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Postmortem Biochemistry and Toxicology

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Abstract

The aim of postmortem biochemistry and toxicology is either to help establish the cause of death, or to gain information on events immediately before death. If self-poisoning is suspected, the diagnosis may be straightforward and all that could be required is confirmation of the agents involved. However, if the cause of death is not immediately obvious then suspicion of possible poisoning or of conditions such as alcoholic ketoacidosis is of course crucial. On the other hand, it may be important to investigate adherence to prescribed therapy, for example with anticonvulsants or antipsychotics, hence sensitive methods are required.

Keywords: Forensic Science, Postmortem, Biochemistry, Toxicology, Cause of Death.

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Blood sampling (needle aspiration, peripheral vein, for example femoral, ideally after proximal ligation) before opening the body minimizes the risk of sample contamination with, for example, gut contents or urine. Other specimens (stomach contents, urine, liver, vitreous humor) may also be valuable and may be needed to corroborate unexpected or unusual findings in the absence of other evidence. The site of sampling should always be recorded. The availability of antemortem specimens should not necessarily preclude postmortem sampling. Appropriate sample preservation, transport, and storage are mandatory.

Interpretation of analytical toxicology results must take into account what is known of the pharmacokinetics and toxicology of the agent(s) in question, the circumstances under which death occurred including the mechanism of exposure, and other factors such as the stability of the analyte(s) and the analytical methods used. It is important to realise that changes may occur in the composition of body fluids, even peripheral blood, after death. Such changes are likely to be greater after attempted resuscitation, and with centrally-acting drugs with large volumes of distribution given chronically, and may perhaps be minimised

by prompt refrigeration of the body and performing the autopsy quickly.

الكيمياء الحيوية وعلم السموم في حالات ما بعد الوفاة المستخلص

يهدف إجراء تحاليل الكيمياء الحيوية وعلم السموم في حالات ما بعد الوفاة إما للمساعدة في تحديد سبب الوفاة، أو للحصول على معلومات حول الأحداث التي سبقت الوفاة مباشرة. وإذا كان هناك اشتباه بتسمم ذاتي self-poisoning، فقد يكون التشخيص واضحاً وكل ما هو مطلوب هو تأكيد المادة التي تم التسمم بها. ومع ذلك، إذا لم يكن بالإمكان تحديد سبب الوفاة على الفور بشكل واضح، فإن الاشتباه باحتمال حدوث تسمم أو بالتعرض لحالات معينة مثل تحمض الدم الكيتو-كحولي سيكون سبباً أساسياً للوفاة. ومن ناحية أخرى، قد يكون من المهم التحري عن أدوية موصوفة ذات علاقة محتملة بالوفاة. على سبيل المثال أدوية مضادات الاختلاج أو مضادات الالتهاب، ما يستوجب العمل بطرق وأساليب دقيقة وحساسة.

إن أخذ عينات من الدم (مثلاً عن طريق سحب الدم بالإبرة من الوريد المحيطي للفخذ بشكل مثالي بعد ربط الجزء السفلي من الوريد (proximal ligation) قبل فتح وتشريح الجسم يقلل من خطر تلوث العينة بسوائل نذكر منها علي سبيل المثال: محتويات الأمعاء أو البول. وقد تكون العينات الأخرى (محتويات المعدة والبول والكبد والجسم الزجاجي في العينة) أيضاً ذات قيمة في تقديم دليل غير متوقع أو غير عادي عند غياب الأدلة الأخرى. ويجب دائماً توثيق مواضع أخذ العينات الحيوية. كما أن توفر العينات الحيوية ما قبل الوفاة لا يجب أن يحول بالضرورة دون أخذ العينات ما بعد الوفاة. كما يُعد حفظ العينات ونقلها وتخزينها بشكل مناسب من الأمور الإلزامية.

ويجب الأخذ في الاعتبار عند تفسير نتائج التحاليل السمية كل ما هو معروف عن الحركية الدوائية والسمية الخاصة بالعقار الذي يُشتبه التسمم به، وكذلك الظروف التي حدثت فيها الوفاة بما في ذلك آلية وطريقة التعرض، وعوامل أخرى مثل ثباتية المادة analyte التي يتم تحليلها والطرق التحليلية المستخدمة. ومن المهم إدراك أهمية التغييرات التي قد تحدث في تركيب سوائل الجسم، وحتى الدم المحيطي، ما بعد

الوفاة، ومن المرجح أن مثل هذه التغييرات تكون أكبر بعد محاولات الإنعاش، وفي حالات التعاطي المزمّن للعقاقير والأدوية ذات التأثير المركزي والتي تتمتع بحجم توزع كبير. ويمكن التقليل من تأثيرات التغييرات ما بعد الوفاة من خلال التبريد السريع للجثة وإجراء تشريح الجثة بسرعة.

