



Obtaining samples at post mortem examination for toxicological and biochemical analyses

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Introduction

The human body can be regarded as a complex assembly of dynamic chemical systems. Maintaining the integrity of these systems is an affront to the second law of thermodynamics and requires the constant expenditure of energy. Once death takes place, the supply of energy from metabolic processes is dramatically reduced, the integrity of the different compartments within the body breaks down at differing rates; complex molecules tend to break down to their simpler subunits and to move down concentration gradients that were maintained in life by the expenditure of metabolic energy. Obviously, these processes do not all occur at once. Thus, for a variable length of time after death the analysis of appropriate samples may yield useful information about the metabolic state of the person in the period immediately before death.

Once death has taken place many drugs are released from their binding sites in tissue as pH decreases on death and as the processes of autolysis proceed. These phenomena can make the interpretation of drug concentrations after death less than straightforward.

Sample collection

It is important that when biochemical analyses, normally carried out on serum, are indicated that the blood sample obtained at necropsy is centrifuged and the serum separated as soon as possible. If there is any possibility that the red cells may require analyses, for example in the investigation of haemoglobinopathies or for the determination of glycated haemoglobin, then either they should not be disposed of after centrifugation or a separate sample should be taken.

Ideally, the decision to take samples for toxicological or biochemical analyses should be made before the post mortem knife touches the skin of the corpse. A few minutes spent studying the case notes or the Police Sudden Death Report before the necropsy is started is time well spent.

Blood samples are best obtained from the femoral artery or vein by percutaneous puncture. Use a 30 or 50 millilitre syringe with a wide bore needle. The femoral artery is

located in the inguinal canal midway between the superior anterior iliac spine and the pubic tubercle. The needle should be inserted perpendicularly at that point. If no loss of resistance is felt when the artery is entered withdraw the needle while aspiration is maintained. The femoral vein may be found a centimetre or so medial to the artery at that point. Aim to collect at least 40 ml of blood. For many analyses, however, a smaller volume may be adequate. Most of the sample should be placed in a fresh (not reused) glass, screw-top universal container filled to the brim and sealed with aluminium foil lined caps. About 10% of the sample collection should be placed in tubes containing a fluoride preservative to reach a final concentration of 1.5 % by weight. This is particularly important if samples are being collected for alcohol, cyanide, or cocaine metabolite analyses. If there is any possibility that a second post mortem examination may be required, take duplicate samples. (The pathologist carrying out the second post mortem examination may wish to obtain his or her own samples, but one cannot obtain, for example, cardiac blood from a dissected heart). Carefully label each sample with the name of the person, the date and time of collection, and the sample site.

It is particularly important that blood should not be collected by being "milked" from a limb. This process can engender dynamic changes in drug concentrations in the expressed blood. Cardiac blood is not, in general, a suitable sample for quantitative toxicological analyses. If cardiac blood is submitted to the laboratory then it should be identified as such. "Blood" collected from the paracolic gutter after evisceration is a less than adequate sample for most metabolic and toxicological investigations. It may be contaminated with gut contents, urine, or other body fluids. Unless no other body fluids are available, as, for example, might be the case in a second post mortem examination, its use should be avoided.

Urine is best obtained by direct puncture with needle and syringe of the exposed bladder once the abdomen has been opened. It may also be obtained by the insertion of a

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urethral catheter before the start of the necropsy. A common practice is to obtain urine by aspiration with a syringe, without a needle, once the dome of the bladder has been opened in the course of the post mortem examination. If this procedure is adopted then great care should be taken to ensure that the urine obtained is not contaminated by blood or other fluids. If the patient was catheterised shortly before the death the laboratory should be so informed. Under such circumstances the urine can be contaminated by lignocaine or other local anaesthetics present in the gel used to lubricate the catheter.

Vitreous humour is obtained by needle puncture of the eyeball. A 17 or 15 gauge needle attached to a 5 ml syringe may be conveniently used. The eyelid should be firmly retracted laterally and the sclera punctured at a latitude of about 60°, taking the pupil as the North Pole, with the needle being directed towards the centre of the eyeball. Gentle aspiration will usually yield 2–3 ml of vitreous humour. Once the sample has been obtained, the syringe should be detached from the needle, leaving the needle in place, and a volume of water or physiological saline equivalent to the amount of vitreous removed should be slowly injected into the eyeball to achieve cosmetic restoration. A portion of the sample aspirated should be preserved with fluoride.

Cerebrospinal fluid is difficult to obtain at post mortem examination by conventional lumbar puncture, but it is relatively easy to obtain by cisternal puncture. With the neck flexed, palpate the atlanto-occipital membrane in the midline and using a needle and syringe, gently introduce the needle through the skin at that point directing the needle towards the bridge of nose. As the atlanto-occipital membrane is punctured at a depth of about 2 cm loss of resistance will be felt at which point cerebrospinal fluid can easily be aspirated. Alternatively, cerebrospinal fluid can be aspirated anteriorly after evisceration by introducing a needle into the spinal theca via the spinal foramina between the first and second lumbar vertebrae.

Toxicological analysis of bile can be extremely useful in some cases of overdose. For example, high concentrations of paracetamol and its metabolites may occasionally be found in bile in the gall bladder after the paracetamol concentration in the blood has fallen to relatively inconsequential concentrations. It may also be useful in cases of opiate overdose. Bile is easily aspirated from the gall bladder before abdominal evisceration. If the patient has undergone cholecystectomy, useful amounts of bile may still be obtained by aspiration of the common bile duct with needle and syringe.

The stomach content is best obtained by either clamping or applying ligatures to the lower end of the oesophagus and the pylorus before the stomach is dissected free. The stomach should then be held inside a large container, such as a 5 litre beaker, while it is opened and the contents removed. If an

attempt is made to make a small opening in the stomach content and to pour the contents into a honey pot or similar sized container then there is a significant risk of loss of stomach content by spillage. Ideally, all the stomach contents should be submitted to the laboratory, but if that is not done then a note of the total volume of the stomach content should accompany the sample that is submitted. In some circumstances samples of small bowel content should also be submitted for toxicological analysis. In cases where the cause of death is not immediately apparent at necropsy it is advisable to take tissue samples in case the necessity for toxicological analysis becomes apparent at a later stage in the investigation. Normally tissue samples of about 100 are adequate for most toxicological analyses. Brain, adipose tissue, liver and kidney may usefully be submitted. The samples should be placed in new glass containers and should under no circumstances be placed in fixative. Care should be taken to avoid contaminating the liver sample with large amounts of bile. Splenic tissue may be useful for DNA analysis. If an inborn error of metabolism is suspected as the cause of death then the advice of the local reference laboratory as to the samples required should be sought as soon as possible after the patient has died. In cases where it seems likely that the death was associated with volatile substance abuse then one lung should be taken and placed inside a nylon bag that is then heat sealed. Most police scene of crime officers will carry a supply of suitable nylon bags that are used for collecting debris at fire scenes for accelerant analysis.

In circumstances where it is suspected that heavy metal poisoning is a possibility or where it may be important to establish whether certain drugs have been used over a prolonged period of time hair or fingernail samples may be useful. Hair samples should be obtained by plucking rather than trimming with scissors. If the samples are obtained by trimming with scissors then the proximal end of the hair sample must be clearly identified.

Faeces may occasionally be useful, principally if porphyria is suspected.

Analyses

The ability of cells to maintain the concentration gradient of electrolytes between the intra- and extra cellular environment is energy dependent. Once death has taken place these concentration gradients can no longer be maintained and there is a rapid flux of the smaller molecules down the appropriate concentration gradients. The most immediate effect is that there is a very rapid rise in serum potassium in the first minutes after death. Within an hour or so the post mortem serum concentration, even in the absence of haemolysis, is at least six times that of the ante mortem concentration. This occurs even in the absence of post mortem haemolysis which, in a cooled body may take some 48 hours or so after death to become clinically

relevant. Serum potassium determinations in post mortem samples are thus of little or no probative value.

Serum sodium concentrations tend to decrease after death but rather more slowly than the serum potassium concentrations. The decrease is somewhat variable from subject to subject and it is unlikely that much useful information can be obtained from analysis of post mortem serum sodium concentrations, although very high or very low values found in samples collected within a few hours may be helpful. The special case of apparent drowning is discussed below.

Glucose concentrations in post mortem blood samples are of little value unless they are high. Glycolysis continues after death and the blood glucose concentration falls extremely rapidly. The finding of a high blood glucose in a post mortem sample by a specific analytical method, such as the hexokinase method, is a useful indication of post mortem hyperglycaemia. The measurement of glycated haemoglobin by an affinity chromatography method may also be useful in circumstances where death due to undiagnosed diabetes mellitus is suspected. The thiobarbiturate method for the assay of glycated haemoglobin is useless, in my experience, for the assay of post mortem blood samples. In the course of the laboratory examination of post mortem samples, often the first clue that otherwise unrecognised diabetes mellitus is present is a large acetone peak found when assaying for ethanol by gas chromatography. The presence of ketones may be demonstrated by placing a drop of vitreous humour or urine on to the appropriate pad of a urine testing stick.

Urea, creatinine, and urate in post mortem serum samples seem to be stable for at least 48 hours, although there may be a slight rise in the urea and urate.

A major problem in the analysis of post mortem blood samples for biochemical variables is that haemolysis seems to take place relatively rapidly *in vitro* unless the sample is promptly centrifuged. By the time the sample has reached the clinical chemistry laboratory it may be so haemolysed as to be unsuitable for analysis. Under such circumstances, vitreous humour may be useful. Although vitreous humour tends to be more viscous than normal human serum, most multi-channel analysers will aspirate samples of vitreous humour satisfactorily. Such samples should be centrifuged before being presented to the analyser and care must be taken to see that the probe does not short sample the specimen. Vitreous humour, for many analytes, can be regarded as being an ultrafiltrate of plasma. Because it is low in protein the Donnan effect operates to ensure that the chloride concentration is about 10%–20% higher than that found in serum. It has been suggested that the potassium concentration in vitreous humour is a useful marker of the time of death, being said to increase by roughly 1.7 mmol/l per hour in the first 12 hours after death. In practice, however, the

rate of increase is extremely variable and such data should be interpreted with great caution. The urea and creatinine concentrations in vitreous humour are a reasonable guide to the urea and creatinine concentrations that pertain in serum at about the time of death. The glucose concentration in vitreous humour tends to fall very rapidly after death and a low vitreous humour glucose concentration in no way indicates ante mortem hypoglycaemia. A normal or high vitreous humour concentration can usually be taken to imply that hyperglycaemia was present at the time of death. An exception is where the body is rapidly cooled after death. Under such circumstances the vitreous humour glucose concentration may be of the same order as that found during life. Bicarbonate concentrations in vitreous humour are usually low in post mortem samples. This may in part be artefact, rather than a reflection of peri- and post mortem acidosis, because it is usual for the laboratory to receive 1–2 ml of vitreous humour sloshing around in a 20 ml universal container. This gives a significant dead space for carbon dioxide to be lost from the sample with a consequent reduction in the total bicarbonate concentration. To avoid this dead space effect 2 ml sample tubes, filled to the brim, should be used for vitreous humour samples. Lactic acid can occasionally be profitably measured in vitreous humour, high concentrations being associated with a prolonged agonal period. Another marker of agonal hypoxia in vitreous humour may be the concentration of hypoxanthine. This is a breakdown product of adenosine monophosphate. During hypoxia there is an increased breakdown of adenosine monophosphate to hypoxanthine that then accumulates in tissues and body fluids. Hypoxanthine can be measured in vitreous humour by high performance liquid chromatography and high concentrations may suggest that death was preceded by a prolonged period of hypoxia. Large molecules, particularly those which are protein bound such as cholesterol and most hormones, cannot be usefully measured in vitreous humour.

A variety of other analytes can be measured in serum separated soon after collection of a blood sample within 48 hours of death. Cholesterol and lipoprotein electrophoresis can usually be carried out on such samples. Any trace of haemolysis does artefactually increase the serum triglyceride concentrations. Under such circumstances lipoprotein electrophoresis can be particularly useful in demonstrating whether or not there are gross increases in the pre β or chylomicra fractions over and above those normally found in non-fasting samples. However, when a young person dies from coronary artery disease it is probably more important to arrange for serum lipids to be measured in members of the immediate family rather than attempting to characterise any disorder of lipoprotein metabolism that the deceased may have sustained using post mortem serum analyses.

