FORENSIC CHEMISTRY

1. Introduction

Forensic chemistry can be defined as the application of chemistry to the law. In American jurisprudence, courts, and judges are established to make factual determinations of matters brought before them. The fact-finding of the courts must often grapple with complex scientific issues and the legal system (1) has a particular way to deal with these technical and scientific matters. The court calls on subject matter experts (2), ie, expert witnesses, to explain and to interpret for the triers of fact the meaning of the scientific concept underlying a case and how it may be interpreted. To testify as an expert witness in a particular field or area of endeavor, the individual must qualify as an expert in a specific area, ie, have special knowledge, skill, training experience, or education. Courts have used subject matter experts for generations (3). The judge sitting on the particular case makes the determination whether an individual is qualified to give expert testimony.

Forensic science is an applied science having a focus on practical scientific issues that come up during criminal investigations or at trial. Some components are unique to the field because it is conducted within the legal arena. Forensic science issues in chemistry and biochemistry in criminal investigations are discussed herein. There are a host of other forensic science areas, eg, forensic medicine, forensic dentistry, forensic anthropology, forensic psychiatry, and forensic engineering, any of which may overlap with forensic chemistry.

2. Physical Evidence

Forensic scientists work with physical evidence, ie, "data presented to a court or jury in proof of the facts in issue and which may include the testimony of witnesses, records, documents or objects". Physical evidence is real or tangible and can literally include almost anything, eg, the transient scent of perfume on the clothing of an assault victim; the metabolite of a drug detected in the urine of an individual in a driving-under-the-influence-of-drugs case; the scene of an explosion; or bullets removed from a murder victim's body.

The courts are the ultimate consumer of the forensic scientist's information. Before expert testimony may be presented to a trier of fact (judge or jury), a legal determination must be made whether the information can be presented. Courts understand that lay juries may place undue reliance on experts and technology. To guard against this possibility, the court employs certain safeguards. Many jurisdictions use the Frye rule (4). In this landmark 1923 case, the Court of Appeals for the District of Columbia considered whether or not the results of a polygraph test were admissible and stated a general rule that courts go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery. However, the theory from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field to which it belongs. The polygraph test was not generally accepted for the detection of deception in the fields of psychology or physiology; consequently, the results of this test were ruled inadmissible. The Frye rule became widely accepted in state and federal courts. In 1993, the U.S. Supreme Court (5), changed the admissibility standard in federal cases, holding that scientific, technical, or other specialized evidence is admissible if it assists the trier of fact to understand the evidence. This results in a lower threshold for admissibility than general acceptance required by many state courts. The Supreme Court indicated that judges should also look at whether the scientific evidence is based on a theory or hypothesis that (*1*) can be tested, (*2*) has been peer-reviewed and published, (*3*) has a known or potential error rate, and (*4*) has become generally accepted in its field. Subsequent Supreme Court decisions (6,7) have extended that application of these guidelines to fields such as engineering.

Examination of physical evidence provides two subtle and different types of conclusion as may be illustrated by the following examples. Consider a hit-and-run case involving an automobile (8) and a decedent. An examination of the victim's clothing turns up some blue paint. A suspect vehicle is located; the vehicle is blue. Infrared (ir) spectroscopy of the surface, solubility tests in various solvents, and microscopic examination of cross-sections demonstrate that the

composition of the paint from the vehicle and from paint samples recovered on the victim's clothing are identical (9). A laboratory report stating that the two specimens are identical is likely to prejudice a jury into concluding that because the paints are identical it was the defendant's car that hit the pedestrian. A more carefully worded laboratory report would explain that the samples are identical and that the paint could have come from the car in question, or any other similarly painted car. Many automobile manufacturers use the same blend of paint on thousands, and likely hundreds of thousands of vehicles. No matter how much testing is done, the results are the same: The samples are indistinguishable. The tests conducted on the paint have provided class or group characteristics. All members of a class or group have identical characteristics. Types of physical evidence that exhibit class characteristics are paint (qv), glass (qv), fibers (qv), fabric, building material, etc. This type of physical evidence is said to be identified. The best that chemical and physical examinations can ever do is to place items into groups of similarly manufactured items. It is not possible to differentiate one item of evidence as being uniquely distinguishable from another.

