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LUMINESCENCE—A REVIEW

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Review: Applications of Luminescence in Forensic Science

Luminescence is defined as the emission from a sample of radiation that falls in the near ultraviolet, visible, or near infrared regions of the spectrum. Conventional luminescence (fluorescence and phosphorescence) is widely used and consists of excitation by electromagnetic radiation, usually of a wavelength greater than 200 nm, and the emission of radiation that has a longer wavelength than the excitation radiation. Other forms of luminescence differ from fluorescence and phosphorescence in that they derive the excitation energy from another source. Cathodoluminescence, for instance, uses a beam of low energy electrons as an excitation source. Chemiluminescence and bioluminescence derive their excitation from chemical reactions, bioluminescence being a special case where the chemical reaction occurs in a biological specimen. Thermoluminescence derives its excitation energy from ultraviolet or ionizing radiation, but the excitation is "trapped" in the material, only to be released when the material is heated.

Luminescence can be used analytically to give a high degree of selectivity because the absorption, emission, and lifetime characteristics of a sample must match those of a control sample. Luminescence is highly selective, nondestructive, and inexpensive. The major advantage of emission spectroscopy over absorption spectroscopy is that an emission band is often observable, whereas the corresponding absorption band is often masked by the background absorption of the sample; thus, the separation of complex mixtures is unnecessary. Furthermore, emission spectroscopy can often detect concentrations of the order of $10^{-8}M$, whereas molecular absorption spectroscopy is limited to concentrations of the order of $10^{-5}M$. Therefore luminescence can be a very powerful tool in the hands of the forensic scientist.

The first use of luminescence in forensic science is thought to have been in the case of *Rex v. Podmore* in 1930 [*1*]. In this case a dirty, oily fragment of paper was found at the scene of a murder, and after it was cleaned with benzene it revealed writing and an address that led to the identification and conviction of the murderer. Although ultraviolet light showed more of the writing than could be seen with the naked eye, it was considered safer to rely on what the jury could see unaided. The judge even excluded an outline photograph of the address.

In 1932 [*2*], in the trial of the case of *New York v. Raymond* on a charge of forgery for fraudulently altering and negotiating a stock certificate, testimony was presented on

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the subject of the use of ultraviolet irradiation as a means of showing what had been chemically erased. In conjunction with this testimony the whole jury looked at the erasure under the ultraviolet lamp and read the erased writing. The trial resulted in conviction of the defendant.

Many uses of luminescence are described below, from the analysis of drugs to the use of infrared luminescence in the questioned document field. A common use of fluorescence is in detector powders often used in an attempt to identify pilferers by tagging an object (for example, a handbag and its contents) with a fluorescent powder that absorbs in the ultraviolet and fluoresces in the blue or green regions and then examining the hands of the suspect under an ultraviolet lamp. Fluorescence is often used to establish that a gemstone is authentic because many synthetic gems fluoresce. Probably the major use of fluorescence is as an aid to detection systems, not only in paper and thin-layer chromatography but also in gas-liquid and high-pressure liquid chromatography.

Drug Analysis

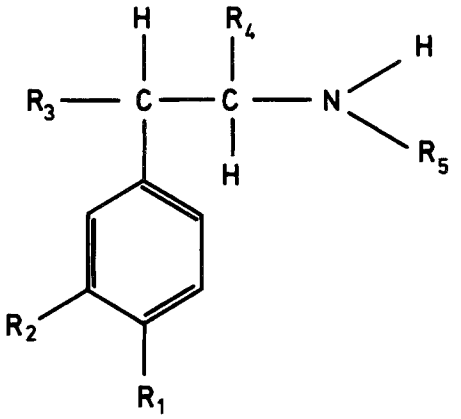
Many drugs have luminescence properties, and these properties provide the forensic scientist with a method whereby unequivocal evidence may be produced. The sensitivity of modern spectrofluorometers is such that a detection limit of 10 ng/ml is not uncommon. The analyses can be conducted on both solid and liquid samples. The solid samples can be vegetable matter or, in the case of suspected hallucinogenic drugs, sugar cubes, blotting paper, or tablets, whereas the liquid samples are usually body fluids. In both cases it is usual to perform an extraction procedure to isolate the drugs and then perform the analysis. As can be imagined, this can be a time-consuming procedure involving much skill, and this makes it desirable to automate the analysis.

The apparatus normally used is similar to that described by Parker and co-workers [*3,4*], although most laboratories use commercially produced equipment. One particular modification especially useful in studying small samples is to replace the sample compartment with a microscope stage [*5*]. Although this does not increase the sensitivity of the equipment it does allow the study of small objects and even single fibers. The experimental procedure of recording an emission spectrum is relatively simple, although it must be borne in mind that photophysical processes are sensitive to impurity quenching, oxygen quenching, the absorption of emitted radiation by another chromophore, and many other complications that are beyond the scope of this essay but are discussed in standard texts [*6*].

