Drugs and Poisons in Humans

A Handbook of Practical Analysis

Osamu Suzuki and Kanako Watanabe

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A Handbook of Practical Analysis

With 236 Figures and 90 Tables

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Foreword

It was with great pleasure that I accepted the invitation to write the foreword for *Drugs and Poisons in Humans. A Handbook of Practical Analysis*. Dr. Osamu Suzuki and Dr. Mikio Yashiki, two outstanding Japanese scientists, first published the Handbook in Japanese in 2002. Specialists throughout Japan contributed analytical methods for a wide variety of therapeutic and illicit drugs, pesticides, and natural toxins and alkaloids. In fact, rarely has such a wide spectrum of analytes and metabolites been addressed within a single reference manual.

At the beginning of the book, general topics are addressed, including instructions on handling biological materials, measurement of drugs in alternative specimens, and guidance on resolving analytical problems that may occur. There are discussions of extraction modalities and detection methodologies and how to select these appropriately based on the physiochemical characteristics of the drug. Analysis of specific classes of drugs and relevant metabolites are covered in subsequent chapters. Clinical, analytical and forensic toxicology and clinical chemistry laboratories will find the volume informative and useful. Toxicologists are often faced with developing methods for new drugs and metabolites with little information available in the literature. This book provides a great starting point for method development providing procedures that have been utilized in real life situations. In addition, toxicologists developing new methodologies may use this volume as a guide to selecting the most appropriate instrumentation to handle the breadth of their analytical workload.

One of the most valuable aspects of the Handbook is the inclusion of specific case studies. Useful also are the discussions on suggested analyte concentration ranges and troubleshooting tips. The 2002 version of the Handbook in Japanese was judged to be highly valuable and led to the production of an English version. This Handbook also has been updated to include additional methods and procedures for this edition.

Despite the value of these methodologies, it is essential for laboratorians to validate fully a method within their own laboratory. Differences in instrumentation, sample size, extraction procedures (such as different solid-phase extraction columns) and experience level of personnel may vary markedly between laboratories. Therefore, these methods provide help and guidance in initiating a new analysis, but do not take the place of independently determining limits of detection, quantification and linearity, and the selectivity and precision of the assay in their own hands. Internal standardization is always the preferred approach, although use of external standard addition may be necessary with difficult matrices, such as decomposed postmortem specimens. Quality assurance and quality control procedures are essential components of accurate and reliable methods and should be included in the analysis of each batch of specimens. Quality control samples should span the linear range of the assay. The issue of method validation cannot be emphasized too strongly and is necessary for the accurate application of these diverse analytical methods.

Dr. Suzuki and Dr. Watanabe have gathered an extensive array of methods for the measurement of xenobiotics and metabolites in biological matrices. *Drugs and Poisons in Humans. A Handbook of Practical Analysis* will be a well-used reference for toxicology laboratorians and will help guide assay development.

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Preface

The readers of this book will immediately realize that all authors are Japanese scientists; this is the English translation of a book which was published in Japanese by Jiho, Inc., Tokyo in 2002. Upon translation, the Editors added five new chapters to the previous 67 chapters in view of international occurences of poisoning by drugs and poisons.

The most important aim of this book is to provide the most reliable and reproducible methods for analysis of drugs and poisons; therefore, the newest methods and ones requiring skills have not been adopted. Each chapter has been written by at least one expert currently engaged in the quantitative analysis of each toxin. This book is arranged so precisely that any fresh analytical chemist can start analytical experiments on a drug or a poison in a crude biological matrix, even if the analyst has no experience of analyzing the compound. Special care has been given to clarify the origins (manufacturers) or synthetic methods for chemicals to be used in reproducing the experiments, and also to present detailed procedures for the extraction of a drug or a poison from complicated matrices such as whole blood, tissues and urine.

Compounds causing cases of poisoning will increase and vary according to events in the world; the technology of analytical instruments is also advancing very rapidly. The Editors do not claim that this book covers all compounds to be analyzed and are well aware of the limitations of the book. The Editors hope that this book will be revised according to feedback received in the near future; some groups of drugs and poisons will then be added in a later edition.

The Editors also hope that this book will be widely distributed in the world and be useful for many analysts affiliated to forensic, environmental, clinical and doping control institutions.

The Editors wish to thank the following people for helping to make the present publication of this book possible: Dr. T. Mager and Mr. A. Spencer, Springer-Verlag, Heidelberg, for undertaking the laborious work of the publication; Messrs. T. Araki, D. Kobayashi and S. Hattori, Jiho, Inc., Tokyo, for kindly encouraging us to translate the original Japanese version; Mr. and Mrs. Kouichi Watanabe, the parents of one of the Editors, for typing extensive pages of manuscripts for the translation.

Osamu Suzuki
Kanako Watanabe
Editors

Notes on the use of this book

Contents

This book is composed of 9 chapters of general nature and 63 chapters of specific toxins. In the latter chapters, compounds with high poisoning frequency have been chosen; detailed procedures of analyses have been presented for each compound or each group. The methods mentioned are relatively new and easily reproducible in every chemical laboratory equipped with the standard analytical instruments. In this book, preliminary tests such as color and immunological reactions are almost omitted; most of them are chromatographic ones.

