NOTE: The following articles:

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Traces of poison

Nothing captures the public’s imagination quite like a murder mystery. In real life, though, a suspicious death rarely sparks off a murder investigation. But until all lines of inquiry have been investigated there is always room for doubt.

Forensic toxicology, with its links to classical 19th century poisoners, is perhaps closest to the popular concept of forensic science. But this Arsenic and old lace image is not accurate for modern forensic toxicology, which is more concerned with the living than the dead. Most of a forensic toxicologist’s work involves securing evidence on whether drugs or alcohol were in the blood of either the suspects or the victims of a crime at a particular time, and on their possible effects. This information is important in many forensic investigations.

The effects of poison

The word toxicology means different things to different people, but whether in medicine, the pharmaceutical industry or forensic science, it concerns the nature and effects of poisons. At the Metropolitan Police Forensic Science Laboratory (MPSFL) it is a non-medical subject—a branch of analytical chemistry in which the analysts interpret their measurements in terms of the dose and time of ingestion. It requires a familiarity with pharmacokinetics—the science which deals with the rate of absorption, distribution and elimination of drugs.

This emphasis on interpretation is important: ordinary members of the public may be asked to provide an account of what they saw, heard or otherwise experienced but only someone formally recognised by the court as an expert witness is allowed to give an opinion on what these facts mean. The expert is required to tell the court which of these interpretations are possible and which are not, without taking sides with either the prosecution or the defence.

As with clinical biochemistry, the analytes in forensic toxicology can vary from simple compounds such as carbon monoxide or ethanol to complex substances such as insulin. Analyte concentrations vary widely: while salicylic acid in blood may exceed 200 μg ml$^{-1}$ after high doses of aspirin, several clinically important tranquillisers are effective in therapeutic doses that give blood levels of around 20–50 ng ml$^{-1}$.

The main difference between forensic toxicology and other branches of analytical chemistry is the matrix in which the analytes are usually found. As well as blood and urine, the contents of the stomach, liver and kidney tissue may also need to be analysed. Often the amount of material is limited and it can sometimes be in poor condition because of the actions, for example, of a fire, or from post-fatalisation. As part of a fire investigation, forensic toxicologists will occasionally estimate the level of cyanide in the victim’s blood. Thus we can ascertain whether the cause of death was as a result of inhaling cyanide gas, which is released when furnishings containing eg polyurethane foams are burnt. First, we need to recover cyanide from the materials brought for investigation—the method used can affect both the specificity of the test and the practical limits of detection. We recover cyanide by using a two-chambered Con-way cell; blood in the outer chamber is acidified and releases gaseous HCN into the alkaline inner chamber, giving a much cleaner solution of cyanide for testing. The presence of cyanide is tested for by using the method of Guibault and Kramer. This test can detect less than 40 ng cyanide in 200 μl of blood.

Vapour phase purification is also used for estimating the levels of alcohol in blood and urine samples. Here, we heat the sample—which is contained in a silicone-stoppered vial—to around 60°C, increasing the partial pressure of the volatile component until it reaches equilibrium. At this stage, we remove the material in the headspace above the liquid for GC analysis.

A question of substance

The main role of forensic toxicologists is to investigate the possible involvement of drugs in an incident. Frequently there is little information on what drugs might be present and we are forced to adopt a broad-brush approach, by using screening methods to detect as wide a range of target compounds as possible. Tentative identities from these preliminary experiments are then checked by more rigorous, compound-specific techniques.

Immunosassays Immunosassays are a group of methods that are widely used as presumptive tests in eg therapeutic drug monitoring by hospitals and clinics, to check that the doses being administered are giving the correct blood levels, and in workplace screening, where employees are required to submit urine samples for testing.

The basis of these methods, known as radioimmunoassay (RIA, Fig. 1) is competition for antibody sites between the molecule of interest and others supplied by the analyst. In the methods used in our laboratory, competition displaces β-emitting $^{125}$I-labelled drug analogues. Immunosay principles are also used in enzyme multiplied immunosassay technique (EMIT) and enzyme linked immunosorbent assay (ELISA) methods, in which competition for an immobilised antibody occurs with a modified form of the drug that has an enzyme bound to it. When this drug-enzyme combination is released from the antibody binding site, the resulting enzyme activity can be correlated with the drug concentration in the solution being tested.

Although each test can only give a result for one drug, or one class of drugs, these methods are sensitive and they are easily...
automated. A battery of tests therefore quickly provides information on several common analytes with only a modest amount of sample (ca 10–20 μl per test). Care has to be taken in interpreting the results, however, because cross-reactivity with closely related metabolites or congeners may be misleading.

**Chromatography.** Chromatographic methods are sensitive for all but the most demanding applications. The separating power of gas chromatography, combined with electron capture, nitrogen-phosphorus detectors, or mass spectrometry, can give a highly specific method. This is important because the techniques used to recover drugs tend to remove unwanted material from the matrix, along with metabolites; chromatography separates the drug from these contaminants.

The formation of urine typically has a concentrating effect and the amounts of most common drugs in urine are high enough to be detected by thin-layer chromatography (TLC). We have a combination of elution systems and reagent sprays that can detect a variety of drugs in two broad classes, according to whether they partition from the aqueous phase into an extracting solvent at an acid or a basic pH. This approach is common in forensic toxicology and kits with all the necessary components—including a colour photograph album to help with the interpretation for standard compounds—are commercially available.

Gas-liquid chromatography (GLC) or high-performance liquid chromatography (HPLC) can be used after TLC as second stage complementary methods for urine analysis. For blood, however, TLC is rarely sensitive enough to permit drug detection and GLC or HPLC are therefore used as primary screening methods. Because TLC is a highly sensitive and selective method, GLC and HPLC are widely used for quantification. For example, we measure the levels of temazepam in blood by GLC with electron-capture detection (ECD), and of morphine by using HPLC with electrochemical detection.

**One man’s medicine**

Temazepam (1), one of the benzodiazepine group of drugs, is frequently prescribed as a sedative. It is sometimes misused, often by injection. Peak plasma levels of around 200–300 ng ml$^{-1}$ usually occur about 90 minutes after taking an oral dose of 10 mg. The drug is recovered by solvent extraction of blood to which an aqueous buffer has been added containing hydroxypropyramiphen as an internal standard. Temazepam does not chromatograph well, because of the hydroxyl group at the 3-position; protecting this as a silyl ether improves both the chromatographic resolution and sensitivity of drug detection. This protection is done by using a mixture of tert-butylmethylchlorosilane and imidazole in dimethyl formamide (Fig. 2).

Morphine (2) is best known as a narcotic analgesic with a prodigious ability to relieve pain. It is also one of the products of diamorphine, or heroin, metabolism.

Diamorphine has a restricted medicinal use in the UK but it is frequently encountered as a drug of abuse.

The levels of unchanged morphine in blood are frequently below 50 ng ml$^{-1}$. In addition, morphine is zwitterionic and is poorly extracted by organic solvents; it is usually recovered from blood at its isoelectric point (pH 8.5) by extraction into a solvent combination with a high proportion of alcohol, such as an ethyl acetate/isopropanol mixture. After resolution by chromatography, the drug is detected at a glassy carbon electrode operating in oxidative mode at +0.6 V with a silver/silver chloride reference electrode.

**Another man’s poison**

In the context of abuse, drugs can be regarded as poisons—substances that destroy life or injure health. Quick to act and difficult to detect, poisons have been used throughout history to commit murder.

In June 1981, a man in his mid-30s was admitted to hospital after complaining of stomach pains. His health deteriorated rapidly and, after a short time in intensive care, he died. Nothing suspicious was reported at that point.

Nearly a year later, however, the similarity of his symptoms to those associated with paracetamol poisoning were noticed in a medical review of the case done as part of a training exercise. Some of his tissue samples, which had been taken at post mortem for histology, were still available and these were sent to a pharmaceutical firm with facilities to carry out a presumptive radioimmunoassay (RIA) test. A strong positive was reported and the case became a police investigation. Further post-mortem samples, some of which had been preserved in formalin, were found, as was a small quantity of serum from a blood sample which had been taken before the patient died. These were sent to the MPPSL, where by performing a further RIA analysis and HPLC we were able to confirm the presence of paracetamol.

Paracetamol is a highly water soluble bis-quaternary ammonium compound (3) and cannot be removed by solvent extraction. We used trichloroacetic acid to precipitate the tissues from these samples, releasing the paracetamol, followed by ferrocyanide oxidation in a strongly alkaline medium to give the dipyrindole (Scheme 1). The dipyrindole can be extracted with a chloroform/isopropanol mixture and is chromatographed on a conventional system, with fluorescence detection at 400 nm after excitation at 340 nm.

The police never discovered the source of the paracetamol that had been ingested but the dead man had been a landscape gardener and it appears that he had once taken a paraquat home to kill weeds in his garden. His wife later admitted to having put some of the weedkiller into a Sunday lunch and was subsequently convicted of murder.

**References**

'Every contact leaves a trace' is more pertinent than Edmond Locard could have realised when he made this observation in the 1920s. With the aid of high resolution microscopy, forensic scientists today have access to the minutest details at the scene of a crime.

When chemists developed modern synthetic fibres, forensic scientists gained a new opportunity. Much of forensic science involves finding and identifying trace evidence, for—as one of the first forensic scientists, Edmond Locard, taught in the 1920s—'every contact leaves a trace'. Textile fibres are useful for detecting links between a suspect and a crime because fibres transfer easily between items of clothing or between clothing and a car seat, for example. Many armed robbers leave distinctive fibres in getaway cars. Similarly, fibres are often left behind when clothing brushes against rough objects or sharp edges. Large tufts of fibres are easily dealt with but many cases hinge on the examination of microscopic contact traces.

**Taped**
The most effective way to recover extraneous fibres from a garment is to use clear adhesive tape. The tendency for fibres to adhere to sticky tape may be a nuisance in everyday circumstances but it is turned to good effect in crime laboratories. Simply applying and removing strips of tape sequentially to the whole of a garment surface removes a high proportion of the extraneous fibres. The fibres are recovered from the tape by softening the adhesive with a little solvent and, after washing, they are mounted individually on slides for more detailed examination and comparison with control samples of fabric from the suspected source.

Low magnification microscopy \((\times10-40)\) provides a first screening, but greater discrimination and a preliminary identification often requires higher power \((\times100-400)\). At this stage it is possible to distinguish natural from man-made fibres and to observe fine details such as the structural features of animal hairs (Fig. 1). If suspect fibres do not match the control at any stage of the examination, and no plausible reason for this can be found, then they are eliminated. This is the scientific method: we apply more and more discriminating methods to test the hypothesis that the crime scene fibres and the control might have a common origin and it is only our continued failure to find differences that supports the hypothesis that the fibres match. At no stage is a forensic scientist trying to prove that the fibres have a common origin.

More critical examination requires a comparison microscope, which consists of two high power microscopes linked together by an optical bridge and a single pair of eyepieces (Fig. 2). Samples are illuminated with either visible or ultraviolet light. The image, which consists of half of each microscope field, allows materials from different sources to be looked at side by side under the same conditions (Fig. 3). Fibres are compared on the basis of features such as colour, diameter, cross-sectional shape, fluorescence and generic class, as far as it can be ascertained.

High power bright field microscopy is, in fact, largely ineffective for distinguishing the various generic classes of man-made fibres. However, the difference between refractive indices parallel and perpendicular to the longitudinal axis of the fibre—its birefringence—often varies with polymer type. The birefringence is quickly estimated from the interference...
Fig. 3. A split field image for comparing control (top) and suspect wool fibres.

colours these materials show when placed between crossed polars on a polarising microscope. For quantitative work a tilting compensator or quartz wedge is used to establish path difference between polarised light which has been resolved in directions parallel and perpendicular to the fibre axis. The birefringence is then calculated from published tables after taking into account the thickness of fibre through which the polarised light has passed. Reference to published birefringence values or comparison with authenticated samples is the basis for identifying most generic classes of polymer.

The next level of detail is provided by microspectrophotometry, which allows visible spectra to be obtained from very small samples or from small inclusions in larger objects. Natural fibres have a somewhat greater spectral variation than man-made fibres because of differences in diameter, dye uptake and the natural yellowness of the raw material—and so more control spectra are recorded (at 10) for wool and cotton than for man-made fibres (at five). Normally about one third of all the microscopically similar suspect fibres are examined by microspectrophotometry, with an upper limit ordinarily set at 10 fibres. When less than five microscopically similar fibres are found, all of them are checked.

Complementary chromaticity coordinates, which are used by colour chemists as an approximate measure of colour, are generated from the control fibres and a mean value is calculated automatically. This is used for data storage purposes, together with other parameters such as the diameter, the cross-sectional shape and the fibre type; the database is therefore a guide to the frequency of fibre occurrence.

**Threads of evidence**

When microscopic comparison and visible spectrophotometry are complete, man-made fibres are usually examined by using infrared spectroscopy, linked to a microscope. This provides clearer molecular discrimination than the preliminary identification of fibre type by using polarising microscopy. Measuring the birefringence is not a waste of time, however, because this simple experiment is a cost-effective way of quickly disproving a match.

The coupling of a microscope and a Fourier transform IR instrument has greatly simplified fibre analysis. Results can be obtained from a few millimetres of a single fibre, typically 20 μm in diameter—the only limiting factor being an inability to manipulate the sample. FTIR can also subdivide some generic classes of fibre on the basis of their chemistry. For example, acrylic fibres can contain up to 15 per cent copolymer, which is easily identified in the IR spectrum. The two most common acrylic fibres consist of polycrylonitrile with copolymers of either vinyl acetate or methyl acrylate (Fig. 4). Polyamide polymers can be discriminated on the basis of their constituent monomers. Nylon 6, for example, is made from caprolactam but Nylon 6.6 is made from hexamethylene-diamine and adipic acid.

With coloured fibres, characterisation may be complemented by examining the dye. Dyes are classified by using a number of well established procedures; at the MPFSL we place single fibres or small tufts in capillary tubes which have been sealed at one end and treat them sequentially with various reagents. The classification scheme depends on whether or not

Fig. 4. The infrared spectra of acrylonitrile copolymerised with (a) vinyl acetate; and (b) methyl acrylate.
dyes are extracted or the fibres change colour. Occasionally, simple thin layer chromatography (TLC) is needed to complete the dye classification.

Once the class of dye on the control fibres is known, a good TLC eluent is sought for analytical purposes using an extract from a further tuft. The dye has to move from the origin but must not run with the solvent front and for maximum discrimination it should separate into as many coloured bands as possible. In terms of sensitivity, and for use in practical crime casework, these bands need to be visible in tests on single fibres and, where necessary, a TLC examination will be done on single control fibres to ensure that this is the case.

At this stage the suspect fibres are examined. A final experiment is set up in which dyes from crime scene fibres and dyes from control fibres are run on the same TLC plate. This completes the usual comparison process although, occasionally, other methods may also be used. Special problems may occur, for example, if clothing has been burnt in an attempt to destroy evidence or where arson is suspected. It is difficult to identify fibre constituents in a carbonised state because most thermoplastic fibres will have melted and cellulose fibres, such as rayon and cotton, may disintegrate when they are handled. In these cases scanning electron microscopy (SEM) can sometimes help.

Whole pieces of fabric can be placed in the SEM chamber and although a definitive identification may not be possible, the material’s appearance at different magnification may give information on the composition of the fibres which is useful to a fibre expert. Occasionally the SEM does provide analytical information to complement that from other sources. Chloro-fibres, such as those made from polyvinyl chloride, are difficult to identify by a combination of optical microscopy and infrared spectroscopy. A high proportion of plasticiser usually dominates the IR spectrum. This can still be used for comparison but not for chemical identification. If the polymer is found to be rich in chlorine atoms, however, using the energy-dispersive X-ray analysis facility on the SEM, this is good evidence of the fibre type. Mass spectrometry, hot stage microscopy and density measurements are also used in fibre examination.

Besides the analytical results on fibres and dyes, forensic scientists have to keep many factors in mind when evaluating the evidence: whether there could have been innocent contact between the items submitted for examination; the persistence of fibre fragments on different types of fabric; the time interval between the incident and recovery of the suspect samples; information from manufacturers of textile materials and clothing on the rarity of particular fibre types and their most common use; and the frequency of random occurrence of common fibre types on garment surfaces.

**Slash and grab**

Fibre analysis is important in many investigations and the following case is typical of the way it is used. A suspect was alleged to have slashed open several bank security bags to check if money was stolen during a security van robbery. He had a Stanley knife with him when he was arrested, with 73 fibres—microscopically similar to the control fibres from a damaged bag (Fig. 5)—on the blade. Fifteen of these were examined by microspectrophotometry but only five were found fully to match the bag fibres. However, the forensic scientist realised that the slashed bags could have comprised fibres from two different dye batches and she asked for other bags, similar to those stolen, to be brought to the laboratory. Examination of a further six security bags revealed that all 15 fibres matched one or other of them.

The Bullion Centre was asked about the origin of the bags. It had dealt with only one supplier, who described the bag material as cross-woven, mauve-dyed nylon, all purchased from a single company, which had dyed the material exclusively for them. A dye batch was normally 500 m, enough for 2000 bags. While small colour variations were expected between batches, they thought that the same dye had been used for the past six or seven years. However, from cloth samples they supplied from different dye batches, it was obvious that two different dye recipes had been used. The court accepted prosecuting counsel’s argument that the suspect’s Stanley knife had been used to cut open the security bags and the suspect was given a 16 year jail sentence.

