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Clinical Biochemistry 39 (2006) 498–510
CLINICAL BIOCHEMISTRY

Toxicology: Then and now

Loralie J. Langman [a], Bhushan M. Kapur [b,*]

[a] Division of Clinical Biochemistry and Immunology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA
[b] Department of Clinical Pathology, Sunnybrook Health Sciences Centre, Department of Laboratory Medicine and Pathobiology, Faculty of Medicine and Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, 2075 Bayview Avenue, Toronto, Ontario, Canada M4N 3M5

Received 11 January 2006; received in revised form 9 March 2006; accepted 12 March 2006

Abstract

Toxicology is "the science of poisons"; more specifically the chemical and physical properties of poisons, their physiological or behavioral effects on living organisms, qualitative, and quantitative methods for their analysis and the development of procedures for the treatment of poisoning. Although the history of poisons dates to the earliest times, the study and the science of toxicology can be traced to Paracelsus (1493–1541) and Orfila (1757–1853). Modern toxicology is characterized by sophisticated scientific investigation and evaluation of toxic exposures. The 20th century is marked by an advanced level of understanding of toxicology. DNA and various biochemicals that maintain cellular functions were discovered. Our level of knowledge of toxic effects on organs and cells is now being revealed at the molecular level.

This paper will review the historical progress of clinical and forensic toxicology by exploring analytical techniques in drug analysis, differing biological matrices, clinical toxicology, therapeutic drug management, workplace drug testing, and pharmacodynamic monitoring and pharmacogenetics.

Keywords: Analytical methods; Clinical toxicology; Therapeutic drug management; Work place drug testing; Pharmacogenetics

Introduction

The history of poisons dates to earliest times. Ancient man likely observed toxic effects in nature partly by accident. He noted the harmful or fatal effect following the causal ingestion of some plant or animal product by one of his fellow tribesmen and possibly used their extracts for hunting or in warfare [1]. By 1500 BC, written recordings indicated that hemlock, opium, arrow poisons, and certain metals were used to poison enemies or for state executions [1]. With time, poisons became widely used and with great sophistication. Notable poisoning victims include Socrates, Cleopatra, and Claudius [2]. By the time of the Renaissance and the Age of Enlightenment, certain concepts fundamental to toxicology began to take shape.

The traditional definition of toxicology is "the science of poisons." More specifically, toxicology is concerned with the chemical and physical properties of poisons, their physiological or behavioral effects on living organisms, the qualitative and quantitative methods for their analysis in biological and nonbiological materials, and the development of procedures for the treatment of poisoning. A poison (or toxicant) is regarded as any substance that when taken in sufficient quantity will cause sickness or death. The key phrase is "sufficient quantity" [2]. As the sixteenth century physician Paracelsus (1493–1541), who was clearly aware of the dose–response relationship, observed, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy." Paracelsus determined that specific chemicals were actually responsible for the toxicity of a plant or animal poison. He also documented that the body's response to those chemicals depended on the dose received. His studies revealed that small doses of a substance might be harmless or beneficial whereas larger doses could be toxic. This is now known as the dose–response relationship, a major concept of toxicology. For example, minute quantities of cyanide, arsenic, lead, and dichlorodiphenyltrichloroethane (DDT) are regularly ingested from food sources or inhaled as environmental

* Corresponding author. Fax: +1 905 849 4389.
E-mail address: b.kapur@utoronto.ca (B.M. Kapur).

0009-9120/$ - see front matter © 2006 The Canadian Society of Clinical Chemists. All rights reserved.
doi:10.1016/j.clinbiochem.2006.03.004

contaminates and retained by the human body [2]. However, the amounts of toxicants are insufficient to cause obvious deleterious effects [2]. On the other hand, a substance as apparently innocuous as pure water will, if ingested in sufficient quantity, cause incapacitating electrolyte imbalance or even death [2]. There is often no difference between the mechanism of action of a drug and a poison. A drug is administered in doses that alter physiological function to produce a desired therapeutic effect. If administered in greater than therapeutic quantities, a drug may produce toxic (harmful) effects. Thus, toxicology is a quantitative discipline that seeks to identify the amount of a substance that, in particular exposure situations will cause deleterious effects in a particular animal or patient [2].

In the late 18th century a Spanish physician, Matthieu Joseph Bonaventure Orfila, working at the University of Paris, first prepared a systematic correlation between the chemical and biological properties of poisons of the time. He demonstrated effects of poisons on specific organs by analyzing autopsy materials for poisons and their associated tissue damage. He stated that investigations of deaths were too important to be left to the untrained and emphasized that toxicology should be a separate science and chemical analysis must be part of its foundation [1]. He wrote a text, *Traité des Poisons Tirés des Regnes Minéral Végétal et Animal, ou Toxicologie Générale Considérée Sous les Rapports de la Pathologie et de Médicine Légale* (1814), which went through five editions and was a landmark in not only toxicology but in scientific history. Modern toxicology is characterized by sophisticated scientific investigation and evaluation of toxic exposures [1].

The 20th century is marked by an advanced level of understanding of toxicology. DNA and various biochemicals that maintain cellular functions were discovered. Our level of knowledge of toxic effects on organs and cells is now being revealed at the molecular level. It is recognized that virtually all toxic effects are caused by changes in specific cellular molecules and biochemicals.

Toxicology is a multidisciplinary field that freely borrows from the basic sciences. Because of a diversity of concerns and applications, modern toxicology has developed into three specialized branches: environmental, clinical, and forensic [2]. Environmental toxicology is concerned primarily with the harmful effects of chemicals that are encountered incidentally because they are in the atmosphere, in the food chain, or present in occupational or recreational environments. Clinical toxicology, a subspecialty of medicine, is concerned with the harmful effects of chemicals that are intentionally administered to living organisms for the purpose of achieving a specific effect. The desired effect achieved may be beneficial to the organ (therapeutic), in which case the toxicologist is interested in the adverse or side effects of the agent in question. Forensic toxicology is the branch of toxicology that is concerned with the medicolegal aspects of the harmful effects of chemicals or poisons [2]. This paper will review the historical progress of clinical and forensic toxicology by exploring analytical techniques in drug analysis, differing biological matrices, clinical toxicology, therapeutic drug management, workplace drug testing, and pharmacodynamic monitoring and pharmacogenetics.