Each chapter on specific toxin is composed of: 1 Introduction; 2 Reagents and their preparation; 3 Instrumental conditions; 4 Procedure; 5 Assessment of the method; 6 Poisoning cases, toxic and fated concentrations; 7 Notes; and 8 References.

Especially, protocols for experimental procedure are headed by small letters of Roman numerals.

For notes, small alphabets are shown on the right shoulder of a corresponding word in the text. For references, Arabic numerals in brackets are shown in the text.

Symbols, units and expressions

Length: 10^{-9} m has been expressed as nm (not mμ); volume: 10^{-6} m^3 expressed as mL (not cc); concentration: mol in 1 L volume expressed as M (not mol/L); NMR shift: δ values (not γ values); fraction: for example g/mL (not g mL^{-1}).

In GC analysis , when the initial oven temperature is 50 °C with 1-min hold, followed by its elevation at 5 °C/min up to 150 °C; after 5-min hold at the latter temperature, it is again elevated at 20 °C/min up to 280 °C. These steps of the procedure are simply described as follows.

50 °C (1 min) → 5 °C/min → 150 °C (5 min) → 20 °C/min → 280 °C.

Abbreviations

There are a number of abbreviated words being commonly used in the field of analytical toxicology. The following abbreviated words can be used in the text of this book without explanation.

CI: chemical ionization
CID: collision-induced dissociation
EI: electron impact ionization
FID: flame ionization detector
GC: gas chromatography or its instrument
GC/MS: gas chromatography/mass spectrometry or its instrument
GC/MS/MS: gas chromatography/tandem mass spectrometry or its instrument
HPLC: high-performance liquid chromatography or its instrument
IS: internal standard
LC: liquid chromatography = HPLC or its instrument
LC/MS: liquid chromatography/mass spectrometry or its instrument
LC/MS/MS: liquid chromatography/tandem mass spectrometry or its instrument
NPD: nitrogen-phosphorus detector
SIM: selected ion monitoring
TIC: total ion chromatogram or total ion current
TLC: thin-layer chromatography
UV: ultraviolet (detection)

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I. Chapters of general nature

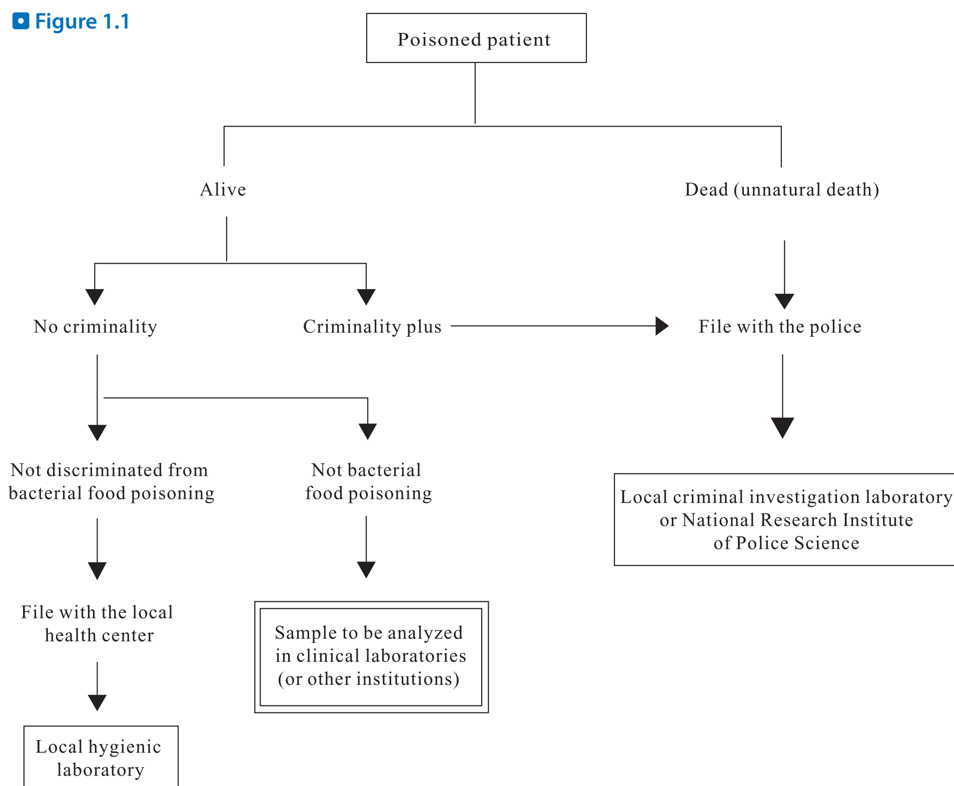
I.1 How to handle biological specimens

By Osamu Suzuki

Some knowledge to be required before handling specimens

The flowchart for how to handle specimens obtained from poisoned patients is shown in *Figure 1.1*. When a poisoning incident takes place and a patient is sent to hospital, medical doctors and co-medical staffs should concentrate their efforts on the intensive care of the patient. However, at this point, the discrimination whether it is a poisoning case or not is, of course, very important. If the patient dies and the death is judged due to poisoning, the responsible doctor should file the death with the police located at the district within 24 h according to a law in Japan.