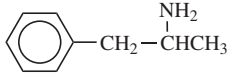
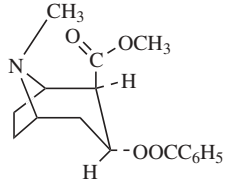
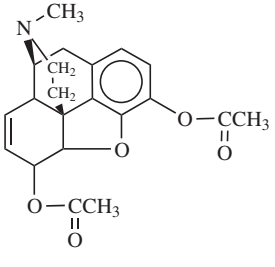
Some types of physical evidence, because of the manner in which the material is made, are unique; such evidence can be individualized. Examination can show an item of individualized evidence is unique and comes from one, and only one source. The classic example is fingerprints. No two individuals, even identical twins, have the same fingerprints. An examiner's report stating that a suspect's fingerprints are identical to latent fingerprints discovered on a weapon would prove, without doubt, that the suspect held the gun. Other categories of evidence exhibiting individualization are handwriting, markings on bullets fired from the same gun, and broken pieces of glass or plastic that can be physically fit together again. In cases involving these types of evidence, the forensic examiner can state that the physical evidence came from a single source, to the exclusion of all others (10).

Historically, physical evidence has taken on increasing importance in criminal matters. Courts have often looked askance at a defendant's admissions of guilt and even on occasion questioned eyewitness testimony. Physical evidence has traditionally been viewed as impartial and unbiased, and not subject to the problems associated with confessions made by an accused or the testimony of witnesses.

Physical evidence serves three purposes. In some cases, it is used to prove a component or element of a crime. For example, in a case involving trafficking in cocaine [50-36-2], the prosecutor must prove that the white powder found in the criminal's possession was cocaine (Table 1). The forensic chemist tests the substance and issues a report. If the powder is methamphetamine [537-46-2], the charge must be amended.

Physical evidence is also used is to develop associative evidence in a case. Physical evidence may help to prove a victim or suspect was at a specific location, or that the two came in contact with one another. In one case, building material debris (wooden splinters, tar paper, insulation material) was found on a blanket used to wrap a body that was found dumped at the side of a road. The evidence suggested an attic and eventually led detectives to the location where the murder occurred. Finally, physical evidence may be used to reconstruct the events that took place during the commission of a crime. For example, at the scene of

Table 1. **Commonly Encountered Drugs of Abuse**

Common name	CAS Registry number	Chemical name	Molecular formula	Structure
amphetamine	[300-62-9]	α-methylbenzeneethanamine	$C_9H_{13}N$	
cocaine	[50-36-2]	{1*R*-(exo,exo)}-3-(benzoyloxy)-8-methyl-8-azabicyclo{3.2.1} ocatane-2-carboxylic acid methyl ester	$C_{17}H_{21}NO_4$	
heroin	[561-27-3]	7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6,α-diol diacetate	$C_{21}H_{23}NO_5$	

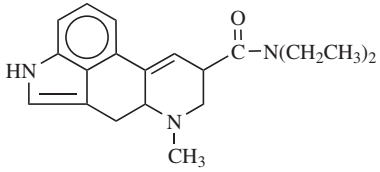
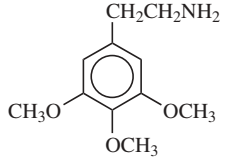
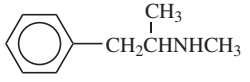
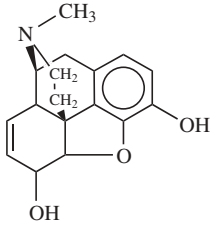
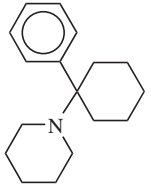
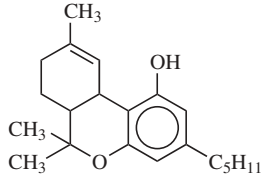
lysergic acid diethylamide (LSD)	[50-37-3]	9,10-didehydro-*N*,*N*-diethyl-6-methylergoline-8β-carboxamide	$C_{20}H_{25}N_3O$	
mescaline	[54-04-6]	3,4,5-trimethoxybenzeneethanamine	$C_{11}H_{17}NO_3$	
methamphetamine	[537-46-2]	*N*,α-dimethylbenzeneethanamine	$C_{10}H_{15}N$	
morphine	[57-27-2]	7,8-didehydro-4,5-epoxy-17-methylmorphanin-3,6-diol	$C_{17}H_{19}NO_3$	

Table 1 (*Continued*)

Common name	CAS Registry number	Chemical name	Molecular formula	Structure
phencyclidine (PCP)	[77-10-1]	1-(1-phenylcyclohexyl)piperdine	$C_{17}H_{25}N$	
Δ-9-tetrahydrocannabinol (Δ-9-THC)[a]		tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-debenzo{*b*,*d*}pyran-1-ol	$C_{21}H_{30}O_2$	

[a]The active ingredient in marijuana and hashish.

a multiple murder bloodtyping of dried bloodstains found in various rooms indicated that two of the victim's bodies had been moved from where they had been attacked. The movement of the bodies was part of a attempt on the part of the murderer to make it appear that the victims had been killed by intruders.

