A system of screening for the presence of a number of common drugs

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SYNOPSIS A method is described to provide a rapid screening technique for the presence of barbiturates, glutethimide, carbromal, meprobamate, salicylate, phenothiazine derivatives, bromide, carbon monoxide, and alcohol.

Phenothiazines are detected by a spot urine test. The first four drugs are identified, within 60 minutes of blood collection, on thin-layer chromatoplates of microscope slide dimensions. The estimations of bromide, salicylate, carbon monoxide, and of alcohol levels are started in that period so the overall time for the screening is less than two hours, and the amount of blood required is only 10 ml.

With the increasing incidence of drug addiction and drug poisoning it has become essential for clinical biochemical laboratories to provide a service for the rapid detection of a wide selection of exogenous toxic compounds. Our investigations have been aimed at producing a simple routine, yielding in the shortest possible time information of extreme importance in the diagnosis and treatment of the comatose patient. A somewhat similar approach to the problem is taken by Curry (1966) and some of his methods are useful supplements to those reported here.

After consultation with physicians and the pharmacy department of Royal Perth Hospital it was decided to screen patients for the presence of barbiturates, salicylates, glutethimide, carbromal, meprobamate, bromide, phenothiazine derivatives, carbon monoxide, and alcohol.

The requirements of any technique to be developed as a suitable routine service are rapidity, sensitivity, and robustness: rapidity for the urgent cases of overdose which present frequently at a casualty department; sensitivity for the cases of drug addiction forwarded from the psychiatric clinic; and robustness for the non-specialist to handle on emergency duty. To fulfil these requirements the rapid thin-layer chromatographic technique is used with the modification of Hofmann (1962), *i.e.*, the preparation of microscope slides for the chromatography. Before chromatography the drugs in the blood are separated into two groups: the neutral group, including glutethimide, carbromal, mepro-

bamate; and the acidic group containing barbiturates. Blood carbon monoxide and alcohol levels are both measured by diffusion methods and plasma bromide by a gold chloride method. The basic phenothiazine derivatives are detected in the urine.

MATERIALS

PREPARATION OF CHROMATOPLATES The microscope slides are spread with a slurry of silica gel G (Merck, for T.L.C.) to a thickness of 12×10^{-3} inch. (A small template was constructed by the Instrument Department of the hospital for the preparation of the plates and a feeler gauge was used to determine the silica gel thickness.)

The plates are allowed to dry on the template for five minutes, then carefully separated. After a further 15 minutes at room temperature the plates are dried in an oven with a draught at 45°C. for 30 minutes. Finally they are activated at 100 to 105°C. for 30 minutes.

By warming at 45°C. preparatory to activation the cracking, due to differing amounts of shrinkage of the silica gel and the binding agent (Dohmann, 1965), is greatly decreased and does not present any problems in the running of the chromatograms.

The plates are stored in a desiccator and retain their activity for several weeks.

REAGENTS The following are the general reagents: NaCl 0.9%, HCl 0.5N, NaOH 0.45N, chloroform (Baker analysed), and methanol A.R. (Merck) although one bottle of Merck methanol had enough amine contamination to interfere in the colour development of chromatoplates.

Chromatography solvent Methanol : chloroform (9 : 91 v/v) (stable five days).

Hypochlorite solution NaOCl (containing 10–13% available chlorine), 20 ml., is diluted to 100 ml. with H₂O.

Potassium iodide-starch reagent Starch (2 g.) is softened with a little water and added to 90 ml. boiling water containing 1 g. KI. The solution is boiled for one minute with constant stirring, allowed to cool and made up to 100 ml. with H₂O. It is stored at 4°C.

Potassium chloride-boric acid buffer (0.6M) Boric acid 9.3 g., and 11.2 g. KCl are dissolved in water and the volume made up to 250 ml. (It may be several hours before the boric acid goes completely into solution even with frequent shaking.)

Standards Two are made up: mixed standard A, glutethimide (100 mg.), meprobamate (200 mg.), and carbromal (400 mg.) in 100 ml. acetone; mixed standard B, pentobarbitone (100 mg.) and phenobarbitone (100 mg.) in 100 ml. chloroform.

METHODS

PREPARATION OF EXTRACTS (NEUTRAL AND ACIDIC FRACTIONS) The extraction procedure is outlined in the flow sheet (scheme 1). Plasma (2 ml.) is adjusted with 0.5N HCl to pH 6 and extracted with 15 ml. chloroform in a 50 ml. separating funnel. The chloroform layer is filtered (Whatman paper no. 1) into a second separating funnel. The extraction is repeated with a further 15 ml. chloroform. The combined chloroform extracts are then shaken with 10 ml. 0.45N NaOH for 2 to 3 minutes. The chloroform layer is filtered into a 50 ml. Quickfit tube and gently evaporated to dryness under vacuum at a temperature below 70°C. to avoid decomposition (Goldbaum, Williams, and Koppanyi, 1960). This fraction contains the neutral drugs glutethimide, carbromal, and meprobamate, and is called extract 1.

Of the aqueous alkaline solution in the separating funnel, 5 ml. is pipetted into a glass tube and kept for measurement of the barbiturate concentration if required.

The remaining 5 ml. of the aqueous alkaline layer is acidified and extracted with 25 ml. chloroform. The chloroform layer is filtered into a Quickfit tube and gently evaporated to dryness. This fraction contains the barbiturates and is called extract 2.

CHROMATOGRAPHY At a position 15 mm. from the end and 8 mm. from one side of a microchromatoplate 10 μ l. of mixed standard solution A (meprobamate, carbromal, glutethimide) is applied, using a disposable pipette (microcap). Extract 1, taken up in 20–30 μ l. acetone, is spotted on level with the standard and 8 mm. from the other side of the plate. On a second microchromatoplate 10 μ l. of mixed standard B (pentobarbitone, phenobarbitone) is applied. Extract 2, taken up in 20–30 μ l. chloroform, is spotted on level with the standard.

A screw-top jar serves as the chromatography tank and a Conway unit base as the holder for the plates. The two plates are placed in the outside well of the unit in the tank. The solvent (10 ml.) is pipetted into the unit, then the lid is quickly screwed on the tank. After 16 minutes the plates are removed and dried at room temperature.

IDENTIFICATION A non-specific colour reaction developed by Rydon and Smith (1952) is used for the identification of glutethimide, carbromal, meprobamate, and barbiturate derivatives.

The two plates, supported almost vertically, are finely sprayed with sodium hypochlorite solution from a modified glass nebulizer. (The outer glass wall of the nebulizer is cut off at the level of the spray unit.) Approximately 30 seconds later the chlorine has evaporated from the inert silica gel. The plates are then sprayed with the potassium iodide-starch solution, using a pressure pack. An intense blue colour develops due to the liberation of iodine by the N-chloro compounds formed in the chlorination step. The drugs are identified by blue spots on a white background, the colour fading to brown in a few hours.

Timing the second spraying so that the background is clear but the unstable N-chloro compounds have not decomposed is the critical step in the detection of these drugs. The plates can be tested for excess chlorine by spotting 1–2 μ l. of the KI-starch solution on trial portions of the plate (e.g., the extreme lower section) at intervals of two to three seconds. When the trial spot is very pale blue or colourless the plates are then sprayed with the KI-starch solution. The microchromatoplates are suited to this method of colour development because even spraying is ensured over the small area.

MEASUREMENT OF THE BARBITURATE CONCENTRATION If barbiturate is detected by the chromatographic technique the level is measured by the method of Broughton (1956) in the 5 ml. aliquot put aside during the extraction procedure.

A standard, of 0.2 ml. of 0.1% solution of diethyl barbituric acid and 2 ml. saline, is extracted in the same way as the patient's plasma. Having already identified barbiturate the spectrum from 225 to 265 m μ is not read but only the difference in optical density of the pH 13.4 and pH 10.0 solutions at 260 m μ .

It is known that salicylate interferes with the measurement of barbiturate by this method and, although it is common elsewhere for these two drugs to be present simultaneously, only one such case has been detected at this hospital in the last six months.

SALICYLATE The plasma salicylate level is measured by the method of Trinder (1954).

BROMIDE The plasma bromide level is determined by the method of Barbour, Pilkington, and Sargent (1936) with modifications (Dunlop, 1966). The volumes are: 1 ml. plasma, 6 ml. 0.5% sodium chloride, and 1 ml. 30% trichloroacetic acid. After 10 minutes the mixture is filtered through Whatman no. 42 paper and 0.5 ml. 0.5% gold chloride solution is added to 4 ml. filtrate. The test solutions and appropriate standards are read in a colorimeter at 470 m μ .

PHENOTHIAZINES IN URINE Phenothiazine derivatives are detected by a spot test developed by Forrest, Forrest, and Mason (1961) using a ferric chloride, perchloric acid, nitric acid (F.P.N.) reagent which reacts with the

partially oxidized highly reactive intermediary metabolites of phenothiazines found in urine.

ALCOHOL Alcohol is measured by the method of Williams, Linn, and Zak (1958) with one modification, that of temperature. For convenience the incubation temperature of 56°C. was changed to 37°C. and it was found that the diffusion equilibrium was still obtained in 60 minutes.

CARBON MONOXIDE Carbon monoxide is detected and measured by the method described by Conway (1962), based on the diffusion of carbon monoxide into palladium chloride with the formation of a palladium mirror and hydrochloric acid. This method is also modified. The diffusion temperature is increased to 37°C. Diffusion is complete in 60 minutes and the level of carbon monoxide is measured by titration of the acid formed.

In an emergency two units are set up initially, one with diluted blood to diffuse for 60 minutes and the other with undiluted blood to diffuse for 15 minutes at 37°C. After the 15 minutes this second unit is examined for the formation of a palladium mirror indicating the presence of carbon monoxide in the blood.

RESULTS

Figure 1 shows the separation of the neutral drugs glutethimide (R_f 0.96), carbromal (R_f 0.87), and

meprobamate (R_f 0.27) located by NaOC1-KI-starch reaction.

Seven barbiturates (pheno-, pento-, amyo-, hexo-, quinal-, butobarbitone, and barbitone) were studied, representing the most commonly dispensed barbiturates at this hospital.

The thin-layer chromatography of these seven barbiturates showed two groups: (1) phenobarbitone and barbitone, the slow acting barbiturates, running slowly with an R_f of 0.44 on these small plates; and (2) the intermediate or fast acting barbiturates, running faster than the first group, with an R_f of 0.54.

Figure 1 illustrates the separation of these two groups, located by the NaOC1-KI-starch reaction.

The sensitivity obtained is 1 µg. for barbiturates and glutethimide, 5 µg. for meprobamate, and 10 µg. for carbromal.

A recovery of 90% for diethyl barbituric acid at the level equivalent to 10 mg./100 ml. plasma has been obtained by this extraction procedure. In patients, levels up to 15 mg./100 ml. plasma have been measured with negligible loss into the neutral extract.

Similarly, glutethimide levels of approximately 15 mg./100 ml. plasma have been measured, the acidic extract remaining clear. (The glutethimide

FIG. 1. Scheme showing the treatment of plasma to produce the fractions for chromatography.

FIG. 2. Chromatogram of neutral and acidic extracts. G glutethimide, C carbromal, M meprobamate, Pe pentobarbitone, