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## Simple Tests to Detect Poisons

*Prepared by a Joint Working Party of the Association of Clinical Biochemists and the Association of Clinical Pathologists*

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This review is a revised version of the Association of Clinical Pathologists' Broadsheet No. 52 (Curry, 1966); it is intended to be a practical guide for use in the hospital laboratory, and not a survey of the symptomatology and treatment of poisoning. Tests requiring elaborate apparatus have not been included, nor have details of analyses (e.g. iron, glucose) with which the clinical chemist must already be familiar.

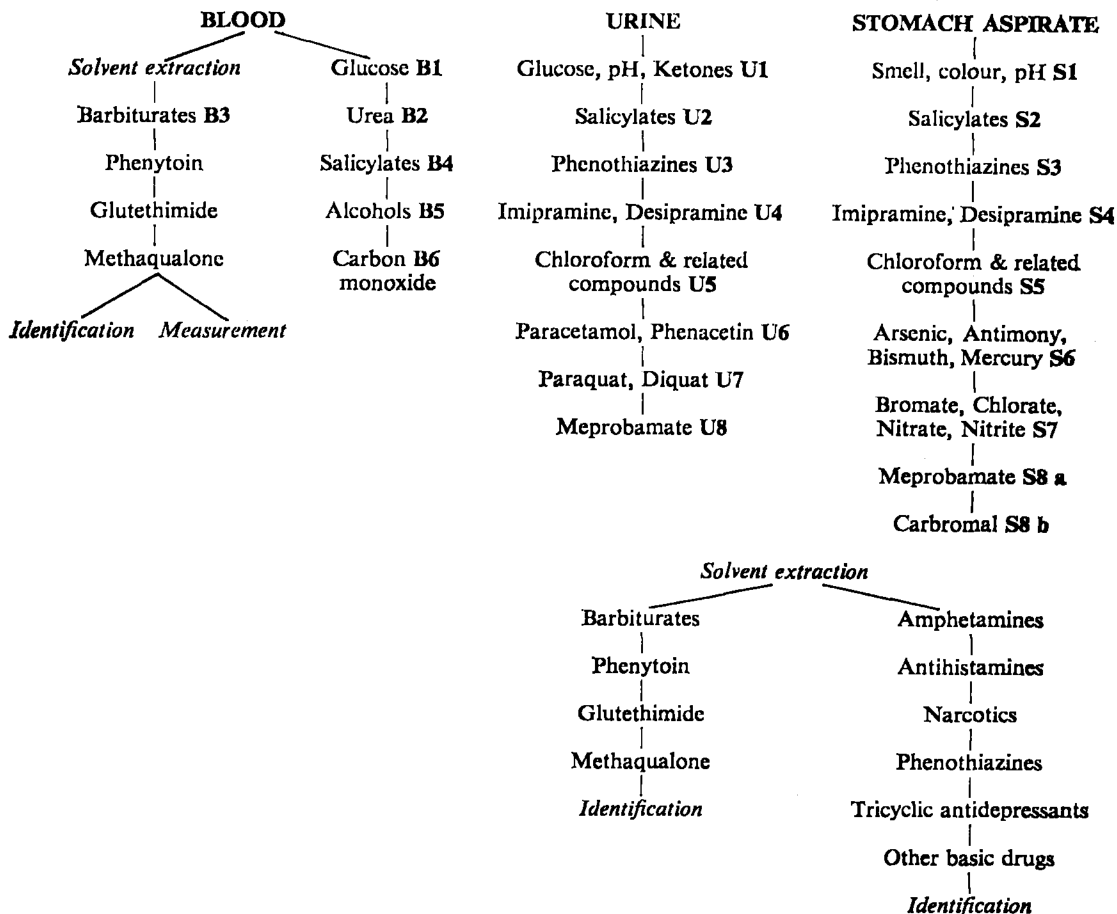
Comprehensive schemes of poison detection have been published (Curry, 1969; Sunshine, 1969) but complete identification and estimation is not always practicable—or necessary—if results are to be obtained quickly enough to have clinical relevance. The tests described here are intended to provide the maximum of useful information in the minimum time, and represent a compromise between speed, sensitivity and specificity. Whether the findings are sufficient to suggest or confirm a diagnosis of poisoning in a particular patient depends on the circumstances. A history of chronic drug ingestion or exposure to a toxic substance should be considered in relation to the clinical features. In any event, *discussion between the clinician and the clinical chemist is essential if limited laboratory facilities are to be used to the best effect.*

With the severely poisoned patient, biochemical investigations such as blood gases, plasma electrolytes or serum enzymes may have greater bearing on the immediate problems of management than toxicological screening, and these aspects have been referred to in the tables below. This is because symptomatic therapy, which is not specific for the poison in question, must form the basis of treatment (Matthew and Lawson, 1970). In the few types of poisoning, viz. carbon monoxide, cyanide, opiate and iron, where truly specific therapy is available, the clinician may well use it without any toxicological investigation. By contrast, it is rarely justifiable to resort to forced diuresis, or to peritoneal- or haemodialysis unless qualitative *and* quantitative information about the poison is available.