**Future prospects**

What of the future? Dyes are more difficult to extract from natural fibres such as cotton and wool because they are sometimes covalently bound. But biochemistry may supply a remedy: we are exploring the benefits of breaking up the fibres with enzymes and, for cotton at least, we already have the promise of a simple TLC based analytical procedure following digestion with a cellulase enzyme which can cope routinely with a high throughput of fibre samples.

Locard would be amazed at the discriminating power of modern chemistry. Certainly many villains are unpleasantly surprised by how much information we can extract from the tiniest quantities of materials left at crime scenes.
Size is no object

The high resolution imaging power of the scanning electron microscope (SEM) means that even the tiniest scrap of evidence can prove conclusive in many forensic investigations.

Forensic scientists spend a lot of time simply looking at objects before beginning any process of dismantling or analysis that might affect their integrity. The Metropolitan Police Forensic Science Laboratory (MPFSL) provides a number of aids, ranging from simple lenses to scanning electron microscopes (SEMs).

Imaging in SEM
The SEM can provide images with much higher resolution and greater depth of field than conventional light microscopes and this remarkable imaging capability is used frequently in casework. In one of the earliest applications in UK forensic science, one of our biologists was able to distinguish between possible sources of feathers in a case where it was important to know whether they were duck feathers or chicken feathers.

Scanning electron microscopy has also been used to identify alterations to coins in numismatic frauds, determine the sequence of writing on documents in forger cases, and produce identifiable fingerprint images from newspapers and other textured surfaces.

However the SEM is much more versatile than this. It can be operated in a variety of different modes, each of which offers different types of information. The main approach in forensic science laboratories is to combine the imaging power with energy-dispersive X-ray spectrometry (SEM/EDX, Fig. 1) to provide multi-element particle analysis. In this mode the electron beam ejects inner electrons from atoms of the material being studied and outer electrons then fall into the electron holes, emitting radiation with a wavelength that is characteristic of the element under electron bombardment. Wavelengths are normally in the X-ray region of the spectrum. This combination of electron imaging and elemental analysis can be performed on very small samples.

Particle analysis
Fragments of glass are an example at the upper end of the size range for materials analysed routinely at the MPFSL. Glass often becomes trapped in the hair and clothing of burglars, for example, when they break windows, and submillimetre-particles of 50 μg or less may be recovered for laboratory examination.

The point of the analysis is to compare these materials with control samples from the crime scene to see whether or not they match. Much useful information is obtained simply from refractive index measurements, but on occasions elemental analysis is required for greater discrimination. Several options are available, such as plasma emission spectroscopy, but SEM/EDX provides an alternative—and it is non-destructive.

The particles are encased in a block of Bakelite and the upper face of the block is polished to give a flat surface which can be illuminated by the electron beam. Good
precision is obtained for major elements in glass but on the whole this electron microprobe analysis technique has poor relative sensitivity. The detection limit for an element in the matrix is in the region of 1000 ppm, so minor impurities and trace components are not detected.

Going down the scale in particle size, bullets have been identified from fragments deposited along wound tracks (Fig. 2) in cases where the bullet passes right through the victim and is not recovered. This is a feature of high velocity weapons often used by terrorists. Fragments may be removed by excising the wound track at the post mortem and treating the tissue with a proteolytic enzyme to break it down. This method has also been used to identify small fragments of material recovered from wounds caused by explosions. The observation of any characteristic high-explosive deformation then provides information on which fragments could have been part of an explosive device as distinct from secondary artifacts produced by the blast.

**Gunfire**

Perhaps the most important application of SEM/EDX in forensic science is some of the work done under higher magnification with particles that are released when guns are fired. These are called cartridge discharge residues (CDRs). They are about 1–10 µm in size. The crime-related use of firearms is much more prevalent in many other parts of the world but a worrying trend for both the police and the government in the UK is a steady increase in the association of firearms with crime.

Firing a gun initiates an energetic event. When the trigger is pulled, the hammer strikes the percussion cup at the back of the cartridge, which contains a shock-sensitive priming mixture. The percussion primer ignites and sends a stream of hot gases into the main charge of propellant, which in turn ignites, generating large volumes of gaseous products and forcing the projectile down the barrel and out towards the target. A typical primer formulation contains lead (tinitrosocarbonate as the shock-sensitive material), antimony sulphide as the pyrotechnic fuel and barium nitrate as an oxidiser. Propellants, on the other hand, are usually entirely organic and are frequently a mixture of nitroglycerine, nitrocylolulose and a stabiliser.

The high-speed photograph (Fig. 3), which was taken at the moment of discharge, shows a bullet emerging with a cloud of smoke and hot particles. As this material cools, components in it condense and they may be deposited on the firer. Paricles may be inhaled and deposited in the nose, or they may come to rest on the firer's skin, hair and clothing. They may also settle on surfaces or on other people in the immediate vicinity. Amongst this discharge debris may be small inorganic particles that come from the constituents of the primer, and these form the basis of the evidence obtainable by SEM/EDX analysis.

Primer discharge residues vary in com-

**Fig. 3. A high-speed photograph of a weapon at the time of firing.**

position according to the formulation of the original mixture. Although none of the heavy elements usually present is itself specific to primer residues, the combination of lead, antimony and barium in a small discrete particle (Fig. 4) which is clearly a condensate from a high-temperature fusion process is widely accepted as good evidence that the particle had its origin in a cartridge discharge event.

The first stage of the scientific investigation is to collect samples for analysis using strips of plastic coated with adhesive tape. The manner in which these are then examined is a good illustration of how technology can provide efficiency gains and how forensic scientists also have to fit in with requirements of the criminal justice system.

