanagan (*24*) have developed a GC method for the detection and identification of some volatile organic compounds in whole blood, plasma, or serum. Qualitative and quantitative analyses of tissues and body fluids for multiple volatile organic compounds were performed by Bellanca et al. (*25*). The authors used a combination of packed and open tubular capillary GC along with gas chromatography/mass spectrometry (GC/MS) in their analyses. A comparative study of ethchlorvynol levels in blood vs. bone marrow has been reported by Winek et al. (*26*).

Morphine and Related Narcotics. Of the major drugs of abuse, the morphine aklaloids are one of the more important classes of drugs to be investigated. Wyatt et al. (*27*) have reviewed the physical properties, synthesis, stability, metabolism, and analysis of heroin. Analysis and identification of heroin and related opiates have been accomplished by a variety of techniques such as mass spectrometry (MS) (*28*), high-performance liquid chromatography (HPLC) (*29–37*), GC (*38*, *39*), thin-layer chromatography (TLC) (*40*, *41*), nuclear magnetic resonance (NMR) (*42*, *43*), and circular dichroism (*44*, *45*). Also, Schwartz et al. (*46*) have used a glassy carbon electrode for the voltrammetric determination of morphine in poppy straw concentrate.

A volumetric procedure for the determination of morphine in urine was developed by Tahir et al. (*47*) and the determination of morphine and morphine 6-nicotinate by an in situ reaction on chromatographic plates with dansyl chloride was reported by Wintersteiger (*48*). Quantitation of morphine alkaloids in biological fluids is an important part of forensic toxicology and many methods have been developed to perform this task. WuChen et al. (*49*) developed a method for the quantitative determination of free morphine and codeine utilizing multiple ion detection mass fragmentography. A rapid method for the simultaneous quantitation of morphine, codeine, and hydromorphone by GC/MS has been reported by Saady et al. (*50*) and the detection and measurement of opium alkaloids and metabolites in urine of opium eaters have been accomplished by methane chemical ionization (CI) mass

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fragmentography (*51*). Edlund (*52*) has determined opiates in biological samples by capillary GC with electron capture detection. Demedts et al. (*53*) combined the use of fused silica capillary columns and nitrogen–phosphorus detection for the analysis of illicit heroin samples. Other authors have reported the use of HPLC for the detection of morphine in biological samples (*54*, *55*). Radioimmunological screening has been applied also to morphine in biological fluids (*56–58*) and Fredenberg et al. (*59*) used thin-layer immunoassay for the detection of opiates.

Cannabinoids. Analytical studies on marijuana have been reviewed by Harvey et al. (*60*). Mass spectrometric differentiation of cannabinoid-containing samples have been performed by a direct-inlet probe mass spectrometry/multiple regression procedure (*61*). Turner et al. (*62*) studied 52 cannabinoids by selected ion monitoring mass spectrometry. The Δ^9-THC content of cannabis products has been determined by several investigators using GC (*63*, *64*), HPLC (*65*), and HPLC high-resolution GC (*66*). Neuninger et al. (*67*) also separated cannabinoids and semiquantitated THC by using reverse-phase TLC. Characterization of the basic fraction of marijuana smoke has been done by capillary GC/MS (*68*).

A monograph containing state-of-the-art analytical methodology pertaining to the analysis of cannabinoids in blood, urine, and breath has been published (*69*). Marijuana metabolites found in human urine have been detected with TLC (*70–72*). Whiting et al. (*73*) developoed a new method for confirming the major urinary metabolite of THC, 11-*nor*-Δ^9-tetrahydrocannabinol-9-carboxylic acid. This was accomplished by derivatizing it to the methyl ester, methyl ether and analyzing by GC. Determination of cannabidiol in plasma by electron-capture GC has been reported by Jones et al. (*74*) and postcolumn derivatization was used by Borys et al. (*75*) for the colorimetric analysis of tissue cannabinoids separated by HPLC. Detection of cannabinoids in human urine by GC/MS has been reported (*76*) and compared with radioimmunoassay (RIA) procedures (*77–79*). EMIT has been evaluated by Law et al. (*80*) for the detection of cannabinoids in urine. Also, an EMIT procedure for the detection of cannabinoids in 1 mL of blood has been described (*81*).

Cocaine. Rapid screening for cocaine by capillary GC has been reported with the use of a nitrogen selective detector (*82*). Quantitation of cocaine has been described by Clark et al. (*83*) using GC/MS with a pentadeuterated internal standard. This procedure has a relative deviation of ~2.3% and is applicable to samples containing a wide variety of diluents and adulterants. Quantitation of cocaine from a variety of matrixes has also been accomplished by HPLC (*84*). In this procedure a reverse-phase packing with UV detection enabled the authors to quantitate cocaine in a variety of mixtures. HPLC was utilized by Noggle et al. (*85*) for the identification of *cis*- and *trans*-cinnamoylcocaine in illicit cocaine samples. Cocaine and *cis*- and *trans*-cinnamolycocaine were determined in cocoa leaves by a GC procedure (*86*). Cocaine can be determined by circular dichroism (*87*, *88*), but this technique is usually not applied to the routine illicit samples submitted to a forensic laboratory. The differentiation of the distereoisomers of cocaine is a problem that forensic chemists often encounter. Allen et al. (*89*) discussed the techniques used to make this distinction and suggested that a logical sequence for analysis was to first identify the diastereoisomer (via IR or MS) and then to determine the chirality with crystal tests, IR, melting points, or optical rotation measurements.

HPLC analysis of cocaine in human plasma has been reported by Masoud et al. (*90*). Methods of analysis for the detection of benzoylecgonine in urine were reported (*91*, *92*). Potential errors in the extraction of benzoylecgonine and cocaine from urine have been discussed (*93*).

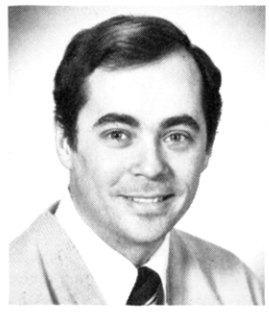
Amphetamines. Sinnema et al. (*94*) discussed the nature of impurities present in illicit amphetamine preparations. Other authors have also reported the identification and structural elucidation of amphetamine impurities (*95*, *96*). The microcrystalline identification of amphetamines and other drugs of abuse found in the illicit street drug preparations known as "white crosses" has been reported by Julian and Plein (*97*). Identification of amphetamine and related illicit drugs, such as phentermine, meperidine, and ephedrine, by second derivative ultraviolet (UV) spectrometry has also been reported by Lawrence and MacNeil (*98*). The authors identified these compounds by comparing their absorption pattern in aqueous acid and basic solutions. Analysis of the carbon-13 NMR spectra of amphetamine related compounds, such as mono- and dimethylamphetamines (*99*), mono- and dimethoxyamphetamines (*100*), and trimethoxyamphetamines (*101*) has also proved to be helpful in the identification of these compounds for forensic purposes. NMR has been applied to the determination and differentation of methamphetamine and amphetamine enantiomers (*102*, *103*). Chiral and achiral capillary GC/MS have been applied to differentiate the enantiomers of these compounds (*104*, *105*).

Amphetamine and six related drugs were determined in urine by GC, EMIT, and RIA. The advantages and disadvantages of these three methods were compared (*106*). HPLC methods have been described for the determination of amphetamine and related drugs in urine (*107*, *108*). Rapid and sensitive methods have been described for the determination of amphetamine-related drugs in urine using electron-capture GC (*109*), capillary GC (*110*), and TLC (*111*). Detection of amphetamines in biological fluids and stains is also of importance in forensic science. Smith (*112*) has described an RIA method for detection of amphetamine in bloodstains, semen, seminal stains, saliva, and saliva stains. Low nanogram quantities of amphetamine were detected in 100-μL samples of dried bloodstains.