Most of the forensic science or crime laboratories located in North America are associated with law enforcement agencies, medical examiner–coroner departments, or prosecutors' offices. There are a small number of private laboratories that provide forensic testing in specialized areas such a DNA profiling, forensic toxicology and fire debris analysis. There are a large number of independent consultants, also. Laboratories exist at the municipal, county, state, and federal levels of government. There are ~300 government-operated forensic science laboratories in the United States. Many of the government-operated and private forensic science laboratories are now accredited by the American Society of Crime Laboratory Directors–Laboratory Accreditation Board (ASCD–LAB).

Forensic science laboratories are generally divided into separate specialty areas. These typically include forensic toxicology, solid-dose drug testing, forensic biology, trace evidence analysis, firearms and tool mark examination, questioned documents examination, and latent fingerprint examination. Laboratories principally employ chemists, biochemists, and biologists at various degree levels. In some specialty areas, eg, firearms examination, questioned documents, and fingerprint examination, experts may not have an academic degree. Some laboratories employ examiners that specialize only in one area, whereas other laboratories maintain a generalist philosophy, rotating examiners through several forensic science disciplines during a practitioner's career. The generalist approach has become difficult to impliment as more and more specialized education is required for one to become a forensic analyst or examiner in certain areas. For example, under the educational guidelines of the DNA Advisory Board DNA analysts are expected to have had college level course work in biochemistry, molecular biology, and genetics. Forensic analysts and examiners may be certified by organizations such as the American Board of Criminalistics. Certification generally requires passing a general knowledge examination about forensic science.

The bulk of the scientific testing in crime laboratories involves the analysis and characterization of either synthetic or biochemical organic substances or both. Additionally, there are a number of evidence categories classified as inorganic. Most assays are simply qualitative and designed to answer the questions: what is it, where did it come from, and could it have come from a specific source? Quantitative analyses may also be carried out on samples involving drug evidence, toxicology evidence, and blood alcohol testing, wherever such information has probative value to the investigation or to the court in its deliberation.

3. Forensic Testing

3.1. Toxicology. Psychoactive substances, illicit and ethical (licit) drugs and alcohol (ethanol), are the greatest source of physical evidence analyzed in state and local crime laboratories (see PSYCHOPHARMACOLOGICAL AGENTS). Drug testing falls into two categories: solid dose samples and toxicology (qv) related

cases, eg, blood, urine, or tissue specimens in postmortem cases or cases involving driving under the influence of alcohol or drugs, as well as workplace or employee drug testing.

3.2. Alcohol. The number of driving under the influence of alcohol (DUI) cases reflects the enormity of the drunken driving problem in the United States (11). Tests to measure blood alcohol concentration are conducted on blood, urine, or breath (12). In the case of urine and breath, the alcohol concentration measured is reported in terms of the equivalent blood alcohol concentration. Most states in the United States presume that a person is under the influence of alcohol with respect to driving a motor vehicle at a blood alcohol concentration of 0.10%, ie, an ethanol concentration of 10 g/100 mL of blood. Some states have set a lower concentration of 0.08%. In some European countries levels are as low as 0.05%. A blood alcohol concentration of 0.10% in a 68-kg (150-lb) person is the equivalent to the alcohol in about four drinks of 80 proof alcoholic beverage or four 340-g (12-oz) beers (see Beer; Beverage spirits, distilled; Wine). Ethanol is metabolized at a rate equivalent to about one drink per hour (13).

Blood and urine are most often analyzed for alcohol by headspace gas chromatography (gc) (qv) using an internal standard, eg, 1-propanol. Assays are straightforward and lend themselves to automation (see AUTOMATED INSTRUMENTATION). Urine samples are collected as a voided specimen, ie, subjects must void their bladders, wait ~20 min, and then provide the urine sample. Voided urine samples provide the most accurate determination of blood alcohol concentrations. Voided urine alcohol concentrations are divided by a factor of 1.3 to determine the equivalent blood alcohol concentration. The 1.3 value is used because urine contains one-third more water than blood and, at equilibrium, there is about one-third more alcohol in the urine as in the blood.