Searching tapes manually used to be laborious and extremely time-consuming. Sticky tape removes a lot of material from skin and clothing and large numbers of particles have to be screened to discover whether there are any of interest. Much of the material on the tapes is fibrous but a range of micron-sized metallic particles is frequently present. Some examples are gold and silver alloys from jewellery; rare earths from lighter flints; lead from petrol; barium—as the mineral barium sulphate—from a variety of sources, and bismuth from some cosmetics.

At the MPFSI the searching procedure has been automated by using back-scattered electrons from the elements of higher atomic number. These enable the instrument to identify particles of potential interest that should be investigated further. Full analyses are then done automatically on each of these particles. The output for the analyst is a summary sheet on which certain combinations of elements are highlighted. It is crucial for court reporting that the expert witness should have examined the analytical results personally before forming an opinion on their likely significance. One of the most important design criteria for the system, therefore, is that it allows the analyst to locate and return to any particle on the tape to re-examine it and to form a personal view about the results. The selection criteria for the searching routines are therefore deliberately set quite wide. A reasonable number of false positives is acceptable at the automatic searching stage because the definitive opinion will later be formed by the operator, whereas false negatives could lose potentially important evidence. The result is a system which deals with the labour intensive searching process but which leaves the critical decision-making to the expert witness, who is then able to testify accordingly.

**A case example**

In many cases the only issue is whether or not the suspect has been in the vicinity of a recently fired gun. On some occasions valuable evidence is also obtained from variations in the formulations of the prim-
ers used by different ammunition suppliers. One recent case in London illustrates both the application of the technique and the additional information which can sometimes be obtained.

In this case two armed men arrived on motorcycles outside a bank in central London and they attacked guards loading money into a security van. Eight shots were fired and one of the guards was hit in the leg, the bullet severing his femoral artery. He was saved from bleeding to death on the pavement only by prompt first aid from a nurse who happened to be passing. The men escaped with more than £200,000, abandoning a revolver, a pistol and gloves at the scene. Police arrested two suspects later on that same day. The questions the police were asking were: is there any evidence connecting the men with the weapons and, if so, which one of them fired the gun?

In the laboratory, tiny metallic fragments were found on wool fibres from around the bullet hole in the guard's trousers. They were identified as being gilding metal with a thin nickel plating (Fig. 5). It was clear from the appearance of the ends of the fibres that the materials of interest had been abraded from the surface of a bullet as it passed through the fabric.

The bullets from the weapons were of three types. The revolver held 0.32 caliber ammunition with uncoated lead bullets whereas the pistol contained two types of 7.65 mm ammunition, both with nickel plated bullets. This was evidence indicating that the near fatal shot came from the pistol rather than from the revolver.

Percussion primer residues from test firings of ammunition in the two weapons were chemically distinguishable. The revolver ammunition had a standard three-component primer with particles containing lead, barium and antimony (Fig. 6a). The pistol ammunition, on the other hand, contained tin in addition to the other three elements (Fig. 6b). Tin is often found in ammunition supplied for use in self-loading and automatic weapons. Analysis of residues recovered from the suspects' clothing indicated which suspect was the likely firer of which weapon. Putting all of this together, the laboratory was able to give useful information to the court on which of the two suspects could have fired the near-fatal shot, and with which of the weapons.

This case also underlines the courts' desire to have corroboration for eyewitness evidence bearing in mind that this frequently comes from frightened people who catch only glimpses of fast moving events. On this occasion the scientific analysis was compatible with eyewitness accounts. Faced with a strong case, of which the scientific evidence was an important part, the accused pleaded guilty.
High and dry?

The number of drug seizures in the UK has more than trebled over the past decade and, with the demand for court cases to be heard more quickly, drug analysts are looking to modern instrumentation for productivity benefits.

Although Sir Robert Peel's government in the 1840s did little to stem the opium trade in the Far East, the Metropolitan Police, which he formed as Home Secretary in 1829, now fights the battle in London. Governments today are less ambivalent about drugs and in the UK the main legislation for dealing with their abuse is The Misuse of Drugs Act, 1971. This meets some of our international obligations and it supports one strand of government policy in tackling the problem of law enforcement.

Several hundred substances are listed as Controlled drugs in a schedule to the Act, a handful of which account for most of the world's illicit market, including household names such as cannabis (1), heroin (2), cocaine (3), amphetamine (4), LSD (5) and Ecstasy, MDMA (6). The role of forensic scientists in drug-related work at the Metropolitan Police Forensic Science Laboratory (MPFSL) is to help London's police to detect drug crime and to provide impartial expert evidence to the courts. At its simplest the question is does this package contain a controlled drug? At the other end of the scale chemists can be called upon to advise officers at illicit drug factories, helping to recover evidence about the method of synthesis and the capacity of the operation. Either way, the job requires analytical chemistry skills and an understanding of both statute and case law.

Plant evidence

Cannabis, heroin and cocaine are all derived from plants. Illicit cannabis products are normally encountered in one of three forms: a herbal material made from parts of the plant close to the flowers; a resin recovered from the plant; or a viscous oil obtained by solvent extraction of the plant material. The most common form in the UK is the resin, which is imported from North Africa or the Middle East in blocks of about 0.25-0.5 kg. These are broken up for sale on the street and forensic scientists can be asked for help with seizures ranging from a few milligrams to several tonnes. Cannabis plants grow as bushy annual shrubs (Fig. 7) in many parts of the world. Cannabis plants are dioecious (every plant has a distinct sex) and the female plant is believed to give better yields of the resin and its active component, tetrahydrocannabinol (THC).

