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ISOLATION AND IDENTIFICATION OF DRUGS

in pharmaceuticals, body fluids, and post-mortem material

VOLUME 2

（内部交流）

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Preface

In the six years that have elapsed since the publication of the first volume of *Isolation and Identification of Drugs* the overall problems facing the toxicologist have become no easier. The annual number of deaths from poisoning in England and Wales, after increasing steadily for several years, has now levelled off at about three thousand:

Year	*1966*	*1967*	*1968*	*1969*	*1970*	*1971*	*1972*
Suicide	1840	1840	1926	1922	1787	1847	1771
Accident	844	873	900	1101	1122	1217	1151
Total	2684	2713	2826	3023	2909	3064	2922

But fatal cases of poisoning do not alone give a true representation of the position, as the great majority of cases recover. When we consider the total admissions to hospitals we find a much more marked increase. In one hospital in England, for example, the number of cases of poisoning admitted went up by 75 per cent between 1965 and 1971.

Similar trends are seen in other countries. For example, the figures for cases of accidental poisoning among children under five years of age reported by the Poisons Control Centres in the United States were:

1969	*1970*	*1971*	*1972*
76,155	70,897	84,370	105,018

Even figures such as these, however, do not give a really true picture of the increasing work of toxicological laboratories. Not only the case-load but also the number of potential poisons has increased, while the problem of drug addiction has assumed even more alarming proportions.

On the other hand, to offset this worsening situation, new and powerful weapons have become available to the toxicologist and increasing experience with older techniques has increased their usefulness.

It is against this background that this supplementary volume has been prepared.

In Part 1, the chapter on Screening Tests for Common Drugs has been completely rewritten in order to meet the changing pattern of drugs found in overdosage and the increasing use made by hospital laboratories of sophisticated equipment. New chapters have been added on the important new techniques of Mass Spectrometry and Radioimmunoassay, while the chapters on Extraction Methods, Gas Chromatography, Infra-red Spectrophotometry, and the Metabolism of Drugs have been supplemented and brought up to date by their original authors. I am most grateful to D. I. Chapman, G. Higgins, P. C. Hirom, J. V. Jackson, H. Leach, A.C. Moffat, M. S. Moss, A.W. Scaplehorn, and R. L. Smith for their contributions.

Part 2 contains 261 monographs, mainly on new drugs, but including revised monographs on eleven of the substances described in the first volume and

monographs on some older drugs for which insufficient information was available when the first volume went to press. I am greatly indebted to A. S. Curry and his staff at the Home Office Central Research Establishment at Aldermaston for supplying ultraviolet and infra-red spectra for most of these compounds, to the Home Office for allowing this work to be done there, to M. S. Moss and his staff, especially P. E. Haywood, for similar data on many of the remaining compounds, to Racecourse Security Services for making these available from their Newmarket laboratory, and to C. Daglish and his staff of the laboratories of the Pharmaceutical Society for providing data on the thin-layer chromatography of the steroids.

Once again, the various pharmaceutical manufacturers listed on page x have been unstinting in their help, both in the gift of drugs and in providing information about them, and again I gratefully acknowledge their co-operation.

Finally, I must express my thanks to Frances Desmond for technical assistance, to my wife for secretarial help, and to the following members of the staff of the Department of Pharmaceutical Sciences of the Pharmaceutical Society for their editorial help: to G. R. Brown for checking the structural formulae, to E. S. Greenfield for his collaboration in the preparation of the chapter on screening tests and in compiling and correlating the ultraviolet and infra-red data, to P. Forbes for assisting with the proofs, and especially to R. G. Todd, Mildred Lang, and K. G. Marriott for their patience and expertise in channelling scientific enthusiasm into the discipline that goes to the making of a book.

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November 1974

Introduction

The arrangement of this supplementary volume closely follows that of the first volume. Such minor modifications as have been considered advisable are recorded at appropriate places in the text. For convenience in cross-referencing and indexing, the page numbers of this volume follow on after those of the first volume, starting at page 871.

Part 1 includes supplementary information on seven of the chapters in Part 1 of the first volume, a rewritten and expanded chapter on Screening Tests for Common Drugs, and new chapters on Mass Spectrometry and the Radioimmunoassay of Drugs. The supplement to the chapter on Paper Chromatography describes an additional system for cannabis. Three new systems for cannabis and steroids are described in the supplement to the chapter on Thin-layer Chromatography which also includes some minor amendments to four of the thin-layer systems described in the first volume.

Part 2 includes monographs on 250 additional substances and revised monographs on eleven of the substances in the first volume—cannabis, hyoscine butylbromide, and the nine substances described in the addenda on pages 599 and 600. The system of presentation of the monographs is the same as that described in the Introduction to the first volume.

Part 3 is arranged in the same way as the corresponding section of the first volume and presents the more important analytical data of Part 2 of the present volume in sequential order and tabular form. The tables are designed to be used in conjunction with the corresponding tables of the first volume.

Appendixes 1 and 2 supersede the corresponding appendixes of the first volume and Appendix 3 is a bibliography of the 490 references cited in the present volume. For references cited in the first volume, the bibliography on pages 810 to 823 of that volume should be consulted.

The general index is a cumulative index to both volumes but, to avoid confusion, references have been omitted to those parts of the first volume that have been superseded in this supplementary volume.

Acknowledgements

Gifts of drugs and information about them are gratefully acknowledged from Abbott Laboratories Ltd, Allen & Hanburys Ltd, Laboratoires André Guerbet, Aspro-Nicholas Ltd, Astra Chemicals Ltd, Ayerst Laboratories Ltd, Bard Pharmaceuticals Ltd, Bayer Agrochem. Ltd, Bayer Pharmaceuticals Ltd, Beecham Group Ltd, Berk Pharmaceuticals Ltd, Boehringer Ingelheim Ltd, Boots Company Ltd, Bracco Industria Chimica S.p.A., Bristol Laboratories Ltd, Brocades (Great Britain) Ltd, Burroughs Wellcome & Co., Calbiochem. U.S.A., Calmic Ltd, Carlo Erba (U.K.) Ltd, Cela Landwirtschaftliche Chemikalien GmbH, Chemie Grünethal GmbH, Chemie Linz AG, Ciba-Geigy (U.K.) Ltd, Ciba Laboratories, Consolidated Chemicals Ltd, Continental Pharma (Brussels), Crookes Veterinary Ltd, Crown Chemical Co. Ltd, Dales Pharmaceuticals Ltd, Delandale Laboratories Ltd, Laboratoires Diamant SA, Dista Products Ltd, Dow Chemical Co. Ltd, Duncan Flockhart & Co. Ltd, Duphar Laboratories Ltd, E. I. du Pont de Nemours & Co., Elanco Products Ltd, Endo Laboratories Inc., F.B.A. Pharmaceuticals Ltd, Fisons Ltd, Gebr. Guilini GmbH, Geigy (U.K.) Ltd, Geistlich Sons Ltd, Glaxo Laboratories Ltd, Hoechst Pharmaceuticals (U.K.) Ltd, Hoffman La Roche Inc., Horlicks Ltd, F. W. Horner Ltd (Montreal), Imperial Chemical Industries Ltd, International Laboratories Ltd, Janssen Pharmaceutica, Kabi Pharmaceuticals Ltd, Knoll AG, Lakeside Laboratories Inc., Lederle Laboratories, Lennig Chemicals Ltd, Leo Laboratories Ltd, Leopold Charles & Co. Ltd, Lepetit Pharmaceuticals Ltd, Lloyd-Hamol Ltd, Lloyds' Pharmaceuticals Ltd, J. M. Loveridge Ltd, Lundbeck Ltd, 3M (U.K.) Ltd, Maggioni & C. S.p.A., May & Baker Ltd, MCP Pharmaceuticals Ltd, McNeil Laboratories Inc., Mead Johnson Laboratories (U.S.A.), E. Merck Ltd, Merck Sharp & Dohme Ltd, Richardson-Merrell Ltd, Monsanto Chemicals Ltd, Moore Medical Products Ltd, Napp Laboratories Ltd, Organon Laboratories Ltd, Parke Davis & Co., Mr. H. Peterson (N.Y.), Pfizer Ltd, Pharmax Ltd, Pharmazell Diamalt AG, Pharmitalia (U.K.) Ltd, Philips Roxane Laboratories Inc., Promonta, Reckitt & Colman Ltd, Richter (Ital.), Riker Laboratories Inc., Roche Products Ltd, Rona Laboratories Ltd, Rybar Laboratories Ltd, Salsbury Laboratories, Sandoz Products Ltd, Schering Chemicals Ltd, G. D. Searle & Co. Ltd, Selpharm Laboratories Ltd, Smith Kline & French Laboratories Ltd, Smith & Nephew Ltd, Specia (Société Parisienne d'Expansion Chimique), SPOFA (Czechoslovakia), E. R. Squibb & Sons Ltd, Stiefel Laboratories (U.K.) Ltd, Syntex Pharmaceuticals Ltd, Unilabo Fr., Upjohn Ltd, Van Dyk & Co. Ltd, Ward Blenkinsop & Co. Ltd, Warner Lambert Pharmaceutical Co., The Wellcome Foundation Ltd, Winthrop Laboratories (Sterling-Winthrop Group Limited), John Wyeth & Brother Ltd, and Zyma (U.K.) Ltd.