Analytical techniques in drug analysis

Things have come along way since Marsh (1794–1846) discovered a test for arsenic in 1832. First publicly used during a murder trial, Marsh's test makes use of the formation and ready decomposition of arsine by addition of hydrogen sulfide and hydrochloric acid. Although Marsh was able to detect arsenic as yellow arsenic trisulfide, when it came to show it to the jury it deteriorated, allowing the suspect to be acquitted due to reasonable doubt. Annoyed by this, he developed a much better test by combining a sample containing arsenic with sulfuric acid and arsenic-free zinc, resulting in arsine gas. The gas was ignited, and it decomposed to pure metallic arsenic, and when passed to a cold surface, it would appear as a silvery-black deposit. The test could now detect arsenic as little as one-fiftieth of a milligram. He first described this test in *The Edinburgh Philosophical Journal* in 1838 [3].

In 1965, the routine method for determination of phenytoin and phenobarbital was a liquid–liquid extraction followed by the Bratton–Marshal coupling reaction [4]. Additionally, ultraviolet spectrophotometry studies correlating drug concentration with therapeutic effects were carried out in the late 1950s and early 1960s [5]. These procedures required large sample volumes, extraction techniques were time-consuming, and complex and the assays were subject to much interference; therefore, drug assays were usually carried out in basic research laboratories rather than in routine clinical chemistry laboratories. Solid phase extraction (SPE) using small columns [6,7] simplified this extraction procedure significantly. It not only made the extraction procedure simpler but also improved by standardizing the method, thus improving reproducibility from sample to sample. It also reduced the organic solvent volumes required in the extraction process. More recently, solid phase microextraction (SPME) has been introduced and applied to a large variety of drugs and chemicals [8–10]. The drug of interest is adsorbed onto a fiber [11,12] and subsequently desorbed into the injector port of a gas chromatograph. This solventless sample preparation technique [13] holds a lot of promise for the future as it simplifies the extraction protocol significantly.

The concept of immunologic methods was first discovered by Berson and Yalow [14]. They found that humans treated with insulin always developed anti-insulin antibodies. This led to their development of radioimmunoassay (RIA) by taking advantage of the sensitivity of radioisotopes and specificity of immunologic methods [15,16]. Their concept showed that the concentration of a substance in a biological liquid, typically serum or urine, is measured using the reaction of an antibody or antibodies to its antigen. The assay took advantage of the specific binding of an antibody to its antigen. Since then, antibodies against many drugs have been developed and RIA has found its way in the analysis of many analytes including some endogenous substances. The high specificity and sensitivity of these antibodies permitted the quantitation of

drugs in small volume of serum. Unfortunately, the complexity of the technique, requirement of special instrument, as well as the lack of radioimmunoassay for a wide variety of drugs prevented its widespread adoption for routine drug monitoring. Making drug assays available to all laboratories and physicians required simple technology that could be performed by a technician without special training or instrumentation. This was achieved with the development of the homogeneous enzyme multiple immunoassay technique (EMIT) [17]. Instead of using a radioisotope as a tracer attached to an antibody, as is the case in RIA, an enzyme tracer was linked to the antibody. This obviated the need for radioisotope handling and the associated instrument and safety issues. The significant benefit of EMIT was the accuracy, rapidity, and ease of operation of the assays. Other immunoassay techniques soon followed. Among these were substrate-labeled fluorescent immunoassays (SLFIA) [18], fluorescence polarization immunoassays (FPIA) [19], cloned enzyme doner immunoassay (CEDIA) [20], kinetic interaction of microparticle in solutions (KIMS) [21], and enzyme-linked immunosorbent assay (ELISA) [22] assay methods. These developments brought many drug assays, once restricted to major hospital laboratories, to the laboratories of the community hospitals. A distinct disadvantage of immunoassays is the potential lack of specificity. Because these are "antibody–antigen" reactions, substances that may have a similar chemical structure as the antigen may cross-react and give erroneous results [23,24]. Typically, immunoassays test for a "class" of compounds and cannot distinguish drugs from within its class, for example, opiate "class" consists of morphine, codeine, and a few other narcotic derivatives that have similar molecular structure. The advantage of immunoassay is that the drug of interest does not have to be extracted or separated from other substances in the biological fluid. However, a large number of drugs, typically smaller molecular weight drugs, exist for which antibodies are not available but which must be therapeutically monitored. Analyses of these substances require a different analytical approach. Extraction and separation of the drug are the prerequisites of almost all non-immunoassay methods.

In 1903, a Russian botanist, Mikhail Semenovich Tswett, used a chalk column to separate the pigments of green leaves [25,26]. He called this technique as "chromatography." Chromatography comes from Greek words, *croma* (chroma) and *grafeih* (graphein), together meaning, "color writing." Tswett is sometimes referred to as the father of a large family of separation methods we characterize today as differential migration processes [27]. He used physiochemical principles such as spectroscopy, liquid–liquid extraction, and adsorption, including dynamic adsorption (chromatography) for solving biological problems. Although Tswett described chromatography in 1903, it was a German graduate student Fritz Prior (1941) who developed solid state gas chromatography. The foundation for developing liquid–liquid (1941), paper (1944) chromatography, and gas–liquid chromatography was laid by Archer John Porter Martin. For the invention of partition chromatography, he shared Nobel Prize in 1952 with Richard Synge [28].