Figure 1.1



Flowchart for how to handle specimens obtained from poisoned patients.

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The death due to bacterial food poisoning should be classified into intrinsic one (disease death), and be discriminated from the death due to drugs or poisons (extrinsic death); it is not necessary to file with the police, but it should be filed with a local health center. Irrespective of being dead or alive of the patient, both bacterial food poisoning and drug poisoning should be filed with a local health center. It should be mentioned here that deaths due to ingestion of puffer fish and mushrooms are classified as extrinsic ones.

After the police accepts the file of an unnatural death, the analysis of a causative toxin is made, according to the need, at a local criminal investigation laboratory of police headquarters in each prefecture in Japan. When the analysis at the local laboratory is difficult, the specimens are sent for analysis to the National Research Institute of Police Science in Kashiwa City, Chiba Prefecture. When a cadaver of unnatural death is subjected to judicial autopsy at a department of legal medicine of a medical school, the toxin specimens obtained from the cadaver is analyzed at the department in case that the department is capable to analyze it.

When a patient survives and criminality is suspected in a poisoning case, it should be filed with the police immediately; in such a case, the police is absolutely responsible for the toxin analysis.

Only when a poisoning patient is alive with no criminality, and also no discrimination between poisonings by bacterial food and by drugs/poisons can be made, a request addressed to a local hygienic laboratory for toxin analysis is possible *via* a health center. The main duties of the hygienic laboratory are bacterial tests on foods and analysis of environmental pollutants; the laboratory is usually equipped with expensive analytical instruments such as mass spectrometers, and seems to sufficiently meet the analysis of drugs and poisons. However, at the present time in Japan, such request is usually rejected by the laboratory after the possibility of being bacterial food poisoning is excluded. Therefore, a problem arises concerning which institution undertakes the analysis of specimens collected from a poisoning patient admitting at a hospital, who survives and shows no criminality (suicide trial or accident). The best way is that the clinical laboratory of the same hospital undertakes the analysis of the specimens; however the analysis of drugs and poisons is almost impossible at a local hospital, because it is usually not easy, and requires a skill of analysts. Unfortunately, in Japan, the so-called poison control centers for undertaking the toxin analysis are not available; while in the US and Europe the poison control centers are active for analysis of such specimens. Our problem is not due to the scientific delay in analytical chemistry in our country, but is due to the delay in measures to be taken by Japanese Government. To overcome the above problem, much efforts are being made at non-governmental levels [1]; one of the efforts is presented in Chapter I.7.

Sampling of specimens on a clinical scene

Blood

Blood specimens are now being collected from the vein using vacuum sampling tubes; EDTA, citrate or heparin is usually contained in the tube as an anticoagulant. Some tubes contain sodium fluoride as a preservative. The analysts should be aware of the presence of such additives.

Larger amounts of blood are preferable to be sampled for toxin analysis; however, in view of the stress to patients, 5–10 mL of blood is to be sampled. If a situation permits, multiple samplings at different intervals are desirable. The time-course analysis is very useful for deciding the therapeutic policy in poisoning cases. When plasma is required, the supernatant fraction is obtained by centrifuging the tubes containing whole blood at 2,000–3,000 rpm.

Urine

Also for urine, larger amounts are preferable to be sampled. When urine is obtained by catheterization from a patient, it should be taken into mind that a jelly containing a local anaesthetic had been applied to the catheter; urine is usually contaminated by such a drug. Also for urine, the samplings according to time intervals are preferable. According to the need, sodium fluoride or sodium azide is added to urine samples at a concentration of 1 mg/mL as a preservative.

According to the kinds of drugs and poisons, large amounts of metabolites are sometimes excreted into urine. Before analysis, some knowledge on the metabolism and excretion for a possible toxin is needed; a useful dictionary was published for such a purpose [2].

Vomitus and gastrolavage fluid

After oral ingestion of a drug or a poison, there is a possibility that gastric contents contain a high level of an unchanged toxin. The vomitus and gastrolavage fluid should be stored in amounts as large as possible; their volumes should be strictly recorded. Also according to the need, sodium fluoride or sodium azide can be added as a preservative.

Hair and nails

When chronic intoxication by a drug or a poison (especially heavy metals and basic drugs) is suspected, after getting the consent from the patient, several pieces of long hair are sampled by cutting off at their roots, put in a dry polyethylene bag with a fastener and kept at room temperature or 4° C.

Nails are also good materials for detection of a drug or a poison which was ingested in the past, and can be an alternative specimen, especially when the scalp hair is too short or not available. They can be kept also at room temperature or 4° C.