Abbreviations

A—ampere(s)
Å—ångström(s)
AMA—American Medical Association
a.m.u.—atomic mass unit
ASTM—American Society for Testing and Materials
AWRE—Atomic Weapons Research Establishment
B.P.—British Pharmacopoeia
b.p.—boiling-point
B.P.C.—British Pharmaceutical Codex
cal—calorie(s)
Ci—curie(s)
cm—centimetre(s)
cm^3—cubic centimetre(s)
cm^{-1}—reciprocal centimetre(s)
d.c.—direct current
e—electron
ed.—editor; edited by
Edn—edition
e.g.—*exempli gratia*, 'for example'
et al.—et alii, 'and others'
eV—electron volt(s)
F—Fahrenheit
FAO/WHO—Food and Agricultural Organisation *and the* World Health Organisation
f.p.—freezing-point
g—gramme(s)
gal—gallon(s)
GC—gas chromatography
GC/MS—gas chromatographic and mass spectrometric linked system(s)
g/kg—gramme(s) per kilogram
GLC—gas–liquid chromatography
GSC—gas–solid chromatography
i.e.—*id est*, 'that is'
in—inch
I.R.—infra-red
KeV—Kiloelectron volt(s)
kg—kilogram(s)
l—litre(s)
lb—pound(s)
LD50—a dose lethal to 50% of the specified animals
λ—wavelength
M—molar
m—metre(s)
m^2—square metre(s)
max—maximum
m/e—mass-to-charge ratio
mEq—milliequivalent(s)
mg—milligram(s)
mg/kg—milligram(s) per kilogram
mg/kg/hour—milligram(s) per kilogram per hour
mg/l—milligram(s) per litre
mg/min—milligram(s) per minute
mg/ml—milligram(s) per millilitre
mg%—milligram(s) per 100 grammes or per 100 millilitres
l mg%—1 milligram per 100 grammes or per 100 millilitres = 10 p.p.m.
ml—millilitre(s)
ml/kg—millilitre(s) per kilogram
mm—millimetre(s)
mmHg—millimetre(s) of mercury
mμ—millimicron(s)—in first volume; replaced by **nm**(nanometre) in this volume
mol—mole(s)
m.p.—melting-point
MS—mass spectrometry
mU—milliunit(s)
mU/ml—milliunit(s) per millilitre
μCi—microcurie(s)
μCi/μg—microcurie(s) per microgram
μg—microgram(s)
μg/kg—microgram(s) per kilogram
μg/l—microgram(s) per litre
μg%—microgram(s) per 100 grammes or per 100 millilitres
μl—microlitre(s)
μsec—microsecond(s)
N—normal
NCIB—The National Collection of Industrial Bacteria (maintained at the Torry Research Station, PO Box 31, 135 Abbey Rd, Aberdeen, Scotland)
ng—nanogram(s)
ng/ml—nanogram(s) per millilitre
nm—nanometre(s)—in this volume; in place of **mμ** (millimicron) used in the first volume
nmol—nanomole(s)
oz—ounce(s)
PEG—polyethyleneglycol
pg—picogram(s)
pg/ml—picogram(s) per millilitre
pH—the logarithm of the reciprocal of the hydrogen ion concentration
pK_a—the logarithm of the reciprocal of the dissociation constant of the acid
p.p.m.—parts per million
p.v.c.—polyvinyl chloride
q.v.—*quod vide*, 'which see'
Rf—relative flow (see p. 32)
r.f.—radiofrequency
RRT—relative retention time (retention time of the sample relative to that of a reference compound)
Supp.—Supplement
$t_{\frac{1}{2}}$—half-life
TLC—thin-layer chromatography
U.S. *and* U.S.A.—United States of America
UV *or* U.V.—ultraviolet
viz.—*videlicet*, 'namely'
vol—volume(s)
v/v—volume in volume
w/v—weight in volume
w/w—weight in weight
wt—weight