Barbiturates. Barbiturates continue to be abused and methods for their identification and determination have appeared in numerous publications. Analysis of barbiturates by GC has been reviewed by Pillai et al. (*113*). Predictions of the GC retention characteristics of barbiturates from molecular structure have been reported by Stead et al. (*114*). HPLC has also been used for the identification and determination of barbiturate preparations. Hoogmartens et al. (*115*) used HPLC to determine 5-ethyl-5-(1-ethylpropyl)-barbituric acid in samples of pentobarbital. White et al. (*116*) used dual-wavelength UV detection in the HPLC identification of 29 barbiturates. Anion MS of barbiturates has been reported by Jones et al. (*117*). In this report, the negative ion mass spectra of 30 derivatized barbiturates were reported and these were used for the identification of specific barbiturates. Gill et al. (*118*) measured chromatographic data for a group of barbiturates on seven chromatographic systems including GC, TLC, and HPLC systems. The values of these systems for barbiturate identification were discussed and the authors concluded that column chromatographic techniques with lipophilic phases, GC with SE-30, and HPLC with ODS-silica were the most suitable for barbiturate identification.

Therapeutic, toxic, and fatal phenobarbitone blood concentrations can be interpreted by the use of concentration–response and the toxicity probability curves (*119*). Structure–activity correlations were studied as a method for interpreting blood levels of barbiturates in fatal cases (*120*). GC/MS has been used to obtain such levels for different barbiturates (*121–123*). Phenytoin, phenobarbital, and primidone have been determined in plasma by flash-methylation followed by GC analysis (*124*). Budd et al. (*125*) studied barbiturate degradation following methylation with dimethyl sulfate. The GC determination of secobarbital in human plasma and urine has been reported (*126*). Micromethods for the identification and quantitation of barbiturates in plasma and blood serum have been described by using GC procedures (*127*, *128*). Kinberger et al. (*129*) have also determined barbiturates in serum by the use of capillary GC. Analysis of barbiturates in biological materials using HPLC has been reported by several workers (*130–132*). HPLC procedures have also been compared to EMIT for the quantitative assay of some barbiturates (*133*). EMIT and RIA procedures for the detection of barbiturates in whole blood and urine have been described (*134–136*). RIA has been used to measure barbiturates in bloodstains, seminal stains, saliva stains, perspiration stains, semen, and saliva (*137*). Employing an automated sample processor, Balkon et al. (*138*) described a procedure for the isolation of barbiturates from postmortem specimens prior to their analysis by GC and HPLC. The identification of a methyprylon metabolite which interfers with the UV differential spectra of barbiturates was accomplished by TLC, GC, and GC/MS in urine and blood extracts (*139*).

Phencyclidine. Phencyclidine has been reviewed by Labianca (*140*) with emphasis on illicit synthesis, drug classification and effects, duration of effects, metabolites, and its damaging effects on the young. A paired ion reverse phase



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HPLC separation of synthetic phencyclidine mixtures has been reported by Jones et al. (*141*). Similarly, Wall et al. (*142*) have reported an HPLC separation of alkylaminecyclohexanecarbonitriles from phenycyclohexylamines related to phencyclidine. 1-Piperidinocyclohexanecarbonitrile (PCC) is a common contaminant of illicit phencyclidine. Soine et al. (*143*) reported a study which showed one-third of the phencyclidine cases analyzed by GC contained PCC. Baker (*144*) reported a colorimetric procedure for the measurement of PCC in phencyclidine samples based on the reaction of the nitrile with *p*-nitrobenzaldehyde and *o*-dinitrobenzene. A color test for the detection of PCC in illicit phencyclidine was developed by Dulik et al. (*145*). The technique described could detect 10 μmol of PCC with minimum interference from other common drugs of abuse. The identification of a new phencyclidine analogue, 1-(1-phenylcyclohexyl)-4-methylpiperdine, has been reported by Soine et al. (*146*). The HCl salt was isolated, purified, and compared to standards by melting point, IR, ^{1}H NMR, GC, and GC/MS.

The stability of drugs in stored blood is important to the forensic toxicologist, especially today when large workloads are plaguing many forensic laboratories. With knowledge of this fact, Clardy et al. (*147*) studied the stability of phencyclidine in stored blood and concluded that in bloods stored unrefrigerated for 8–15 months phencyclidine was not affected to any large degree by the deterioration of blood. Several methods have been reported for the detection of phencyclidine and related compounds in biological fluids. Kelly et al. (*148*) identified phencyclidine and its analogues at low concentrations in urine by selected ion monitoring and Budd (*149*) used TLC for the analysis of 1-(1-phenylcyclohexyl)pyrolidine in urine. EMIT and RIA procedures have been described for the detection of phencyclidine in biological fluids (*150*, *152*). Likewise, GC procedures have been described for the analysis of phencyclidine in biological materials (*153–155*). Schaldenbrand et al. (*156*) have shown that doxylamine may cause false-positive GC results for phencyclidine. Measurement of phencyclidine in saliva has also been reported (*157*).

Miscellaneous Drugs and Poisons. A method has been described for the analysis of psilocybin and psilocin in dry and preserved mushrooms using reverse-phase HPLC (*158*). This method provides for a good separation of the two hallucinogens. Christiansen et al. (*159*) also used HPLC in conjunction with MS to analyze psilocybin mushrooms. Quantitative procedures for psilocybin using reverse-phase HPLC have also been reported (*160*, *161*). Quantitative procedures for the determination of LSD have been described by using synchronous spectrofluorimetry (*162*) and circular dichroism (*163*). The sensitivities of each method were $\sim 10^{-10}$ g/mL and ~ 2 μg, respectively. Analysis of LSD in urine has also been reported by RIA (*164*). The identification of dyes in illicit tablets can supply further confirmation of similarities between illicit samples and hence can be of significance in relating seizures from different locations. A series of articles by Joyce et al. (*165–167*) has reported the identification of such dyes in illicit tablets and the detection of trace impurities in soluble food dyes.

"China White", an illicit heroin substitute which has caused numerous poisonings, has caught the attention of the forensic community. 2-Methylfentanyl has been identified as a component of "China White" by NMR and GC/MS (*168*). Structural information derived from tandem MS for "China White" and related fentanyl derivatives has been reported by Cheng et al. (*169*). 2-Methylfentanyl has been isolated, identified, and quantified in an overdose victim by GC fitted with a nitrogen–phosphorus detector (NPD) and has been confirmed by MS (*170*). Despropionylmethylfentanyl was also detected in the same extracts of the victim's tissues.

Methaqualone and some of its chemical analogues and positional isomers have been synthesized and identified by IR spectroscopy, MS, and NMR (*171*). A direct injection of HPLC determination of methaqualone in blood plasma has been described by Hux et al. (*172*). This method uses precolumns of Amberlite XAD-2 which allows for a relatively large volume of undiluted plasma to be injected directly onto the column. Separation and characterization of standard propoxyphene diastereomers and their determination in pharmaceuticals and illicit preparations have been reported by Soni et al. (*173*). Methods for the determination of propoxyphene in biological fluids have also been described (*174*, *175*).

The illicit manufacture of tryptamines is also a problem occasionally encountered by the forensic chemist. Cowie et al. (*176*) have reported the identification of the major impurities in the illicit manufacture of tryptamine and related compounds. The determination of pentazocine and tripelennamine in the blood of "T's and Blues" addicts has been done by GC (*177*). A method for the determination of pentazocine in blood has been developed by using HPLC (*178*). Analysis of methadone and metabolites in biological fluids has been reported by using GC/MS (*179*) and GC/NPD (*180*). GC/NPD has also been used to determine amitryptyline (*181*) and tricyclic antidepressant drugs in postmortem fluids and tissues (*182*, *183*). Methylenedioxyphenylacetone, a precursor of the drug MDA, is commercially available under the name piperonylacetone. Shulgin et al. (*184*) warned that this compound can give rise to a homologue of MDA which is largely uninvestigated and may represent an imminent toxicological problem. Another arising problem in forensic toxicology is the analysis, identification, and quantification of benzodiazepines in biological fluids. Several authors have reported methods for their analysis. These methods include GC/MS (*185*), EMIT (*186–188*), HPLC (*189*), and derivative spectroscopy (*190*).