Collection of informations on a possible drug or a poison administered or ingested

Inquiries on a drug or a poison to the patient and his/her family are essential. Efforts should be made to find a cup or a bottle left on a poisoning spot, because there is a possibility that pure or clean solution of a toxic compound is contained in it. It is a good method to ask members of the emergency services to look for such items on the spot.

Sampling from cadavers

Observation on the spot of poisoning

When a medical doctor is requested to make postmortem inspection, he/she should arrive at the spot of poisoning to achieve the duty. Before the inspection of a cadaver, the doctor should observe the surrounding situations as carefully as possible, and also should sniff the air. There are many fatal cases of organophosphorous pesticide and cresol poisonings, in which a strong aromatic smell is given from the vomitus and from the mouth of a cadaver. When no finding of vomiting is observed, it is sometime useful for the doctor to try to sniff the smell by keeping doctor's nose closer to the nose and mouth of the cadaver and by pushing its chest slowly. Many of cadavers due to poisoning show dirty mucous fluids, froth or degeneration of the lip or around the mouth. It is also essential to look for a bottle or a cup containing a toxic compound. When they are found, they should be carefully stored until analysis. The packages and plastic cases for tablets and capsules should be looked for especially in a trash can or other places in the room. If vacant packages or cases are found in the trash can, they should be carefully lined up according to upper-to-lower layers. In case of failure to find out them inside the room, such search should be extended to nearby places, where trash is gathered outdoors.

When it is disclosed that the victim has visited a clinic or a hospital, detailed informations can be obtained on the kinds of drugs and their amounts prescribed; informations on the diagnosis of diseases and the time of the last visit can be also obtained by making inquiries to the responsible doctor. On every package or case for tablets or capsules, code numbers or special marks are usually shown; it is easy to identify a drug by the code numbers or marks using a drug-list book [3]. In most cases of poisoning, victims usually ingest multiple kinds and large amounts of drugs to commit suicide. The final judgement whether a death is due to drug poisoning should be made by counting the number of drugs and by considering the toxicity of each drug ingested.

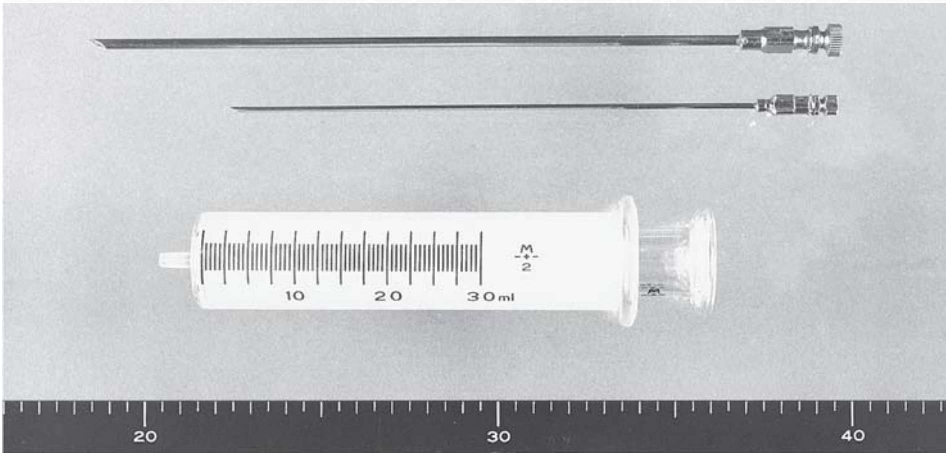
Sampling at postmortem inspection

When vomitus and gastrolavage fluid are available, it is preferable to keep all of them. Since the froth or saliva fluids attached to the lips or their surrounding skin may contain high concentrations of a drug or a poison, they should be sampled by wiping them carefully with gauze and be kept in a sealed case at room temperature or 4° C.

The author et al. usually sample about 10 mL blood at every postmortem inspection, even if the cause of death is strongly suggested to be only disease; we keep it at –80° C for at least 1 year. This is because any unpredictable matters may be disclosed by further investigation of the police. Especially in rural areas without a medical examiner system (this system is active only in some big cities in Japan), the cadavers, which are considered not involved in criminality, are usually not autopsied, but subjected only to postmortem inspection. Therefore, the storage of a blood sample for a long time seems very important, because there is a possibility that the sample will serve as an effective evidence in the future.

Since the punctures for samplings are based on the request of a judicial police officer, they are not illegal; but it is preferable to get the consent of family members upon samplings.

Figure 1.2



Needles and a glass syringe for punctures. The upper big needle is used for stomach puncture; the smaller one for cardiac, suboccipital and urinary bladder punctures.