الكلمات المفتاحية: علوم الأدلة الجنائية، حالات ما بعد الوفاة، الكيمياء الحيوية، علم السموم، سبب الوفاة.

1. Introduction

The aim of postmortem biochemistry and toxicology is either to help establish the cause of death, or to gain information on events immediately before death. If self-poisoning is suspected, the diagnosis may be straightforward and all that could be required is confirmation of the agent(s) involved together with some indication of the amount ingested. If the cause of death is not immediately obvious, then suspicion of possible poisoning or conditions such as alcoholic ketoacidosis is of course crucial. On the other hand, it may be important to investigate adherence to prescribed therapy, for example with anticonvulsants or antipsychotics, or prior use of psychoactive or incapacitating agents as in drug facilitated crime [1], hence sensitive and selective analytical methods are required. However, it is not possible to look for all poisons in all samples even in the best equipped laboratories, hence it is important that all those involved in death investigations understand what the laboratory can and cannot do in individual cases.

The value of providing as full a clinical/occupational/circumstantial history as possible together with a copy of the postmortem report (when available) when submitting samples for analysis cannot be overemphasized [2] (Box-1). Not only might this help target the analysis to likely poisons, but also interpretation of any analytical results may be greatly simplified. Herbal medicines, novel (new) pharmaceutical (psychoactive) substances (NPS, 'legal highs', 'designer drugs'), many volatile substances, and of



Box 1- *Information that should accompany a request for postmortem biochemistry or toxicology.*

- Name, address and telephone number of clinician/pathologist and/or coroner's officer, and address to which the report and invoice are to be sent. A post-mortem (reference) number may also be appropriate.
- Circumstances of incident (including copy of sudden death report if available)
- Past medical history, including current or recent prescription medication, and details of whether the patient suffered from any serious potentially infectious disease such as hepatitis or tuberculosis
- Information on the likely cause, and estimated time, of ingestion and/or death and the nature and quantity of any substance(s) implicated
- If the patient has been treated in hospital, a summary of the relevant hospital notes should be supplied, including details of emergency treatment and drugs given both therapeutically and incidentally during investigative procedures.
- Note of occupation/hobbies
- A copy of any preliminary pathology report, if available

course radioactive poisons such as polonium-210 (^{210}Po) are some areas where all laboratories struggle to give a service let alone a comprehensive service [3-7]. Of all the new drugs of abuse, the synthetic cannabinoids are emerging as amongst the most dangerous [8].

Some poisons, for example hydrogen sulfide and aluminium phosphide, are so reactive when in contact with tissues that any attempt to identify, let alone measure, them is meaningless and the diagnosis is based on the circumstances under which illness or death occurred, clinical or post-mortem observations, and the exclusion of other potential causes of illness/death. Although measurement of hydrogen sulfide in lung tissue after fatal hydrogen sulfide poisoning has been described [9], there is the possibility of hydrogen sulfide production postmortem by putrefaction in people dying from other causes [10].

Other poisons, notably paracetamol (acetaminophen), may have been cleared from the circulation before toxicity becomes manifest [11-12].

Some compounds, for example lithium, digoxin, and carbon monoxide (measured as carboxyhaemoglobin saturation), may not be part of a normal laboratory 'screen' and thus the laboratory needs to be informed if they should be looked for, assuming an appropriate sample is available.

Particular problems may arise when the need to assess exposure to drugs or other poisons is raised after the autopsy has been completed, since specimens may not have been collected, or not all appropriate specimens may have been obtained. If the patient was admitted to hospital there may be ante-mortem specimens available for analysis, but the availability of such samples should not preclude appropriate sampling at autopsy. However, if a patient has been maintained for several days on supportive measures such as mechanical ventilation the analysis of specimens obtained postmortem has little chance of detecting poison(s) that may have been present initially.