The detection of carbon monoxide (CO) in blood is usually performed spectrophotometrically or by headspace GC. Several spectrophotometric procedures have been reported for the detection of CO (*191–195*). Winek and Prex (*196*) have compared spectrophotometric and gas chromatographic procedures when analyzing 21 autopsy blood samples for CO. The authors found that all of the methods examined gave reproducible results when fresh samples were analyzed, but for stored, aging blood calculated CO levels based on iron determination were the most consistent. Guillot et al. (*197*) have reported an improved GC headspace technique to measure CO released from blood. To improve the sensitivity, the authors reduced CO to methane prior to its detection by GC.

A detailed procedure for a new fluorometric assay of cyanide in human blood, stomach contents, and urine has been re-

ported (*198*). This method requires neither diffusion nor distillation from samples and is capable of determining 1.0 nmol of cyanide. RIA detection of Digoxin in tissues has been reported by Sedgwick et al. (*199*). Smith (*200*) has also reported the use of RIA in the detection of digoxin in bloodstains. Experiments have been carried out to evaluate the variation in the amount of insulin residue in syringes and needles under different circumstances. These results indicate that unless syringes and needles are thoroughly and carefully washed, the insulin residues remaining will be detected by current RIA methods (*201*). Regarding insulin as a toxic chemical, another report has described a method employed to demonstrate that a fatal hypoglycemic episode was due to marked beef hyperinsulinemia (*202*).

General Procedures. Finkle has discussed the role of the analytical chemist in forensic toxicology in a recent review (*203*). Foltz et al. (*204*) has published a thorough monograph on a collection of methods for the quantitative analysis of several important drugs of abuse by GC/MS. Gough et al. (*205*) has published a comprehensive review of the identification of major drugs of abuse, using chromatography. Daldrup et al. (*206*) reported relative retention times for 570 drugs and related compounds on eight chromatographic systems by using a combination of TLC, GC, and HPLC for the rapid detection of these drugs. The use of retention indices for the identification of common drugs-of-abuse in forensic toxicology has been described (*207*, *208*). Identification and quantification of neutral and basic drugs in blood by GC and MS have been reported (*209*). McCurdy et al. (*210*) presented a GC procedure for the identification and quantification of certain commonly abused weak acid and neutral drugs from whole blood. A method for analyzing urine for the presence of basic drugs and their metabolites using two fused silica capillary columns has been described (*211*). Applicability of capillary GC to drug identification in toxicology by means of retention indices was found to be unreliable by Schepers et al. (*212*). Caddy et al. (*213*) has illustrated the use of capillary GC for the identification of drugs-of-abuse. Rapid analysis of some commonly abused drugs has also been accomplished by HPLC (*214*). Other authors have applied HPLC to the analysis of abused drugs in biological fluids especially when quantitative data are needed (*215*, *216*). Analysis of drugs encountered in fatal poisonings with HPLC and fluorescence detection has been reported by King (*217*). Moffatt (*218*) reported the combined use of HPLC and immunoassays for drugs and metabolites in biological fluids. Lurie et al. (*219–221*) has reported on the optimization of the reverse-phase ion-pair chromatographic separation for drugs of forensic interest with emphasis on variables affecting capacity factors and selectivity. Urine screening for drugs by EMIT has also been performed (*222*). The specificity of the EMIT drug abuse urine assay methods has been evaluated by Allen et al. (*223*). EMIT assays for abused drugs have been applied to postmortem brain tissue (*224*) and vitreous humor (*225*). The authors report good correlation with other techniques but caution against false positives. The optimization of TLC for toxicology screening has been reported by Franke et al. (*226*) and an improved separation of basic drugs extrated from tissue by TLC has also been described (*227*). Other tests have been applied to the identification of drugs in biological fluids such as derivative UV–visible spectroscopy (*228*), colorimetry (*229*), and the TRI "Dipstick" test (*230*). The authors reported that the data obtained from the TRI "Dipstick" were clearly unreliable. The detection of drugs in bloodstains has been reported by Hammond (*231*) and a review of the use of saliva in the forensic detection of drugs and other chemicals has also been published (*232*). A variety of extraction methods for the analysis of drugs of abuse in forensic toxicology have appeared recently. Removal of phenolic compounds which interfere in the spectrophotometric and/or GC analysis has been studied and a procedure evolved which eliminated the interference (*233*). Decreases in yields of extracted drugs after exposure to 1% HCl in methanol have been demonstrated (*234*). A method has been reported for separating drugs from serum lipids on special thin-layer chromatographic media (*235*). Nakamura et al. (*236*) described a method for the separation and determination of neutral compounds in postmortem tissues. The stability of drugs to the conditions used in the enzymatic hydrolysis of tissues using Subtilisin Carlsberg has been discussed (*237*). The mineral content of Subtilisin Carlsberg and an associated buffer reagent has been established by spark source mass spectrometry. The concentration of the elements detected were too low to contaminate tissue samples that form the bulk of toxicological work in the forensic laboratory (*238*). The effectiveness of several digestion procedures has been compared by simultaneous determination of several drugs by GC in liver extracts after enzymic digestion (*239*). Klug et al. (*240*) found that the use of Subtilsin A for the enzymic digestion of liver tissue in the determination of barbiturates, benzodiazepines, and other drugs was no more effective than a modified acetone extraction method with ultrasound precipitation. Enzymic digestion of biosamples has been performed as a method of sample pretreatment before XAD-2 extraction (*241*, *242*). A rapid isolation technique for drugs from tissues and fluids with the use of the Du Pont Prep 1 System has been reported by Balkon et al. (*243*) and a microextraction has also been described by these same authors (*244*).

FORENSIC BIOCHEMISTRY

Testing of bloodstains is an important part of forensic biochemistry as has been illustrated in the chemical investigation of the Shroud of Turin (*245*). Reagents for the preliminary screening of bloodstains have been evaluated by Yatomi (*246*) for sensitivity, stability, and side reactions. The author recommends the use of 3,3′, 5,5′-tetramethylbenzidine. The effects of the argon ion laser on the presumptive testing of bloodstains has been reported by Platt (*247*). Here, the bloodstains showed greatly reduced reactions or none at all with presumptive reagents applied by fine mist spraying. On the other hand, filter paper rubs on the same stains followed by presumptive testing yielded the appropriate oxidation reactions of the indicator reagents. The use of reflectance analysis for direct colorimetric determination of the age of bloodstains has been reported by Lins et al. (*248*). The idenfication of human bloodstains by means of a microthin-layer immunoassay procedure has also been reported (*249*, *250*). The use of isoelectric focusing of hemoglobin and the red cell enzyme, superoxide dismutase (SOD_A), has been described for the species identification of deer blood (*251*). A method for the discrimination of human hemoglobin from animal sources has been described using isoelectric focusing (*252*). The determination of the sex of an individual from a dried bloodstain has been reported by Brown (*253*) using RIA. Sex determination from dried bloodstains was also accomplished by monitoring the ratio between testosterone and progesterone (*254*). Thomsen (*155*, *256*) has studied Y-Chromosome detection for sex determinations in bloodstains. Clausen and Rose (*257*) have developed a pyrolysis-GC method for the differentiation of adult and fetal bloodstains. This differentiation is based on the peak height ratio of two long retained peaks appearing in the pyrograms. A sensitive and simple procedure has been established for the forensic detection of pregnancy in bloodstains. This technique is based on the heat stability of placental alkaline phosphatase (*258*).