Contents

Screening Tests for Common Drugs

G. Higgins and H. Leach

This chapter replaces the chapter on Screening Tests for Common Drugs on pages 3 to 15 of the first volume

The large increase in the use of drugs previously noted (page 3) has continued and is reflected in the constantly increasing numbers of patients suffering from drug overdosage now admitted to hospitals. This increase has been experienced in all the major countries of the world and drug overdosage now constitutes the largest single reason for admission to adult medical wards.

In 1965 in one town in the Midlands of England, 18·9% of the patients admitted to the medical wards of one hospital were suffering from the ingestion of an overdose of drugs (Ellis, Comish, and Hewer, 1966), whilst in a town in Southern England, the number of overdose cases admitted to hospital increased from 330 in 1965 to 580 in 1971 (Higgins, unpublished data). Smith (1972) has reported a similar increase in the Sheffield area of England. It is estimated that in the U.S.A. the total number of non-fatal poisonings, deliberate and accidental, exceeds a million a year. In addition, the problem of the drug-addicted patient has very greatly increased.

The treatment of these cases, both of overdosage and of drug addiction, demands the identification and, frequently, the estimation of the ingested drug.

Whilst in many cases the cause of coma can be recognised easily by the discovery of tablets or capsules on the person or in the bed of the patient or by reference to the prescribing physician, in others no such cause is obvious and many drug addicts deny the use of any drugs. Under these circumstances the cause of the condition must be sought by biochemical analyses and the role of the hospital laboratory has become important in the diagnosis and treatment of such cases.

Frequently the ingestion of excessive amounts of drugs can be assumed or proved, but every case of coma demands expert clinical and particularly neurological examination since the symptoms displayed in coma caused by disease processes and by drug overdosage may be very similar. It is also recommended that the blood sugar and urea content of the patient's blood should be estimated. 'Filter-stick' methods, such as 'Dextrostix' and 'Urastrat', are easily applicable when laboratory facilities are not available.

A review shows that the drugs ingested in the majority of cases of overdosage are limited to one or more of about twenty kinds. In a hospital in Southern England, 1002 women were admitted to a single ward in 1971. Of these women, 122 had taken drugs in overdose and, of these, 28% had taken drugs of the benzodiazepine group, 16% of the barbiturate group, 11% of the aspirin group,

10% of the triptyline group, 8% of the methaqualone group, 6% of the phenothiazine group, 4% codeine, and 3% other drugs. In 14% of these cases the drugs were not identified (Higgins, unpublished data). Brown (1971) has reported that in Edinburgh, with a total population of 750,000, there are about 1000 admissions yearly of cases of poisoning; in 1968, 52% were due to hypnotic drugs, half of these being barbiturates.

It has become apparent that during the past five years a change has occurred in the type of drugs used. In most fatal cases of poisoning the barbiturate group of drugs is still implicated, but the number of patients being admitted to hospital suffering from barbiturate overdose is decreasing, whilst the number ingesting excessive amounts of tranquillisers and antidepressants is markedly increasing. Furthermore, the number of patients taking more than one type of drug is on the increase.

It is the purpose of this chapter to describe a series of tests that can be applied rapidly to biological material to identify the drugs most commonly encountered. In many cases the simple screening tests should be confirmed by the more elaborate techniques which are also described. Cases of drug abuse present different problems and, accordingly, a separate section has been devoted to these (see p. 908).