Breath alcohol testing is accomplished by a number of techniques. The oldest reliable procedure involves bubbling a measured volume of deep-lung air containing alcohol through an acidic solution of potassium dichromate, $K_2Cr_2O_7$. Deep-lung air is the last portion of expired breath. It is collected in breath alcohol testing to ensure that the alcohol concentration in the breath is in equilibrium with the alcohol in the alveolar blood supply. Products from a simple oxidation–reduction reaction forming Cr^{3+} are measured photometrically. The amount of Cr^{3+} formed is directly proportional to the alcohol concentration of the breath which is in turn proportional to the blood alcohol concentration. Newer instruments rely on infrared spectroscopy to measure the blood alcohol concentration in breath. A fixed quantity of breath is captured and the alcohol concentration measured by determining the absorbance at the C–H stretch of ethanol (14) (see INFRARED AND RAMAN SPECTROSCOPY, INFRARED TECHNOLOGY).

3.3. Other Substances. Driving under the influence of alcohol cases are complicated because people sometimes consume alcohol with other substances (15–17). The most common illicit substances taken with alcohol are marijuana and cocaine (see Table 1) (18). In combination with alcohol, some drugs have an additive effect. When a blood or urine sample is tested for alcohol and the result is well below the legal concentration threshold yet the arresting officers observed that the subject was stuporous, further toxicological tests for the possible presence of drugs are indicated.

Forensic toxicology laboratories having large caseloads rely on immunoassay (qv) techniques to screen specimens. Immunoassay technology involves the manufacture of antibodies that are specific to particular drugs or to a class of drugs. For example, morphine (Table 1) can be chemically bound to a protein and injected into a host animal, such as a goat. After several weeks the animal is bled and antibodies for morphine and related drugs can be isolated and purified (19).

There are several immunological techniques in use. In these tests, antibodies combine with the drug or drug metabolites present in blood or urine, in competition with a labeled drug or metabolite that is in the reaction mixture. Radioimmunoassay (RIA) uses reagents tagged with radioactive isotopes such as ^{125}I (20); enzyme multiplied immunological technique (EMIT) employs an enzyme label (21,22); and fluorescence polarization immunoassay (FPIA) uses drugs or drug metabolites labeled with fluorescein (23). A competing reaction between tagged and untagged drug or drug metabolite molecules provides a semiquantitative result by allowing the determination of the amount of unbound radioactively labeled reagent (RIA), the extent of an enzyme-catalyzed chromogenic reaction as determined colorometrically (EMIT) or the degree of fluorescence polarization (FPIA). These values are related to the concentration of the drug. These procedures yield results in a matter of minutes and are easily automated. However, one drawback is that there is cross-reactivity between similar drugs. For example, most of the opiate alkaloids (qv) cross-react with the morphine antibody to some extent. Thus, using this test alone, it would be impossible to differentiate between codeine [76-57-3] and morphine. As a result, immunoassay procedures are best used as screening techniques and their results must be confirmed by other, more selective analytical procedures.

Thin-layer chromatography (tlc) (24) is frequently used. The procedure allows rapid screening for most drugs of abuse using simple, inexpensive technology. One drawback to tlc, however, is that the technique has a high detection limit and low levels of drugs may be missed. Another drawback is that a single tlc separation does not suffice for an identification of an unknown drug or drug metabolite. In tlc identifications are made through the comparisons of the retardation factors (or Rf values) of the unknown substance and a standard. The Rf value of a substance is the ratio of the distance it moves from the location where it was spotted on the tlc plate to the distance the mobile phase moves. Only 100 Rf values can be determined, which means that many of the millions of organic compounds known must have the same Rf values. The discriminating power of tlc can be improved through the use of chromogenic visualization reactions. The developed tlc plate is sprayed with a reagent that reacts with a certain drug or class of drugs to produce a specific color. The confidence with which drug identifications can be made using tlc may also be increased by employing two tlc separations: one with a normal phase tlc plate and a mixture of organic solvents as mobile phase and the other with a reversed phase tlc plate (ie, a tlc plate coated with silica gel modified with aliphatic or aromatic groups) and a mixture of water and alcohols as mobile phase (25,26). If the Rf values of the unknown drug and a drug standard match in both separations it is highly probable that the unknown drug and the standard are the same.

Gas chromatography and gas chromatography–mass spectroscopy (gc/ms) are the most common analytical procedures used in modern forensic toxicology

laboratories (27–29) (see Analytical methods, hyphenated instruments). Drugs are separated from their biological matrices, ie, blood, urine, and liver, by liquid–liquid or solid-phase extraction (qv) using the distribution of the suspect drug between an acid or alkaline aqueous solution and an immiscible organic phase (30–33). As quantitative analysis is often required, an internal standard is added to the assay at the beginning. For gas chromatography analysis, a chemically similar compound is used, whereas for gc/ms analysis, a deuterated version of the questioned drug is often added to the specimen as an internal standard (34–36).

The interpretation of forensic toxicology (37) results is often challenging. Courts frequently ask if an amount of drug detected in a specimen could cause a specific type of behavior, ie, would someone be under the influence of a drug at a specific concentration, would a particular drug concentration cause diminished capacity, or was the drug the cause of death? In a random employee drug testing case, a worker screened positive for opiates by EMIT and gc/ms analysis of the urine specimen showed low levels of morphine. Although one possibility was that the individual was a heroin user, a review of foods eaten in the prior 24 h suggested a more innocent cause: a poppy-seed bagel.

3.4. Solid-Dose Narcotics and Dangerous Drugs. Solid-dose drug testing (38) differs from forensic toxicology in that the solid form of the drug is tested, rather than a biological specimen containing the drug and its metabolite. The typical drugs of abuse (Table 1) in North America are heroin; cocaine, ie, free-base, crack, and the HCl salt; marijuana; hashish, a concentrated form of marijuana; amphetamine; methamphetamine; phencyclidine; and LSD. There are also many other illicit and legitimate pharmaceuticals (qv) (39) that find their way into the illegal drug market and thus must be analyzed in forensic science laboratories.

Forensic science laboratories may have different missions and therefore conduct different types of testing on samples (40,41). For example, the United States Department of Justice, Drug Enforcement Administration (DEA) forensic laboratories assist authorities in criminal intelligence-gathering efforts. As such, DEA chemists routinely analyze both the illicit drug and excipient, the material used in the cutting or diluting of the pure drug, in a given specimen. The excipient may provide information as to where the sample was produced.

Local and state forensic laboratories generally do not engage in excipient testing. Most provide qualitative and quantitative analysis of the evidence to determine if an illegal substance is present and if so, the amount of the drug present. The quantity of drug seized by the authorities may be important in jurisdictions that give enhanced sentences for larger amounts of the pure drug, or in some cases the total weight of the drug and diluent in possession of the defendant.

The large numbers of drug trafficking arrests made by police agencies and the resulting high volume of cases submitted to most crime laboratories make rapid analytical schemes the norm. Laboratories usually rely on a combination of screening tests followed by instrumental-based confirmatory analyses. Functional group color spot tests (42–44) are followed by microcrystalline tests (45) and then often a combination of tlc, gc, gc/ms, and Fourier transform ir spectroscopy for further identity confirmation (46–49). Microcrystalline tests are unique

to drug testing in forensic science laboratories. These tests involve adding a drop of a solution of a heavy-metal salt, eg, gold or platinum, to a few milligrams of the drug and observing the formation of characteristic crystals under the microscope. Microcrystalline tests are highly specific and very slight structural changes within a family of drugs can easily be detected.

The most common illicit drugs in the United States today are heroin, cocaine, marijuana, hashish, phenyclidine, LSD, and methamphetamine. These make up at least 90% of the total drugs seized.

3.5. Trace Evidence. Trace evidence (50) refers to minute, sometimes microscopic material found during the examination of a crime scene or a victim's or suspect's clothing (see TRACE AND RESIDUE ANALYSIS). Trace evidence often helps police investigators (51) develop connections between suspect and victim and the crime scene. The theory behind trace evidence was first articulated by a French forensic scientist: the Locard Exchange Principle notes that it is not possible to enter a location, such as a room, without changing the environment. An individual brings trace materials into the area and takes trace materials away. The challenge to the forensic scientist is to locate, collect, preserve, and characterize the trace evidence.

Searching a crime scene is a complex process (52), involving police, crime scene technicians, and forensic scientists. The procedure requires careful documentation, collection, and preservation of the evidence. Trace evidence (53) in criminal investigations may consist of hairs (54,55); both natural and synthetic fibers (qv) (56,57), fabrics; glass (qv) (58,59); plastics (60); soil; plant material; building material such as cement (qv), paint (qv), stucco, and wood (qv) (61), flammable fluid residues (62,63), eg, in arson investigations; and explosive residues, eg, from bombings (64,65) (see EXPLOSIVES AND PROPELLENTS).

Perhaps the simplest examination done is the physical match. A small fragment of glass, wood, plastic, or other material is recovered and fitted into a large piece found on the suspect or at the scene of the crime (66). Other examinations result only in demonstrating class characteristics (67). Such information may be used in a prosecution as circumstantial evidence in a trial. However, it is important that the forensic scientist neither inflate nor minimize (68,69) the significance of matching class characteristics.