Studies in the United States indicate that driving under the influence of drugs is widespread. Finkle et al [*7*] report that of over 3000 drinking driver arrests 21% indicated some kind of concurrent drug use. Some of these drugs were relatively nontoxic, while others were obviously potential hazards to safe driving. About 2% of the cases indicated use of a drug which unless administered under medical supervision would have constituted an offense. In a small survey Turk et al [*8*] showed that 5% of drivers killed in single-vehicle incidents had both alcohol and a drug present in the bloodstream and that 15% of pedestrians killed in road traffic accidents had detectable amounts of drugs other than alcohol, and 6% had both alcohol and drugs present in the bloodstream. Garriott and Latman [*9*] reviewed the procedure for dealing with suspected cases of driving under the influence of drugs. The methods used did not analyze for opiates, cannabinoid, or lysergic acid derivatives but did analyze for the sedative, hypnotic, and stimulant drugs. They do note that in 12% of all cases in 1974 and 14% in 1973 "cannabis" was mentioned in the arresting officers' reports.

Phenylethylamines

This class of compounds has the general structure shown in Fig. 1. The important members of the group are amphetamine (I), epinephrine (II), and ephedrine (III). These



I	amphetamine	$R_1 = R_2 = R_3 = R_5 = H$; $R_4 = CH_3$
II	epinephrine	$R_1 = R_2 = R_3 = OH$; $R_4 = H$; $R_5 = CH_3$
III	ephedrine	$R_1 = R_2 = H$; $R_3 = OH$; $R_4 = R_5 = CH_3$
IV	methamphetamine	$R_1 = R_2 = R_3 = H$; $R_4 = R_5 = CH_3$
V	phenylethylamine	$R_1 = R_2 = R_3 = R_4 = R_5 = H$

FIG. 1—*General structural formula of phenylethylamines.*

drugs are used as stimulants, and it is thought that drug dependence may occur with excessive usage. Although the phenylethylamines have a natural luminescence it is usual to enhance the luminescence by means of modifications to the drug. Miles and Schenk [*10*] have shown that the fluorescence of phenylethylamines is sufficient to allow detection of 2 μg/ml amphetamine and ephedrine and 10 ng/ml epinephrine. They have studied the application of fluorescence and phosphorescence to the problem of identification of the phenylethylamines, but there is insufficient variation in their luminescence characteristics to form the basis of a method of identification.

Among the methods of preparation of highly fluorescent derivatives of the phenylethylamines is that of Nix and Hume [*11*], who formed the lutidine derivative of amphetamine, which has an order of magnitude higher quantum yield of fluorescence than amphetamine. They noted that structurally related drugs might interfere with the determination and report that methamphetamine (IV) and phenylethylamine (V) do interfere, the latter having a detection limit of comparable magnitude to that of amphetamine.

Stewart and Lotti [*12*] investigated the formation of a highly fluorescent coumarin-amine salt by reacting 3-carboxy-7-hydroxycoumarin with various phenylethylamines. They report detection limits of 0.3 μg/ml for amphetamine and 0.15 μg/ml for methamphetamine. Although the coumarin reagent is itself fluorescent and will react with any amine having a pK value greater than 8, they report that the coumarin-amine salt can be detected even in the presence of a 30-fold excess of coumarin reagent. This method does not provide a means of identifying the various phenylethylamines. Neither does the method of Loh et al [*13*], who used the highly nonspecific dansylchloride to determine amphetamine.

Montforte et al [*14*] used 7-chloro-4-nitro-benzo-2-oxa-1,3-diazole (NBD), which reacts with primary and secondary amines to produce highly fluorescent derivatives of several phenylethylamines. Although this method of analysis agreed very well with a gas chromatographic and a colorimetric method for a trial using therapeutic dosage levels, it is noted that if more than one drug is present fluorometric analysis is unsuitable for quantitative work. Montforte et al [*14*] also note that their method does have an advantage over that of Stewart and Lotti [*12*] and of Loh et al [*13*] in that NBD is not itself fluorescent.

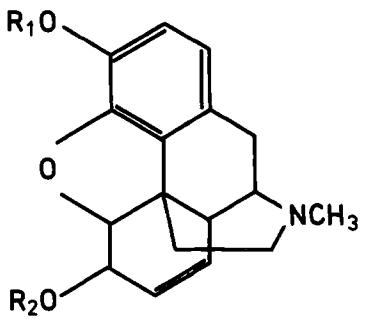
This advantage is shared by the reagent fluorescamine [*15*], which has the added advantages of only reacting with primary amines and being hydrolyzed in water to form nonfluorescent products. Nowicki [*16*] has shown that this reagent will differentiate between amphetamine and methamphetamine. The fluorescamine-amine reaction product has an intense blue-green fluorescence, and the fluorescamine reagent can be used to visualize thin-layer and paper chromatograms.

The above methods all use various manual separation techniques; Hayes [*17*], however, has successfully automated the whole procedure for the fluorometric determination of amphetamine (a primary amine). Hayes attained a flow rate of 30 samples per hour and showed that this method could determine 1 μg/ml. The only compounds tested that yielded false positives were phenylethylamine and tuaminoheptane, both of which are unlikely to be encountered in urine analysis.

Harbaugh et al [*18*] have applied time-resolved phosphorimetry to the analysis of drugs and report that methamphetamine and amphetamine can be neither spectrally nor temporally resolved.

Morphine and Its Derivatives

Morphine was until recently widely used as an analgesic in many parts of the world. Its disuse has come about because it is now recognized as a drug of dependence, and this forms the basis of a major social problem in the United States. The important members of the morphine group of drugs (Fig. 2) are morphine itself (VI), methylmorphine (codeine) (VII), ethylmorphine (VIII), and heroin (IX), whose major metabolite in man is morphine.



VI	morphine	$R_1 = R_2 = H$
VII	methylmorphine	$R_1 = CH_3$; $R_2 = H$
VIII	ethylmorphine	$R_1 = C_2H_5$; $R_2 = H$
IX	heroin	$R_1 = R_2 = OCCH_3$
X	6-acetylmorphine	$R_1 = H$; $R_2 = OCCH_3$

FIG. 2—*General structural formula of morphine derivatives.*

Kupferberg et al [*19*] have shown that morphine may be extracted from biological tissues and estimated with a high degree of specificity. The basis for their method is the conversion of weakly fluorescent morphine to the highly fluorescent dimer pseudomorphine by using alkaline potassium ferricyanide. Kupferberg et al note that although the method is sensitive to 0.1 μg/ml it is subject to interference from normorphine, *N*-allyl-normorphine, dihydromorphine, and 6-acetylmorphine (X). They note that it is not sensitive to morphinans, codeine, heroin, dihydromorphinone, or apomorphine. Takemori [*20*] has described a modification to the method of Kupferberg et al [*19*] which increases the sensitivity tenfold by reducing the reaction volumes.

Mulé and Hushin [*21*] have described a method for detecting morphine and quinine (a frequent adulterant of illicit morphine and heroin) that consists of a rapid oxidation procedure with an autoclave. They claim that the detection of quinine by fluorometry could be a first step in large-scale screening of the urine of suspected morphine and heroin users. They show that quinine is detectable in urine up to ten days after ingestion of 325 mg of quinine sulfate. Such a method would not be useful if the suspect had consumed a tonic water containing 10 mg of quinine sulfate in the preceding 24 h. The detection limit for morphine is 0.2 μg/ml, and only normorphine and *N*-allyl-normorphine were found to interfere with the method. Mulé and Hushin [*21*] compared the spectral properties of pseudomorphine with the fluorophore produced by their method and conclude that although similarities exist there are sufficient differences to indicate that pseudomorphine and their fluorophore are not identical. Cordova and Banford [*22*] have modified the method of Mulé and Hushin [*21*] by including a charcoal extraction step in the determination of morphine in urine. They report a 20-fold increase in sensitivity, allowing the determination of 10 ng/ml.

Blackmore et al [*23*] and Sansur et al [*24*] have described automated methods of analysis for morphine in urine. Their methods are sensitive to 0.2 μg/ml and both used pseudomorphine as the fluorophore. Sansur et al [*24*] showed that their analyzer gave excellent agreement with the results obtained by thin-layer chromatography on 500 samples from a methadone maintenance program. Neither group indicates that false positives were recorded in their work. Sohn et al [*25*] have compared more than 3000 urine samples chosen from over 50 000 on the basis that they were positive for quinine and have found that automated morphine analysis employing fluorometric techniques (AMA), thin-layer chromatography (TLC), and spectrofluorometry yielded 24.3%, 19.9%, and 27.0% morphine-positive samples, respectively. The AMA technique employed pseudomorphine as the fluorophore, but the spectrofluorometry technique used the fluorophore of Mulé and Hushin [*21*]; the AMA technique had a detection limit of 0.1 μg/ml as opposed to 0.2 μg/ml for the spectrofluorometry technique.

Harbaugh et al [*18*] studied the time-resolved phosphorimetry of morphine, codeine, and ethylmorphine and concluded that the three could not be spectrally resolved, although morphine could be temporally resolved from the other two. Bowd and Turnbull [*26,27*] have reported the luminescence characteristics of many morphine derivatives, in particular the triplet state of heroin.

Cannabis

Forrest et al [*28*] prepared the fluorescent dansyl derivatives of nine cannabinoids and then separated them by TLC and visualized the spots using the fluorescence of the derivatives. Although they claim a sensitivity of approximately 1 ng they did not apply their technique to body fluids and it must be borne in mind that dansylchloride is highly nonspecific, reacting with amines and phenols. Bowd and co-workers [*29,30*] have reported the luminescence characteristics of some *Cannabis* constituents and also noted that photochemical effects produce certain characteristic spectroscopic changes. A detection limit of 10 ng/ml is claimed by using the intense fluorescence emission peaks at 366 and 382 nm that develop on irradiation. An extraction procedure has been developed for the treatment of mouthwash of a suspected *Cannabis* smoker, and although only 60 to 90% of the *Cannabis* derivative is recovered a detection limit of 15 ng/ml is claimed (this represents the extraction of 0.75 μg of *Cannabis* from the mouth of the suspect). The same method could be used to investigate the presence of *Cannabis* constituents in the urine. Under the present United Kingdom Road Traffic Act a person who is suspected of driving under the influence of alcohol or drugs has the choice of providing a blood or urine sample for analysis. This choice means that the forensic scientist may be faced with a blood sample to analyze rather than a urine sample, which is generally easier to analyze.