Heroin was originally a trade name used for the diacetylated form of morphine (2). More recently it has become associated with illicit material to distinguish it from the legitimate medicinal compound, which continues to be known by the approved name diamorphine. Heroin is produced by acetylation of morphine, which is recovered from opium—a dried exudate from the seed capsule of opium poppies (Fig. 2). It continues to be one of the major drugs of abuse worldwide. Heroin in the UK comes mainly from south west Asia. It consists of diamorphine base together with smaller quantities of the other opiate alkaloids such as thebaine and papaverine. Admixtures with other drugs such as phenobarbitone, methaqualone and phenacetin are common and chemical adulterants such as phenylphenethylamine have also been found, as have substantial proportions of chalk. Heroin in this country is a crude and at times quite complex mixture containing between 30 and 60 per cent diamorphine by weight. There is no evidence to support a widely held view that serial dilution occurs routinely as heroin is passed along the distribution chain.

Cocaine is recovered by solvent extraction from the leaves of the coca bush
Erythroxylon coca. Whereas cannabis is ubiquitous, all of the world's illicit trade in cocaine originates in the Andean region of South America, which has been the only area to grow large amounts of coca successfully. Cocaine is almost always imported into the UK as the high purity hydrochloride salt. However, police seizures show that cocaine, unlike heroin, is often diluted with inert materials before sale. Electrically neutral molecules generally cross biological membranes more readily than ionic species; the hydrochloride can be readily converted to the base, and when it is inhaled in this form cocaine takes effect rapidly. Although recent emphasis has been on what is colloquially termed crack, an alternative means of producing the base has been used for some time. In 'free basing' as it is called, the user isolates the base by solvent extraction from aqueous solution, taking risks with flammable solvents, and smokes the result. Crack cocaine, on the other hand, can be produced easily and without risk by using common utensils and materials. The other three important drugs of abuse seen most frequently in forensic science laboratories in the UK are synthetic chemicals: amphetamine, methyleneoxy methylamphetamine (MDMA or Ecstasy), and lysergide (LSD). Some of the amphetamine is imported but substantial amounts are also manufactured illegally in the UK.

Amphetamine is normally a powder containing about 5 per cent of the drug by weight. It contains an inert diluent such as a sugar and is sometimes admixed with other drugs such as caffeine. MDMA is supplied in tablet or capsule forms, but LSD solution is almost invariably soaked onto paper and, after drying, the product is subdivided into 5 mm² portions. These contain about 70 μg of the drug and they are usually printed with distinctive characters or designs. They appear in phases and in effect the design becomes a colourful 'sell-by date' showing the age of the product.

Fig. 1. The bushy cannabis plant.

Colourful characters
Cannabis is usually identified by using a combination of a simple colour test and by observing characteristic structures under low magnification after cleaning the material with chloral hydrate solution—a botanical technique for removing the colour and revealing underlying structures. In particular, analysts look for resin-producing glands, which are thought to be diagnostic. However, cannabis grown in this country is often sold as finely chopped foliage leaves which lack the resin glands. Thin-layer chromatography (TLC) is then used to check extracts of the material for the presence of cannabinoids. The spray reagent Fast Blue BB reveals cannabinoids as a range of vivid red, orange and purple TLC spots in a test which is both rapid and sensitive.

Fig. 2. An incised poppy capsule.

Other drugs have been identified for several years by using a combination of chemical spot-tests and TLC as presumptive tests, followed by infrared spectroscopy for confirmation. Spot tests and TLC are capable of giving a considerable amount of information quickly and cheaply. In the MFSIL we use the Marquis reagent—a weak solution of formalin in concentrated sulphuric acid. It gives a deep violet colour with heroin, an orange colour with some of the amphetamines and a blue–black colour with their ring-substituted derivatives such as MDMA. An infrared spectrum is then capable of giving unambiguous identification.

The use of IR spectroscopy also illustrates our need to respond to judgements in court. In the late 1970s, judgements from the Court of Appeal were such that forensic scientists felt obliged to identify the form of the drug, wherever reasonably possible. Was it the free base or was it a salt for example, and, if so, which salt? Courts were not concerned with pharmacokinetics and the safety of individuals, but with legal arguments in an adversarial legal system over whether someone could be convicted if the charge were one of supplying, say, cocaine and chemical analysis showed it to be cocaine hydrochloride. Infrared spectroscopy was one of the few readily available techniques which could provide that information.

Infrared spectroscopy gives interesting variations in the way that samples are prepared for analysis. Most of them are analysed in discs formed by compression with potassium bromide and, provided that the purity of heroin seizures is high enough, a recognisable spectrum can be obtained directly without pre-treatment. Otherwise, extraction with chloroform usually recovers material capable of giving a spectrum of acceptable quality. The proportion of amphetamine in street seizures, however, is generally too low for either technique to be successful. Fortunately, and as pharmacists are taught during their training, it is not simple to mix powders well and the amphetamine samples are often sufficiently inhomogeneous that particles of the amphetamine sulphate can be physically separated from the adulterants under a low power microscope.

Instrumental changes
The number of drug seizures and the quantities of drugs seized by the authorities have risen steadily over the past 10 years. London accounts for a substantial proportion of national police seizures and some figures for submission to the MFSIL are shown in Fig. 3.

Demands on drug analysts in the past 20 years have followed the rising trend in drug abuse, with court cases being heard much earlier. As a result, productivity gains have been sought through modern instrumentation. One method uses benchtop gas chromatography/mass spectrometry (GC/MS) with capillary columns and automatic sample injection. It is supported with software which identifies analytes of interest using a library of reference spectra and is operated as a

Fig. 3. A comparison of the number of drug seizures in 1981 and 1991.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td></td>
</tr>
<tr>
<td>1981</td>
<td>300</td>
</tr>
<tr>
<td>1991</td>
<td>500</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1000</td>
</tr>
<tr>
<td>Heroin</td>
<td>1500</td>
</tr>
<tr>
<td>MDMA</td>
<td>500</td>
</tr>
<tr>
<td>LSD</td>
<td>1000</td>
</tr>
</tbody>
</table>

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'walk-up' service by staff who are not MS specialists. An alternative is to use high performance liquid chromatography (HPLC) in combination with ultraviolet diode array detection. Analysts using the GC/MS service identify their samples to the computer when they place their vials in an autosampler. The mass spectra from each run are compared with a library of 39 compounds prepared in-house from 'best-available standards'. The output consists of a summary together with a full spectrum of any analyte likely to be a controlled drug, a copy of the library standard spectrum and a statistical assessment of the match. If a mass spectrum does not correspond to one of the primary compounds of interest the computer goes on to test it against a further 2000 spectra in a commercial database.

One instrument has been in service for two years, in which time it has dealt with about 200–300 cases a month. Along with the blank and quality control analyses which are part of the standard protocol for court reporting, this means that ca 600–700 samples a month are being processed.

**Under the floorboards**

Some drug seizures and investigations can be quite mundane but there are some cases that require the full range of talents of the forensic scientist. Detectives raided a garage in North London, where they discovered a number of empty solvent drums, various reagents and travelling trunks holding laboratory apparatus (Fig. 4). No drugs were found, although small quantities of reaction residues were detected on the apparatus. In particular, intermediates and by-products of an amphetamine synthesis were found in a dark brown gum sticking to the blades and boss of an extractor fan in one of the trunks.

Within a few days police investigations had led to an isolated cottage in Lincolnshire and the scientist who had examined the items from the garage went to assist police officers at the scene. His attention became focused on one of the upstairs bedrooms in the unoccupied farmhouse. The room had been recently redecorated but a large rectangular mark could still be seen on the newly varnished floor. It was subsequently found to coincide with the shape and size of a greenhouse which was stacked in sections in an outbuilding. On lifting some of the floorboards the scientist found a small deposit of a white crystalline material on the ceiling of the room below (Fig. 5). There was an area of charring close by which could have been caused by an acid. Later, analysis showed that the white material was crude amphetamine sulphate. Intermediates and by-products from the Leuckart process for synthesising amphetamine were also found on what appeared to be scorched grass close to a manhole access to the cesspit of the cottage.

The scientist also noted scratch marks on the bricks of the fireplace in the room and on looking at some circular metal ducting in the outbuilding he noted traces of what appeared to be brick dust—this was subsequently confirmed by a colleague at the laboratory. When this ducting was taken back to the laboratory, one end was found to correspond exactly with marks on the casing of the fan found in North London.

A detailed search of the debris in the outbuilding revealed scraps of paper which could be fitted to torn labels on containers from the first scene. The scientist later told the court that in his opinion the greenhouse had been set up within the bedroom to provide a laboratory environment and that this had been ventilated by an extractor fan through a duct inserted into the chimney. The court accepted that the material found under the floor, on the fan and in the cesspit, was good evidence that manufacturing had taken place at the farmhouse.

Sherlock Holmes would have been proud of the scene reconstruction, but he might be astonished at today's analytical chemistry technology. However, as a regular abuser of opium and cocaine, we would prefer him to concentrate on physical evidence and to keep clear of the drug laboratory.
A low profile

With the emergence of molecular biology, forensic scientists acquired a powerful and discriminating new tool. Combined with better detection systems, DNA profiling will become a routine part of forensic investigations.

DNA-containing materials are often left at the scenes of violent crimes. Blood and semen are common examples, particularly in cases of violent assault, including murder and rape.

As molecular biology began to flourish, after the discovery of the double helical structure of DNA and the triplet base code was deciphered, forensic scientists realised that sequence differences in these DNA bases might in principle allow villains to be identified. But nobody knew how to unlock and read this molecular detail; determining the entire base sequence of a suspect is not possible because the human genome comprises about 3000m base pairs.

Then, in 1985, Alec Jeffreys and his group discovered that several sections of the DNA were so highly characteristic that they alone could be used to identify a suspect. These sections of DNA contain short, repeating sequences of the bases A, T, G and C, and the number of repeats is very variable between individuals. The necessary chemical and biochemical tools were already available to excise these variable number tandem repeat (VNTR) sequences and to determine their molecular size.

‘Nicked’ at both ends

Enzymes are used to cut both strands of the DNA, with great specificity, at particular base sequences. The enzyme used in UK forensic science, and throughout Europe, is from the bacterium Haemophilus influenzae, known as Hinf I. No one yet knows the fine details of the remarkable molecular recognition process by which restriction enzymes such as Hinf I identify a sequence several bases long, given that the bases face inwards in double stranded helical DNA. Although the mechanism by which this enzyme works is also unclear, it is known to involve two concerted hydrolyses, cutting both strands of the DNA:

\[ 5' \ldots G\text{A}NT\ldots 3' \]
\[ 3' \ldots C\text{T}N\text{A}I\ldots 5' \]

Hinf I has recognition sequences close to—but not in—the VNTR regions, and therefore releases the latter sequences intact. In the US, it is more common to use the enzyme Hae III from Haemophilus aegyptius, which cuts at the sequence GC-C*, where C* is 5-methyl cytosine.

The DNA fragments released by Hinf I are anionic polymers which can be separated according to size by agarose gel electrophoresis. The smaller the fragments, the further they travel through the gel in the time allowed. After electrophoresis, the DNA fragments are denatured in situ to give single-stranded DNA fragments which are then transferred to a nylon membrane, where they remain firmly bound during the subsequent analysis.

\[ ^{32}P \text{-labelled DNA probes that have a base sequence complementary to one of the VNTR sequences are used to identify the fragments of interest from the thousands of others on the membrane. When the membrane is bathed in a solution of the probe, the probe binds through specific hydrogen bonds to the recognition sequences. The } ^{32}P \text{ labels are then accurately located by placing an X-ray film over the membrane (Fig. 1).} \]
The present technology uses probes and experimental conditions—in temperature and ionic strength—to ensure that the X-ray film contains bands only from a single region or locus of the DNA. The probes are called single locus probes. However, our chromosomes come in pairs and we have two copies of each locus, one from each parent; a single locus probe therefore produces two bands (occasionally overlapping) on the X-ray film.