Microscopy (qv) plays a key role in examining trace evidence owing to the small size of the evidence and a desire to use nondestructive testing (qv) techniques whenever possible. Polarizing light microscopy (70,71) is a method of choice for man-made textile fibers and crystalline materials such as minerals. Microscopy and microchemical analysis techniques (72,73) work well on small samples. They are relatively nondestructive and fast; in the hands of an experienced microanalyst they can be highly discriminating. Evidence such as soil, minerals, synthetic fibers, explosive debris, foodstuff, cosmetics (qv), and the like, lend themselves to this technique as as well as to comparison microscopy, refractive index, and density comparisons with known specimens. Other microscopic procedures involving ir, vis, and ultraviolet (uv) spectroscopy (qv) also are used to examine many types of trace evidence.

More traditional analytical techniques (74) also are used. Capillary column gc is the method of choice for characterizing flammable fluid residues (75) in arson cases. Trace residues may be collected by heated headspace techniques

or absorption–deabsorption of the residue from an appropriate solid matrix such as activated charcoal strips. The challenge in arson cases is interpreting the resulting chromatograms. Flammables subjected to high temperatures or weathering, eg, exposure to the elements over a period of time, appear significantly different from a sample of the original flammable fluid. It is also important to consider the effects of high heat and subsequent distillation on the arson scene's components such as carpeting, paints, wood products, various foams, etc. These can sometimes be confused with flammable residues (76). To reduce the interference of pyrolysis products from materials present at the fire scene some forensic laboratories are beginning to use gc/ms (77–79). If target compound analysis is used certain compounds commonly found in flammable liquids (eg, alkylbenzenes) are identified in the suspected arson accelerant residue using retention time and mass spectral data. Extracted ion chromatograms can be used to categorize flammable liquid residues (as a gasoline, a little petroleum distillate, etc). Computer software is used to generate chromatograms for clusters of ions that are abundant in the mass spectra of classes of compounds commonly found in flammable liquids (aliphatics, olefins, aromatics, etc).

Scanning electron microscopy (sem) and energy dispersive X-ray analysis (edx) are used frequently in gunshot residue examination (80–82) and to characterize evidence of an inorganic origin. When a gun is fired, gases from the explosion of the primer condense to form particles that settle on the shooter's hands (83). These particles may also be found on the shooter's face and hair. If the primer contains compounds of lead, antimony and barium these elements will be detected in the spherical primer residue particles. Lead-free primers have begun to replace lead-based primers (84,85). Some residue particles produced by lead-free primers are virtually indistinguishable in morphology and elemental composition from residue particles produced by fireworks. Collecting these residues and examining them by sem/edx is a straightforward way to determine whether someone recently fired a firearm. Sem/edx is also widely used in the comparison of the paint layers in paint chips.

Pattern recognition examinations are important in footwear (86) and tire impression cases. Often, tire impressions and shoeprints (87) are left at crime scenes and forensic scientists are asked to compare the impressions with shoes or tires. Interpretation of such evidence requires an understanding of the manufacturing process (88), a critical study of the large variety of different patterns, and experience in the way these items wear. One concern in footwear examination is whether a feature on a shoe sole is a wear mark, a mark unique to that shoe alone, or simply a defect caused during the manufacturing process. For example, if an injection molding process is used, tiny imperfections in a sole may be found on each and every shoe sole and not be a unique mark imperfection. Thus it is critical for the examiner to have a clear appreciation of the manufacturing processes involved. Three-dimensional tire and shoe impressions may be preserved by casting with dental stone, a highly refined plaster of paris containing a binder. Dental stone casts preserve minute details of the impression better than ordinary plaster of paris. Three-dimensional impressions in snow require special treatment: The heat released by the setting of the dental stone can melt such impressions. Impressions in snow may be sprayed with Snow-Wax, which consists of paraffin wax in a volatile vehicle. After the wax has

hardened dental stone or plaster of paris may be poured into the impression. The impressions should be carefully photographed before casting is attempted.

Two-dimensional impressions are of two types: positive impressions and negative impressions. Positive impressions are produced when dust, grease, blood, paint or some other material is transferred to a surface; negative impressions result when a shoe, tire, or object picks up material from a coating (eg, dust) on a surface. Positive and negative impressions in dust may be lifted with an electrostatic dust lifter, with adhesive or gel lifters or with fingerprint lifting tape. Before lifting is attempted two-dimensional impressions should be carefully photographed. These impressions may be treated chemically to icrease the contrast between the impressions and their background. Two-dimensional impressions in soil may be enhanced with ammonium pyrrolidonedithiocarbamate, ammonium thiocyanate, β, β′-dipyrridyl, potassium ferrocyanide, potassium thiocyante, Alizarin red S, Arsenazo III, 8-hyrdoxyquinoline, or bromphenol blue. Most of these reagents produce colored products with iron in the soil. Impressions in oil or grease can be enhanced by iodine fuming or with small particle reagent.

Chemistry has also become an important tool for the development of latent fingerprints. There are three types of fingerprints: plastic prints, patent (or visible) prints, and latent fingerprints. Plastic prints are three-dimensional impressions in a soft, plastic material such as glazing compound. Patent fingerprints are made when the finger transfers blood, paint, or ink to the surface touched. Plastic and patent fingerprints are usually preserved for later comparison with known fingerprints by means of scaled photographs. Latent fingerprints are made when the finger deposits fingerprint residue on a surface. Latent fingerprints, as their name suggests, are difficult to see or photograph. Fingerprint residue consists of mainly of water with 1 to 2% solids. The inorganic conponent of these solids consists of sodium, potassium and calcium cations and chloride, phosphate, carbonate, and sulfate anions. The organic fraction of the solids consists of lactic acid, a variety of long-chain fatty acids, glucose and other sugars, ammonia, urea, creatinine, amino acids, proteins, riboflavin, and pyridoxin. The oldest chemical method for the development of latent fingerprints is the silver nitrate method: Latent fingerprints on porous surfaces such as paper or cardboard are treated with a dilute aqueous solution of silver nitrate; chloride ions in the fingerprint residue react with silver ions to form insoluble silver chloride; exposure of the print to uv light or photographic developer reduces the silver ions in the silver chloride to metallic silver, producing a dark brown fingerprint. Ninhydrin will react with the proteins and amino acids in fingerprint residue to form Ruhemann's purple. The resulting pinkish purple fingerprint may not have sufficient contrast with its background to render it visible or photographable. Ruhemann's purple is capable of forming complexes with metal ions. If ninhydrin-developed latent fingerprints are treated with solutions of the chloride, iodide or nitrate salts of zinc, cadmium, or mercury the resulting metal complexes will fluoresce at liquid nitrogen temperatures. Ninhydrin analogues such as benzoninhydrin and 5-methoxyninhydrin may also be used to develop latent fingerprints. DFO (1,8)-diaza-9-fluorenone) forms a fluorescent compound with the proteins in fingerprint residue (89). It was accidently discovered that latent fingerprints on nonporous surfaces (eg, metal or glass) could be developed with

cyanoacrylate adhesives. Cyanoacrylate vapors condense on the fingerprint residue and residual moisture in the residue promotes polymerization of the cyanoacrylate. If the fingerprints are on a white surface they may be treated with stains such as gentian violet, rhodamine 6G, BBD (4-benylamino-7-nitrobenzofuran), or MBD [4-(4-methoxybenzylamino)-7- nitrobenzofuran].

Lasers (qv) and other high intensity or alternative light sources are useful in crime laboratories to visualize latent fingerprints, seminal fluid stains, obliterated writings, and erasures, and to aid in specialized photographic work. Infrared and uv light sources are also used to view items of evidence.

3.6. Forensic Biology. Stains of blood and other body fluids can be powerful physical evidence in crimes against the person (eg, homicide and rape). Blood is mixture of cells (red blood cells and white blood cells), cell fragments (platelets), proteins (including a number of enzymes), inorganic salts, and water. The process for the forensic testing of suspected bloodstains proceeds as follows: (*1*) the suspected stain is identified as blood; (*2*) the species of animal from which the blood came is determined; and (*3*) DNA is extracted from the stain and profiled.

Chemical tests are relied upon for the identification of bloodstains. Presumptive tests are first applied to the suspected stain. Presumptive tests have low detection limits but lack complete specificity; they are also simple, quick, and economical so that large numbers of suspect stains can be rapidly screened. The presumptive tests for blood make use of the peroxidase activity of hemoglobin. Hemoglobin catalyzes the release of atomic oxygen from oxidants such as hydrogen peroxide and perborate ion. The oxygen atoms react with a colorless compound (eg, benzidine, tetramethylbenzidine, leucomalachite green, phenolphthalin) to produce a colored product. Blood contamination, chemical oxidants (eg, fertilizers, household bleaches, explosive residues), salts of copper and nickel and plant peroxidases all can potentially produce false positive reactions. The results of the presumptive tests are confirmed with a Takayama crystal test for the heme moiety in hemoglobin. The Takayama reagent contains reducing agents that remove oxygen from the reagent and reduce methemoglobin (the oxidized form of hemoglobin) to hemoglobin, hydroxide ions to hydrolyze the globlin proteins and pyridine, which complexes with heme to form pink, birefringent, leaf-like crystals (90).