Vinson et al [*31*] have recently reported the derivatization of Δ^9-tetrahydrocannabinol using 2-*p*-chlorosulfophenyl-3-phenylindone, which reacts with the phenol group to form a derivative. After extraction of the derivative from either blood or serum it is visualized on thin-layer chromatograms utilizing its fluorescence under long wavelength ultraviolet light after the chromatogram has been sprayed with methylate. Although only phenols appear to react with the derivatizing reagent common analgesics containing phenolic groups such as salicylic acid, salicylamide, and acetaminophen do not react. Although detection limits claimed are similar to those obtained by using gas chromatography this method requires several extraction procedures and the development of a thin-layer chromatogram, because several likely interfering substances give fluorescent products, and is therefore more time-consuming than the gas-chromatographic method.

Recently there have been reports in the press [*32,33*] of a device which would provide the necessary screening test for the presence of *Cannabis*, similar to the Breathalyzer® for the detection of excessive alcohol levels in the body. Such a device would use the photochemical method of detection, but as yet the underlying chemistry is still not fully understood. The need for such a device has not yet been fully realized, but with successful prosecutions for the possession of *Cannabis* in the United Kingdom increasing from 588 in 1962 to 13 827 in 1973 it is difficult to imagine that driving under the influence of *Cannabis* is an isolated offense.

Other Important Drugs

Of the many other drugs available to the medical profession two of the important groups are the antidepressants and the tranquilizers. Both are available mixed with an analgesic such as aspirin or acetaminophen, which will hinder rapid identification in body fluids.

Hill and Smith [*34*] have described an automated analytical method for the salicylate ion in blood and tissues using the intrinsic fluorescence of the salicylate ion; the method is sufficiently sensitive to operate with microlitre quantities of blood and to detect 0.1 μg/ml salicylate. Of the tranquilizers the most well known are probably Librium® and Valium®, which Koechlin and D'Arconte [*35*] estimated to 0.1 μg/ml by a photochemical conversion to the highly fluorescent epoxide.

Recently de Silva et al [*36*] have reported on the low-temperature luminescence of tetrahydrocarbazole, carbazole, and many 1,4-benzodiazepines. They also reported on the use of TLC plates immersed in liquid nitrogen as a simple method for establishing whether a compound showed luminescence at 77 K. This innovation is of limited application as portable ultraviolet lamps emit at only 254 or 366 nm and thus only species that absorb these wavelengths will respond. Stewart and Williams [*37*] used fluorescamine to determine Librium in both urine and plasma after acid hydrolysis to give a detection limit of 0.25 μg/ml.

Mellinger and Keeler and others [*38–40*] oxidized members of the phenothiazine group (chlorpromazine, trifluoperazine, and thioridazine) to give highly fluorescent derivatives and reported detection limits of the order of 20 ng/ml. Forrest et al [*41*] used the highly nonspecific fluorescent reagent dansylchloride to prepare the fluorescent derivative of chlorpromazine, which they characterized by TLC, fluorometry, and mass spectroscopy. Using the *o*-phthalaldehyde reaction Rieder [*42*] formed a fluorescent product with nitrazepam which gave a detection limit of 10 ng/ml. The tranquilizing alkaloid reserpine has been determined by its reaction with vanadium pentoxide in phosphoric acid down to 4 ng/ml [*43*].

The barbiturates, another often used and abused group of drugs, have been the subject of an intensive study by King and co-workers [*44–46*] and also by Miles and Schenk [*10*]. The latter observed that although the detection limit for barbiturates was of the order of 100 ng/ml neither fluorescence nor phosphorescence could be used to identify

members of this group of compounds. Phenobarbital was noted by both groups as having a phosphorescence of shorter wavelength than the room-temperature fluorescence, which has been attributed to an energy-transfer process [*45*]. Time-resolved phosphorimetry has been used to estimate mixtures of phenobarbital and amobarbital [*18*].

Antidepressant drugs are often primary or secondary amines and thus if they are not themselves fluorescent can be made so using dansylchloride or fluorescamine. Turner et al [*47*] showed that tranylcypromine, a potent inhibitor of monoamine oxidase and a widely used antidepressant, could be detected in urine down to 100 ng/ml. Dansylchloride was used by Forrest et al [*41*] to characterize nortriptyline and imipramine, although no application to biological fluids was reported. The high fluorescence of imipramine has been used to estimate its presence in urine down to 100 ng/ml [*48*].

Many hallucinogenic drugs are related to the ergot alkaloids, which contain the indole nucleus, probably the most well known being lysergic acid diethylamide (LSD). Martin and Alexander [*49*] described several possible sources of illicit LSD, from powders, sugar cubes, and liquids to tablets. The characteristic fluorescence emission of LSD at 432 nm in ethanolic solution is used to identify as little as 1 ng/ml. Bowd et al [*50,51*] have reported the luminescence characteristics of nine LSD derivatives, all showing a high quantum yield. A detection limit of the order of 1 ng/ml can be derived from their data. The use of microspectrofluorometry has been applied to determination of LSD in solid samples [*52*].

Another important group of hallucinogens is the dimethyltryptamines; psilocin (4-hydroxydimethyltryptamine) and psilocybin, its phosphate ester, both occur naturally in the Mexican mushroom, and bufotenin (5-hydroxydimethyltryptamine) occurs naturally in the toad and in a fungus. Clarke [*53*] has reported that psilocin and bufotenin fluoresce when irradiated at 254 nm, whereas psilocybin does not; this observation is not supported by the work of Gillespie [*54*], who reported a detection limit of 1 μg/ml for psilocybin.