A factor that must not be neglected is the selectivity and reliability of the analytical method used in any analysis and the training and competence of those performing the analysis. Separate measurement of major blood drug metabolites is important if only to ensure that they have not been quantified together with the parent drug. The drug: metabolite ratio might help indicate whether exposure was acute or chronic. Immunoassays designed simply for drugs of abuse testing are widely used for screening purposes and simply indicate the need for further analysis using a more selective method because of the risk of false-positive results. Amphetamine immunoassays, for example, are notoriously



prone to giving false-positive results. False negative immunoassay results due to the presence of non-physiological concentrations of water, salts, acid, or bleach can also occur. Enzyme-based ethanol assays are unreliable for forensic purposes because of the risk of interference. Many laboratories use gas chromatography-mass spectrometry (GC-MS) with GC for alcohols and high performance liquid chromatography (HPLC) for certain analytes such as paracetamol as their primary analytical system, although increasingly liquid chromatography-mass spectrometry (LC-MS) has a part to play [13-15].

LC-MS instruments are not only very expensive (high resolution instruments are even more expensive), but also require highly trained and experienced operators and robust vendor support in day-to-day operation, prerequisites that are often lacking. Moreover, LC-MS may suffer from either ion enhancement, or ion suppression from co-eluting sample components [16], phenomena that may not be compensated for by the use of (expensive) stable-isotope labelled internal standards [17]. It should be remembered that many drugs are administered as either single enantiomers, or racemic mixtures, and yet achiral analytical methods are often all that is available for the analysis of biological specimens. Mass spectrometry is achiral, a limitation hardly if ever mentioned by those who sell such instruments. Chiral chromatography is not widely used in analytical toxicology.

Box 2- *Information that should be recorded on chain of custody documents.*

- Name of the individual collecting the specimen
- Name and signature of each person or entity subsequently having custody of the specimen, and details of how it has been stored
- Date and time the specimen was collected or transferred
- Specimen or post-mortem number
- Name and date of birth of the subject or deceased
- Brief description of the specimen
- Record of the condition of tamper-evident seals

Similarly, inductively coupled plasma-mass spectrometry (ICP-MS) measures (isotopes of) elements, and cannot differentiate between toxic and non-toxic arsenic species, for example, without a prior separation step [18]. Even then, phenomena such as adduct formation and/or spectral interference from isotopes of different elements can confuse an analysis, unless special precautions are taken [19].

These considerations notwithstanding, the analysis of specimens obtained postmortem is especially challenging owing to the range and variable composition of the specimens that may be submitted, the wide variety of compounds and metabolites that may be encountered, and the invariably limited amount of sample available. Pre-analytical (sample collection, transport and storage) and post-analytical (interpretation of results) aspects are especially important. Use of appropriate, properly validated procedures as part of a formal quality management system, including staff training, participation in external quality assessment schemes, and in research, is mandatory if reliable analytical results are to be obtained and appropriate clinical interpretation provided [20-21].

2. Sample Collection, Transport, and Storage

Sampling for postmortem biochemistry and toxicology is not always straightforward, yet is of vital importance if all subsequent analytical work is not to be invalidated [22-23] (Table-1). The use of disposable hard plastic (polystyrene) sample containers, for example Sterilin™ tubes, is



recommended. If these are not available, then containers with secure closures appropriate to the specimen volumes being collected should be used, i.e. excessive headspace in the container should be avoided if possible. Some laboratories provide specimen containers for collecting postmortem blood, urine, and other specimens. Vitreous humor is preferred for much postmortem biochemistry because it lies in a relatively protected part of the body and is less susceptible to postmortem change than fluids such as blood [22].

It may be important to note if urine was obtained by use of a catheter. As there is little information on drug distribution within solid tissues in man, collection of approximately 50 g specimens from several sites from organs such as

the brain is recommended if the whole organ is available. For liver, the sample should be taken from deep inside the right lobe as this is furthest from the gastrointestinal tract.

When death has occurred in hospital, any residual antemortem specimens should be obtained as a matter of urgency from the emergency department or pathology laboratory (not only chemical pathology and haematology, but also immunology, transfusion medicine, and virology departments may be a source of such specimens) and submitted for possible analysis in addition to postmortem specimens. Similar considerations apply if there may have been a drug administration error.