### *Interpretation of results*

Occasionally the detection of a foreign substance, such as arsenic in gastric aspirate or paraquat in urine, is diagnostically definitive. Today, however, most cases of poisoning involve drug overdosage and caution is needed in interpreting qualitative analytical results. A patient may have taken a therapeutic dose of a drug quite legitimately, and its detection in a urine or blood specimen will not in itself indicate poisoning; this is most likely to happen with analgesic drugs such as aspirin, codeine or paracetamol which can be obtained without prescription. Alternatively, patients may have been on long-standing regimes of sedative, tranquillising or anti-epileptic drugs; poisoning may then occur on account of chronic toxicity or an acute overdose, or because of the ingestion of some quite unrelated substance. All of these possibilities should be considered if the tests yield a positive result.

Several other aspects of the problem are also important. There is a host of less common toxic substances whose detection requires more elaborate analytical methods than those described here. Furthermore, a positive finding does not automatically rule out the possibility of concurrent disease, and in particular a cerebrovascular accident. Negative findings do not necessarily exclude a diagnosis of poisoning. Much depends on the amount of active substance absorbed, on individual sensitivity and on




the lapse of time before the specimen is taken. Thus gastric aspirate may have been taken too late after ingestion, or urine too soon. Careful enquiry should be made about therapy administered before the patient reaches hospital, or before the first specimen is taken, because this may confuse or even invalidate toxicological screening.

*Collection of specimens*

It is essential to obtain those specimens which are most likely to yield useful information. When the poisoning is thought to be associated with the ingestion of some specific material, any residual solid or fluid found alongside the patient should be submitted for analysis; such substances may have played no part in the episode, but subsequently it may be important to try to establish their chemical nature.

The following specimens should be sent for analysis, *each in a separate container*, clearly marked with the patient's full name, the date *and* time of collection, and the nature of the specimen. A properly completed and signed request form should of course also be submitted.

1. Vomit, stomach aspirate and the first portion of the lavage.

2. A 10 ml specimen of heparinised blood, 2 ml of fluoridated blood and 10 ml of blood, without preservative or anticoagulant, taken on admission. The use of swabs containing alcohols and of heparin containing phenolic preservatives should be avoided.

3. The first urine specimen voided after admission

and preferably before administration of diuretic drugs which may interfere with the tests.

4. Further samples of urine and blood should be taken if the first analysis has proved negative and there remain sound clinical reasons to suspect poisoning.

These recommendations perhaps represent a counsel of perfection; clearly in some cases blood or urine specimens may not be readily available. Nevertheless, if medical and nursing staff are aware of the needs of the laboratory and the complexity of the analytical problem, there is a much better chance of a useful outcome to the investigations.

## Analytical Scheme

The analytical scheme involves preliminary tests followed by solvent extraction procedures. Thin-layer chromatography (TLC) and ultra-violet (UV) spectrophotometry are used for further identification or measurement.

### *Uses of the scheme*

The order in which the tests are performed will depend to some extent on the information provided by the clinician. It should not be forgotten that aspirin and barbiturates are still the commonest poisons; test for these first, preferably in blood. In the difficult case the whole scheme should be followed systematically. With the exception of the barbiturates, for which a TLC procedure is recommended, the spot tests should be carried out initially; other TLC procedures are intended only as a further screen for the presence of drugs and for use in cases where a request for the detection of a particular drug has been made. Valuable information may be derived from UV spectra (Clarke, 1969; Sunshine and Gerber, 1963) but such findings should be interpreted with discretion.

In every case a known negative sample should be tested at the same time and, where indicated, the reaction of the unknown should be compared with that of a positive prepared with the authentic compound.

Reference has been made in the appendix to well-established quantitative methods for some common poisons.

There are, as yet, no simple specific tests for the detection of the benzodiazepins and related compounds (Librium, Valium, Mogadon, Nobrium).

### *Flow sheet*

B, S and U stand for blood, stomach aspirate and urine respectively, and the figures refer to the numbered tests in the list of Preliminary Tests.

### *Index of substances*

Common proprietary drug names are italicised; many others are indexed in Clarke (1969) or in Todd (1967).


| | | *Page* |
|---|---|---|
| Acetone | **B5, U1** | 40 |
| Alcohols | **B5** | 40 |
| *Allegron* (Nortriptyline) | TLC (U, S) | 45 |
| Amitriptyline (Tryptizol) | TLC (U, S) | 45 |
| Amphetamine | TLC (U, S) | 45 |
| Antimony | **S6** | 42 |
| Arsenic | **S6** | 42 |
| Arvynol (Ethchlorvynol) | **S1** | 42 |
| Aspirin | **B4, U2, S2** | 40–42 |
| *Aventyl* (Nortriptyline) | TLC (U, S) | 45 |
| Barbiturates | **B3**, TLC (B) UV (B) | 40–46 |
| Bismuth | **S6** | 42 |
| Bromate | **S7** | 43 |
| Bromvaletone | **S8b** | 43 |
| Camphor | **S1** | 42 |
| Carbon Monoxide | **B6** | 40 |
| *Carbrital* (Carbromal and Pentobarbitone) | **B3, S8b** | 40, 43 |
| Carbromal | **S8b** | 43 |
| Chloral Hydrate | **U5, S5** | 41, 42 |
| Chlorate | **S7** | 43 |
| Chlorodyne | **U5, S5** | 41, 42 |
| Chloroform | **U5, S5** | 41, 42 |
| Chlorpromazine (*Largactil*) | **U3, S3**, TLC (U, S) | 41–45 |
| Codeine | TLC (U, S) | 45 |
| Cyanide | **S1** | 42 |
| Desipramine (*Pertofran*) | **U4, S4**, TLC (U, S) | 41–45 |
| Dichloralphenazone (*Welldorm*) | **U5, S5** | 41, 42 |
| Diquat | **U7** | 42 |
| *Disipal* (Orphenadrine) | TLC (U, S) | 45 |
| *Doriden* (Glutethimide) | **B3**, TLC (B), UV (B) | 40–46 |
| *Epanutin* (Phenytoin) | **B3**, TLC (B) | 40, 44 |
| *Equanil* (Meprobamate) | **U8, S8a** | 42, 43 |
| Ethanol | **B5** | 40 |
| Ethchlorvynol (*Arvynol*) | **S1** | 42 |
| Glucose | **B1, U1** | 39, 40 |
| Glutethimide (*Doriden*) | **B3**, TLC (B), UV (B) | 40–46 |
| *Gramoxone* (Paraquat) | **U7** | 42 |
| Imipramine (*Tofranil*) | **U4**, TLC (U, S) | 41, 45 |
| Insulin (overdose of) | **B1** | 39 |
| Iron | **S1** | 42 |
| Ketones | **U1** | 40 |
| *Largactil* (Chlorpromazine) | **U3, S3**, TLC (U, S) | 41–45 |
| Lysol | **S1** | 42 |
| *Mandrax* (Methaqualone and Diphenhydramine) | TLC (B), UV (B) | 45, 46 |