The needle usually used for lumbar puncture can be used at postmortem inspection. However, we are using so-called "a contrast medium needle", which is thicker and longer than that for lumbar puncture (*Figure 1.2*); it is about 16 cm long and its internal diameter is about 1 mm; it is useful for cardiac, urinary bladder and suboccipital punctures. For stomach puncture, we are using even thicker and longer needles (20 cm long and 1.5 mm internal diameter) (*Figure 1.2*), because the stomach usually contains solid contents. The marking with an oil-based marker pen at the sites of 5 and 10 cm from the tip of a needle is useful to estimate the depth of puncture. A conventional glass syringe of a 10–30 mL volume is recommendable rather than a plastic disposable syringe, because the glass syringe easily gives subtle touch sense to be transmitted to the finger upon drawing blood.

For cardiac puncture, the needle should be stuck rapidly at the depth of about 10 cm on the following location of the chest; on the straight line combining both nipples, on an intercostal space and at the left margin of the sternum. After removing the inner needle, the glass syringe is connected to the needle; together with pulling the plunger, the needle position is moved back and forth slowly. When blood is present in the heart, it is easily withdrawn into the syringe; at least 5 mL blood is sampled and stored.

For urine sampling, the pubic symphysis is palpated, and the needle is stuck into the urinary bladder at the upper margin of the pubic bone at an angle of about 45 degree against the abdominal skin surface. When a large amount of urine is present in the bladder, it is easily withdrawn into a syringe. Of course, the sampling of urine by catheterization *via* the urethra is possible like in the case of a living patient. Larger amounts of urine are preferable for the case in which poisoning is suspected.

For sampling of stomach contents, the above large needle is rapidly stabbed toward the stomach at the inner margin of the left costal cartilages. When a large amount of stomach contents is present, it is easily obtainable. However, it is not easy when their amount is small; it is difficult to inject the needle through the stomach wall, because the latter is too movable in the absence of a large amount of stomach contents.

According to the need, cerebrospinal fluid (CSF), hair and nails are sampled. CSF is sampled by suboccipital puncture as follows. The neck is bent forward, and the needle is stuck from the backside at the level between the foramen magnum and the first jugular vertebra to reach the cisterna magna; more than 10 mL of CSF can be obtained by such puncture. As stated before, there are many cases in which basic drugs or poisons are relatively stably retained in hair or nails for a long time ; hair and nails sometimes become good alternative specimens for analysis of drugs and poisons in putrefied cadavers, and may be also useful for detection of toxins which had been taken or administered in the past. The utility of hair and nails is presented in Chapter I.2 entitled "Alternative specimens" of this book.

Puncture needles should be kept clean; after their use, blood attached to the needles should be immediately removed by washing with tap water by moving the inner needles back and forth. The bloody needles should not be left to dryness after use.

Sampling at autopsy

When death by poisoning is suspected, stomach contents, right and left heart blood and urine are collected as much as possible (10–100 mL) and stored. More than 20 g of each tissue of the brain, lung, heart, liver, kidney and spleen should be sampled. In case of a putrefied cadaver, the skeletal muscle in the thigh may become a useful specimen for analysis, because the tissue of this part is most resistant to putrefaction and contains levels of drugs and poisons almost equal to those in blood. The tissues from different organs should not be put in the same container or polyethylene bag; they should be kept separately. Special care should be taken for the stomach contents, because it may contain a very large amount of a drug or a poison, which can contaminate other specimens.

When subcutaneous or intramuscular injection of toxins is suspected, the probable injection site is incised, and the skin is carefully removed to sample the corresponding subcutaneous adipose tissue or muscle, which may contain high levels of unchanged drugs or poisons.

Storage of samples

Blood or urine obtained from a living patient or a cadaver is kept in a glass vial (or tube) with a Teflon screw cap; the vial should be sealed completely. When a polyethylene or plastic tube is used, the contamination of the sample by a plasticizer and other compounds should be taken into mind. Solid samples (organs and tissues) are separately put in small polyethylene bags with fasteners to prevent them from drying. It is preferable that every sample is prepared in duplicate; one is kept at 4° C for analysis within a few days and the other kept at −80° C for a long storage. When glass tubes are kept at temperatures below 0° C, the rupture of glassware due to expansion of frozen fluids should be avoided by leaning or laying the tubes.

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I.2 Alternative specimens

By Fumio Moriya

Introduction

Blood, urine and stomach contents (including gastric lavage fluid and vomitus) are usually used as specimens for analysis of drugs and poisons for living subjects. A blood concentration of a toxin can be an indicator for estimation of intoxication degree. Urine sometimes contains large amounts of metabolites and/or an unchanged form of a toxin; it contains low levels of proteins, which usually interfere with analysis, and thus is suitable for screening tests using immunoassays without tedious pretreatments. Stomach contents can be a useful specimen for identification of a toxin, only when the time after ingestion is short; it contains a large amount of an unchanged form of a compound ingested. However, there are many cases, in which neither blood, urine nor stomach contents can be obtained, because of various reasons. Even with urine, illegal drugs become undetectable several days after their administration. Recently, according to marked development of analytical technologies, possibilities are being extended to ultra-sensitive analysis of toxins in hair, nails, saliva and sweat; these specimens are proving to be useful for toxin analysis, because many toxins are excreted into these specimens [1].