At the Metropolitan Forensic Science Laboratory (MPFSI), our interpretation of the X-ray spectrum is helped by referring to a database of many hundreds of previously encountered DNA profiles—this indicates how often bands of those molecular sizes have been recorded and gives an estimate of their frequency in the population. Typically we use four different DNA probes, each specific for a different DNA locus, generating a total of eight bands. If any of these is clearly different when we compare the DNA profiles from a crime scene and a suspect, the investigating police officer is told that the suspect could not have been the source of the crime scene material.

If a full match does occur, we estimate the chance of this occurring among unrelated individuals in London, taking into account factors such as whether, and how, to allow for ethnic origin. Different calculations are made if the suspect has close relatives—especially brothers or sisters—who may have committed the offence. Experts differ about how to allow for these factors, and the courts do show concern if these differences are pronounced, but there seems to be a growing acceptance that DNA profiling is immensely valuable to the criminal justice system. Any debate is now largely aimed at refining and improving the fine details of the current technique.

**Mistaken identity**

Figure 2 illustrates an early use of DNA profiling at the MPFSI. The case involved a young woman who had told the police that she had been raped by someone who had her school some years ago. She gave the police his name and subsequently picked him out from an identification parade.

A DNA profiling membrane typically contains about 12 tracks, including three molecular size calibration ladders of standardized DNA fragments and also a standard control sample from an immortalised human cell line. This leaves room for about eight DNA samples. As a matter of good practice, crime scene samples and suspect samples are not run in neighbouring lanes.

For simplicity, Fig. 2 shows only the tracks for two calibration fragments and for the three samples of interest. Track a shows the two bands from a vaginal swab, track b shows bands from a blood sample provided by the suspect, and track c shows DNA bands from a control sample of blood provided by the victim. Clearly the bands in tracks a and b could not have come from the same person and, in spite of the woman's evidence, the suspect was eliminated from the enquiry.

The scientist noticed, however, that one of the bands in b coincided with one of the bands in a. This occurred with two of the probes used in this case and the scientist asked the police whether the original suspect had a close relative—in particular, a brother—who could have committed the crime. In fact, the suspect did have a brother, who subsequently confessed and pleaded guilty to the charge of rape. This illustrates how the discriminating power of DNA profiling is used to protect the innocent.

Another feature of DNA profiling is that guilty suspects may be more inclined to admit their guilt in the face of DNA evidence, with corresponding savings in expensive legal procedures. Although the benefits have not been quantified—surprisingly, scientists often do not know the outcome of a trial—there is anecdotal evidence that the remarkable discriminating power of DNA profiling has reduced the need for victims to give evidence in court. Because all of the legal protections are designed to prevent the chance of a wrongful conviction, rape victims, for example, may often feel that they have been harshly treated.

Being a witness is a testing experience; as forensic scientists it is accepted as an occupational hazard that we may have to be cross examined by barristers who will often attack the scientist instead of the science.

**Throwing some light**

Recently, instead of using 3²P, we have been chemically linking the DNA probes to alkaline phosphatase—an enzyme that hydrolyses phosphate acid monoesters. The probe–enzyme adducts are then attached to the VNTR sequences on the nylon membrane as before. In principle, the sequences should be found simply by spraying with a substrate that generates a coloured product on reacting with the enzyme, but in practice this method is limited because of its poor sensitivity. Instead, we employ the substituted dioxane phosphate substrate that was introduced by Cellmark Diagnostics. This substrate is relatively stable until it comes into contact with the enzyme whereupon the phosphate group is removed and the ensuing product decomposes with the emission of light—a phenomenon described as chemiluminescence (Scheme 1).

The film is therefore exposed by photons rather than by the β-particles emitted during ³²P decay.

But although at least one of the substrate breakdown products is in an excited state, this will tend to lose energy as molecular vibration and rotation instead of light and only a few photons emerge per
million molecular disintegrations: the quantum yield is low. Alkaline phosphatase has a high turnover number—i.e. a large number of catalytic cycles per second—but this is still not enough to give the sensitivity needed.

An ingenious extension of this technology is to add a carefully designed surfactant to the substrate solution. The surfactant contains fluorescein molecules attached to a hydrocarbon tail and these form mixed micelles with cetyltrimethylammonium bromide (Fig. 3). The dephosphorylated product partitions into the micelles before chemical breakdown occurs. In this environment, the excited state breakdown product can transfer energy to fluorescein, which emits it as fluorescence.

This results in more than a 300-fold increase in light output. Apart from removing the need to work with radioactive materials, the overall sensitivity is at least as high as with 32P labelling; the chemiluminescence method is now used routinely at the MPFL.

**Amplification cycles**

Already the technology of detection is rapidly progressing. Natural biochemical mechanisms for duplicating DNA, which use the enzyme DNA polymerase, have been adapted to turn many cycles of duplication into a cascade of amplification. The polymerase chain reaction generates a greater quantity of starting material for DNA analysis, provided that care is taken to avoid amplifying contaminants instead of the target material. In the future, it will be possible to use simpler fluorescence methods to identify specific DNA fragments and perhaps to determine the sequence of individual DNA fragments. DNA profiling will benefit from the technology being developed for the Human Genome Project; the improved sequencing methods that are likely to emerge from this might ultimately allow us to use the technique routinely. The project may also define other informative regions of the DNA that could be useful in profiling.

Another possibility is to use the DNA in mitochondria—organelles which consume oxygen and produce energy for the cell. Unlike genomic DNA, mitochondrial DNA is inherited from the mother. This has some disadvantages for forensic science because paternal information is missing, but one of the benefits of mitochondrial DNA is that each cell contains many copies and, if genomic DNA is hard to find, mitochondrial DNA might be used as an alternative. The shaft of a hair, for example, does not contain useful amounts of genomic DNA but it has already been shown that mitochondrial DNA can be isolated in adequate quantities. With better detection systems and more readily available sources of DNA coming onto the scene DNA profiling looks set to have a bright future.

**References**


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