During the past century, there have been remarkable advances in the technological and analytical aspects of drug analysis. Gas–liquid chromatography (GLC) represented a breakthrough. It provided a method of separating endogenous substances [29] and measuring toxins [30] in biological fluids. GLC techniques were further refined and improved so that by the early 1970s, GLC analysis of various therapeutically monitored drugs were performed routinely in many clinical chemistry laboratories [5,31]. GLC permits separation of parent drug from metabolite(s) and differentiation from coadministered drugs, other drugs in a class of drugs, and endogenous compounds. Disadvantages include the need for a relatively large sample volume to achieve biological sensitivity, and in some instances chemical derivatization to ensure that the analytes have the prerequisite volatility. Advances in the development of detectors, particularly the nitrogen–phosphorus detector, and application of capillary columns have improved sensitivity of the instruments to such an extent that microliter volumes of sample can be used.

High performance liquid chromatography (HPLC) offers versatility with minimal sample preparation. Its specificity and sensitivity, relatively small sample requirements, and ease of operation make it a viable alternative [31–34]. In addition, HPLCs' adaptability to simultaneously quantitate a large variety of drugs, as well as their metabolites, a capability not available with immunoassays, was demonstrated early in its development [32,35]. This makes HPLC a valuable tool for not only establishing correlations between drug and drug metabolite concentrations in biological fluids, but also in pharmacokinetic studies.

In 1965, Buttery et al. [36] showed that GLC could be combined with mass spectrometry (MS) to take advantage of two devices, the first as a separator of complex mixture and the latter as an identifier of the separated component. The limitations of GC/MS are the same as that of GLC. The combination of HPLC with mass spectrometers, either singly (LC/MS) or in tandem (LC/MS/MS), has revolutionized analytical approaches to therapeutic drug monitoring (TDM) and clinical toxicology. It allows for analysis of biological specimen with minimal samples preparation, high sensitivity, specificity, precision, and high throughput. However, several limitations of LC/MS(/MS) should be kept in mind when establishing them in the laboratory. The choice of chromatographic systems and thus the separation power is often limited in LC/MS(/MS) because only certain volatile buffers and mobile phase additives can be used. Moreover, the spectral information of electrospray ionization (ESI) and/or atmospheric pressure chemical ionization (APCI) mass spectra is often limited compared to electron ionization mass spectra in GC/MS. However, collision-induced dissociation (CID) can lead to the formation of structure related fragments, which can considerably vary between different instruments. Reproducibility of production spectra measured in different apparatus types, as reported by some authors, could not be confirmed by others [34]. In clinical toxicology, this poses an interesting problem in that a computerized drug identification library developed by one laboratory may not be portable to another instrument or

laboratory. Another important problem in LC/MS(/MS) is the possible reduction of the ionization of an analyte by co-eluting compounds, the so-called ion suppression, because in these cases a relevant toxicant might be overlooked and the measurement uncertainty may increase [37,38].

Capillary electrophoresis (CE) was first reported in details by Stellan Hjertén [39] in 1967. In this pioneering monograph he writes, "The free zone electrophoresis method described in this monograph can be used for the fractionation of small molecules, large molecules, and particles. Its versatility is illustrated in runs with inorganic ions, organic ions, nucleic acid bases, nucleosides, nucleotides, proteins, nucleic acids, subcellular particles, viruses, and erythrocytes." When a high-voltage DC field is applied, charged solutes begin to separate and are swept through the capillary by the combined action of electrophoresis and electroosmotic bulk flow and are on-column detected toward the end of the capillary column. Highly efficient separations can be performed in CE using different modes, such as capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC or MECC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF), capillary gel electrophoresis (CGE), and capillary isotachophoresis (CITP) [40]. This allows for the possibility of carrying out determinations of the same analytes based on different separation modes with the same instrumentation simply by changing the buffer composition. This provides for the possibility of 'internal' confirmation of the results [41]. Because of its versatility, CE has found applications in almost all fields of toxicology [41–47] excepting TDM. Although most applications have used UV detection, recently this sophisticated technique has been coupled with mass spectrometry [48–50]. It is undoubtedly a very versatile technique. The sample requirements in CE are minimal, so as it becomes more widely established as an alternative and complementary to HPLC, it holds promise in TDM applications.

Biological matrices

Over the years, blood, urine, saliva, and hair have been considered as possible biological samples for drug analyses. By its very nature, hair is a retrospective medium that can give an indication of drug concentrations in the weeks or months that precede sampling. Hair has been used for the exposures to metals [51–53]. Because hair can provide "historical" information, it has been used successfully in the identification of many drugs [54–56]. Another important application is in detection of exposure in utero to the fetus from maternal drug use [57,58] and child abuse [59]. Hair analysis has little relevance to the individualization of therapy. Applications of hair analysis to TDM are therefore limited at present, although there have been studies on the long-term monitoring of antipsychotic drugs, such as haloperidol [60–62] in psychiatric inpatients. Similarly, the variation of drug concentrations in urine with the state of hydration affecting drug concentration means that there are no direct applications in TDM in which urine is preferred to plasma as a sample matrix, although this matrix is useful in identifying poisonings.

Plasma (or serum) is normally the preferred sample for TDM analyses but requires an invasive procedure for its collection (venipuncture). Some drugs (e.g., many immunosuppressants) are concentrated in the red cells, and whole blood [with an appropriate anticoagulant; e.g., ethylenediaminetetraacetic acid (EDTA)] is more suitable than plasma. When plasma samples are used, care must be taken with anticoagulants and the use of gel separation barriers, both can cause interference with some drugs or assay systems [63–65]. However, in the absence of such effects, there are no clinically significant differences between serum and plasma, and either may be used. Plasma also contains a considerable amount of protein, and many drugs of interest in the TDM field (e.g., phenytoin, valproic acid) show significant protein binding. As a result, the concentration of the free fraction or drug not bound to protein (pharmacologically active) varies with protein concentration, although the total (free fraction plus the bound fraction) concentration remains constant. This has led to moves to measure only the circulating free (unbound) drug. Free drug concentrations can either be achieved in vitro by determining the concentration of drug in a plasma ultrafiltrate (obtained by centrifugation of plasma through an appropriate filter or by equilibrium dialysis across a semi permeable membrane) or by sampling an in vivo ultrafiltrate.