Various methods for the antigenic grouping of bloodstains exist today. The use of microtitre plates for Lewis typing of blood, saliva, and semen in liquid or stain form has been shown to increase the sensitivity of typing to permit considerable reductions in the amounts of both test material and antisera (*259*). A modified procedure for absorption–elution of A and B antigens with ammonia extraction was presented (*260*). A method has been described for determining A, B, H, Le^a, and Le^b antigens in parallel on saliva or seminal stain extracts (*261*). The effect of blood group active microorganisms on the ABO grouping of human whole saliva has been assessed (*262*). The paradoxical secretion and aberrant secretion of the ABH substances have been studied in the saliva of a number of subjects. A standardized hemagglutination-inhibition method was used and the results were scored by an inhibition index (*263*). The detection of Rh antigens on bloodstains by a microelution technique using low ionic strength solutions and papain-treated red cells has been examined (*264*). The grouping reactions of seminal stains and mixtures of semen and vaginal material have been collected from approximately 400 sex cases. ABO was found to be the most successful while Phosphoglucomutase (PGM) slightly less successful and Gloxylase I (GLO I) was the least often to succeed (*265*). Two cases in which bloodstains and seminal stain evidence were important were subjected to the identi-

fication of Gm antigenic determinations. The results of these two cases are interpreted with regard to the Gm results obtained (*266*). A modification of the MN typing of dried bloodstains and a study to identify the reasons for false negatives with MN typing have been conducted (*267*).

The identification of HLA-A9 in dried stains of blood and body secretions has been reported (*268*), as well as the utility of HLA and six erythrocyte antigen systems in excluding paternity (*269*). The typing of the antigens HLA-A1, -A3, -A9, -B7, and -B8 in bloodstains was investigated. In this study, more than 90% of the samples were correctly typed (*270*). Hillman and Shaler (*271*) have reported the extraction of leukocytes from dried bloodstains on a variety of surfaces in yields high enough to make sex determinations, polymorphic enzyme typing, and human lymphocyte and surface antigen typing feasible in the future. Shulter and Dawson (*272*) have outlined a simple method for concentrating a limited quantity of bloodstains and seminal fluid stains using a vacuum diffusion technique.

The search for semen stains and presence of spermatozoa can be important for proving the commission of a sexual assault. Cocheiro et al. (*273*) have described a reliable and simple technique for the location of spermatozoa in seminal stains. The persistence of spermatozoa on vaginal, anal, and oral swabs after intercourse has been reported (*274*), as well as the frequency of azoospermia in seminal stains from case swabs (*275*). The identification of seminal stains based on the presence of choline using an enzymatic method has been demonstrated (*276*). This method is based on the reaction of choline oxidase with choline. Studies on the specific microdetermination of free choline in semen have been reported (*277*). Joshi et al. (*278*) studied the effects of water immersion of seminal stains on cotton cloth and concluded that prolonged immersion in water does not affect the detection of spermatozoa.

A study of leucine aminopeptidase (LAP) as a possible means of identifying semen has been reported. LAP activity was investigated in a number of human body fluids, vegetable extracts, casework stains and vaginal swabs by means of either a spot test or photometric assay (*279*). A rapid test for the quantitative detection of seminal acid phosphatase which uses 4-nitrophenyl phosphate as the substrate has been described. The authors claim this method excludes many common interferences such as saliva, nasal mucous, and vaginal secretions (*280*). An RIA method was described for the detection of human prostrate-specific acid phosphatase in forensic semen samples in which sperm cels could not be seen microscopially (*281*). The detection of the sperm-specific lactate dehydrogenase isoenzyme X (LDH-X) for the identification of testicular tissues was demonstrated by starch gel electrophoresis (*282*). A new method for the detection of human semen by monitoring the electrophoretic pattern of glycylprolyl dipeptide aminopeptidase (GP-DAP) has been reported. In the GP-DAP electrophoretic pattern, semen showed an active band at the origin which is absent in other fluids and was also detected even in 10 month old seminal stains (*283*). The presence of human seminal plasma proteins in biological stains has been demonstrated by an absorption test using anti-human seminal protein rabbit serum. The authors claim this test to be sensitive and highly specific (*284*). The estimation of time since intercourse has been reported by Rutter et al. (*285*). This was accomplished from data generated from the plot of coital time interval vs. the acid phosphatase activity/UV absorbance at 270 nm from extracts recovered from 283 vaginal swabs. Alkaline phosphatase activity was measured in six sites from a cadaver circulating system. This was compared with sodium and potassium levels in the serum and time after death (*286*). Utilizing affinity chromatography, an attempt was made to produce an antiserum for the detection of saliva in forensic investigations. Crossed-over electrophoresis was used to evaluate the antiserum (*287*). The use of polymorphic red cell enzymes in forensic science has been reviewed by Sensabaugh (*288*). Application of the principles of genetic inheritance for linking a bloodstain to a missing victim has been discussed by Kuo (*289*). The author explains that genetic marker typing can determine whether the parents of the missing victim are possible parents of the bloodstain source. An isoelectric focusing method has been described for typing salivary amylase in liquid saliva and saliva stains (*290*). The storage stability of phosphoglucomutase (PGM) in blood under laboratory conditions was determined by observing its electrophoretic pattern (*291*). Attempts were made to detect phenotypes of the enzymes PGM and phosphogluconate dehydrogenase (PGD) in human skin and adipose tissues (*292*). Detection of the rare PGM_1^3 allele has been reported and the criteria for the electrophoretic separation and accurate typing of the PGM_1^3 isoenzymes have been outlined (*293*). The cautions in interpreting ABO, Lewis, and PGM typing in seminal stains and postcoital vaginal swabs have been discussed (*294*). Isoelectric focusing has been successfully applied to the subtyping of PGM with a charge-balanced agarose (*295*). A method for isoelectric focusing of PGM_1 using agarose had significant advantages over published acrylamide techniques (*296*). The rare phenotypes of PGM were examined by starch gel electrophoresis and cellulose acetate gel isoelectric focusing and were compared with the commonest phenotypes of PGM (*297*). Ultra-thin-layer isoelectric focusing was modified for the determination of PGM isoenzymes in bloodstains (*298*). The determination of Carbonic Anhydrase II (CA II) phenotypes in dried bloodstains by cellulose acetate electrophoresis was described by Noppinger and Morrison (*299*). A simple and rapid procedure has been presented for the identification of CA II in fresh blood and bloodstains by using cellulose acetate membranes (*300*). A screening method has been developed for the simultaneous detection of CA-II with Esterase D (EsD), PGM, and GLO-1 on the same starch agarose system (*301*). A gradient gel electrophoretic technique for the subtyping of haptoglobin (Hp) in serum samples and bloodstains has been developed (*302*). Some possible explanations have been presented for an unusual group specific component (Gc) pattern in bloodstains (*303*). Five new Gc variants have been detected by Thymann et al. (*304*) using isoelectric focusing in agarose gel. Cleve (*305*) also reported observing six new Gc variants by isoelectric focusing and polyacrylamide gel electrophoresis. Serum and bloodstain samples were typed for Gc using isoelectric focusing on ultra-thin polyacrylamide gels with detection by immunofixation (*306*). The techniques of isoelectric focusing in ultra-thin polyacrylamide gels as a method of phenotyping erythrocyte acid phosphatase (EAP) has been applied to a large number of red cell lysates and dried bloodstains. This paper presented the results and discussed some features of the phenotype patterns and problems with their interpretation (*307*). A polyacrylamide gel isoelectric focusing technique has been described for the determination of α-antitrypsin (Pi) phenotypes in human bloodstains (*308*). A method for the identification and quantitation of hemoglobin derivatives by means of electrofocusing has been presented (*309*).

Studies were carried out on semen, seminal stains, and vaginal swabs for the detection of Peptidase A (Pep A) by starch gel electrophoresis. The results indicated that Pep A typing could be carried out on semen and seminal stains. However, it was possible to determine the Pep A type on vaginal swabs only when they had been taken within about 3 h after intercourse (*310*).

A great deal of population frequency data has been generated over the past couple of years which have involved a number of different genetic markers. These include the Pm and Ph system in saliva (*311*), PGM (*296–298, 312, 313*), GLO-1 (*314–316*), CA-II and EsD (*317*), Gc (*318*), Hp (*302, 319*), transferrin (Tf) (*320*), α-1-antitrypsin (PiM) (*321*), α-fucosidase (E.C. 3.2.1.51) (*322*), and phenotypes of salivary amylase (*290*).

TRACE EVIDENCE

Petroleum Products. Efforts at identifying petroleum accelerants in debris recovered at suspicious fires constitute a significant portion of the tasks routinely confronting forensic science laboratories. While the gas chromatograph (GC) remains the instrument of choice for detecting and identifying petroleum residues, there has been a significant effort at developing sample collection procedures designed to augment heated headspace analysis. A number of alternative approaches have been proposed. One procedure involved concentration of accelerant vapors through exposure of fire debris to a copper wire or Plexiglas beads coated with charcoal. The adsorbed vapor was eluted with carbon disulfide prior to GC analysis (*323*). Kubler et al. (*324*) and Tontarski et al. (*325*) transferred volatile organics from fire debris onto a charcoal

trap which was eluted with carbon disulfide prior to GC analysis. Kubler and Stackhouse (*326*) reported their charcoal trapping procedure to be 100 times more sensitive than headspace isolation for the recovery of hydrocarbons. Twibell et al. (*327*) exposed fire debris to a carbon-coated ferromagnetic wire. This wire was then thermally desorbed onto a GC column. Twibell et al. (*328*) also constructed a heated splitless inlet system enabling the ferromagnetic wire to be thermally desorbed onto a capillary column. This method proved 20–100 times more sensitive than a heated headspace approach. Concentration of volatile hydrocarbons was also accomplished with Tenax-GC. Fire debris contained within nylon bags was sampled by inserting one end of the Tenax trap in the bag and removing a volume of air through the trap with a syringe. The trap was then desorbed in the injection port of a GC (*329*). A purge-and-trap procedure for accelerant residues was also reported utilizing Tenax-GC as an adsorbent. Sorbent trap concentration on Tenax-GC followed by thermal desorption directly onto a GC column enhanced by thermal desorption directly onto a GC column enhanced sensitivity for the detection of common accelerants by 2 orders of magnitude over a static headspace technique. Air drawn through a Tenax-GC trap with a protable pump allowed monitoring of a fire scene atmosphere for trace levels of organic substances (*330*).

Recovery of accelerants by headspace and steam distillation was discussed by Kubler et al. (*331*). Extracting the water condensate collected from steam distillation with pentane was recommended. Computer-assisted programs for gas chromatographic analysis of accelerants and chromatogram interpretation have been suggested by a number of authors (*325*, *332–333*). Heated headspace-capillary GC has been employed to detect and identify gasoline in the brain, blood, lung, and gastric contents of a deceased individual. The interpretation of resultant gasoline chromatograms was discussed (*334*). Accelerants extracted from fire debris were also examined by NMR (*335*). However, two drawbacks to this approach are that NMR is less sensitive than GC and that separation of the accelerant from the burned debris is a necessary prerequisite to analysis.

Liquid gasoline samples were examined by capillary GC for comparison purposes (*336*). No attempt was made to discriminate between gasoline brands as they are known to vary from lot to lot. It was also demonstrated that different types of petroleum-based lubricants can be readily distinguished by capillary GC. This technique was used to distinguish ten commonly used automobile engine oils (*337*). Capillary GC was applied to the characterization of isoparaffinic solvents and was capable of distinguishing them from petroleum distillates (*338*). TLC was used to characterize gasoline dyes isolated from wood and soil samples. Subtle differences in the dye mixtures were noted between different brands of gasoline (*339*). A GC linked to an atomic absorption (AA) spectrophotometer (*340*) and an electron capture detector (*341*) was used to determine tetraalkyllead compounds present in gasolines. Generally, a significant variation in the lead alkyl content was noted between random gasoline samples. The electron capture method was used for the identification of samples collected from sewers, soil, and asphalt. Aldridge (*342*) has recommended the utilization of a preparative TLC procedure to separate and identify turpentine and gasoline mixtures.

Chemicals associated with the manufacture of PCP were detected after a simulated fire of a drug laboratory. Chemical residues were detected in debris and in air samples collected at the scene (*343*). Letini and Waters (*344*) cautioned about the interpretation of GC patterns obtained from debris containing roof shingles or tar paper. The presence of these materials may lead to a false conclusion regarding the presence of accelerants. Furthermore, the importance of receiving control samples from fire scenes, and the necessity of burning the control, especially when dealing with polymeric materials, was demonstrated (*345*). DeHaan (*346*) found polyester/polyolefin heat-sealable pouches to be convenient and adequate containers for storage of materials suspected of containing volatile accelerants.

The characterization of brake fluid mineral oils was undertaken by Lloyd (*347*). A column chromatography technique using silica gel was used to recover small quantities of mineral oil from automobile brake fluids. Rubber seals used in braking systems strongly absorb mineral oil which ultimately leads to their degradation and subsequent brake failure.

Explosives and Lachrymators. A systematic procedure for the identification of postexplosion samples of commercial blasting explosives was reported. The proposed scheme was based upon a detailed microscopic examination followed by chemical analysis of residues and solvent washings made on materials suspected of containing explosive residues. Chemical tests included color spot tests, GC, and TLC (*348*). Chrostowsti et al. (*349*) described the utilization of a color test for the detection of perchlorates in explosive residues. An OV-101 and OV-17 (1:1) GC column linked to an electron capture detector was recommended for the routine screening of volatile explosive samples by headspace analysis. Weak specimens required a preconcentration step using a silica gel adsorbent (*350*). Capillary GC used in combination with an electron capture detector (ECD) was found capable of detecting explosives at low picogram levels. The minimum detectable limits of nine explosives examined ranged from 1 pg for ethylene glycol dinitrate to 100 pg for octogen (*351*). Nitroglycerine absorbed into a poly(vinyl chloride) will survive for a considerable time whether the plastic is exposed to aeration or water. Recovery of the nitroglycerine by solvent extraction was found to be superior to a sorbent trapping technique (*352*).

HPLC in combination with an electron capture detector was applied to the detection and identification of explosive residues as was HPLC/GC/ECD. In the latter case, the GC oven was solely utilized for the vaporization of HPLC effluents with a small portion of the eluent being directed into the ECD of the GC (*353*). Likewise, HPLC used with a UV detector was found suitable for identifying common high-order explosives such as TNT, PETN, and RDX. Nitroglycerine present in gunpowder was also quantitatively determined by this HPLC technique (*354*). The detection limits for common explosives were significantly enhanced over HPLC/UV by linking an HPLC to an electrochemical detector (*355*). Another effective detector for the HPLC proved to be a direct interface to a negative ion chemical ionization (NICI) mass spectrometer. This interface was used for the analysis of commercial and military explosive mixtures (*356*). A liquid introduction interface allowed about 1% of the solvent/sample effluent into the ion source. The minimum detectable amount of TNT was 100 ng injected on column, or approximately 1 ng to the ion source. The combination of GC and MS also proved to be an effective approach for detecting explosives. Twelve explosives were analyzed by GC/MS with different ionization modes, i.e., electron impact and positive and negative chemical ionization. Samples were also introduced directly into the ion source with a solid probe (*357*). Zitrin (*358*) reported on the fragmentation pathways of RDX in a chemical ionization ion source using different reagent gases. The relationship of spectra to the strength of reagent gas ions was discussed. A study of collision-induced dissociation of RDX and HMX by mass spectrometry was reported. High-resolution mass spectra and mass-analyzed ion-kinetic energy/collision-induced dissociation spectra of RDX and HMX were recorded in the electron impact and positive and negative ion chemical ionization modes (*359*). The NMR characterization of trace levels of the impurity trinitroanisole was useful for furnishing information regarding the source and manufacturing process of the explosive trinitroaniline (*360*).