Aaron et al [*55*] have made an analytical study of some important hallucinogens by combining fluorometry and phosphorimetry. They observed fluorescence from both psilocin and psilocybin and report detection limits ranging from 15 ng/ml for *N,N*-dimethyltryptamine to 1 ng/ml for bufotenin. They report that phosphorescence spectra and lifetimes can be used in a complementary mode to identify hallucinogens; for example, LSD and lysergic acid exhibit phosphorescence at a much longer wavelength than the other hallucinogens they studied and can be distinguished by the different vibrational structure of their phosphorescence bands. Psilocybin and ibogaine show very similar vibrational structure and position of their phosphorescence bands but can be characterized by their different phosphorescence lifetimes.

Aaron et al [*55*] report phosphorescence lifetimes of 4 and 100 ms for LSD and lysergic acid, respectively, whereas Bowd et al [*50,51*] have reported lifetimes of the order of 20 ms. The lifetimes reported by Bowd et al have been shown to be incorrect because of instrumental limitations and are being redetermined; preliminary results indicate a phosphorescence lifetime of 0.5 s for LSD in an ethanolic glass at 77 K.[2]

Dal Cortivo et al [*56*] have determined the synthetic narcotic meperidine down to 0.3 μg/ml by reacting meperidine with formaldehyde and concentrated sulfuric acid. They note that phenothiazines, benzodiazepines, dibenzazepines, and opiates do not interfere with the determination procedure. McGonigle [*57*] has described a modification to the method of Dal Cortivo et al [*56*] for the determination of methadone. A detection limit of 1 μg/ml is quoted and while morphine, heroin, codeine, and cocaine do not interfere with this method, amphetamine, quinine, and meperidine do. The successful use of methadone to wean patients from heroin has led to its widespread use in the United States and it is now the major prescribed drug of dependence used by registered addicts

[2] A. Bowd and E. P. Gibson, unpublished report.

in Great Britain and therefore is possibly subject to drug abuse. Caddy et al [*58*] have described an oxidative procedure for drugs which contain the diphenylmethylidene group, such as methadone, which yields benzophenone quantitatively and thus allows phosphorimetric determination.

The hypnotic drug flurazepam can be converted into the highly fluorescent acridone derivative to give a sensitivity of the order of 3 ng/ml of blood or urine [*59*]. The highly addictive drug cocaine has been spectrally resolved from procaine and both have a sufficiently high phosphorescence yield to be detected down to 1 μg/ml [*18*].

Contact Material Characterization

Contact materials are an important class of evidence, whether in the form of a fiber or a hair linking an assault victim to a suspected assailant, or a blood stain, or an oil spill. Often such evidence is characterized by conventional optical techniques, but luminescence can be helpful on occasions.

Oils and Related Materials

All the luminescence characteristics of such substances are due to the complex mixture of polynuclear aromatic hydrocarbons present in them. Originally such characterization was performed after a separation, usually by paper or thin-layer chromatography. Bayes-Cope [*60*] described a test for bitumen, pitch, and mineral grease based on such a procedure that used their natural luminescence, estimating that as little as 1% bitumen in a 1-mg sample can be detected. Parker and Barnes [*61*] investigated the fluorescence emission and excitation spectra of a variety of lubricating oils, all of which showed an intense fluorescence in the ultraviolet region. On this basis less than 1 μg of oil mist per litre of air was determined. Matthews [*62*] used TLC for the separation and fluorescence for the systematic identification of petroleum and coal-tar oils and residues and successfully applied the method to water pollution problems.

Lloyd [*63*] described a major innovation in experimental technique in 1971: the synchronized excitation of fluorescence emission spectra. The fluorescence emission spectra of complex mixtures such as petroleum products are often complicated and cannot be successfully resolved with the conventional technique of excitation at various wavelengths selected specifically for individual components. Considerable improvement can be made when the excitation and emission wavelengths are varied together a fixed wavelength interval apart; strictly speaking the interval should be a fixed energy, but over a restricted wavelength region the variation is small. While the spectra obtained are not very meaningful for identifying the fluorescent species concerned, they do provide a useful "fingerprint" for comparison purposes. Lloyd [*64–66*] has applied synchronous excitation to the examination traces of mineral oils, fuels, bitumens, pitch, and soots. The fluorescence is due in all cases to polynuclear aromatic hydrocarbons [*66*], a number of which were previously unknown to occur in petroleum products and alkylated benzyl analogues of thiophene.

Longhran et al [*67*] have reported the discovery and first application of the fluorescence of tire prints in a successful murder prosecution. The discovery that tires could leave a luminescent print led to the characterization of rubbers, rubber contact traces, and tire prints by synchronous excitation fluorescence [*68,69*]. The fluorescence is due mainly to extender and process oils, to antioxidants, and to polynuclear aromatic hydrocarbons. Lloyd reports that the variation in these components in tire treads, caused by variation in manufacture and wear, enables tread rubbers and their prints to be effectively distinguished from one another. Tire sidewall rubbers are distinguished from tread rubbers by differences in the extraneous polynuclear aromatic hydrocarbon emissions, and in the case of radial tires, principally by differences in extender oil emissions. Lloyd [*69*] has

noted that since phosphorescence occurs at longer wavelengths than fluorescence and since fluorescence occurs in the violet or blue region of the spectrum in which the eye is not as sensitive as it is at longer wavelengths, then phosphorescence rather than fluorescence could be used to visualize tire prints and possibly to photograph the tire prints.

John and Soutar [*70*] have shown that the use of synchronous excitation fluorescence spectroscopy can allow firm conclusions to be drawn concerning the identity of an unweathered crude oil sample. Bentz [*71*] has recently reviewed the use of fluorescence spectroscopy in oil spill identification, without, surprisingly, including the technique of synchronous excitation fluorescence.

Biological Materials

One of the most common biological materials with which the forensic scientist has to deal is blood, usually in the form of bloodstains. The information that could be obtained from a bloodstain was until recently limited to the determination of the blood group. A specific fluorescent staining of the human Y chromosome was accomplished by Zech [*72*] in 1969 and others subsequently [*73*]. The Y chromosome has been stained by quinacrine hydrochloride or quinacrine mustard and is now routinely used in sexing procedures. Phillips and Webster [*74*] improved the fluorescence from quinacrine-stained Y chromosomes in extracts of human bloodstains by the addition of magnesium ions. They showed that a fivefold increase in the intensity of fluorescence was obtainable and that it was possible to type bloodstains up to eight weeks old, as opposed to only one week old without added magnesium ions. Ishizu [*75*] has shown that some 65% of male leukocytes are stained with quinacrine hydrochloride as opposed to only 1% of female leukocytes. He also reported successful use of the technique on such materials as saliva, hair, urine, and teeth. Furthermore, Ishizu has determined that this method produces significant results on bloodstains up to six months old.

Phillips and Gitsham [*76*] have made a study of the possible determination of the XYY chromosome, which occurs in only about 1:600 of the Caucasian population. Although Pearson and Bobrow [*77*] have reported an incidence of 0.5% of normal male nuclei showing two bright spots, indicating two Y chromosomes, Phillips and Gitsham [*76*] report an incidence of 28% of nuclei from XYY genotypes showing two bright spots.

King [*78*] has used the identification of antiparasitic antibodies in bloodstains using an indirect fluorescent antibody technique to show that by typing only five antigens the discriminating power of the technique is greater than the theoretical discriminating power of the ABO system. This method can provide additional information on the age of the source of the blood sample; for instance, most adults carry antibodies to tuberculosis but young children rarely do. The use of the fluorescent antibody technique for the grouping of human hair has been shown by Brown [*79*] to be insufficiently sensitive both microfluorometrically and macrofluorometrically because of the high background fluorescence level of the amino-acid residues in the hair.

Jones [*80*] has recently reported on the possible use of the phosphorescence characteristics of hairs for their individualization. He reported differences in the excitation and emission spectra and in the phosphorescence decay lifetimes of hairs from different individuals. He concluded that although differentiation through phosphorescence characteristics was possible in the limited sample that he took, for a large population such properties could not be expected to vary sufficiently from one individual to another to be useful.

Calloway et al [*81*] have developed a technique in which the low-temperature phosphorescence from seminal stains on fabric has been used to make the stains and their flow patterns visible. Previously the natural fluorescence of semen had been used for these purposes, but the widespread use of fluorescent additives in detergents and some

clothing fibers has limited the usefulness of this technique. Calloway et al note that while phosphorescence is observed from perspiration, it is not observed from blood or saliva.

In the more usual test for seminal stains, which relies on the action of the enzyme acid phosphatase found in semen, 7-monophosphate-4-methylcoumarin or naphthyl phosphate is added to a damp filter paper, which had prevously been placed on the suspected stain, and the acid phosphatase converts 7-monophosphate-4-methylcoumarin into 7-hydroxy-4-methylcoumarin and naphthyl phosphate into α-naphthol, which are highly fluorescent. Such a test shows that semen may be present, and it is usual to test for the presence of sperm cells by staining with acridine orange and viewing under a fluorescence microscope.

The dating of human bones has always been a difficult task for the forensic scientist. The dating is important to determine whether the remains are recent enough to warrant further inquiries or whether the bones are only of antiquarian or archaeological interest. Knight and Lauder [*82,83*] have reviewed the methods of dating bones and show that the fluorescence of freshly sawed cross-sectional surfaces of femurs, humeri, or tibiae can be correlated with the age of the bone. In these bones a central zone of fluorescence, sandwiched between concentric zones of nonfluorescent material, is observed. Although the central zone is by no means progressively reduced with age and is obviously influenced by environment, generally it can be assumed that a bone showing fluorescence across the whole cross section is less than 100 years old.

Fiber Transfer

The use of microspectrofluorometry to examine fibers in forensic work has not yet been fully developed, although the possibility has been recognized [*52*]. Fibers may be characterized by the fluorescence and absorption spectra of any dyestuff on the fiber, and by the fluorescence of any optical brighteners present, usually from washing powders. Eckhardt and Hefti [*84*] have made a comparison of the fluorescent brighteners used in the production of synthetic polymer fibers and in the textile industry. Gasparic [*85*] has tabulated the R_f values of over 20 optical brightening agents used in fiber production. The transfer of fibers between clothing materials during simulated contacts and their persistence during wear has been studied by Pounds and Smalldon [*86,87*]. Thus, although the underlying research has been performed there has been little or no application in the field of forensic science because of a lack of commercially available microspectrofluorometers.