Saliva is sometimes used as the ultrafiltrate [66], although care must be taken to ensure that the saliva–plasma concentration ratio is constant and unaffected by salivary pH or salivary flow rate. This is not always the case, but where these conditions are satisfied (for drugs that are not ionizable or unionized within the salivary pH range; e.g., theophylline, carbamazepine, and phenytoin), saliva can provide an effective, noninvasive sample matrix to determine the pharmacologically active component of a drug in plasma. Saliva sampling can be particularly useful in children or in adults with needle phobia, although there are still problems with collection and potential contamination [66]. Drug concentrations in saliva are normally lower than in plasma or serum, and the matrix itself provides some analytical challenges. For these reasons, salivary monitoring still has not found wide application, although it undoubtedly has a role in some circumstances [67].

Breast milk is another biological matrix that has been used to assess drug exposure in a newborn via lactation [68]. Some of the pharmacologic properties useful in predicting drug transmission during lactation include lipid solubility, ionization constant, and molecular size that predisposes a drug to crossing membrane barriers to the milk. A carrier-mediated transport system has recently been described as a mechanism of drug transport [69]. The rather high fat (4%) content of breast milk poses interesting analytical challenge in extracting the drug(s) out of this very complex matrix. As with saliva, breast milk–plasma ratio is of importance in assessing infant's exposure to drug(s) [70].

Clinical toxicology

Traditionally, clinical toxicology has been regarded as a specific discipline of the broader field of toxicology. It is

concerned with the toxic effects of agents/drugs that were intended for treatment, or to improve, change, or prevent disease states. A liberal definition of clinical toxicology addresses issues such as accidental exposure to animal bites (snakes, spiders), or chemicals used nontherapeutically and environmental exposure to chemical by-products of industries such as gases (CO, H_2S etc), hydrocarbon (solvents), radiation, and/or components of agriculture, such as pesticides, insecticides, and herbicides. Toxic effects of drugs used as a result of societal behavior (alcohol and drugs of abuse) are also of concern to the toxicologist.

The effects of most drugs result from interaction with their receptor at the site of action. Because plasma concentration generally reflects drug concentration at the site of action, it is used in assessing the efficacy of treatment, for example, TDM, or in assessing toxicity. Pharmacokinetic factors such as dose, volumes of distribution, and elimination half-life are important when laboratory aspects of clinical toxicology are being considered. The larger the volume of distribution, the smaller the amount of drug circulating in the peripheral blood; conversely, the lower the volume of distribution, the larger the amount of drug available in the peripheral plasma for testing purposes. Drug dosage also correlates with the concentration in the peripheral blood. A single dose of aspirin or acetaminophen is in multiples of 100 mg/dose and their plasma concentrations are in the micromolar range; whereas a dose of digoxin is a few milligrams, and as a result the plasma concentration is in the nanomolar range. Plasma level is inversely correlated to the sophistication required in the clinical laboratory to measure plasma concentrations.

It is not surprising that until the sophisticated methods, such as immunoassays and chromatographic procedures, were developed, drugs that had relatively low volume of distribution and prescribed in relative large amounts (e.g., aspirin) were measured using spectrophotometeric methods. Because the plasma concentrations are in the micromolar range, even today many clinical laboratories use these methods. Spectrophotometeric assays for barbiturates and hypnotics, the frequently abused drugs of the 1960s and 1970s, were developed in 1963 [71,72].

Micro diffusion method described by Conway in 1950 [73] was combined with colorimetry and adapted for bromide [74] and ethanol [75]. Bromides poisoning commonly seen in the 1970s is not seen today. Because of its legal implications, ethanol received early attention from the forensic community that addressed both analytical [76–78] and clinical [79] aspects. The enzymatic method using alcohol dehydrogenase (ADH) developed in the 1960s is still being used in many clinical laboratories. Although ADH method is specific for ethanol, false positive results caused by elevated serum lactate and lactate dehydrogenase when present together, as is often the case in autopsies, have been reported [80]. Methanol, if present, is not recognized by the ADH method. Methanol poisoning can be lethal if not recognized early. Unfortunately, in some instances, a latent period can be as long as 12–24 h [81] before toxicity is recognized making laboratory identification of this poisoning critical. Development of the gas chromatographic method for volatiles in 1964 [82,83] was a significant development in the recognition and treatment of this very toxic alcohol. Most laboratories today have a separate procedure for ethylene glycol, another very toxic alcohol. Recently, a modification of the GC procedure has been described that has the potential of combining both the toxic alcohols in a single GLC analysis [84].

Identifying an unknown substance in a biological fluid is a daunting task. It involves numerous steps: extraction, separation, and identification of the separated compound. Urine has been the biofluid of choice as it is easily obtainable in relatively large volume by noninvasive procedure, and most drugs and their metabolites are excreted in this biofluid. The presence of metabolites aids the identification process. Although the extraction of the acidic, neutral, and basic compounds is achieved by differential extraction, it is the separation and identification of the isolated compounds that pose the greatest challenge. Identification of the drug is a prerequisite for optimal treatment.

In the early 1970s, a scientist at the National Institute of Standards and Technology (NIST) developed a database that has mass spectra from a wide range of compounds [85]. The National Institutes of Health (NIH) and the U.S. Environmental Protection Agency (EPA) maintain it. In 1971, Law et al. [86], in their manuscript *Identification of Dangerous Drugs by Mass Spectrometry*, described how to use this library database to identify the drugs in patients who have overdosed themselves. Since the development of the NIST database, numerous other databases have been developed.