Chain of custody procedures (a record of who has had custody of the sample and how it has been stored) are im-

Table 1- Sample requirements: General postmortem biochemistry and toxicology¹.

| Sample | Notes ² |
|-------------------------------------|---|
| Heart whole blood (right ventricle) | 20 mL unpreserved (qualitative toxicology only) |
| Jugular vein whole blood | 20 mL unpreserved (qualitative toxicology only) |
| Peripheral whole blood | 20 mL from femoral or other peripheral site ensuring no contamination from urine or from central or cavity blood. Collect one portion into 2% w/v sodium fluoride and another into a plain tube |
| Urine | 20–50 mL if available (plain tube, no preservative unless a portion required for ethanol measurement) |
| Gastric contents ³ | 25–50 mL (plain bottle, no preservative; keep a note of total volume) |
| Vitreous humour | Maximum available, plain tube, separate specimens from each eye if feasible. Avoid excessive suction to minimize risk of aspirating retinal fragments. Collect one portion into 2% w/v sodium fluoride if for ethanol measurement |
| Cerebrospinal fluid | 5–10 mL, plain tube |
| Pericardial fluid | Maximum available, plain tube |
| Synovial fluid ⁴ | Maximum available, plain tube |
| Liver and other tissues | Liver 50 g (deep inside right lobe), other tissues 50 g as appropriate |
| Scene residues ⁵ | As appropriate |

¹ See Belsey and Flanagan [22] and Dinis-Oliveira et al. [24] for detailed discussion on samples and sampling with especial reference to postmortem biochemistry and forensic toxicology, respectively.

² Smaller volumes may often be acceptable, for example in the case of young children.

³ Includes vomit, gastric lavage (stomach washout, first sample), etc.

⁴ Alternative if vitreous humor not available.

⁵ Tablet bottles, drinks containers, aerosol canisters, etc. should be packed entirely separately from biological samples, especially if poisoning with volatiles is a possibility.



portant (Box-2).

The possibility of specimen contamination is always a concern. Sampling through tissues containing high concentrations of analyte may lead to contamination of the sample. Use of lidocaine is common in association with emergency procedures, but lidocaine is sometimes misused, for example in association with misuse of propofol. Fluoride added as a preservative to blood obviously invalidates a fluoride assay and may enhance decomposition of organophosphorus compounds, and lithium added with an anticoagulant invalidates a serum lithium assay. Collection of vitreous humour into dipotassium EDTA invalidates potassium and by association sodium assay. All organ and tissue samples, and any tablet bottles or scene residues, should be placed in separate containers to minimize the risk of cross-contamination of samples such as blood or vitreous humour during transport to the laboratory. However, even if sampling and transport has been performed with all due care, the post-

sampling stability of the analytes that may be encountered varies considerably, ranging from a few minutes in blood for volatiles such as butane and for protease-sensitive peptides and esters such as acetylsalicylic acid (aspirin) and diamorphine (heroin) to several years for some other drugs and pesticides [25] (Table-2).

3. Interpretation of Quantitative Analytical Results

Clinical interpretation of postmortem biochemistry and toxicology results is a complex area [22, 26, 27]. It used to be assumed that concentrations of drugs and some other poisons measured in blood obtained at autopsy reflected the situation at the time of death, hence interpretation of results could be made simply by comparison with 'normal' or 'therapeutic' plasma concentration data. However, we now know that interpretation of postmortem toxicology results must take into consideration the clinical pharmacol-

Table 2- Examples of substances that may exhibit *in vitro* instability in blood or plasma.

| Volatile substances | Non-volatile substances |
|--|---|
| Aerosol propellants, anesthetic gases, carbon monoxide, ethanol, ethchlorvynol, hydrogen cyanide, mercury, methanol, nicotine, organic solvents, paraldehyde, volatile nitrites (amyl nitrite, etc.) | Acyl (ester) glucuronides, amiodarone, bupropion, gamma-butyrolactone, carbamate esters (physostigmine, pyridostigmine), cyclosporine ¹ , cyanide ion, esters (e.g. acetylsalicylic acid, benzocaine, cocaine, diltiazem, heroin, meperidine, methylphenidate, 6-monoacetylmorphine, procaine, succinylcholine), nitroglycerin and other nitrites, <i>N</i> -glucuronides (e.g. nomifensine <i>N</i> -glucuronide), <i>O</i> -glucuronides (e.g. morphine glucuronides), insulins, proinsulin, insulin C-peptide, lysergic acid diethylamide (LSD), nitrobenzodiazepines (clonazepam, lorazepam, nitrazepam), nitrophenylpyridines (e.g. nifedipine, nisoldipine), olanzapine, <i>N</i> -oxide metabolites, <i>S</i> -oxide metabolites, paracetamol, peroxides and other strong oxidizing agents, phenelzine, phenothiazines ² , quinol metabolites (e.g. 4-hydroxypropranolol), rifampicin, sirolimus ¹ , <i>N</i> -sulfate metabolites (e.g. minoxidil <i>N</i> -sulfate), tacrolimus ¹ , thalidomide, thiol- (sulfydryl)-containing drugs (e.g. captopril), thiopental, zopiclone |