| | | Page |
|---|---|---|
| *Melsedin* (Methaqualone HCl) | TLC (B), UV (B) | 45, 46 |
| Meprobamate (*Equanil*) | **U8, S8a** | 42, 43 |
| Mercury | **S6** | 42 |
| Metaldehyde | **B5** | 40 |
| Methadone (*Physeptone*) | TLC (U, S) | 45 |
| Methanol | **B5** | 40 |
| Methaqualone (*Mandrax, Melsedin*) | TLC (B), UV (B) | 45, 46 |
| Methylamphetamine | TLC (U, S) | 45 |
| Methyl salicylate | **B4, U2, S1, S2** | 40, 42 |
| Morphine | TLC (U, S) | 45 |
| *Nardil* (Phenelzine) | **S1** | 42 |
| Nicotine | TLC (U) | 45 |
| Nitrate | **S7** | 43 |
| Nitrite | **S7** | 43 |
| *Norflex* (Orphenadrine) | TLC (U, S) | 45 |
| Nortriptyline (*Aventyl, Allegron*) | TLC (U, S) | 45 |
| Orphenadrine (*Norflex, Disipal*) | TLC (U, S) | 45 |
| *Panadol* (Paracetamol) | **U6** | 41 |
| Paracetamol (*Panadol*) | **U6** | 41, 46 |
| Paraldehyde | **B5, S1** | 40, 42 |
| Paraquat (*Gramoxone, Weedol*) | **U7** | 42 |
| *Pertofran* (Desipramine) | **U4, S4,** TLC (U, S) | 41–45 |
| Phenacetin | **U6** | 41 |
| Phenelzine (*Nardil*) | **S1** | 42 |
| Phenothiazines | **U3, S3,** TLC (U, S) | 41–45 |
| Phenytoin (*Epanutin*) | **B3,** TLC (B) | 40, 44 |
| *Physeptone* (Methadone) | TLC (U, S) | 45 |
| Salicylamide | **B4, U2, S2** | 40, 42 |
| Salicylate | **B4, U2, S2** | 40, 42 |
| Surgical Spirit | **B5** | 40 |
| *Tofranil* (Imipramine) | **U4, S4,** TLC (U, S) | 41, 42 |
| Trichloroethylene | **U5, S5** | 41, 42 |
| *Tryptizol* (Amitriptyline) | TLC (U, S) | 45 |
| Urea | **B2** | 40 |
| *Weedol* (Paraquat) | **U7** | 42 |
| *Welldorm* (Dichloralphenazone) | **U5, S5** | 41, 42 |


*Index of reagents*

Except where indicated, these reagents are stable at room temperature.

*Acetic acid* (glacial)

*Ammonium chloride solution*
Dissolve 16 g of ammonium chloride in 100 ml of water.

*Ammonium hydroxide*
Make the following volumes of '880' ammonia up to 100 ml with water: 85 ml (12 mol/l); 14 ml (2 mol/l).

*Copper foil*

o-*Cresol*
Dissolve 1 g of o-cresol in 100 ml of water.

*Dichromate reagent*
Dissolve 0.5 g of potassium dichromate ($K_2Cr_2O_7$) in 5 ml of water and add to a previously prepared solution (CARE!) of 60 ml of concentrated sulphuric acid in 40 ml of water.

*Diphenylamine reagent*
Dissolve 0.5 g of diphenylamine in 100 ml of concentrated sulphuric acid.

*Fluorescein reagent*
A saturated solution of sodium fluorescein in ethanol.

*FPN reagent* (Forrest and Forrest, 1960)
Add 36 ml of 72% perchloric acid to 144 ml of water; add 100 ml of concentrated nitric acid to 100 ml of water; dissolve 1 g of ferric chloride in 100 ml of water. Mix all three solutions.

*Furfural reagent*
Add 1 ml of furfural to 9 ml of absolute ethanol; this reagent must be freshly prepared.

*Hydrochloric acid*
Make the following volumes of 36% hydrochloric acid up to 100 ml with water: 18 ml (2 mol/l); 9 ml (1 mol/l).