Thin layer chromatography (TLC) developed in the 1950s had a significant impact in the identification of both known and unknown substances in biological fluids. TLC applications in the identification drugs in biological fluids started to appear in the 1960s [87,88]. Extraction and TLC procedures for drugs in urine, described by Kaishta and Jaffe in 1972 [89], are still being used with some subtle modifications. TLC was the mainstay for many laboratories who could not afford the expensive GC/MS equipment. TLC, although a relatively easy technique because of subjective interpretation, is dependent on the skills of the analyst. The development of an automated emergency drug profiling system REMEDi-HPLC [90] very elegantly resolved this and objectified the identification of the “unknown” substance. REMEDi-HPLC system is a HPLC system with multiple columns linked in series that is coupled with a library database containing UV spectra of drugs and their metabolites. The current database consists of more than 900 UV spectra of different drugs and their metabolites. Urine samples, without any pretreatment, can be automatically introduced into this system. UV spectra of the separated peaks are compared with the extensive drug library and a probability match is suggested. Because no sample preparation is involved, this system became the mainstay in many laboratories offering emergency drug screening. To the consternation and disappointment and of hundreds of REMEDI users worldwide, this very elegant system is not going to be supported by its manufacturer, Bio-Rad of Hercules, California, by the end of 2007. No

alternatives have been suggested. Although HPLC/MS/MS is being suggested, the issues of availability and portability [34] of drug library still need to be resolved. Recently Herzler et al. [91] published a UV library of more than 2600 compounds and demonstrated its use in identifying unknown substances/toxins in biofluids. The authors do, however, use multiple other systems in conjunction with their HPLC system.

Immunoassays for the various drugs of abuse were introduced soon after the workplace drug testing was initiated in the early 1990s. Most of these drugs have a half-life long enough that their use can be detected for a few days, if not weeks, as is the case with cannabis use. Many of these assays are also available in rapid test formats. Because of simplicity of use and lack of alternatives, these methods have found their way in the toxicology laboratory and emergency rooms of hospitals. Many of these immunoassays cross-react with other drugs and can lead to misdiagnosis [23,24]. The concerns about using immunoassays in medical settings, their utility, and cost-effectiveness have been eloquently discussed by Hammett-Stabler et al. [92].

Dole and Nyswander (1968) demonstrated the viability of methadone as a replacement therapy for the heroin abuser. Regular urine drug screening by immunoassay is a *modus operandi* in clinics that participate in methadone management treatment programs. The objective here is, of course, to monitor patient's compliance to treatment objectives. Various methods of sample tampering and diversion of the treatment drug methadone are of great concern with this group of patients. "Fingerprinting" [93] and markers of adulterations [94,95] have been suggested. At steady state the plasma drug levels fluctuate within a narrow range, implying that the amount of (drug) intake is equal to the amount eliminated. This suggests that urinary excretion window (UEW) or profile of the amount of drug excreted would be parallel to the plasma profile. Hydration, missing dosage, or diversion of the drug would be the variables that affect this UEW. This concept has been suggested [96] as a method of detecting sample tampering by patients on methadone maintenance program.

With the availability of TLC followed by GC and HPLC, toxicology laboratories have been providing drug analysis to the various clinical programs since the mid-1960s. As pharmacotherapy has changed so has the diversion of some drugs on the street. The drug scene has been continuously changing thanks to the increasing sophistication of the garage chemist. These changes are manifested in what is presented in the emergency rooms of hospitals and the toxicology laboratory. Over the years, the authors have seen changes in the patterns of drugs seen in the emergency rooms of hospitals (Table 1). Barbiturates seen in the 1970s and 1980s are rarely seen today. Increases over the years in cocaine, MDMA and oxycodone use are most disturbing. Drugs seen in the local community and in the emergency room of hospitals should assist in developing the tests menu a toxicology laboratory plans to provide [92]. In poisonings where the initial presentation of the toxidrome may not be revealing (e.g., acetaminophen or methanol), the toxicologist in the clinical laboratory can play a critical role in identifying these poisonings.

Table 1
Trends in drugs seen in the emergency room of Toronto

	October 1972 to June 1973[a] (%)	January 1986 to July 1987[b] (%)	November 2003 to October 2004[c] (%)	November 2004 to October 2005[c] (%)
Serum				
Ethanol	37.1	44.72	36.02	32.20
Isopropanol	0.9	0.85		
Methanol		1.48	4.70	3.08
Acetone		3.73		
Ethylene glycol		0.20	18.39	13.04
Barbiturates	39.5	8.76	1.30	1.05
Acetaminophen		8.51	7.32	8.93
Salicylates	6.6	7.27	2.58	2.75
Urine (EIA)				
Opiates	1.9	0.15	15.57	14.37
Cannabinoids		1.33	13.19	16.89
Benzodiazepines		13.99	13.23	13.36
Barbiturates		11.23	0.88	0.68
Urine analysis		TLC	REMEDI-HPLC	REMEDI-HPLC
Codeine		10.10	2.63	6.39
Morphine		2.28	5.90	6.05
Cocaine		5.13	14.44	18.35
Amphetamine		0.18	5.25	4.95
Methamphetamine		0.13	1.20	0.77
MDA		0.01	1.25	1.83
MDMA			0.69	1.14
Oxycodone		0.40	5.72	13.74

[a] Kapur B. Patterns of drug abuse and their relationship to traffic accidents. In: Israelstam S, Lambert S, editors. Alcohol, Drug and Traffic Safety. Proceedings of the sixth international conference. Toronto: Addiction Research Foundation; 1975. pp. 69–72.

[b] Statistics from clinical laboratory; Addiction Research Foundation. Urine = 9593; serum = 12295.

[c] Annual statistics from St. Michael's Hospital in Toronto. Average number of urine = 4700; serum = 4800 for the respective years.

Therapeutic drug management or monitoring (TDM)

Since antiquity, physicians have adjusted the dose of drugs according to the characteristics of the individual being treated and the response obtained. This practice is easiest when the response is readily measurable, either clinically (e.g., antihypertensive drugs, analgesics, hypnotics) or with an appropriate laboratory marker (e.g., anticoagulants, hypoglycemic agents, lipid-lowering drugs). Dose adjustment is much more difficult (but no less necessary) when drug response cannot be rapidly assessed clinically (e.g., in the prophylaxis of seizures or mania), or when toxic effects cannot be detected until they are severe or irreversible (e.g., nephrotoxicity or ototoxicity). Clinicians have long recognized the limitations of empirical standard or fixed-dose regimes. They responded by utilizing their clinical skills and knowledge of basic pharmacology to attempt to individualize each patient's drug dose. Knowledge of serum concentrations is most helpful when the drug in question

requires individualized dosing for optimal efficacy and more routine measures of therapeutic success are unavailable [97]. It must be stressed that TDM is not simply the provision of an analytical result, but a process that begins with a clinical question and continues by devising a sampling strategy to answer that clinical question, determining one or more drug concentrations using a suitable method, and interpreting the result appropriately.