The use of non-therapeutic drugs, by pregnant women is now a problem, because of their bad effects on the fetus. To assess the effects of maternal use of drugs on the fetus, data obtained from a newborn baby together with the mother sometimes become necessary. In that case, blood and urine are, of course, usually used. Recently, however, meconium to be excreted by a newborn baby has become an object of interest [2].

At autopsy, any body fluid and tissue can be used for analysis; blood, urine, bile, stomach contents and the liver are being well used. For assessment of intoxication degree, the blood levels of drugs and poisons are usually used; however, we occasionally encounter the cases, in which sufficient amounts of blood cannot be collected, because of exsanguination. In place of blood samples, pericardiac fluid, cerebrospinal fluid, vitreous humor and skeletal muscle can be used [3, 4].

Hair

Hair consists of its shaft and root; the cross section shows the cuticle, cortex and medulla. The cortex part consists of keratine and melanin, and the part counts 80–90% of the whole weight. At the hair bulb, there is the hair papilla with a bundle of capillary vessel, where drugs and poisons are transported from blood to hair cells. The cells are keratinized during their growth. Through this procedure, drugs and poisons are incorporated into hair, resulting in their stable storage in it. The growth rate of hair is dependent to some extent on age, sex, race and health conditions; the rates are about 10 mm and 6 mm per month for scalp and pubic hair, respectively [5].

Hair had been used for detecting its exposure to heavy metals by chemical analysis from the 1950s [6]. The first use of hair for drug analysis is not so old; Baumgartner et al. [7] were

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Table 2.1

Segmental analysis of methamphetamine in hair and nails, obtained from a habitual abuser at autopsy, by mass spectrometry in the CI mode*

Specimen	Length from the root (cm)	Methamphetamine concentration (ng /10 mg)
Scalp hair (parietal region)	0–0.2	10.8
	0.2–1.0	1.38
	1.0–2.0	2.19
	2.0–3.0	0.68
Pubic hair	0–0.2	25.2
	0.2–2.0	0.76
	2.0–5.0	0.08
Finger nail (left thumb)	0–0.5	0.83
	0.5–1.0	0.76
	1.0–1.5	0.38
	1.5–2.0	0.08
Toe nail (left big toe)	0–0.5	1.51
	0.5–1.0	0.60
	1.0–1.5	0.23
	1.5–2.0	0.23

* Cited from reference [9]; methamphetamine could not be detected from any body fluid or organ.

first to detect opiate from hair of a heroin abuser by radioimmunoassays in 1979. Thereafter, many kinds of drugs were reported to accumulate in hair. Nowadays, hair analysis is recognized to be a useful tool for detection of drugs use or abuse. It is possible to detect drug use history of several months by making segmental analysis of hair, when it is sufficiently long [8]. For example, the authors et al. [9] could detect repeated abuse of methamphetamine until the time 3–5 days before his death by segmental analysis of both hair and nails obtained at autopsy (*Table 2.1*); in this case, methamphetamine could not be detected from blood, urine and organs.

For samplings of hair, the scalp hair at the posterior part of parietal region is said to be best, because of its constant growth rate 10]. Prior to analysis, it is necessary to remove environmental (exogenous) compounds attached to the surface of hair. Some surfactants, 0.05–1% sodium laurylsulfate, organic solvents such as n-hexane, dichloromethane, methanol, ethanol and acetone, and 0.01–0.1 M HCl are used for washing the hair surface. To enhance the washing efficiency, an ultrasonic cleaner is often used. To extract a target compound from hair, the sample is put in methanol, 0.1 M HCl or 0.1 M NaOH and incubated at 40–60° C. There are also methods employing digestion with 2.5 M NaOH or proteinase K. These extracts are subjected to liquid-liquid extraction or solid-phase extraction to purify target compounds; the final analysis is usually made by immnnoassays, HPLC, GC or GC/MS [1, 11].

On the basis of extensive data of hair analysis, the cutoff values when measured by GC/MS were presumed to be 1.0 pg/10 mg hair for marijuana metabolites, 5 ng/10 mg hair for cocaine, opiate and methamphetamine, and 3 ng/10 mg hair for phencyclidine [12].

Hair is a good specimen for long-term detection of drugs and poisons; it is possible to analyze a compound many days later. However, we should keep it in mind that the drug use within 3 days cannot be detected by hair analysis.

Nails

The nail consists of nail body (plate) and root; its growth takes place at the nail root and the Malpighian layer of the nail bed. The nail contains hard keratin and its growth process is similar to that of the hair cortex. Drugs are considered to be transported from blood to nail matrix cells at capillary vessels located around the nail root. Drugs incorporated into nails are very stable like in hair. Growth rates of nails were reported to be 3–5 mm [13] and 1.1 mm [14] per month for the fingers and toes, respectively, although they differ to some extent according to seasons. In spite of the fact that similar mechanisms do exist in nails for transportation and accumulation of drugs to those in hair, the reports on nail analysis are not many. In 1984, Suzuki et al. [15] first reported detection of amphetamines from the nails of methamphetamine abusers. Since then only a few reports on methamphetamine detection from nails were reported [9, 16].