Analysis of post mortem serum samples for cortisol may occasionally be useful; very high

concentrations may suggest a stressful agonal period and low concentrations may be associated with adrenal hypofunction.

Measurement of total thyroxine may occasionally be useful as an adjunct to histological examination of the thyroid.

Catecholamines are best measured in urine. If it is thought that the measurement of catecholamines might be appropriate then an aliquot of the urine collected at post mortem examination should be acidified to a pH of less than 2.0 by the dropwise addition of concentrated hydrochloric acid. High concentrations of catecholamines in urine have been reported in patients dying of hypothermia.

The determination of enzyme activity in post mortem samples is not usually useful. Autolysis rapidly releases intracellular enzymes into blood and makes the interpretation of enzyme activity in post mortem samples extremely difficult in practice. Some workers claim that measurement of creatine kinase and its isoenzymes in peripheral blood, right atrial blood, and pericardial fluid may be used to calculate a discriminant function that distinguishes those who have died from myocardial infarction, before microscopic or macroscopic changes in the myocardium are apparent at necropsy, from those who have died suddenly from non-cardiac causes. The methodology is rather complex for routine practice. An exception to the general rule that post mortem enzymology is unhelpful may be the determination of vitreous humour amylase activity, which is occasionally increased in deaths associated with hypothermia.

Toxicological analyses

Apart from the submission of samples appropriately labelled with the name of the deceased, the nature of the sample, the site from which the sample was collected and date and time of sampling, the most important item that should be sent to the laboratory is a proforma bearing the name and age of the decedent and an estimate of the time at which death took place; the time at which the post mortem examination was carried out; and an account of the final illness; and a list of all the drugs to which the deceased had access.

Special circumstances

THE PUTREFIED BODY

Biochemical analyses have little to offer in the examination of a putrefied body. Where no blood can be obtained, tissue samples, including samples of skeletal muscle, may be of great value for toxicological analyses.

When assays for ethanol are carried out on blood removed from a putrefied body then questions inevitably arise as to the validity of the results obtained. The question obviously arises as to whether or not the ethanol present in the sample has risen as a result of ante mortem alcohol ingestion or post mortem fermentation. If the alcohol analyses have been carried out by head space gas liquid chromatography and there are several peaks present on the tracing other than ethanol and the

internal standard, the implication is that other volatiles are present. It is likely, therefore, when the sample has come from a putrefied body, that they are present as a result of post mortem fermentation. Culture of the sample may reveal the presence of alcohol producing organisms. A simpler technique may be to leave the sample at room temperature and to repeat the analysis a week or so later. If alcohol producing organisms are present in the sample then the alcohol concentration will normally have increased in the interim. If blood samples are taken from multiple sites in the body and there is a large discrepancy in their blood alcohol concentrations then the implication is that post mortem fermentation is confusing the issue in the absence of the possibility that alcohol present in the stomach at the time of death might have diffused to the sampling site. Thus, if blood alcohol analyses are to be carried out in samples collected from a putrefied body, then blood samples should be obtained from several sites, those sites being carefully specified on the sample tubes; the analyses should be carried out by head space gas liquid chromatography and aliquots of the samples should be submitted for microbiological examination.

Occasionally examination of larvae and other entomological samples associated with a putrefying body may yield useful toxicological information. Samples of such material can be preserved by refrigeration prior to toxicological analyses. They should not be placed in any fixative solution.

When a body is partially skeletonised the process may be less advanced in the feet where they have been protected by substantial footwear. It may be possible to recover sufficient soft tissue from the foot for worthwhile toxicological analyses under such circumstances. Marrow from the long bones may also be available and is worth submitting to the toxicological laboratory.