¹ Redistributes between plasma and erythrocytes on standing – use whole blood

² Particularly those without an electronegative substituent at the 2 position



ogy and toxicology of any agents in question, the age of the individual, the circumstances under which death occurred including the mechanism of exposure (route by which a poison entered the body) and other factors such as whether prolonged resuscitation was attempted, how the body was stored prior to sampling, and how the samples were collected. Further important considerations include the changes that might occur in the composition of blood or other body fluids after death (Table-3) and the suitability of the analytical methods employed [28]. The data compilation of Baselt [29] gives invaluable information on the clinical pharmacology and toxicology, including postmortem toxicology, of most commonly-encountered compounds and many others to help with case work.

Blood obtained postmortem is highly variable in com-

position. There is always a degree of hemolysis and sedimentation of cells, clot formation, contamination with tissue fluid, or putrefaction/bacterial degradation may have occurred [30]. Dehydration may have resulted from exposure to heat during a fire, or dilution may have occurred in bodies recovered from water, a phenomenon perhaps more apparent in bodies recovered from fresh water than from sea water. Nevertheless, whole blood is commonly used in postmortem toxicology because it is relatively simple to collect and is relatively homogeneous making it easier to dispense in the laboratory. In addition, and there are often data on the plasma or serum (or sometimes even whole blood) concentrations of many analytes measured during normal therapy in adults to provide at least some basis for the interpretation of results. However, any comparison

Table 3- Factors influencing the likelihood of postmortem change in blood analyte concentrations.

| Factor | Comments |
|---|---|
| Body storage temperature | The higher the temperature, the greater the potential for change. |
| Headspace in specimen tube | Volatile analytes will equilibrate between sample and headspace; opening the tube when cold (4 °C) will minimize losses. |
| Medical intervention | Attempted resuscitation may result in aspiration of stomach contents or movement of blood from central to peripheral sites after death, and possibly release of drug from traumatised tissue into blood. |
| Nature of poison | Lipophilic compounds more likely to show increase than hydrophilic compounds; volatile or otherwise unstable compounds likely to show decrease; ethanol concentration may increase, or decrease depending on circumstance. |
| Presence of poison in the airways, GI tract, or bladder | Postmortem diffusion may alter concentrations in blood and in adjacent tissues (sample liver from deep inside right lobe as furthest from stomach). |
| Position of body when found | May result in blood draining from central sites to peripheral sites. |
| Site of sample collection | Central sites (heart, vena cava, or 'subclavian' blood) more likely to show changes than peripheral sites (e.g. femoral vein after appropriate isolation). Blood from left ventricle of heart more likely to show change than blood from right ventricle. |
| Specimen preservation | Sodium fluoride needed to help stabilize certain analytes (e.g. ethanol, cocaine, 6-monoacetylmorphine) - does not reverse any pre-collection changes. |
| Time between death and specimen collection | A longer elapsed time gives more potential for changes as tissue pH-decreases and autolysis proceeds. |
| Transport of the body | May promote movement of blood from central site to peripheral sites. |
| Volume of blood collected | A larger specimen volume less likely to be influenced by localized changes in blood composition. |



with plasma analyte concentrations measured in life must be performed with considerable caution, in part because the plasma:whole blood ratio may not be unity.

Many tables of 'fatal' blood concentration data have been published, including a compilation of postmortem femoral blood concentrations observed in poisoning fatalities [31-33].

Most such tables do not state the criteria used to establish whether a death was indeed due to poisoning, the site of blood sampling, and indeed the interval between death and sampling. Moreover, simply relying on data from previous cases does not always recognize the possible magnitude, direction, and time dependence of the changes in blood analyte concentrations that may take place after death [34]. Uncritical reliance on tabulated 'fatal concentration' data is likely to mislead sooner rather than later [35,36], especially when, as is often the case, more than one poison is present. With clozapine, for example, postmortem blood concentrations of clozapine and its *N*-desmethyl metabolite norclozapine in clozapine-naïve subjects dying from acute clozapine poisoning are likely to be considerably lower, perhaps 5-10 fold lower, than in patients treated chronically with clozapine dying from causes unrelated to clozapine [37-38].