The analytical procedure for nails can be similar to that for hair. Before extraction, nails should be washed with methanol and water to avoid exogenous contamination. The extraction can be made after dissolution in 2.5 M NaOH with heating or after crushing in 0.6 HCl. Although the reports on nail analysis are not many, its usefulness seems comparable to that of hair analysis, in view of identification ability of a drug previously administered and estimation of both amount and time (period) of administration [9]. Nails seem worth considering as a good alternative specimen for both antemortem and postmortem subjects.

Saliva

It was in the middle of 1950s when drugs were reported movable from blood to saliva [1]. Since then many researchers examined the usefulness of saliva analysis, and clarified that drug concentrations in saliva reflected those in blood, showed close relationship with the pharmacological effects and could be used for calculation in pharmacokinetics. Recently, saliva is being tried for therapeutic drug monitoring and for detection of the driving under the influence of a drug in the world. Drugs are usually excreted into saliva in their unchanged forms. The concentration ratio of saliva to blood tends to be less than 1 for acid and neutral drugs, and more than 1 for basic drugs; the ratio is also dependent on pH values of saliva [17]. The ratio for alcohol is about 1.1 and not influenced by pH of saliva [18].

Saliva can be easily sampled by directly spitting to a tube; a small cotton ball, which had been weighed, can be placed just under the tongue and kept there for a while for absorption of saliva into the cotton. These are all noninvasive. It is possible to enhance saliva secretion by biting a Teflon plate or rubber bands; citric acid is also useful for stimulating the secretion. However, it should be kept in mind that during the change in the secretion rate, the amount of a drug excreted into saliva may change according to changes in its pH [17].

A close relationship between drug concentrations in blood and in saliva can be found only under strictly controlled conditions. This means that it is difficult to determine blood drug concentrations from the results of saliva analysis in actual cases. However, the drug analysis using saliva is qualitatively useful for proving drug use, when contamination is excluded.

Sweat

Sweat is a fluid excreted from the sweat glands (eccrine and apocrine types). The eccrine glands are widely distributed at the surface of the whole body. The apocrine glands are located in the axillary, mammary, genital and perianal regions. The glands are under the control of sympathetic nerves; but a majority of the glands is cholinergic and a small part is adrenergic. The maximal excretion volume was reported to be about 2 L/day in healthy subjects and about 4 L/day in trained sport athletes; but the volumes and components are greatly different according to individuals, types of the gland and various stresses (emotional, physical and thermal) [19].

The sweat analysis started in about 1970, and showed that various drugs can be detected from sweat [19]. Johnson and Malbach [20] reported that there was close relationship between p*K*a of a drug and its amount of excretion into sweat, and also between drug concentrations in sweat and in plasma. However, the sampling of sweat is a problem; it is difficult to collect it quantitatively. In actual cases, the sweat components are collected by wiping the skin surface with cotton, gauze or towel; PharmChek™ sweat patch (PharmChem Lab. Inc, Menlo Park, CA, USA) is commercially available for absorbing sweat components [19]. Underwears, which absorbed sweat components, were used for detection of amphetamines [21]. The components absorbed could be eluted with water, followed by extraction of drugs before instrumental analysis.

The sweat is not suitable for quantitative analysis of drugs, because of its problem for samplings. However, only advantage of the use of sweat is the longer periods of drug excretion into sweat; drugs could be detected from sweat even 1–4 weeks after single administration [1].

Meconium

Meconium is dark-greenish/green-black and muddy, but does not smell unlike feces of children and adults. It contains meconium vesicles, downs, squamous cells, lipid droplets and cholesterol crystals. It begins to accumulate in the large intestine at week 16 of pregnancy, and is not excreted before birth; it is excreted 1–3 days after birth [22]. Ostrea et al. [23] first reported that meconium was suitable as a specimen for drug analysis in newborn babies. A drug, which has been administered to a pregnant woman, passes through the placenta, reaches the fetus, and is metabolized in the fetal liver. The drug together with its metabolites is partly excreted into bile and finally stored in meconium [24]. Amniotic fluid, which may contain a maternal drug and its metabolites, is swallowed by the fetus, also resulting in the accumulation of compounds in meconium [25].

The samplings of meconium is easy; meconium excreted in diapers is put to a container. The volume of meconium to be analyzed is usually 0.5–1 g. Liquid-liquid extraction and/or solid-phase extraction are employed [2]. The author et al. [26] made drug analysis for meconium and urine of 50 newborn babies delivered from mothers, who had been suspected for their drug abuse, at University of Southern California Medical Center; as results benzoylecgonine could be detected in 12 cases; 5 cases positive for both meconium and urine, 3 cases positive only for meconium and 4 cases positive only for urine. Opiate was also detected in 7 cases; 3 cases positive for both meconium and urine, 2 cases positive only for meconium and 2cases positive only for urine. In addition, phencyclidine was detected from meconium in one case [26].