Gas chromatography/electron capture detection was found to be the most accurate and sensitive technique for analyzing hand swab extracts for the presence of nitroglycerine. The lowest limit of detection was 10 ng of nitroglycerine and residues could be detected over 20 h after handling the raw explosive (*361*). Ethanol appears to be the best organic solvent for removing nitroglycerine from hands with cotton swabs (*362*). Douse (*363*) suggested using a preliminary cleanup with Amberlite XAD-7 to remove interfering lipid material from the hand swab extracts. This was followed by detection of explosives in the concentrated extracts by capillary GC with electron capture detection.

A quick cleanup for tear gas formulations containing the lachrymators capsaicin, chloroacetophenone (CN), and o-chlorobenzalmalononitrile (CS) was reported. The active ingredients were isolated from the bulk product using a silica Sep-PAK cleanup. Determination of the lachrymators was achieved by reverse-phase HPLC (*364*). Nowicki (*365*) has reported analyzing various tear gas sprays using GC/MS in the electron-impact mode. Liquid sprays containing CS and

CN were readily extracted with methanol. A basic aqueous extraction procedure was recommended for sprays containing capsaicin. Another approach for identifying small quantities of capsicinoids in tear gas is reverse-phase HPLC followed by GC/MS on the trapped eluents. An alternative procedure of solvent extraction and preparative TLC followed by IR was recommended for large samples (*366*). Avdovich et al. (*367*) have reported MS, IR, and NMR data for CS.

Gunpowder and Primer Residue Detection. The development of reliable procedures aimed at characterizing and identifying gunpowder residues on the hands of suspect firers has been a perennial problem for forensic science laboratories and continues to occupy the efforts of researchers. A combination of neutron activation analysis (NAA) and atomic absorption (AA) analysis was found to be the most efficient method for the determination of trace quantities of antimony and barium on the hand swabs of firers. NAA was found to be superior to AA for antimony detection, while the sensitivity and accuracy of AA for barium was superior to that of NAA. Based on the results of hand blanks, minimum concentrations of 0.5 μg and 0.05 μg for barium and antimony, respectively, were estimated as being consistent with gunshot residue. If one element fell below its value or was not detected at all, the other had to be at the minimum level of 1.0 μg for barium and 0.1 μg for antimony for a positive finding (*368*). An automated AA procedure for the analysis of antimony, barium, and lead on cotton hand swabs was proposed. Not all weapons are found to emit primer residues, but on the average, hands swabbed after the discharge of a firearm show amounts of these elements that are greater than occupational levels (*369*). A photoluminescence technique for detection of lead and antimony in gunshot residue was evaluated. A significant number of suicide cases investigated exceeded the threshold levels that were set for both these elements (*370*). Also investigated for applicability to gunshot residue detection was a proton-induced X-ray emission technique. Studies of firearm discharge element profiles around bullet holes indicated that the firing distance could be estimated by this technique. Furthermore, studies of hand swabs from firers showed that the high sensitivity of this procedure (0.1–1 ng) can help screen out suspects who have not handled a weapon (*371*). Another approach examined was anodic stripping voltammetry. It was used to quantitatively detect lead and antimony deposits on the hand swabs of firers (*372*). Bratin et al. (*355*) were able to detect nitroglycerine and diphenylamine on the hand swabs of firers by utilizing an HPLC linked to an electrochemical detector. Primer residues recovered from ammunition manufactured in India was analyzed by NAA for their elemental content (*373*).

Gunshot residue derived from a large variety of weapons and ammunition were examined by X-ray diffraction and scanning electron microscopy-energy dispersive analysis (SEM-EDX). Lead, in its metallic form, was found to be the main constituent in all gunshot residues tested (*374*). A compact and inexpensive kit was devised for removing gunshot residue particles from the hands for laboratory examination by SEM (*375*). SEM-EDX analysis was used to substantiate the view that gunshot residue particles have a characteristic structure. In this study both the surfaces and cross sections of gunshot residue were examined for morphology and elemental distribution (*376*). By use of bone material fragmented by a bullet, EDX was successfully employed to identify lead and antimony deposits. X-ray mapping also determined the relative proportion of the lead and antimony present (*377*). SEM-EDX and AA were applied to detecting and characterizing primer residues around bullet holes in cotton cloth. Primer particles were also detected and characterized by SEM-EDX on the base of the recovered bullets (*378*).

Smokeless gunpowders may be characterized by means of color spot test reagents containing sulfuric acid. Sulfuric acid, alone or with modifying materials, will react with diphenylamine stabilizer to give a considerable degree of interclass variation among smokeless powders (*379*). A spectrophotometric technique was developed for quantitating nitrates present in extracted gunshot residue. The procedure was found to be sensitive enough to detect submicrogram quantities of nitrates (*380*).

Fibers and Hair. The prominent role fiber evidence played in the arrest and conviction of Wayne Williams in the Atlanta murder cases has served to focus public attention on the role and significance of fiber evidence in criminal investigations. While the general public may have been surprised by its importance, practitioners of forensic science have long recognized that fiber evidence is among the most frequently encountered contact trace evidence in criminal investigations. To illustrate this point, Home and Dudley (*381*) reported that 3836 items submitted to a forensic laboratory for examination during two periods of 2 months yielded 10034 different fibers. The use of adhesive tape to remove fibers transferred during contact between garments is a standard practice in many forensic laboratories. Grieve and Garger (*382*) discussed a procedure used for searching such tape strips. Likewise, Kidd and Robertson (*383*) studied the transfer of fibers to and from clothing typically encountered in criminal case situations. The persistence of fibers transferred from acrylic, wood, and polyester garments to a large range of recipients has been examined (*384*).

The microscope continues to be the most important analytical technique utilized for the forensic characterization of textile fibers. Fong (*385*) has proposed an analytical scheme for distinguishing common synthetic fibers by their refractive indices using a polarizing microscope equipped with a dispersion-staining objective. A wide range of mountants useful for the microscope examination of textile fibers were evaluated by Cook and Norton (*386*). In the authors' opinion XAM was the most suitable mountant tested. Interference microscopy was shown to be a feasible technique for classifying fibers. Differences between fibers were determined by measuring refractive indices with an interference microscope and entering the values into standort diagrams. Interference patterns also proved useful for differentiating some fiber products belonging to the same chemical class (*387*). Petraco has reported as a simple method for cross-sectioning hairs and fibers (*388*).

Fifteen different types of acrylic fibers were examined by small-angle light scattering (SALS) and SEM. Each fiber was found to have its own characteristic SALS pattern. SALS proved to be more sensitive than microscopy for differentiating fibers with subtle differences in surface topography arising out of exposure to different finishing treatments such as fiber softening (*389*). Similarly, SALS patterns provided a means for discriminating among different commericial polyester fibers. SALS was able to distinguish identical fiber types that had been subjected to different laundering practices or exposure to outdoor weather. However, the question still remains as to whether SALS can yield meaningful similarities among fibers from a single source (*390*).

Ten paired TLC solvent systems were evaluated for use in the comparison of extracted fiber dyes. The solvent systems were evaluated by correlation coefficients and scatter diagrams. Additionally, the resolving power of individual solvent systems was evaluated through their ability to resolve major and minor colored components in commercial dyes. The results of this study show that the proper selection of TLC solvent systems produces a tool of very high discrimination power for the analysis of dyed textile fibers (*391*). Beattie et al. (*392*) have described a simple procedure for the extraction and classification of dyes from 1 to 2 cm lengths of acetate and triacetate fibers. Similarly, TLC systems for the characterization of disperse, acidic and basic dyes extracted from polyester, nylon, and polyacrylonitrile fibers were evaluated and recommendations made regarding the selection of TLC systems for each dye class (*393*). Also, TLC solvent systems were recommended for direct and reactive dyes extracted from cellulosic fibers (*394*). Dyes extracted from single black wool fibers were characterized by TLC and used to discriminate a majority of the fibers examined. On the other hand, microscopy and microspectrophotometry did not yield satisfactory results (*395*). A two-dimensional TLC method was used to compare dyes extracted from purple-colored wool fibers. The technique was found to be a reliable screening procedure for fiber comparisons (*396*).