The specimens generally available from the living patient are blood and gastric contents, collected before the customary stomach washout, but in many of the tests described the use of urine is indicated. It is suggested that a specimen of urine be obtained from all patients if possible.

Since it is apparent from the figures given above that analysis for benzodiazepines, barbiturates, aspirin or salicylates, triptylines, methaqualone, and phenothiazines should elucidate the nature of the drugs in 75% of the cases, it is suggested that, in any scheme devised for the identification of drugs causing coma, these drugs should be first sought for.

Scheme for Rapid Identification of Drugs Commonly Taken in Overdosage

This scheme has been devised to give a simple method for indicating the identity of the drug or drug-type which may be present in samples of blood, urine, or stomach contents after poisoning or overdosage. It includes drugs which are commonly found in these cases. It should be borne in mind that the scheme may need to be adapted to the circumstances of any particular case.

The scheme is divided into five sections: tests performed directly on samples (see p. 875), extraction procedures (p. 878), tests performed after an extraction procedure (p. 881), individual tests for specific drugs (p. 893), and tests for some acid radicals (p. 905). The tests include colour tests, spectrophotometry, and chromatography; they are not necessarily sufficiently sensitive to detect drugs taken in medicinal doses.

The scheme should be followed in cases where there is no clue to the identity of the drug. When there is evidence of its nature, reference may be made directly to the individual tests for specific drugs (pp. 893–905).

The direct tests (pp. 875–877) can be applied quickly, with a minimum of

equipment, and can provide a useful preliminary examination. However, prior solvent extraction is often necessary for urine and stomach contents and is usually essential for blood samples.

Reference Standards

A collection of reference standard drug samples is essential, as it is always advisable to run a reference standard in parallel with the test, especially for spectrophotometry and chromatography.

It is possible to obtain samples of drugs from the manufacturers, but it is better if a reference collection is built up by extracting the substances from tablets or capsules. If each sample is subjected to the complete extraction procedure a great deal of useful information will be obtained. By subjecting the extracts so obtained to spectrophotometry and thin-layer chromatography, a collection of spectra and thin-layer data can be built up.

DIRECT TESTS ON SAMPLES OF URINE, STOMACH CONTENTS, AND BLOOD

This section describes a number of tests which may give an indication of the nature of the drug present. It should be noted that tests for inorganic substances are applied preferably to stomach contents as the amount of substance in urine will usually be too small for satisfactory analysis without some form of separation.

Direct Tests on Urine

1. *'Clinistix' test for glucose*—dip a 'Clinistix' strip into the urine and read the colour after 10 seconds.
2. *Rothera's test for ketones*—saturate 3 ml of urine with ammonium sulphate crystals and add 3 drops of strong ammonia solution and a few drops of freshly prepared 5% sodium nitroprusside solution. A purple colour develops if acetone or acetoacetic acid is present.

   A 'Ketostix' strip can also be used.
3. *Ferric chloride test for salicylates*—to 2 ml of urine add 3 drops of 5% ferric chloride solution. A violet colour develops if salicylate is present.
4. *Dichromate test for volatile reducing substances* (including alcohols and aldehydes)—place 1 ml of urine in a test-tube; apply 1 drop of 2·5% potassium dichromate in 50% sulphuric acid to a strip of Whatman glass-fibre filter paper, insert the paper in the neck of the test-tube, lightly cork the tube, and place in a water-bath at 100° for 2 minutes. A colour change to green indicates a positive result. Ethanol, the most commonly found member of this group, gives a positive reaction if present above 40 mg%.
5. *Forrest test for phenothiazines*—to 1 ml of urine add 1 ml of *FPN reagent*. A variety of colours ranging through pink, red, orange, violet, and blue indicates the presence of a phenothiazine derivative.
6. *Forrest test for imipramine, desipramine, and trimipramine*—to 0·5 ml of urine add 1 ml of *Forrest reagent*. A greenish-yellow colour darkening through dark green to blue, depending on the amount of drug present, immediately appears.

7. *Fujiwara's test for trichloro-compounds*—mix 1 ml of 10% sodium hydroxide solution and 1 ml of redistilled pyridine and heat in a boiling water-bath for 2 minutes; if no colour develops, add 2 ml of urine and repeat the heating. The development of a pink to red colour in the pyridine layer indicates the presence of trichloro-compounds, e.g. chloroform, chloral, chlorbutol, trichloroethane, trichloroethylene, trichloroethanol, and trichloroacetic acid. Blank and control tubes must be treated similarly because contamination of the atmosphere with laboratory reagents may give positive results. Metabolites of carbon tetrachloride may also give a positive result with this test.
8. *Cresol–ammonia test for phenacetin and paracetamol*—to 0·5 ml of urine add 0·5 ml of hydrochloric acid and heat for 1 hour at 100°; to 2 drops of the mixture add 10 ml of water, 1 ml of 1% *o*-cresol in water, and 4 ml of 2N ammonium hydroxide. A blue colour appears if paracetamol or phenacetin is present.

   In this test the *o*-cresol solution can be replaced by 1% phenol in water. The test is sensitive and can detect the conjugated metabolite and drug for several days after ingestion.
9. *Dithionite test for paraquat and diquat*—to 5 ml of urine add 0·1 g of sodium bicarbonate and 0·1 g of sodium dithionite. A blue colour which develops immediately indicates paraquat and a yellow-green colour indicates diquat.

Direct Tests on Stomach Contents

1. *Diphenylamine test for oxidising agents*—filter a portion of the sample and to 2 drops of the filtrate add one drop of 0·1% diphenylamine in sulphuric acid. A deep blue colour, appearing instantaneously, indicates the presence of an oxidising agent such as chlorate or hypochlorite. A light blue colour, caused by organic matter, should be ignored. Note that nitrate and nitrite from meat products will give a positive reaction.
2. *Ferric chloride test for salicylates*—boil a portion of the sample for 10 minutes with an equal volume of 0·1N hydrochloric acid, filter if necessary, neutralise the filtrate with 0·1N sodium hydroxide, and add 3 drops of 5% ferric chloride solution. A violet colour develops if salicylate is present.
3. *Ferricyanide–ferrocyanide tests for ferrous and ferric iron*—to each of two aliquots of the sample add 0·25 volume of 1N hydrochloric acid, boil, and cool. To one solution add one drop of potassium ferricyanide solution; in the presence of ferrous iron a deep blue precipitate is produced. To the other solution add one drop of potassium ferrocyanide solution; in the presence of ferric iron a deep blue precipitate is produced.
4. *Fujiwara's test for trichloro-compounds*—carry out the test as described for urine (above) using a suitable volume of the filtered sample. Note that nitrazepam gives a yellow colour in the pyridine layer.
5. *Dithionite test for paraquat and diquat*—filter a portion of the sample and, using 5 ml of the filtrate, carry out the test described for urine (above).
6. *Test for organophosphorus compounds* (inhibition of cholinesterase)—to each of two tubes add 3 ml of *dithiobisnitrobenzoic acid solution*, 0·1 ml of 5% acetylthiocholine iodide solution, and 20 µl of normal serum; to the first tube add 0·2 ml of water and to the second tube add 0·2 ml of the filtered sample and allow to stand for 2 minutes. Any significant difference in colour

between the tubes is suggestive of the presence of an organophosphorus compound or other cholinesterase inhibitor. Alternatively, the sample tube can be examined spectrophotometrically as described for the determination of cholinesterase in blood (below).

Direct Tests on Blood

1. *Estimation of sugar*—by a standard method or, approximately, by the 'Dextrostix' method—to exclude diabetic or hypoglycaemic coma.
2. *Estimation of urea*—by a standard method or, approximately, by the 'Urastrat' method—to exclude uraemia.
3. *Carboxyhaemoglobin test for carbon monoxide*—dilute a sample of the blood 1 in 20 with 0·01N ammonia and compare the colour with a sample of normal blood treated similarly. A pinkish tint suggests the presence of carboxyhaemoglobin.
4. *Ferric nitrate test for salicylates*—to 0·5 ml of plasma add 4·5 ml of 0·55% ferric nitrate in 0·4N nitric acid. A purple colour is obtained in the presence of salicylates; the colour is proportional to the concentration of salicylate.
5. *Gold chloride test for bromide*—mix 2 ml of plasma, 6 ml of water, and 2 ml of 20% trichloroacetic acid, filter, and to 5 ml of the filtrate add 1 ml of 0·5% gold chloride solution. In the presence of bromide a gold to brown colour, depending on the amount present, develops.
6. *Dichromate test for volatile reducing substances* (including alcohols and aldehydes)—use the method described for urine (p. 875).
7. *Nitrite, chlorate, and other oxidising agents*—methaemoglobin occurs in blood when significant amounts of these substances have been ingested. This gives the blood a characteristic 'chocolate' colour. Confirmation can be obtained by spectroscopic analysis of the diluted blood when an α-band at 610 to 620 nm is obtained.
8. *Test for organophosphorus compounds* (inhibition of cholinesterase)—to each of two tubes add 3 ml of *dithiobisnitrobenzoic acid solution* and 0·1 ml of 5% acetylthiocholine iodide solution; to the first tube add 20 µl of normal serum and to the second tube add 20 µl of the sample serum and allow to stand for 2 minutes. Any significant difference in colour between the tubes is suggestive of the presence of an organophosphorus compound or other cholinesterase inhibitor.

   Cholinesterase determination—adjust the temperature of 3 ml of *dithiobisnitrobenzoic acid solution* to 25°, add 20 µl of the sample serum and 0·1 ml of 5% acetylthiocholine iodide solution, mix well, and record the absorbance of a 1-cm layer at 405 nm at ½-minute intervals for 2 minutes. If the change in absorbance exceeds 0·2/30 seconds, dilute the sample 1 in 10 with normal saline and repeat the measurements (the readings must then be multiplied by 10).

   Milliunits/ml of cholinesterase (at 25°) = change in absorbance/30 seconds × 23400

   Normal values in serum = 1900 to 4000 mU/ml

   Test kits based on similar methods are commercially available. An alternative method is given under Organophosphorus Pesticides on page 901.

EXTRACTION PROCEDURES

Simple general methods for the extraction of samples are given. Where a particular drug is sought, a specific method may give better results.

Extraction of Urine

To 10 ml of urine add sufficient phosphoric acid or tartaric acid to adjust the pH to 3, extract with two 30-ml portions of ether, combine the ether extracts, wash with 5 ml of water, add the washing to the sample, and retain the aqueous solution for later extraction. Extract the combined ether extracts with 5 ml of saturated sodium bicarbonate solution and retain the aqueous solution for possible examination for the presence of salicylate (Strong Acid Fraction 'A').

Extract the ethereal solution with 5 ml of 0·45N sodium hydroxide and retain the extract for examination for the presence of barbiturates and other weakly acid substances (Weak Acid Fraction 'B')—see TABLE 1, p. 880.

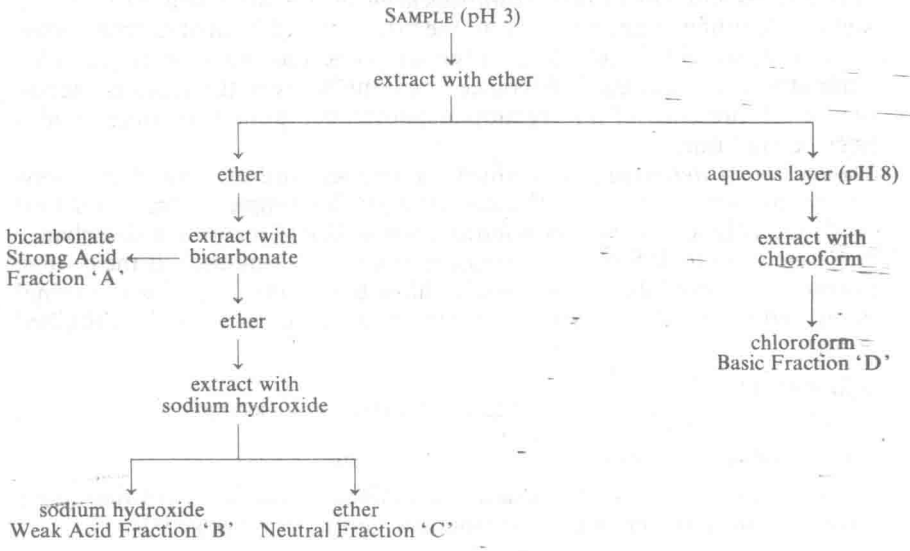
Wash the ethereal solution with water, discard the washing, dry the solution with anhydrous sodium sulphate, and evaporate to dryness. The residue may contain neutral drugs (Neutral Fraction 'C')—see TABLE 1, p. 880.

To the aqueous solution retained after the first extraction add sufficient ammonia solution to adjust the pH to 8·0, extract with two 10-ml portions of chloroform, wash the combined extracts with water, filter, add a little tartaric acid to prevent the loss of volatile bases, and evaporate to dryness. The residue may contain basic drugs (Basic Fraction 'D')—see TABLE 1, p. 880.

Extraction of Stomach Contents

Any fragments of capsules or tablets or any powdery material should be removed and suspended in water for extraction. If the sample contains much

SCHEME FOR THE EXTRACTION OF DRUGS FROM URINE AND STOMACH CONTENTS



food residue or mucus, stable emulsions will be produced during the extraction procedure and the following pre-treatment is necessary:

Add an excess of solid ammonium sulphate together with a few drops of 10% phosphoric acid, heat and stir well, and filter. The filtrate is then extracted by the method described for urine, above.

NOTE ON EXTRACTION PROCEDURES. The techniques are rather elaborate and may be simplified, if desired, by using a single acid–ether extraction so that any acid, weakly acid, or neutral drugs will appear in one extract. This is adequate for screening purposes. Salicylate, if present, may interfere but can be removed by bicarbonate extraction. Bases may be extracted in a similar way after the test solution has been made alkaline with ammonia.

Extraction of Blood

Because blood, plasma, or serum samples are small and only a limited number of drugs may be easily detected and identified in them, this extraction procedure differs slightly from that for urine and stomach contents.

The initial extraction is carried out at pH 7·4 as many basic drugs are recovered by chloroform extraction at this pH. As a result, the substance looked for is most likely to be found in either fraction B or C, and preparation of fraction D is only necessary either to ensure that nothing has been missed or where no drug has been found in fractions B and C.

To 4 ml of the sample add 2 ml of *phosphate buffer (pH 7·4)* and 40 ml of chloroform, shake vigorously, add about 2 g of anhydrous sodium sulphate, and shake again to produce a solid cake. Decant the chloroform through a filter, extract the cake again with a further 20 ml of chloroform, and combine the chloroform extracts. Retain the sodium sulphate cake.

If salicylate has been found in the preliminary tests, it should be removed from the chloroform by extraction with sodium bicarbonate.

To the chloroform extracts add 8 ml [equivalent to twice the volume of sample taken] of 0·45N sodium hydroxide, shake for 2 minutes, and centrifuge. The sodium hydroxide solution may contain barbiturates and other weakly acid substances (Weak Acid Fraction 'B')—see TABLE 1, p. 880.

Wash the chloroform solution with a little water, discard the washing, dry the chloroform with anhydrous sodium sulphate, and evaporate to dryness. The residue may contain neutral drugs together with a number of bases (Neutral and Basic Fraction 'C')—see TABLE 1, p. 880.

If sufficient of the original sample is available, make a further portion alkaline with ammonia solution, extract with two 10-ml portions of chloroform, dry the chloroform with anhydrous sodium sulphate, and evaporate to dryness. The residue may contain basic drugs (Basic Fraction 'D')—see TABLE 1, p. 880.

If there is not sufficient of the original sample for this extraction, the following procedure may be carried out. After fraction C has been examined by the methods described for tests performed after an extraction procedure (p. 881), dissolve any remaining residue in chloroform and extract with 1N sulphuric acid. Add this extract to the sodium sulphate cake retained after the first extraction, make alkaline with ammonia solution, and extract with two 10-ml portions of chloroform. Dry the chloroform with anhydrous sodium sulphate and