*Hydrogen peroxide solution* (100 volumes)

*Imipramine reagent* (Forrest, Forrest and Mason, 1960)
Dissolve 0.2 g of potassium dichromate in 100 ml of water; cautiously add 30 ml of concentrated sulphuric acid to 70 ml of water; add 20 ml of 72% perchloric acid to 80 ml of water; add 50 ml of concentrated nitric acid to 50 ml of water. Mix all four solutions.

*Iodoplatinate spray*
Dissolve 0.25 g of platinic chloride in 100 ml of water and add 5 g of potassium iodide. Add 2 ml of concentrated hydrochloric acid.

*Mercurous nitrate spray*
A saturated solution of mercurous nitrate ($Hg_2(NO_3)_2 . 2H_2O$) in water.

*α-Naphthol reagent*
Dissolve 100 mg of α-naphthol in 5 ml of 2 mol/l sodium hydroxide solution. This reagent must be freshly prepared.

*Palladium chloride solution*
Dissolve 10 mg of palladous chloride ($PdCl_2$) in 100 ml of water.

*Phosphate buffer* (pH 5.7)
Dissolve 6.2 g of sodium dihydrogen phosphate ($NaH_2PO_4 . 2H_2O$) in 200 ml of water (solution A); dissolve 7.1 g of disodium hydrogen phosphate

($Na_2HPO_4.12H_2O$) in 100 ml of water (solution B). Mix 47 ml of A with 4.0 ml of B and make up to 100 ml with water. The buffer is best freshly prepared from the component solutions.

*Potassium carbonate solution*
A saturated solution of potassium carbonate in water.

*Pyridine* (analytical reagent grade)

*Salicylate standard* (salicylic acid, 50 mg/100 ml)
Dissolve 58 mg of sodium salicylate in 100 ml of water.

*Sodium dithionite solution*
Dissolve 0.1 g of sodium dithionite ($Na_2S_2O_4$) in 10 ml of 1 mol/l sodium hydroxide solution. This solution must be freshly prepared.

*Sodium hydroxide solutions*
Cautiously dissolve the following quantities of sodium hydroxide in water and make up to 100 ml: 2 g (0.5 mol/l); 4 g (1 mol/l); 8 g (2 mol/l); 20 g (5 mol/l).

*Sodium nitrite solution*
Dissolve 0.1 g of sodium nitrite ($NaNO_2$) in 10 ml of water. This solution must be freshly prepared.

*Sodium phosphate solution*
Dissolve 10 g of trisodium orthophosphate ($Na_3PO_4.12H_2O$) in 10 ml of water.

*Sodium sulphate* (anhydrous)

*Solvents* (analytical reagent grades)
Chloroform, dichloromethane, ethanol, *i*-propanol n-amyl methyl ketone, petroleum spirit (b.p. 60–80°).

*Sulphuric acid*
Cautiously add the following volumes of concentrated sulphuric acid to 50 ml of water: 50 ml (10 mol/l); 5 ml (2 mol/l).

*Trinder's reagent* (Trinder, 1954)
Dissolve 10 g of mercuric chloride ($HgCl_2$) in 200 ml of water; add 12 ml of 1 mol/l hydrochloric acid and 4 g of ferric nitrate ($Fe(NO_3)_3.9H_2O$). Make up to 250 ml with water.

*Suppliers of special materials or equipment*

| | |
|---|---|
| Cavett Flask (Quickfit and Quartz, assembly 2BC) | Scientific Supplies Co. Ltd., Scientific House, Vine Hill, London, E.C.1 (Tel. 01-837 7765) |
| Ethanol Estimation Kit | Boehringer Corporation (London) Ltd., Bilton House, Uxbridge Road, London, W.5. (Tel. 01-567 4551)<br>Sigma (London) Chemical Co. Ltd., 12 Lettice Street, London, S.W.6. (Tel. 01-736 5823) |
| Labstix (No. 2810) | Ames Co., Stoke Poges, Slough, Bucks. (Tel. 01-369 2151) |
| Cellulose thin-layer plates with fluorescent indicator (No. 6065); Eastman Chromatogram Developing Apparatus (Model 104) | Research Chemicals Sales, Kodak Ltd., Kirkby, Liverpool. (Tel. 051-546 2101) |
| Ucon Lubricant | Union Carbide U.K. Ltd., 8 Grafton Street, London, W.1. (Tel. 01-629 8100) |

## PRELIMINARY TESTS

| *Test* | *Technical Notes* | *Remarks* |
|---|---|---|
| BLOOD<br>**B1** *Blood glucose*<br>Carry out a blood glucose determination | | Hypoglycaemia is a feature of diabetes and of overdosage with insulin or hypoglycaemic agents; it may also occur early in hepatic failure caused by severe iron, chlorpromazine or paracetamol poisoning. |

| Test | Technical Notes | Remarks |
|---|---|---|
| B2 *Blood urea*<br>Carry out a blood urea determination. | | |
| B3 *Barbiturates and related compounds*<br>Test the blood for barbiturates and related compounds by one of the TLC methods described on pages 44-46. | Whole blood is best used as the barbiturates are found in both cells and plasma. | Qualitative detection is often all that is necessary except in the severely poisoned patient. In this circumstance identification and quantitative assay may be of value in management, particularly if phenobarbitone is involved. |
| B4 *Salicylates*<br>To 1 ml of plasma add 5 ml of Trinder's reagent; shake well; centrifuge. If the supernatant is slightly turbid (due to lipid material), it may be clarified by shaking with 1 ml of chloroform. | A violet colour in the supernatant indicates salicylate or salicylamide. For measurement treat 1 ml of the salicylate standard in the same way and read at 540 nm (Trinder, 1954). | Measurement of the plasma level is of great value in deciding on the need for forced alkaline diuresis; for adults this should, in general, be instituted if the level exceeds 60 mg/100 ml. A blood-gas analysis must be carried out since severe salicylate poisoning is characterised by respiratory alkalosis and metabolic acidosis. The results of plasma electrolyte determinations before treatment are unlikely to be meaningful because of severe dehydration. Unless central depressant substances have been taken, impairment of consciousness occurs only in very severe poisoning with acidaemia. |
| B5 *Alcohol and related compounds*<br>Place 1 ml of blood with 1 ml of saturated aqueous potassium carbonate solution in the base of a Cavett flask. In the hanging cup put 0.5 ml of dichromate reagent. Mix the flask contents and seal the flask with Ucon lubricant. Incubate at 60° for 1 hour. If strongly positive the blood ethanol level should be measured (see appendix). | Extreme cleanliness of containers and purity of reagents is essential. A green colour developing after ½ to 1 hour indicates ethanol, methanol, met- or paraldehyde or possibly acetone. The presence of ethanol can be confirmed by the quantitative enzymatic method given in the appendix. | Ethanol may be taken with other drugs and may potentiate their toxic effects. Measurement of the blood ethanol level may be helpful in assessing its contribution to the clinical features of drug overdosage. |
| B6 *Carbon monoxide*<br>Place 1 ml of blood with 1 ml of sulphuric acid, 2 mol/l, in the base of a Cavett flask. Put 0.5 ml of palladium chloride solution into the hanging cup. Mix the blood and the acid, seal the top and leave to stand for 30 minutes. | The liberated palladium can be seen as a black film within about ½ hour if carbon monoxide is present. Two quantitative procedures are referred to in the appendix. | This test should not be ignored even if the blood is of normal colour. The severity of poisoning cannot be assessed by quantitative carboxyhaemoglobin determination if oxygen has been administered before the blood specimen is taken. |
| URINE<br>U1 *Glucose, pH and ketones*<br>Dip a 'Labstix' briefly in urine and read after 10–15seconds. | | Correlate with the blood glucose results. A positive ketone test may indicate acetone or isopropanol intoxication. This test may also be positive in starvation or diabetic ketosis. |

| Test | Technical Notes | Remarks |
| --- | --- | --- |
| U2 *Salicylates*<br>To 1 ml of urine add 4-5 drops of Trinder's reagent. | A violet colour indicates salicylate or salicylamide. | A positive reaction may be obtained after the ingestion of a therapeutic dose of a preparation containing aspirin or p-aminosalicyclic acid. A better indication of poisoning is provided by determination of the plasma salicylate level. |
| U3 *Phenothiazines*<br>To 1 ml of urine add 1 ml of FPN reagent. | Blue to violet colours may develop depending on the dose and the metabolites present. | A strongly positive reaction is a good indication of the presence of phenothiazines but not necessarily of overdosage. |
| U4 *Imipramine and desipramine*<br>To 1 ml of urine add 1 ml of imipramine reagent. | Green or blue colours indicate imipramine or desipramine. To obtain a known positive, grind one Tofranil tablet (25 mg imipramine hydrochloride) with 10 ml of water. Take 0.1 ml diluted to 1 ml with water for the test. | If inco-ordination or coma is accompanied by hypertension or hyperpyrexia, the possibility of interaction of a 'therapeutic dose' of a tricyclic antidepressant with a monoamine oxidase inhibitor should be considered although no simple tests are available for the latter group of drugs. |
| U5 *Chloroform and related compounds*<br>To 1 ml of urine add 1 ml of sodium hydroxide, 5 mol/l, and 1 ml, of pyridine; heat in a boiling water-bath for 1 minute. | A red colour in the pyridine layer indicates chloroform, chloral hydrate, dichloralphenazone or trichloroethylene. Chlorodyne and other medicines containing chloroform-water should also be considered. To obtain a known positive use a dilute solution of chloral hydrate. *N.B.* This test is very sensitive and a blank must be carried through the procedure at the same time. Avoid the use of chloroform since a positive result may be obtained due to accidental contamination of the test tubes or sample by this solvent. | Carbon tetrachloride is much more toxic than chloroform or trichlorethylene and is only metabolised in part to trichloromethyl compounds. This test, therefore, may fail in carbon tetrachloride poisoning; if this is suspected, evidence of direct hepatoxicity should be sought by appropriate serum enzyme assays. |
| U6 *Paracetamol and phenacetin*<br>(a) To 1 ml of urine add 1 ml of hydrochloric acid, 1 mol/l, and 2-3 drops of freshly prepared sodium nitrite solution followed by 2-3 drops of α-naphthol reagent. If negative proceed to (b) | A red colour indicates a positive reaction. Aqueous paraminophenol (0.2 g/100 ml) may be used as a known positive. | A positive reaction may be obtained after the ingestion of a therapeutic dose of a preparation containing paracetamol or phenacetin. In overdosage with paracetamol, evidence of direct hepatotoxicity should be sought by appropriate serum enzyme determination for up to 2-3 days after admission. Consciousness is not impaired until hepatic coma ensues. |