Chemical cleaning or laundering with mild detergents did not affect the microspectrophotometric characteristics of synthetic fibers and only slightly increased the total transparency of wool fibers. However, acetone-containing stain removers were found to alter the transparency of acetate fibers. The effect of soiling was also studied for its impact on the microspectrophotometric measurements of textile fibers (*397*). Both IR spectrophotometry (*398*) and X-ray diffraction (*399*) have been applied to the identification of single synthetic

fibers. The utility of solution viscometry for the analyses of short lengths of single fibers was examined and found to be unsuitable for fiber identification, but it was shown to be used to detect changes in single fibers that result from laundering or outdoor exposure (*400*).

Recent advances in enzyme typing of hair root sheath cells have increased the evidential value of hair examinations. However, the majority of hairs examined in forensic laboratories have no sheath cell material and attempts to individualize these hairs must rely on detailed microscopic examination. Robertson (*401*) has reviewed the microscopic aspects of forensic hair examination and discussed the objectivity of such an approach. Gaudette (*402*) and Barnett and Ogle (*403*) have presented conflicting views on the significance of probability estimates relating to human scalp and pubic comparisons arising out of microscopic examinations. A new method for hair identification based on electron microscope observation of the hair's medullary ultrastructure has been proposed (*404*). The ultrastructure morphology of the medulla is characteristic of genus, but does not possess sufficient variation for individual identification (*405*).

The grouping of EsD, PGM, and GLO I from hair root sheaths was shown to be a reliable forensic technique, with PGM found to be the most stable enzyme (*406*). Using starch gel electrophoresis, the enzymes PGM, adenylate kinase (AK), EsD, GLO I, 6-phosphogluconate dehydrogenase, glucose 6-phosphate dehydrogenase, and phosphohexose isomerase could be detected in fresh air sheath cells. Of these all but AK and EsD could be detected in 7-week old sheath cells (*407*). The relationship between the volume of the root sheath and PGM typability has been determined for both pubic and scalp hair (*408*). Successful typing of PGM from hair sheath cells was possible up to 4 months for hairs mounted in XAM. Petroleum ether was found to be the most suitable solvent for dissolving the mountant (*409*). Different methods of extracting hair proteins were tested as part of a program aimed at assessing the value of the electrophoresis of human hair keratin for hair discrimination (*410*). Also, a two-dimensional electrophoretic procedure has been developed which provides a high degree of discrimination between hair proteins (*411*).

The reliability of sexing hair by identifying Y-bodies in hair sheath cells was examined. Ninety percent of the male hairs examined showed a Y-cell index greater than 25%, whereas all hairs from females had a Y-cell index of less than 20% (*412*). In order to determine sex from plucked hair roots devoid of a root sheath, the frequency of the X-chromosome was examined in the nuclei of the hair cortex. Distinct differences were observed between male and female hair stored for 32 weeks in a dried condition (*413*). The question of using the presence or absence of hair sheath cells as a criterion for judging whether hair was forceably removed from the scalp has been examined by King et al. (*414*).

Phencyclidine (*415*), phenobarbital (*416*), cocaine (*417*), and morphine (*418*) have all been detected in hair utilizing radioimmunoassay. There appears to be a correlation between total drug content in the hair and the extent of drug use. The presence of these drugs in hair does provide added discriminating information for evaluating the significance of a hair comparison. The uptake of mercury, copper, and iodine by the hair shaft has been studied. Concentration profiles of these elements could assist in understanding elemental concentration variations in the hair shaft arising from diet and environmental exposure (*419*). A new development in X-ray fluorescence instrumentation that provides automatic longitudinal scanning for some 16 elements in consecutive hair segments was described (*420*). Young's modulus of human scalp hair was determined and found to offer little evidential value in forensic hair analysis (*421*). However, techniques useful for detecting cosmetic hair treatments may have merit for the forensic characterization of hair evidence. Sections of individual hairs have been stained and studied for chemical damage and bleaching. Also, acetone extracts of hair have been examined for lacquers and conditioners. Hair extracts have been examined for dye content (*422*).

Paint and Glass. Color is probably the single most important property of paint from the forensic viewpoint. The comparison of the color of a control and suspect paint is normally the first step in any forensic paint examination. A microspectrophotometer was shown to offer a precise, rapid, and convenient method for defining the color of small paint fragments or a layer within a layer sequence (*423*). The microspectrophotometer provided high discrimination between sets of household paint of the same color (*424*). Transmitted light microscopy was used to discriminate between paints of similar color on the basis of the pigments and extenders present. The following physical properties of pigments were considered: color, crystallinity, shape, size, and refractive index (*425*). The application of solvent tests to the analysis of paint pigments has been reported by Castle (*426*). Also, dichloromethane extracted organic paint pigments were examined by TLC. The technique provided good discrimination between similar color classes of paints (*427*). Two TLC systems have been identified that, when coupled with the reactions of chemical visualization agents, are capable of separating and distinguishing 18 inorganic elements commonly encountered in forensic paint pigments, driers, and metallic alloys (*428*).

Cardosi (*429*) has developed a pyrolysis gas chromatographic (PGC) system for comparing paint samples, and Sansom (*430*) discussed the utilization of PGC for vehicle and household paint analysis. Wheals (*431*) has reviewed the application of analytical pyrolysis techniques to paint and polymer analysis. A discussion of the applicability of pyrolysis-MS to paint comparisons recommended using select ions for classifying and comparing such materials. A statistical method for the comparison of similar IR spectra has been described and applied to paint resin mixtures (*432*). Curry et al. (*433*) have collected X-ray diffraction data on some 71 paint pigments. A rapid system for the identification of vehicles from paint samples was devised. The system entailed collecting microscopic and IR data for representative automotive undercoatings. This classification scheme was designed to narrow the possible sources of paint chips left at hit-and-run scenes (*434*). In an effort to define the statistical significance of a forensic paint comparison, data were compiled on the distribution of automotive topcoat paint colors based on a survey of approximately 45 000 vehicles (*435*). The forensic examination of paints has been reviewed by Raaschou Nielsen (*436*).

The values of density, refractive index, and dispersion for identifying the source of glass specimens were evaluated by Slater and Fong (*437*). Commerical batches of silicone oil were investigated for determining the refractive index of glass particles by an immersion technique. It was recommended that each new batch of commercial oil be carefully monitored and, if necessary, purified (*438*). Annealing of tempered glass was used to produce changes in the glasses' refractive index. This approach can be employed to distinguish tempered glass from other types of soda-line glass (*439*). Data were presented to show that the refractive index of the tin-containing surface of float glass was always greater than that of the bulk glass. The authors concluded that the measurment of the surface refractive index, in addition to the bulk refractive index, is a very useful parameter in the discrimination of float glass (*440*). Float glass fragments were differentiated from other glass surface fragments by their fluorescence excitation spectra. Considerable variation occurs between the fluorescence intensities of different samples of float glass and, to some extent, nonfloat glass (*441*). Computer simulations have been carried out to investigate the interpretation of glass refractive index measurements (*442*). An approach useful for the statistical interpretation of refractive index measurements has also been presented (*443*).

In determining the elemental composition of glass, the spectral sensitivities of different EDX spectrometers may vary to the extent that the same glass fragment will produce noticeably different spectra. To overcome this problem glass standards were prepared for the purpose of standardizing instrument response parameters. These glasses are regularly used to check the response of EDX spectrometers. If any significant changes are found, the new elemental ratios can be correlated to the original standard values (*444*). Quantitative levels of manganese, iron, magnesium, aluminum, and barium have been determined in glass by inductively coupled plasma spectrometry (*445*). A scheme for classifying glass on the basis of refractive index and the five elements was formulated by the application of multivariate statistical procedures (*446*).