TDM offers a scientific approach to selecting a drug regimen to optimize therapy. First it is important to understand what TDM stands for. It is only recently that TDM is considered to be therapeutic drug management; defined as a multidisciplinary clinical activity encompassing specialties such as clinical pharmacology, clinical pathology, clinical chemistry, toxicology, analytical chemistry, and medicine. Historically, TDM referred to therapeutic drug monitoring and was defined as the use of drug or metabolite monitoring in biological fluids as an aid to the management of therapy. TDM has been routinely practiced in clinical laboratories since the mid-1970s, but the scientific foundations of the subject date back to the 1920s. In 1927, Wuth [98] demonstrated the value of monitoring serum bromide levels to differentiate bromide induced from intrinsically psychotic behavior [98]. Marshall and Dearborn [99] then evaluated concept that the activity of a drug is dependent on its plasma concentration [99]. The first studies correlating plasma drug concentrations with their therapeutic efficacy were published in the late 1950s and early 1960s. In 1960, Buchthal et al. [100] showed a relationship between seizure control and plasma phenytoin concentration in patients being treated for epilepsy, and Baastrup and Schou [101] described the plasma concentration–pharmacological effect relationship for lithium in 1967. By the late 1960s, with the rise of clinical pharmacology and the demonstration of the fundamental concepts of pharmacokinetics and pharmacodynamics, which underpin the interpretation of drug concentration measurements [102], TDM became widespread.

The relation between dosage of a drug and its concentration in the serum will be influenced not only by the bioavailability of the dosage form used, but also by a host of factors that affect the degree of GI absorption, body size and composition, distribution through fluid compartments, binding sites of various protein, and by rates of metabolism and excretion. All these determinants are subject to much individual variation due to genetic and environmental factors, to consequences of disease, and to concomitant administration of other drugs [103]. Individual variation in the rate of drug elimination is quantitatively the most important. There are significant genetically controlled differences due to induction or inhibition of drug-metabolizing enzymes. The future of TDM now lies in the fields of analytical technology, drug metabolism, and genetics.

Rapid advances in clinical pharmacology over the past decades are directly attributable to the availability of TDM. TDM, in turn, is directly related to the rapid advancement in technology associated with the quantitation of drug compounds [5]. It was not until development of immunoassays in the late 1960s that TDM became widespread. Assays for drugs that had a narrow therapeutic window and/or index were the first to be developed. In most cases, either GC or HPLC procedure preceded the development of immunoassays. In some instances, where animal data looks promising, both the immunoassays and HPLC procedures get addressed [104]. As clinical trials show efficacy, immunoassays for these drugs have been made available [105,106].

It is interesting that currently available new highly sensitive analytical techniques such as capillary electrophoresis have yet to be applied to their full potential. It is recognized that in general there is a direct correlation between free drug concentrations and clinical response. However, we routinely quantify total drug concentrations and not free drug concentrations. There are clinical situations where monitoring free drug concentration clearly is advantageous. This is particularly true in those patients with hypoalbuminemic renal failure or in cases of drug–drug interactions precipitated in patients who are receiving drugs that are displaced from their binding sites by other highly protein-bound drugs, as well as other altered physiological states. The technology for determining free drug concentrations has been available for several years; however, such determinations are not performed routinely in most hospital laboratories. The laboratories do not measure the free drug because the physicians do not know such assays can be easily performed and therefore do not request them. This is an educational role that the toxicologist or the laboratory scientist could undertake because information on free drug concentrations is of clinical value and should ideally be available from every TDM laboratory. In the 30 years or so that TDM has been practiced in routine clinical laboratories, it has been repeatedly demonstrated that making drug concentration measurements available to clinicians does not in itself result in improved clinical care.

Workplace drug testing

Due to concern about how the use of illegal drugs affected the combat readiness of the armed forces, the U.S. Congress in 1971 directed the Secretary of Defense to devise methods for identifying and treating drug-abusing military personnel [107]. As a result in the United States, the military was the first segment of society to initiate drug testing of their employees. The objective was to provide a “drug-free” federal workplace. During this period, national concern about drug abuse was increasing, and many industries in the oil, chemical, transportation, as well as nuclear power generation began implementing their own drug-testing programs. In 1986, the executive branch of the federal government became actively involved. U.S. President Reagan issued an Executive Order (Federal Register 1986) that required those Federal employees in safety and security-conscious positions to be drug tested [108]. The analytical procedures and standards of these programs varied, and this variability caused much controversy and litigation. American Association of Clinical Chemistry established a task force to address the various analytical concerns that were being raised [109].

The Department of Health and Human Services (DHHS) published the *Mandatory Guidelines for Drug Testing of Federal Employees* (Federal Register 1988a), which became

known as the *National Institute for Drug Abuse* (*NIDA*) *Guidelines* (or today as the *Substance Abuse and Mental Health Services Administration* [*SAMHSA*] *Guidelines* [110]), who currently have responsibility for the *Guidelines*. The rules were amended to cover alcohol testing in 1994 and to change the assay cut-offs for cannabis detection and again in 1998 to change the cut-offs for opiate detection [111]. The *CFR Part 40* was revised again in 2000 (Federal Register 2000); the revision does not extend the scope of drug testing, but has a significant impact on how it is practiced [112]. The Executive Order also set the stage for the introduction of drug-testing programs to the nonfederal workplace, welfare recipients, as well as within the criminal justice system. Drug testing has been expanded to include both professional and amateur athletes who compete at national and Olympic levels. Testing high school athletes is controversial, but today in the United States, parents may privately test their children by collecting samples and sending them to laboratories for anonymous testing. The insurance industry is currently testing life insurance applicants for use of illicit drugs. In medicine, hospital emergency rooms, prenatal clinics, and delivery rooms, testing for illicit drugs has become part of diagnostic care and the results may or may not become forensic, depending on individual circumstances.