| Test | Technical Notes | Remarks |
|---|---|---|
| (b) To 0.5 ml of urine add 0.5 ml of concentrated hydrochloric acid and place in a boiling water-bath for 1 hour. Dilute 0.1 ml of this mixture to 10 ml with water and add 1 ml of *o*-cresol solution and 4 ml of 2 mol/l ammonium hydroxide. | A blue colour is observed if paracetamol or phenacetin were present. Due to the hydrolysis of drug conjugates, which are not detected by the above test (U6a), this test is the more sensitive and is useful in cases where several days have elapsed following ingestion. | Overdosage with phenacetin may cause methaemoglobinaemia. |
| U7 *Paraquat and diquat*<br>To 1 ml of urine add 1 ml of sodium dithionite solution. | A strong blue or green colour indicates the presence of paraquat or diquat or both. Where the natural colour of the urine masks the colour produced, the sensitivity of the test can be improved by shaking with aluminia before adding the dithionite solution. | If this test is positive it is possible that clinical complications (renal, hepatic and/or pulmonary) will occur. |
| U8 *Meprobamate*<br>Take 10 ml of urine and proceed as for the test on stomach aspirate (S8a) | | |
| STOMACH ASPIRATE | | |
| S1 *Smell, colour and pH*<br>Note the smell, colour and general appearance of the stomach aspirate. Test the pH. | Characteristic smells such as those due to lysol, camphor, phenelzine, methyl salicylate, cyanide, paraldehyde, ethanol or ethchlorvynol should be noted. Recognisable tablets or capsules may be present and colours may derive from these. Blue ferrous phosphate following ingestion of 'iron pills' is usually obvious. A high pH may indicate ingestion of alkalies. | |
| S2 *Salicylates*<br>This test is preferably done on blood or urine. If using stomach aspirate proceed as for test U2. | Aspirin itself will not react with Trinder's reagent, since preliminary alkaline hydrolysis to salicylate is required. | |
| S3 *Phenothiazines*<br>Carry out the test described under U3. | | |
| S4 *Imipramine and desipramine*<br>Carry out the test described under U4. | | |
| S5 *Chloroform and related compounds*<br>Carry out the test described under U5. | | |
| S6 *Arsenic, antimony, bismuth and mercury*<br>Place a quarter of the gastric aspirate in a conical flask with an equal volume of hydrochloric acid, 2 mol/l, and 0.5 cm square of copper foil. Boil for 5 minutes. | Clean the copper foil with sandpaper until shiny before carrying out the test. Observe the colour of the foil; a film of metallic mercury is obvious and arsenic, antimony and bismuth give black stains. Confirmatory tests are essential. | |

| Test | Technical Notes | Remarks |
|---|---|---|
| S7 *Bromate, chlorate, nitrate and nitrite*<br>Add 1 ml diphenylamine reagent to two drops of aspirate. | A blue colour indicates the presence of one of these substances. | |
| S8 *Carbromal and meprobamate*<br>Acidify a portion of the gastric aspirate with hydrochloric acid, 2 mol/l, and extract with four times its volume of chloroform. Separate and evaporate the organic solvent to dryness. Dissolve the residue in 0.5 ml of ethanol. | If the sample contains fatty material, this must be removed by extracting with an equal volume of petroleum spirit prior to extracting with chloroform. | |
| (a) *Meprobamate*<br>Evaporate 0.1 ml of the ethanolic extract on a filter paper. Superimpose 0.01 ml of furfural reagent and allow to dry. Expose to the fumes of concentrated hydrochloric acid. | A purple-black colour developing in about 1 minute indicates a carbamate. A positive control may be obtained by extracting one tablet of Equanil (400 mg of meprobamate) with 5 ml of hydrochloric acid, 1 mol/l, and 5 ml of chloroform, and treating 0.1 ml of the chloroform extract as for the unknown. Positive and negative controls on the same piece of filter-paper are essential for purposes of comparison. If the aspirate is very fatty or otherwise unsuitable, 10 ml of urine can be used. | |
| (b) *Carbromal*<br>Heat 0.1 ml of the ethanol extract to dryness on a white porcelain dish with 2 drops of sodium hydroxide, 2 mol/l, using a microburner. Cool, add 2 drops of fluorescein reagent, 4 drops of glacial acetic acid and 4 drops of hydrogen peroxide solution. Evaporate to dryness on a boiling water bath. | A red colour, due to eosin, indicates carbromal or bromvaletone which are the most common bromoureides. To obtain a known positive, extract one tablet of Carbrital (250 mg of carbromal) with 5 ml of 1 mol/l hydrochloric acid and 5 ml of chloroform, and treat 0.1 ml of the chloroform extract as for the unknown. | Very little carbromal is excreted unchanged in the urine and, therefore, this is only easily detectable in gastric contents. The acute clinical effects of overdosage with Carbrital (pentobarbitone and carbromal) are entirely those of barbiturate poisoning. |

## SOLVENT EXTRACTION PROCEDURES

### *Barbiturates and related compounds in blood*

1. To 1 ml of blood in a glass-stoppered tube, add 1 ml of phosphate buffer and 10 ml of chloroform. Shake for five minutes and centrifuge.

2. Remove the upper (aqueous) layer with a Pasteur pipette and filter the chloroform through Whatman No. 90 paper into a dry conical tube.

3. Evaporate the extract to dryness. Redissolve the residue in 0.1 ml of chloroform.

*Note*: If barbiturates are to be measured by the spectrophotometric method, they may be back-extracted from the initial chloroform extract into 1 ml of 0.5 mol/l sodium hydroxide solution, scanned in the spectrophotometer between 220 and 320 nm and re-extracted into 10 ml of chloroform

(after adding 2 mol/l sulphuric acid) prior to TLC. This procedure reduces the amount of sample required for analysis.