Miscellaneous. The use of lasers for the development of latent fingerprints was first reported in 1977 and continues to receive attention by forensic science researchers. Herod

and Menzel (*447*) demonstrated that ninhydrin-treated latent fingerprints not discernible in the conventional way can show fluorescence in the red and near-infrared spectral regions when subjected to continuous-wave dye laser illumination at about 580 nm. A pronounced improvement in the laser detection of latent fingerprints was accomplished when ninhydrin-treated prints were sprayed with a solution of zinc chloride (*448*). Dalrymple (*449*) advocates using a narrow-band-pass filter to isolate laser-induced luminescence of fingerprint residues. Latent fingerprints on skin can be developed by dusting with a fluorescent powder or using evaporative staining with a fluorescent dye, followed by laser examination (*450*). Greer (*451*) discussed the application of an argon-ion laser to the examination of different types of physical evidence including fingerprints. Fingerprints on skin were visualized by fuming the suspected area with iodine (*452*). Almog et al. (*453*) have synthesized a number of ninhydrin analogues to study their effectiveness in developing latent fingerprints. The usefulness of a number of silver salts as alternatives to silver nitrate for the detection of fingerprints was evaluated (*454*).

TLC has been utilized for differentiating fluorescent brighteners and dyes present in white and colored papers. This approach proved valuable for identifying and comparing various types of papers encountered as forensic evidence (*455*). Ziederman (*456*, *457*) has proposed using inverse paper chromatography for comparing paper evidence. This technique uses the questioned paper as a chromatographic sorbent medium on which a known mixture of dyestuffs is separated under standard conditions. The resulting chromatogram designates the paper grade and composition. In addition, PGC was used to distinguish coated and noncoated papers. The method was successfully applied to discriminate papers similar in external appearance and base weight (*458*).

HPLC was applied to the analysis of ball point ink extracted off paper. This approach allowed detection of differences among the formations, as well as differences among batches of the same formulation (*459*). The application of TLC to ink analysis was discussed (*460*, *461*), and TLC was utilized to distinguish ink in carbon paper impressions (*462*).

The lead content of soil provided a useful parameter for comparisons of known and questioned soils (*463*). HPLC also appears to offer a promising forensic approach for comparing soils (*464*). Fourier transform infrared analysis has been successfully applied to the analysis of an automobile rubber bumper guard received from the scene of a hit-and-run (*465*). Additionally, rubber bumper guards were compared by first placing them in the injection port of a GC and separating the volatile components of the material. This was followed by pyrolysis of the sample. The results are a chromatogram of the volatile components and a pyrogram of the nonvolatile polymeric component (*466*). TLC was used to separate and compare the constituents of sealing wax (*467*). A combination of several techniques including, EDX, TLC, and HPLC were used to differentiate lipstick stains. The probability of finding two indistinguishable stains by the techniques employed proved to be less than 1 in 7000 (*468*). X-ray difraction was used to differentiate brasses. The cooper/zinc ratio within a single-phase brass could be estimated to ±1% (*469*). A 0.5%, 2-nitroso-1-naphthol solution was successfully utilized to detect trace metals on hands. A metal object held for approximately 1.5 min could be detected (*470*). NAA was employed to measure trace levels of antimony, silver, cooper, and arsenic in bullet lead for comparison purposes. Several case examples demonstrating the applicability of this technique were described (*471*).

Lloyd and Weston (*472*) described a synchronous fluorescence procedure for the detection of fecal traces. Spectra varied considerably between different samples of human feces and between different mammals. Organic compounds that phosphorese when absorbed on cellulose were used to mark cellulose-containing fiber and papers for identification purposes (*473*). The application of SEM to the examination of light filaments for the determination of whether the filament was on or off at the moment of an accident was reported by Thorson (*474*).

BOOKS

There have been some noteworthy books published on forensic science topics since 1980. For the reader looking for an introductory overview of the forensic sciences, "Criminalistics" by Saferstein (*475*), "Introduction to Forensic Sciences" edited by Eckert (*476*), and "Chemical Criminalistics" by Maehly and Stromberg (*477*) are recommended readings. A more in-depth treatment of many of the topics covered in this review is presented in "Forensic Science Handbook" edited by Saferstein (*478*). "Modern Legal Medicine, Psychiatry and Forensic Science" edited by Curran, McGarry, and Petty (*479*) also provides comprehensive coverage of subjects pertinent to forensic science. Svensson's et al. (*480*) classic text on crime scene investigation has been revised, as has Hilton's (*481*) excellent treatment of document examination. In the area of toxicology and drug analysis "Introduction to Forensic Toxicology" edited by Cravey and Baselt (*482*) provide well-written chapters for both the student and practitioner of forensic toxicology. A must reference for practicing toxicologists is "Disposition of Toxic Drugs and Chemicals in Man" (*483*). Also, Gottchalk and Cravey (*484*) have collected invaluable data for interpretating the significance of pyschoactive drugs that may be present in biological fluids and tissues. The Proceedings of the Eighth International Conference on Alcohol, Drugs and Traffic Safety contains many significant papers on various aspects of blood and breath testing for alcohol (*485*). "The Measurement of Breath Alcohol" by Emerson et al. (*486*) is an in-depth review of state-of-the-art breath testing devices. A comprehensive bibliography of publications on forensic aspects of ethanol has been compiled by Holleyhead (*487*). Finally, two treatments relating to explosion investigation and analysis have recently appeared. Yinon and Zitrin's (*488*) "The Analysis of Explosives" offers in-depth review chapters on analytical chemical techniques suitable for characterizing explosives and their residues. On the other hand, Yallop's (*489*) "Explosive Investigations" is a more general treatment of explosives covering their properties, field investigation techniques, and laboratory analyses. Procedures for the phenotyping of over 25 genetically controlled polymorphic enzyme, protein and antigen systems was published by Grünbaum (*490*). This book also presents a statistical interpretation of the data.

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Solid and Gaseous Fuels

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SOLID FUELS

This section covers methods of sampling, analyzing, and testing coal, coke, and related materials. *Energy Research Abstracts* and *Chemical Abstracts* were used as the primary reference sources. In most categories the volume of material available made it necessary to limit the number of publications in the review.

SAMPLING AND PROXIMATE ANALYSIS

Sampling. The theory and practice of coal sampling and sampling preparation were reviewed by Scholz (*25A*). Graham (*10A*) discussed quality control considerations in coal sampling techniques for laboratory analysis.

Nir-El et al. (*20A*) assembled a new set of 100-kg coal standards for use with prompt neutron activation analysis (NAA) field systems. The standards were calibrated by using the American Society for Testing and Materials (ASTM) methods.

Proximate Analysis. Rapid proximate analysis of coal and coke using thermogravimetry was discussed by Ottaway (*22A*). Althapp et al. (*1A*) described equipment for thermogravimetric analysis of coal along with the working principles and various applications. Cardillo (*5A*), Cumming (*6A*), and Ghetti (*8A*) compared standard ASTM methods for proximate analysis with thermogravimetric analysis techniques. Recent advances in microcomputer-controlled thermogravimetry of coal for proximate analysis were examined by Earnest and Fyans (*7A*).

Jenke and Hannifan (*14A*) determined moisture and volatile content of coal by measuring the pressure change and chromatographically analyzing the gas produced in a constant temperature–volume reactor.

Simultaneous determination of moisture and ash content of coal using γ-emission following thermal neutron capture was demonstrated by Starchik et al. (*28A*).

An apparatus for the determination of the moisture content of coal on a main conveyor using an infrared sensor was described by Kawatetsu (*15A*).

Brown et al. (*4A*) discussed the use of various electromagnetic techniques, including capacitance, microwave attenuation, and nuclear magnetic resonance in determining moisture in coal.