The anticipated outcome was improvement in safety and public health and economic health care costs [113]. The question that underlies these drug-testing programs is, Has this been effective? As the decade progressed, a number of studies were published, the majority of which showed that some benefits were realized [114,115]. For the United States, there are certainly data that suggest a reduction in the number of positives throughout the 1990s (Table 2). It is likely that numerous factors are responsible for the reduction in drug use, including targeted education programs and supply reduction programs, in addition to workplace drug testing [113]. In the United States, it is now an accepted practice with between 30 and 40 million such tests being carried out annually.

In many other industrialized countries, workplace drug testing started to gain increasing acceptance [116] over the past decade, although it has not been universally accepted [117,118]. The International Labor Organization (ILO) of the World Health Organization (WHO) in Geneva, Switzerland, with United Nations International Drug Control Programme (UNDCP), hosted two tripartite conferences that involved labor, employer, and government to address this subject. In the first instance, it was the Maritime industry (Geneva, Switzerland 1992), and the second instance (Oslo, Norway, May 1993), the industry at large was represented to address concerns of various affected parties. Ethical and moral issues [119], alcohol and drug use [120–122], and testing methods [123] were addressed.

Pharmacodynamics monitoring and pharmacogenetics

We began by defining TDM as the use of drug or metabolite measurements in body fluids as an aid to monitoring therapy based on pharmacokinetic principals. In recent years, other methods of controlling drug therapy have been introduced, and although they do not fit this definition of TDM, they merit brief mention as they may become increasingly important. Pharmacodynamics (PD) can be viewed as study of the biochemical and physiologic effects of drugs and their mechanisms of action. PD refers to the relationship between the drug concentration at the site of action (receptor) and the pharmacological response. The utilization of biological effects to assess efficacy and make dosage adjustments is fundamentally not new. Assessment of a patient's mental status in the case of treatment with antidepressants or the measurement of glucose levels in a diabetic patient can be considered pharmacodynamics monitoring. Many drugs produce pharmacologic responses by interacting

Table 2
Changes in pattern of drug use as seen through AACC-CAP Questionnaire Survey of Laboratories[a]

Year	December 1992 to February 1993[1]		January 1995[2]		January 1997[3]		January 1998[4]	
Drug	Number screened	% Confirmed	Number screened	% Confirmed	Number screened	% Confirmed	Number screened	% Confirmed
Cannabinoids	969625	4.12	638676	2.254	1117327	3.206	990688	3.737
Ethanol	208430	6.662	159692	1.330	183620	1.158	185543	2.223
Benzoylecgonine	1200286	2.293	757341	1.024	1248489	1.018	1143820	1.846
Opiate group[b]	968583	1.690	516425	0.688	1114280	0.578	19063	1.099
Oxycodone	25	0.003	583	0.010	999	0.004	1130	0.020
Barbiturate group	863684	0.641	440145	0.241	695597	0.245	536588	1.381
Benzodiazepine group	846411	1.044	435349	0.465	671270	0.425	549089	1.184
Amphetamine group	971013	0.464	686722	0.390	1101973	0.310	989634	0.100
Propoxyphene	501957	0.297	283166	0.096	448732	.0226	435673	0.335
Phencyclidine	935360	0.124	656504	0.075	1060269	0.074	445120	0.478
Methaqualone	597492	0.005	221588	0.004	410615	0.006	324842	0.004
LSD			459	0.283	362	0.829	1042	2.111

[a] Forensic urine drug testing: [1]September 1993, [2]December 1995, [3]March 1998, and [4]June 2001 issues, respectively.
[b] Opiate group: codeine, hydrocodone, hydromorphone, 6-MAM, morphine oxycodone. Barbiturate group: amobarbital, butalbital, pentobarbital, phenobarbital, secobarbital. Benzodiazepine group: alprazolam metabolite (met), flurazepam met, lorazepam met, nordiazepam, oxazepam, temazepam, triazolam. Amphetamine group: amphetamine, methamphetamine.

with (binding to) specific macromolecules, usually complex proteins, on or within cells. Some drug classes react directly with endogenous or exogenous nonprotein substances. With current analytical techniques, these alterations in these molecular targets or receptor functioning have recently been applied in the areas of immunosuppressive therapy and cancer chemotherapy. For example, the biological effect of the immunosuppressant cyclosporine A can be assessed by measuring the extent of inhibition of calcineurin phosphatase [124], or the interleukin-2 concentration of peripheral blood lymphocytes [125]. The advantages of such monitoring are that it gives an integrated measure of all biologically active species (parent drug and metabolites), so therapeutic ranges can be defined more closely, and that it is free from the matrix and drug disposition problems. The main disadvantage of pharmacodynamic monitoring is that the assays involved are significantly more complex and time-consuming than the measurement of a single molecular species by chromatography or immunoassay. It is too early to say whether pharmacodynamic monitoring will have a significant role to play in optimizing therapy. However, it is proving useful in the development of new drugs; calcineurin phosphatase analysis was integral in the development of ISA247 [126].