Patterned impressions in blood (shoe and tire impressions or fingerprints) are often important pieces of evidence. If the impressions are faint or on a dark substrate they may require chemical enhancement before they can be photographed. Blood proteins may be stained with non-specific protein stains such as Coomassie blue or amido black. Other enhancement techniques rely on the catalytic properties of hemoglobin: These include leuco-crystal violet, leuco-fluorescein, 3,3′-diaminobenzidine (DAB), *o*-phenylenediamine (OPD), *p*-phenylenediamine (PPD), and 2,2′-azino-di-(3-ethylbenzthiazolinesulfonate) diammonium salt (ABTS). In most of these procedures the blood proteins are first fixed with an aqueous solution of 5-sulfosalicylic acid. After fixation the impression is treated with the enhancement reagent, which reacts with the hemoglobin in the bloodstain to yield a colored or fluorescent product. The colors range from bright green (ABTS) to orange (OPD) to brown (DAB) to blue (leuco-crystral violet) to purple/black (PPD).

Immunological tests are used to determine the species of animal the blood came from. Antisera for species identification are produced by injecting host animals such as rabbits with blood or blood components from other species (human, bovine, porcine, canine, feline etc). The host animals' immune systems produce antibodies against the foreign blood proteins. Forensic biology laboratories typically use antisera against whole blood or against hemoglobin. The reactions of the antisera with blood proteins from the evidentiary blood stains can be carried out in a number of ways. Blood proteins are first extracted from the evidentiary stains with isotonic saline or a buffer. In the Ring Test, the blood stain extract is carefully layered on top of a layer of antiserum. The blood proteins and the antibodies in the antiserum react at the interface between the two solutions to produce a white precipitate. In the Ochterlony double diffusion test, the blood proteins and the antibodies diffuse toward one another through a agarose gel; a band of precipitation forms within the gel. The results of the Ochterlony test may be preserved by washing unreacted proteins out of the gel, drying the gel to a thin varnish-like layer and staining the band of precipitation. Recently, immunochromatography kits for the identification of human hemoglobin have become available (91,92). These kits make use of the same technology as home pregnancy test kits. The stain extract is introduced at one end of a porous membrane strip where a strip of dye-labeled monoclonal antibodies has been placed. Human hemoglobin reacts with the dye-labeled antibodies and the resulting antigen–antibody complexes are carried along the porous membrane to a strip of immobilized polyclonal antihuman hemoglobin antibodies. The dye-labeled antigen–antibody complexes bind to the immobilized antibodies, producing a colored line on the membrane. The benefits of immunochromatography are numerous: sample preparation is minimal; no reagent preparation is required; and the shelf-life of the kits is several years.

Blood collected as evidence in criminal acts is usually dried and deposited on a variety of substrates. Sample size is usually on the order of a 2- or 3-mm diameter stain. Traditional typing (93,94) involves ABO blood grouping, and characterizing stable polymorphic proteins or enzymes present in blood by means of electrophoresis (see BLOOD FRACTIONATION; ELECTROSEPARATIONS, ELECTROPHORESIS). The dried blood stain is extracted with saline then reconstituted onto a cotton thread. The thread is embedded into a gel and electrophoresed using appropriate control and known samples. Each genetic-type has a known, independent population distribution and, using the product rule, the results of several typing tests on different enzymes and proteins can be multiplied together to determine the likelihood that the blood came from a certain donor.

More recently, the forensic application of DNA testing has dramatically enhanced the ability to determine the source of a blood sample (94–97) (see NUCLEIC ACIDS). Two procedures are in forensic use: restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR). Using RFLP, DNA is extracted from a sample and is cut up into fragments at specific sequence sites using restriction enzymes. The fragments are separated by size by means of electrophoresis and transferred onto a nylon membrane. Radioactive, single-locus probes which recognize specific sequences on DNA are added to the membrane that is subsequently placed in contact with X-ray film to show the location of bands. The procedure is repeated four times using different probes and a

probability of the sample coming from a specific donor is calculated. The resulting calculations often show that a sample is unique to one and only one source.

Utilizing PCR, the analysis works using much smaller samples. Samples of DNA are amplified, which is the biological equivalent of molecularly photocopying the DNA (see GENETICS ENGINEERING). Exceedingly small samples of DNA can be duplicated for subsequent testing. This procedure yields much smaller probabilities of a match.

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