Miscellaneous Uses of Luminescence

Another major field in which luminescence has been exploited is questioned document work. Watermarks can be faked by waxing the paper to make it more translucent, and the wax will show a different luminescence from the paper. Most papers possess their own luminescence that would be altered by chemical erasure. Mechanical erasure alters the surface of the paper, and many fluorescent powders have been developed to show these alterations.

Additions are more difficult to detect, and much work has gone into characterizing the luminescence of inks. Ellen and Creer [*88*] have studied over 100 fountain pen and ball-point inks and found that 39 showed luminescence. Those inks that luminesce do so mainly in the infrared region. This luminescence can be detected on infrared-sensitive film or viewed through an image intensifier. There is a reasonable probability that any additions made to a document using ink of nominally similar appearance may be detected by a difference in infrared luminescence. Faded inks can often be visualized as the luminescing component is usually more stable than the coloring material. Infrared luminescence can often enable chemically, or even mechanically, erased writings to be

read. It is probable that the particular luminescent component in the ink penetrates to a greater depth than the pigments, many of which remain as finely divided solids on or close to the surface of the uppermost fibers of the paper.

Kelly [*89*] has studied the fluorescence of ball-point inks and showed that some emit in the near ultraviolet or the blue region of the spectrum. He notes that some manufacturers of ball-point pens purchase ink from the same source, and therefore the pen brand cannot be identified. Constain and Lewis [*90*] recently reviewed the application of infrared luminescence to questioned document problems and discussed the setting up of a questioned document laboratory.

Jones and Nesbitt [*91*] have developed a luminescence technique for the detection of gunshot residues. Lead, antimony, and barium are the three most characteristic metallic elements found in gunshot residues. These researchers discarded many luminescence procedures for reasons of insensitivity, nonspecificity, or complexity, and they observed the luminescence from lead(II) and antimony(III) ions complexed with chloride ions in 7*M* hydrochloric acid at 77 K. Only five other inorganic ions show luminescence under these conditions: thallium(I), bismuth(III), tellurium(IV), selenium(IV), and arsenic(III), all of which have the same electronic configuration ns^2, and the luminescence appears to be analogous to the $^3P_1 \rightarrow {}^1S_0$ radiative transition in mercury. Barium does not luminesce under these conditions, and lead and antimony can be simultaneously determined with detection limits of 0.1 and 1.0 ng, respectively.

Fluorescence has also been used for the visualization of fingerprints. Patterson [*92*] was the first to propose the use of a fluorescent fingerprint powder, using a finely ground polymeric powder containing a fluorescent material. Patterson reports that good results have been obtained from prints on paper, plastics, steering wheels, gear levers, fine-grain leather, and silicone-polished surfaces. Morris [*93*] has developed a reagent that reacts with the traces of urea present in latent fingerprints. The reagent dimethylaminocinnamaldehyde (DMAC) forms a magenta-colored complex with urea. The most dramatic results are obtained by applying DMAC along with a strong acid to form an image and then treating with alkali to restore the yellow fluorecence of unreacted DMAC. Under long wavelength ultraviolet light this puts the dark magenta print ridges of the developed fingerprint into bold contrast with a yellow background.

Other Luminescence Techniques

Of the other luminescence techniques only chemiluminescence seems to have gained wide use, and although thermo-, bio-, and cathodo-luminescence have been investigated they do not, as yet, appear to have gained wide use.

Ingham and Lawson [*94*] studied the thermoluminescence of glasses, soils, safe insulations, and salts from room temperature to 700 K. Thermoluminescence is due to the release of an electron from a trap to a recombination center. The luminescence is characteristic of the recombination center, and the temperature dependence of the total emission is characteristic of the trap. Originally the electron is ionized from the recombination center by natural or laboratory-produced radiation and is captured by a trap, which can be a vacancy or a lattice defect in a crystal lattice or even an impurity. As thermoluminescence depends on the crystal structure, presence of impurities, and the previous environmental history of the sample the thermoluminescence properties of a sample can be characteristic of that sample, and thus provide evidence.

Although the investigation by Ingham and Lawson [*94*] was of a limited nature the results clearly show that thermoluminescence can be used for comparison of two samples of the same type to show whether they were from the same source, although there is generally a finite probability of coincidental matching of glass or soil samples. In addition, it is well known that polymers contain impurities and that they too are thermoluminescent

[*95*], although usually measurements have to be made in the region 77 K to room temperature. It appears that the forensic application of thermoluminescence to this class of materials has not yet been studied.

Church [*96*] has described a device that uses the ability of certain marine bacteria to exhibit luminescence when exposed to specific vapors, in this instance the vapor from certain explosives. The luminescence is detected by a photocell and thus registers the presence of explosive material. It is claimed that bacteria can be bred that will respond only to a specific vapor (for example, explosive vapors or drug vapors) and that the level of sensitivity is such that 1 part in 10^{20} could be detected. The system is marketed in the United States, but it is unreliable and none of the claims made by its manufacturer have been substantiated. A similar system has been developed for the detection of heroin vapor, and again it has proved unreliable. However, if such systems were developed satisfactorily they would be highly sensitive and specific as well as being easy to operate and therefore would have the wide applicability of the Breathalyzer while reducing the case load of the forensic scientist.

Dudley [*97*] has shown that cathodoluminescence, that is, luminescence excited by electron bombardment, can have an application in forensic work. Although minerals, soils, and glasses do not usually show sufficient variation in their emission for cathodoluminescence to be useful in differentiation between samples, ceramics do. Dudley chose 48 basically white or near-white samples, of which 75% showed cathodoluminescence. The luminescent inclusions varied to such an extent that ceramics with the same bulk luminescence could be subdivided into groups.

Glasses which on visual examination showed little variation in luminescence were found to be easily divided into three groups, window, container, and headlamp, when their emission spectra were compared. Thus cathodoluminescence, which is initially a subjective technique, could with spectral recording equipment be easily converted into an objective technique requiring only the comparison of spectra, whereas the usual method of comparing two samples visually under a microscope normally requires a trained mineralogist. Cathodoluminescence could also be applied to the difficult problem of overlapping white paint layers to compare paint flakes.

The chemiluminescence reaction of luminol with the iron of the heme group in blood has been used since the 1930s to detect bloodstains. Zweidinger et al [*98*] have investigated the use of very fast black and white films to photograph bloodstains visualized by the chemiluminescence reaction. With these films they were able to detect as little as 1 ng of iron. Vladimirov et al [*99*] have studied the chemiluminescence of mitochondrial protein after death. The changes in lipids in the first 24 h after death show a change in the intensity and kinetics of chemiluminescence and a change in protein by the decrease in the intensity of protein chemiluminescence. Such a technique would be a useful complement to the usual technique of estimating time of death from stomach contents.

Future Developments

Looking into the future, especially in a field where economic considerations are an important factor, is always difficult. The uses and limitations of luminescence in the field of forensic science have been described above. Major developments to improve the sensitivity of existing luminescence spectrometers are foreseeable, first by increasing the energy input into the sample and second, by increasing the energy absorbed by the sample without increasing the concentration. The increase in energy input can be achieved by increasing the power of the lamp unit used. Commercial instruments rarely use a lamp more powerful than 250 W, whereas laboratory-built instruments using 2-kW lamps have been described [*100*]. A more powerful excitation source is available in the form of the laser. As the spontaneous emission rate is proportional to the cube of the frequency the

development of a continuous-wave ultraviolet laser has only recently been achieved [*101*]. The next problem is to ensure that the excitation source is tunable and to this end ultraviolet dye lasers are available, although cost has militated against their use in forensic science.

To simply increase the path-length over which the excitation is absorbed by the sample will not increase the emission viewed by the detector optics without a complicated light-gathering system. However, with the use of multiple internal reflection fluorescence [*102*] the effective path-length is increased without increasing the cell size. Multiple exposure of the sample to the exciting radiation is achieved as the incident radiation propagates along a thin plate-like element. Emitted fluorescence from samples on large faces of the element is largely trapped and propagates to a narrow edge of the element, where it emerges concentrated into a narrow beam.

Green and O'Haver [*103*] have shown that the use of derivative techniques in luminescence spectroscopy enhances the resolution of minor spectral features and increases the specificity of fluorescence crude oil fingerprint spectra. The use of derivative techniques is common in electron spin resonance and nuclear quadrupole resonance spectroscopy but does not seem to have been adopted in luminescence work. The derivative spectra show subtle features of emission bands much more clearly than the undifferentiated emission spectrum and therefore would be very much more useful for the qualitative identification of compounds via comparison to a file of reference spectra. However, this technique is only useful in instances where the signal to noise ratio is high because the signal to noise ratio is approximately halved by electronic differentiation.

Recently the importance of solvent correction has been emphasized by workers of the American Instrument Co. using a dual-beam ratio recording instrument in the field of oil spillage identification [*104*]. Such an instrument can be easily modified to produce fluorescence difference spectra, and such spectra have been used to identify oil spills [*105,106*], although the effect of aging of the spill was not considered.

Phosphorescence occurs at longer wavelengths than fluorescence, and if the latter is in the near ultraviolet or violet region it is often difficult to see by eye. Phosphorescence is only rarely observed in fluid media at room temperature as the triplet state is extremely susceptible to oxygen and collisional quenching. Accordingly, the reported phosphorescence of absorbed ionic organic molecules at room temperature is surprising [*107*], especially as such phosphorescence shows the same fine structure as low-temperature phosphorescence (except for slight line broadening). This technique, or the use of an enhancer of spin-forbidden transitions (dimethylmercury) [*108,109*], may be more convenient for measuring phosphorescence spectra than the normal low-temperature method.

Another important area of development is automation. Three automated methods of analysis have been mentioned above [*17,23,24*], and the determination of the alcohol content of blood and urine is now automated. Curry [*110*] asserts that it is not possible to predict what techniques will be discovered in the next few years; however, the development and evaluation of techniques at present at the research stage will continue.

McLellan [*111*] points to the drug field as one in which much progress remains to be made. While thousands of blood or urine samples are analyzed annually for alcohol in connection with road traffic offenses in the United Kingdom, the number analyzed successfully for drugs is much lower. In the manufacture of potent drugs like LSD and heroin there will be a certain vapor pressure of the final product in the premises used for manufacture, and this could be detected using a bioluminescent "sniffer" device, which although not yet reliable offers the ultimate in sensitivity.

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