Clinical observations of inherited differences in drug effects were first documented in the 1950s [127–129]. Person to person variations in response to succinylcholine, primaquine, and isoniazid were first to be intensely scrutinized [130]. Succinylcholine is normally broken down into inactive products by serum cholinesterase [131]. The discovery of hereditary variation in serum cholinesterase followed the widespread use of succinylcholine as a muscle relaxant during surgery [131]. Kalow and Gunn [131] demonstrated the presence of an abnormal form of the enzyme in affected persons and their immediate relatives and explained the sensitivity to succinylcholine. Their landmark studies were the first to show a link between a heritable variant of an enzyme and drug sensitivity. Hereditary variation of red blood cell (RBC) glucose-6-phosphate dehydrogenase (G6PD) was discovered by Brewer et al. [132] after numerous reports of RBC destruction associated with the administration of antimalarial drugs, such as primaquine. The destructive effect was shown to be due to a specific deficiency of RBC-G6PD, and the predilection of males over females was clarified when the enzyme was demonstrated to be a sex-linked trait [130]. An appreciation of the hereditary variation in response to isoniazid grew out of the initial clinical trials. Hughes et al. [133] demonstrated that isoniazid was biotransformed by acetylation and that persons differed severalfold in their capacity to acetylate isoniazid. People could be divided into slow and fast acetylators. It was the slow acetylators who determined to be mutant of *N*-acetyltransferase and were sensitive to isoniazid neurotoxicity [133].

The confluence of genetics and biochemistry and pharmacology recognized in a paper by Motulsky marked the beginnings of pharmacogenetics (PG) as an experiment science [134]. However, it was Vogel who proposed the term PG be applied to the study of the effect of heredity on drug response [135], and Kalow in 1962 published the first systematic account of the field [136]. PG is the study of the hereditary basis for differences in a population's response to a drug. The science of pharmacogenetics links differences in gene structure (polymorphism) with pharmacologic differences in drug metabolism, transport of pharmacodynamic action. PG aims to elucidate the inherited basis for interindividual differences in drug response, using genome-wide approaches to identify genetic polymorphism that govern an individual's response to specific medications giving birth to pharmacogenomics. With the completion of the human genome projects, the knowledge of the impact of genetics on drug disposition developed rapidly in the late 1990s and continues to develop. The ultimate goal of pharmacogenetics research is to help tailor medicines and therapies to people's unique genetic makeups. This will make medicines safer and more effective for everyone.

It is well recognized that different patients respond in various ways to the same dose of the same medication. These differences are often greater among members of a population than they are within the same person or between monozygotic twins [137]. Large population differences with small intrapatient variability are consistent with inheritance as a factor determining drug response. It is now well established that many of the genes that encode proteins dictating the pharmacology of medications display genetic polymorphisms. These polymorphisms in turn may alter the functionality of the protein in response to medication [138–140]. Although many nongenetic factors influence the effects of medications including age, organ function, concomitant therapy, drug interactions, and the nature of disease, it has been estimated that genetics can account for 20–95% of variability in drug disposition and effects [141]. Importantly, inherited determinants differ from other factors influencing drug response, as they remain stable for an individual's lifetime.

Current PG targets included any polymorphisms gene that encodes for the many different proteins involved in the processes of pharmacokinetics; absorption, distribution, metabolism, and elimination. Common features of pharmacogenetics tests of proven usefulness include: The enzyme of interest is the primary pathway for metabolism of the drug; changes in enzyme activity results from the polymorphism have a significant effect on the relationship between dose and plasma concentration; the efficacy and or toxicity of the drug correlates with changes in plasma concentration; and the drugs affected have a narrow therapeutic index. When prescribing drugs, physicians must consider that the inherent capacity to clear a drug may differ among patients. A patient with rapid metabolism may require larger, more frequent doses to achieve therapeutic concentrations; a patient with slow metabolism may need lower, less frequent doses to avoid toxicity, particularly for drugs with a narrow margin of safety. Further pharmacogenetic testing is of particular usefulness for those drugs that require a long period to establish efficacy or to optimize dose.

To date, the best-studied PG targets are those involved in drug metabolism. However, beyond metabolism, PG targets that may become important to TDM and pharmacotherapeutics include genes that encode for many proteins associated with drug handling, such as transporters, drug receptors, or effectors.

Several examples now exist for which the presence of genetic polymorphisms can predict clinical response [139,140,142,143]. These include cytochrome P450 (CYP) 2D6, thiopurine methyl transferase (TPMT), CYP2C9, CYP2C19, *N*-acetyltransferase (NAT), CYP3A5, UDP glucuronosyltransferase 1A1 (UGT1A1), CYP2B6, multidrug resistance (*MDR1*) P-glycoprotein, serotonin transporters (5-HTT), and 5,10-methylenetetrahydrofolate reductase (MTHFR).

It is already clear that pharmacogenetic studies have clear and wide-ranging clinical relevance. The enzymes that are responsible for metabolism of drugs and other compounds exhibit wide interindividual variation in their protein expression or catalytic activity and result in different drug metabolism phenotypes between individuals. This variation may arise from transient effects on the enzyme, such as inhibition or induction by other drugs, or may be at the gene level and result from specific mutations or deletions. The overall response to drug therapy is polygenic and multifactorial [144,145]. Limitations relate to the completeness of knowledge about a medication's pharmacokinetic mechanism of action and which genes are involved.

Summary

The science of toxicology has come a long way in the past centuries with significant advances in analytical technologies as well as in the various disciplines of toxicology. Among the advances that hold promise is the solventless sample preparation technique of solid phase microextraction (SPME) and capillary electrophoresis. Both these techniques have yet to be used to their full potential. We have seen one technology be replaced by another; TLC by REMEDi-HPLC; REMEDi-HPLC by HPLC/MS/MS and GC/MS. Comorbidities are present very often requiring multiple drugs to be prescribed to the patient. Where the potential of drug–drug interaction is possible, it is clearly advantageous to monitor free drug concentration. Clinical utility of free drug levels has been demonstrated, and here is a possibility for a toxicologist to take on an education role that can have an impact on clinical care.

PG is still in its infancy; much attention has been paid to the drug metabolizing enzymes. However, beyond metabolism, PG targets that may become important to TDM and pharmacotherapeutics include genes that encode for many proteins associated with drug handling, such as transporters, drug receptors, or effectors.

Toxicology has developed into a science that brings together many disciplines, analytical chemistry, biochemistry, and genetics, which may have once appeared to be very different. Toxicology is an ever-evolving field; it will be interesting to see what the next century will bring.

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