Blood concentrations of analytes with a relatively small distribution volume (V) such as lithium may change minimally after death, although continued absorption from the gastrointestinal tract may occur postmortem even with such compounds and this may be reflected especially in blood sampled from central sites such as the vena cava. Similarly, although free (unconjugated) morphine concentrations in ventricular postmortem blood are consistently higher than those at peripheral sites, there appears to be on average little change in morphine concentrations with time after death in blood specimens obtained from either central or peripheral sites. On the other hand, it has been known

for many years that heart blood concentrations of digoxin may increase markedly after death if the drug has been given chronically. Marked increases in the concentrations of many lipophilic drugs with a relatively large V such as the tricyclic antidepressants have been documented in central (heart, vena cava) as opposed to peripheral (femoral) blood [39].

Often little information is available as to the elimination kinetics of a substance after overdose. The plasma half-lives after therapeutic or 'normal' doses are not necessarily the same as those after overdose [40-41].

Moreover, for drugs with short plasma half-lives such as the anaesthetic gases, γ -hydroxybutyrate (GHB), zopiclone (itself markedly unstable in postmortem blood) and zolpidem, the concentration measured in postmortem blood may be expected to be appreciably less than the concentration causing incapacitation and coma, owing to distribution, metabolism and/or elimination continuing up until the point of death.

It is sometimes suggested that published values of V can be used to estimate the likelihood of postmortem increase in blood concentration given the availability of information such as daily dose, etc. However, published V values are themselves estimates. For clozapine, for example, literature values of V are 2.5–10 L/kg. For clozapine and its *N*-desmethyl metabolite norclozapine, mean postmortem increases in (presumed femoral) blood concentrations of these compounds of 480 and 360 %, respectively, have been observed in patients not thought to have died from clozapine poisoning [37]. Similar findings have been reported with another centrally-acting drug, fentanyl, in femoral blood [42]. The dangers of using a concentration measured in postmortem blood and a published value of V to attempt to estimate either the perimortem plasma concentration, or even worse the dose taken, are evident without even considering other variables such as the time



course of exposure and the route of administration.

A further problem is that some analytes may be lost from or even produced in the body after death. Analysis of other fluids (urine, vitreous humor) and screening for other possible fermentation products is needed to confirm a blood ethanol result, for example, especially if death involved extensive trauma or the body was beginning to decompose. Analysis of urine and especially of vitreous humour may facilitate detection of 6-monoacetylmorphine thereby giving definitive evidence of diamorphine use. Vitreous biochemistry may also be useful in some situations, such as assessing blood glucose at the time of death, whilst β -hydroxybutyrate (BHB) assay may be helpful in the investigation of alcohol-associated or diabetic ketoacidosis [22]. Vitreous creatinine can be helpful in interpreting blood concentrations of drugs or metabolites normally eliminated in urine, for example morphine conjugates when morphine has been given in palliative care. However, vitreous potassium increases and vitreous glucose falls rapidly after death such that the measurement of these analytes is of no use in attempting to assess perimortem plasma potassium and hypoglycaemia, respectively.

If discovery of a body is delayed, the extent of decomposition can make not only specimen collection, but also the interpretation of qualitative let alone quantitative results very difficult. Ensuring that the body is stored at 4 °C prior to the autopsy and that the autopsy is performed as soon as possible after death may minimize the risk of changes in blood analyte concentrations occurring before sampling, but even then for some analytes postmortem change occurs so rapidly that an analysis is often futile. Embalming or other similar procedures of course render most attempts at analysis futile [23, 43-44].

Collecting blood by needle aspiration from a peripheral site prior to opening the body and after ligating the blood vessel proximally may help minimize the effects of

postmortem change [24], but will not account for changes that may have occurred during attempted resuscitation, for example. However, such precautions are not always taken (even proper documentation of the site of blood collection may be lacking) and blood sampling from a central site such as the heart, or even collection of 'cavity blood' (blood remaining in the body cavity when the organs have been removed), is not uncommon. This latter practice carries the risk of contamination of the 'blood' specimen with fluids from other sources. For many poisons, the blood concentrations associated with severe toxicity are very low, typically in the mg/L (parts per million) or even $\mu\text{g/L}$ (parts per billion) range and thus even trace contamination of a peripheral blood sample with gastric contents or urine, for example, can confound the most careful analytical work. In such instances, toxicological analysis can often do little more than provide evidence of exposure to a particular substance.