THE EMBALMED BODY

In the United Kingdom most pathologists will be asked, from time to time, to examine an embalmed body that has been returned from abroad. The vitreous humour usually remains uncontaminated by the embalming process and may usefully be analysed for ethanol and for urea and creatinine. In such cases skeletal muscle may be the only material available in reasonable amounts for toxicological analyses. Many drugs are oxidised by the embalming process. Occasionally the question of whether or not the embalming fluid used contains ethanol arises. Some embalming fluids do contain ethanol. Where the body has been returned from an EC country, the local MEP may be willing to help in obtaining details of the composition of the embalming fluid used if other channels fail.

DEATH BY DROWNING

When significant inhalation of water takes place in fresh water drowning there is said to

be consequent dilution of the blood in the left ventricle with a reduction in the sodium and chloride concentration. Measurement of sodium and potassium in haemolysed blood samples, such as are currently recovered when left and right blood samples are obtained from bodies recovered from water, is not a trivial procedure for many contemporary clinical laboratories. Sodium and potassium are no longer conventionally measured by flame emission spectrophotometry but rather by ion selective electrodes. Ion selective electrodes, dedicated to the analyses of normal clinical samples, are likely to produce less than reliable results when used to analyse post mortem haemolysed blood samples. A simple alternative is to estimate the water content of the blood samples submitted for analysis by accurately dispensing about 1 ml of blood into a weighing boat, weighing it on an analytical balance, drying the sample to constant weight in a laboratory oven at 70°C and noting the difference in weight. Aspiration of water into the lungs gives a high water content in the left ventricular blood. The reliability of these tests is increased if the blood samples are taken before the heart is removed from the body. High concentrations of magnesium may be found in the left ventricular blood from those who drown in salt water. The colorimetric methods currently used in most clinical laboratories for the determination of magnesium in blood serum are unsuitable for the analysis of grossly haemolysed blood in post mortem samples. Atomic absorption spectroscopy is a suitable analytical method for the determination of magnesium in such cases. If examination for diatoms is thought to be useful, then the spleen, rather than the bone marrow, may be the most useful sample to take. Samples of the water in which the body was found should also be taken, care being taken to ensure that the water samples do not contaminate the spleen or marrow samples during collection or transport.

SUSPECTED NON-THERAPEUTIC INJECTION OF INSULIN

When non-therapeutic intramuscular or subcutaneous injection of insulin is suspected the prudent prosecutor will immediately seek the help of an experienced forensic pathologist. As well as peripheral blood samples, samples of tissue from the putative injection site(s) and a control site should be collected. Unless blood and vitreous humour samples are collected within a few minutes of death, the measurement of glucose in post mortem samples is of no value in such cases. If a death has taken place in hospital every effort should be made to retrieve ante mortem samples, particularly if they have been appropriately preserved. As well as post mortem peripheral blood samples, consideration should be given to collecting blood samples from the inferior vena cava. In cases where exogenous insulin

has not been administered then the concentrations of insulin and C-peptide will usually be higher in blood samples collected from the inferior vena cava than in peripheral blood samples. The blood samples should be centrifuged as soon as possible after collection and haemolysis avoided if at all possible. Enzymes present in red cells are capable of reducing the disulphide bonds that maintain the secondary structure of insulin necessary for its immunological recognition by the antibodies used in immunoassays. The demonstration of the injection of insulin depends on the demonstration of high tissue concentrations of insulin at the putative injection site, together with a low tissue concentration of C-peptide and low concentrations of insulin and C-peptide in the control tissue, and is supported by finding of high concentrations of insulin in peripheral blood in association with low concentrations of C-peptide.

Conclusion

The key to the successful collection of suitable samples for post mortem chemical analyses is to prepare oneself before the necropsy by reading all the relevant papers, to have the necessary sample containers to hand, to ensure that the samples are adequately labelled and that the chain of evidence is properly maintained, and to submit the samples to the laboratory without delay. In difficult cases discussion with the analyst before the samples are collected is worth while. In any case a full history should be submitted to the laboratory along with the samples. Two ml of unpreserved blood in an unlabelled bottle submitted a week after the necropsy, having been kept at room temperature in the interim, along with a note that says “? overdose” are unlikely to yield results of great value to anyone.

This broadsheet is based, with permission, on a chapter in *The Hospital Autopsy* (Editors T Stephenson, D Cotton. Published by Butterworth & Heinmann, in preparation).

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