The author et al. [27] divided the large intestine containing meconium into 5 parts of a still birth baby delivered from a woman, who had been habitually abusing cocaine during pregnancy, and measured benzoylecgonine levels in each part; but we obtained similar levels (1.86–2.24 ng/g) of the metabolite in each part.

Meconium cannot be used for detection of drug use by a mother on a few days before delivery; but it is useful for the use during an earlier period. The merit of the use of meconium is that drug concentration is usually high when a drug was habitually used by a mother and that the amount of meconium obtainable is large enough. It seems to be a better alternative specimen for living newborn babies than hair and nails.

Pericardial fluid

Pericardial fluid exists in the pericardial space; 5–10 mL or more of it can be obtained, if a cadaver is relatively fresh. The fluid can be easily sampled with a syringe after opening the pericardium.

Pericardial fluid has not drawn attention as a specimen for drug analysis until now. However, the author et al. [3] have clarified its usefulness in forensic toxicology by examining autopsy cases. The concentrations (x) of acid, neutral and basic drugs in pericardial fluid were compared with those (y) in blood of the femoral vein using fresh cadavers almost without postmortem changes [4]; there were good correlation between the two body fluids (y=1.03x–0.034, r=0.994, n=16), suggesting that drug concentrations in pericardial fluid is useful for estimation of intoxication degree. The ratio of drug concentration in pericardial fluid to that in blood of the femoral vein was 1.33±0.55 [4]. Other merits are that sufficient amounts of pericardial fluid can be obtained even from a completely exsanguinated body and that the clean fluid can be directly used for drug screening with an immunoassay kit such as Triage™ without any pretreatment. In addition, the author et al. [4] reported that an average value of drug concentrations in pericardial fluid and in cerebrospinal fluid gave more accurate value for estimation of blood drug concentration than the value of pericardial fluid only.

Care should be taken against that pericardial fluid is easily contaminated by postmortem diffusion, when a large amount of a drug is present in the stomach. The mechanism by which a drug is transported from blood to pericardial fluid antemortem is considered to be passive diffusion. The drug concentrations in pericardial fluid seem to change almost in parallel with those in blood; but more precise data on the pharmacodynamic relationship between the interval from the intake of a drug to death and its pericardial fluid concentration are required.

Cerebrospinal fluid (CSF)

CSF is slightly yellowish fluid secreted from the choroids plexus of the ventricle, and fills the ventricles and subarachnoid spaces; its protein contents is as low as about 0.02%. About 400 mL of CSF is produced per day, and transported to the sinus; the total amount of CSF in a adult human is 100–150 mL [28]. CSF can be sampled by lumbar or suboccipital puncture at postmortem inspection, or by introducing a thin vinyl tube into the ventricles after removal of some parts of the brain at autopsy.

There are almost no reports dealing with the relationship between drug concentrations in CSF and in blood except for alcohol. The authors et al. [4] compared the concentrations (x) of acid, neutral and basic drugs in CSF with those (y) in blood of the femoral vein; the equation and correlation coefficient were: $y=1.28x-0.055$ and $r=0.991$ (n=16). The ratio of drug concentration in CSF to that in blood of the femoral vein was 0.55±0.29. Though the value was far less than 1.0, the data of drug concentrations in CSF can be a supporting evidence for judging whether a death is due to poisoning.

Vitreous humor

Vitreous humor is a clear gel-like fluid filling the vitreous body of the eyeball. A 1–2 mL volume of the fluid can be obtained from one eyeball by puncture. Vitreous humor was first used for alcohol analysis in 1966 [29]. Since then, many researchers tried analysis of various abused and therapeutic drugs in vitreous humor, and studied the relationship between drug concentrations in vitreous humor and in blood [30]. The author et al. [4] also made similar experiments; it was disclosed that drug concentrations in vitreous humor were sometimes helpful for assessment of intoxication degree, like those in pericardial fluid and CSF. However, it seemed difficult to estimate a blood drug concentration only with the concentration in vitreous humor. The volume of vitreous humor is limited, and thus it is not suitable for extensive analysis for many drugs.

Skeletal muscle

Garriott [31] and the author et al. [3] clarified that drug concentrations in the skeletal muscle well reflected those in blood. In the case of alcohol, the skeletal muscle-to-blood ratio of alcohol concentration usually show a value of about 1.0. Therefore, when blood cannot be sampled or contamination of blood is suspected, alcohol concentrations in the skeletal muscle can be an indicator for intoxication degree and estimation of the quantity ingested [18, 32]. Although the concentration equality observed for alcohol in the skeletal muscle is not the case for other drugs [33], the drug concentration in the muscle seems very helpful for judgement of poisoning and its degree. In addition, the skeletal muscle is obtainable in large quantities; the specimen is useful in cases in which any body fluid cannot be sampled, and even in cases of mutilated and dismembered bodies.

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