*Barbiturates and related compounds in urine*

Proceed as for blood but using 10 ml of urine. Because of the presence of metabolites, interpretation of TLC plates is difficult and these drugs are preferably sought in blood.

*Basic drugs in urine*

1. To 10 ml of urine in a glass-stoppered tube, add 1 ml of 1 mol/l sodium hydroxide and 10 ml of dichloromethane-isopropanol (9:1) mixture. Shake for 5 minutes and centrifuge.
2. Remove the upper (aqueous) layer with a Pasteur pipette and filter the organic phase through Whatman No. 90 paper into a dry conical tube.
3. Evaporate the extract to approximately 0.1 ml (CARE: some basic drugs, e.g. amphetamines, are highly volatile and may be lost if the extract is taken to dryness).

*Note*: The solvent extraction procedures may be applied to stomach aspirate, but samples may first require centrifugation of particulate material and removal of fat by extraction with petroleum spirit.

## CHROMATOGRAPHIC PROCEDURES

*Thin-layer chromatography*

It is essential to realise the limitations of TLC for the detection of poisons. Rf values are rarely reproducible and should only be estimated in relation to those obtained from simultaneous chromatography of authentic samples. The technique is highly susceptible to interference from extraneous compounds (e.g. nicotine, from the urine of heavy smokers) and from urinary drug metabolites. A positive result in one solvent system and with one spray reagent is not an infallible proof of the presence of a specific drug. For further confirmation, using other solvent systems and spray reagents, reference should be made to Clarke (1969) or Sunshine (1969).

*Barbiturates and related compounds*

*System I: Silica gel/chloroform-acetone*

1. Activate silica gel plates by heating at 100°C for 30 minutes shortly before use.
2. Apply 20 $\mu$l of the concentrate to the plate together with 5 $\mu$l aliquots of authentic solutions of barbiturates or related compounds (1 mg/ml in chloroform). (*N.B.* Do not attempt to use the sodium salts of barbiturates since these are insoluble in chloroform.)
3. Develop the plate in chloroform/acetone (9:1) mixture (*CARE*: chloroform and acetone can form an explosive mixture in the presence of alkali and should be disposed of immediately after use).
4. Air-dry the plate and detect the spots by examination in UV light (254 nm) and subsequently by spraying with mercurous nitrate reagent. In either case, barbiturates and related compounds appear as dark spots on a white background. Approximate Rf values are given in Table 1.

Table 1. *Rf values of barbiturates and related compounds in the two TLC systems*

| *Drug* | *Approximate Rf* System I | System II |
|---|---|---|
| Phenytoin | 0.50 | — |
| Barbitone | 0.60 | 0.20 |
| Phenobarbitone | 0.65 | 0.30 |
| Cyclobarbitone | 0.70 | 0.40 |
| Butobarbitone | 0.75 | 0.60 |
| Pentobarbitone | 0.75 | 0.80 |
| Amylobarbitone | 0.80 | 0.75 |
| Quinalbarbitone | 0.85 | 0.85 |
| Glutethimide | 0.95 | — |

*System II: Cellulose/acetone-water*

1. Cut the cellulose plates to 4 × 10 cm size, and dip in sodium phosphate solution. Oven-dry at 100°C for 30 minutes. Plates prepared in this way may be stored indefinitely.
2. Apply 10 $\mu$l of the chloroform concentrate to the plate alongside 10 $\mu$l aliquots of authentic barbiturate solutions (1 mg/ml in chloroform). Dip the plate in an acetone/water (3:1) mixture and allow the acetone to evaporate at room temperature by holding in a vertical position for 20 to 40 seconds. (CARE: This step is critical since over-drying destroys the resolution.) Develop the plate with n-amyl methyl ketone in a covered beaker (or preferably in an Eastman Chromatogram Developing Apparatus). Air-dry the plate and detect the spots as before. Approximate Rf values are given in Table 1.

*Methaqualone*

Apply 10 $\mu$l of the chloroform concentrate obtained from a blood sample to a silica gel plate alongside 10 $\mu$l of an authentic solution of methaqualone (1 mg/ml in chloroform). Develop the plate in methanol–12 mol/l ammonium hydroxide (100:1.5), air-dry and spray with iodoplatinate reagent. Methaqualone appears as a blue spot with an approximate Rf of 0.8. (*N.B.* Unchanged methaqualone may also be found in urine, and may be extracted as a 'basic drug'.)

*Basic Drugs*

1. Apply 30 $\mu$l of the concentrate to a silica gel plate together with 10 $\mu$l aliquots of authentic sample solutions (1 mg/ml in chloroform).
2. Develop the plate in methanol–12 mol/l ammonium hydroxide (100:1.5).
3. Air-dry the plate and detect the spots by spraying with iodoplatinate reagent. Most basic drugs give blue colours against a pink background. A list of approximate Rf values and colour reactions is given in Table 2.