Tolerance cannot be measured in retrospect, although hair or nail analysis can sometimes be employed in an attempt to assess exposure to toxic metals, illicit drug use, or adherence to prescribed medication in the weeks or months before death. Although hair is well preserved even after burial, analysis gives no information pertaining to acute poisoning and qualitative information on exposure may be all that can be gleaned [45]. Many factors such as differences in hair growth rate depending on anatomical region, age, sex, ethnicity and inter-individual variability in drug/metabolite incorporation taken together mean that interpretation of quantitative results even in samples obtained in life is not easy [46]. Moreover, there is always the possibility of external contamination from, for example, skin secretions, of passive contamination, and of removal of analyte through either excessive washing, or cosmetic hair treatment, or of distributing analyte from the surface to the matrix of hair during sample preparation [47]. As an ex-



ample, studies have shown that synthetic cannabinoids, and indeed their metabolites, can be detected in hair segments seemingly dating from before the compounds in question were available on the drug market [48-50].

Clearly, where there is no obvious indication of poisoning and there are the possibilities that a patient or victim may have developed tolerance and/or that postmortem changes in blood analyte concentrations may have occurred, the availability of additional information such as the results of tissue analyses may provide some information on the nature and magnitude of exposure, although comparative data are sparse [26]. Moreover, it should be remembered that site-to-site variation in postmortem drug concentrations has been reported within certain large organs such as the liver and also in muscle. Site-to-site variation in brain drug concentrations are also likely. Attempts to assess the dose from an isolated tissue measurement, in liver for example, can only be speculative.

Measurement of poison concentrations in a representative specimen of gastric contents can sometimes give an estimate of unabsorbed dose if the total volume of contents is known. However, simply detecting a basic drug in gastric contents does not prove recent ingestion, since ion-trapping of basic drugs that diffuse from blood into the stomach can occur and in any case salivary excretion will have been almost inevitable.

Finally, the possible role of the 'molecular autopsy' in providing additional information to help interpret postmortem toxicology data must be mentioned. In the case of 'sudden cardiac deaths' in those aged less than 40 years, the role of genetic testing has evolved as an important feature in establishing an underlying diagnosis and in screening at-risk family relatives [51,52]. In cases where no definitive cause is identified at postmortem, i.e. sudden unexpected death (SUD, also known as sudden arrhythmic death, SAD), genetic testing, including pharmacogenetic testing,

may emerge as a useful adjunct in the investigation of the cause of death. Whilst the US FDA has required manufacturers to pinpoint relevant pharmacogenomic markers for certain drugs, including carisoprodol, citalopram, codeine, and risperidone, with the aim of identifying individuals who need lower or higher doses, or even a different drug [53], more research into the cost/benefit of such an approach is needed before it can be advocated in routine case work [54].

4. Conclusion

All the available evidence must be taken into account when an attempt is made to interpret postmortem biochemistry and toxicology data. An overall knowledge of the circumstances, time course, clinical and perhaps postmortem observations, substances thought to be involved in an incident and their pharmacology are important, together with knowledge of the specimens available for analysis and the analytical methods used [55]. Bringing together the necessary information may not be easy, especially as many individuals with different backgrounds may be involved: investigating authorities, emergency treatment personnel, former medical providers, postmortem examiners, and analysts. Despite the problems listed above, toxicological and sometimes biochemical analysis is an essential component of many types of enquiries and can often provide evidence of exposure to drugs and other substances and may assist in estimating the extent and timing of the exposure. Extension beyond this requires full knowledge of the case under consideration and appreciation of the pharmacology of the agent(s) in question.

Better training in postmortem biochemistry and toxicology is needed for pathologists and others who may be called upon to interpret biochemical and toxicological data for the Courts. Undue reliance on quantitative results is likely to confuse sooner rather than later, especially in



the case of centrally-acting drugs such as opioids and clozapine. Remember that with drugs and other poisons the question is normally 'was it poisoning?' or 'was it an overdose?', and not 'is it a fatal level?'

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