Table 2. *TLC Rf values and iodoplatinate colours of some basic drugs*

| *Drug* | *Approximate Rf* | *Colour* |
|---|---|---|
| Desipramine | 0.25 | Blue |
| Nortriptyline | 0.35 | Blue |
| Codeine | 0.40 | Purple |
| Morphine | 0.45 | Deep Blue |
| Amphetamine | 0.45 | White |
| Imipramine | 0.50 | Blue |
| Methadone | 0.50 | Purple |
| Methylamphetamine | 0.50 | White |
| Chlorpromazine | 0.50 | Blue |
| Amitriptyline | 0.55 | Blue |
| Orphenadrine | 0.60 | Blue |
| Methaqualone | 0.90 | Blue |

N.B. Nicotine from the urine of heavy smokers gives a strong brown spot with an approximate Rf of 0.52.

## ULTRA-VIOLET SPECTROPHOTOMETRIC PROCEDURES

The ultra-violet absorption spectrum of a drug extract can give a useful lead to its identity but it should be noted that interference may occur when more than one drug is present; interpretation may then be difficult. Below are details of a simple method for obtaining the ultra-violet spectra of three drugs which are fairly readily distinguished by this technique:

Blood (1 ml) or stomach aspirate (1 ml) is shaken for 5 minutes with 10 ml of re-distilled chloroform. After centrifugation, the chloroform layer is separated and shaken for 10 minutes with 2 ml of 0.5M sodium hydroxide. The alkaline extract now contains barbiturates, and the chloroform layer glutethimide and methaqualone.

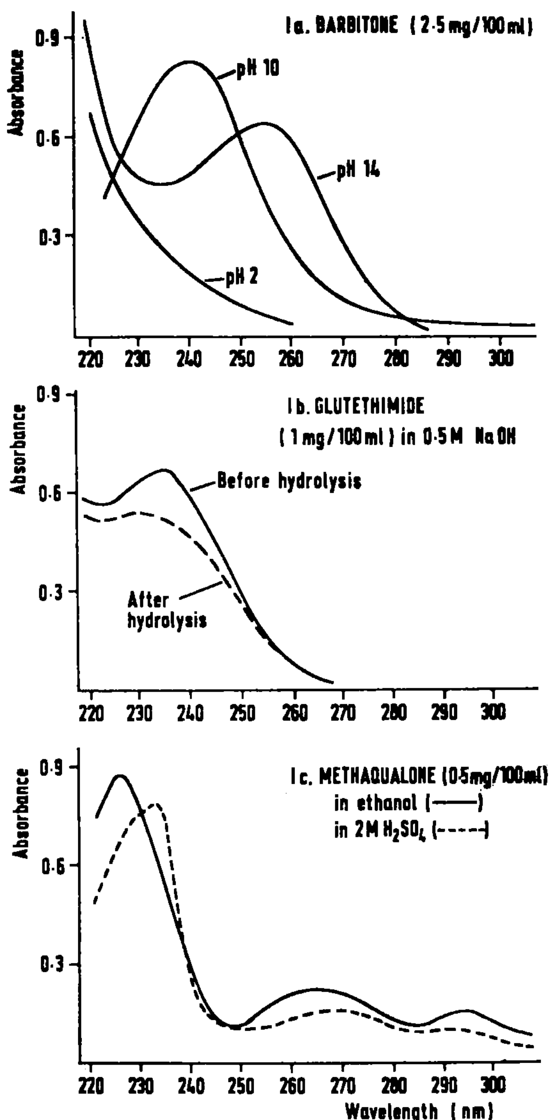



Fig. 1—Ultra-violet absorption spectra of barbitone (1a), glutethimide (1b) and methaqualone (1c).

*Barbiturates*

Scan the alkaline extract immediately from 220 to 320 nm against a blank of chloroform-saturated 0.5 mol/l sodium hydroxide. Compare the spectrum with that shown in Fig. 1a. Re-scan after leaving for 10 minutes at room temperature and note a change in absorbance at 235 nm; if there is a distinct fall, suspect glutethimide. Add 1 ml of ammonium chloride solution to both the sample and the blank solution and re-scan the spectrum at this lower pH of 10. The common barbiturates display a peak of 240 nm. Finally, add a few drops of 10 mol/l sulphuric acid to both cells to give pH 2 or less, when the absorption at 240 nm is reduced almost to zero.

*Glutethimide*

This is not quantitatively extracted into alkali, and should be sought in the chloroform fraction. Dry the chloroform phase with 2 g of anhydrous sodium sulphate, evaporate to dryness and redissolve in 2 ml of ethanol. Add 1 ml of 0.5 mol/l NaOH and scan immediately from 220 to 320 nm. A peak at 235 nm which is considerably reduced after 10 minutes (Fig. 1b) due to alkaline hydrolysis is indicative of glutethimide.

*Methaqualone*

Dry the chloroform phase with 2 g of anhydrous sodium sulphate, evaporate to dryness and redissolve in 2 ml of absolute ethanol or 2 ml of 2 mol/l sulphuric acid. Scan from 220 to 320 nm. Methaqualone has the characteristic absorption spectra shown in Fig. 1c.

## APPENDIX

*References to simple quantitative methods of assay for some common poisons*

*Barbiturates*—Garvey and Bowden (1966), colorimetric; Broughton (1956), and Curry (1969, p. 59), spectrophotometric.

*Carbon Monoxide*—Curry (1969, p. 179); Whitehead and Worthington (1961).

*Ethanol*—The Boehringer or Sigma enzymatic estimation kits; note that this method is unsuitable for medico-legal purposes.

*Glutethimide*—Dauphinais and McComb (1965), spectrophotometric.

*Methaqualone*—Lawson and Brown (1967), spectrophotometric.

*Paracetamol*—Routh *et al.* (1968) spectrophotometric.

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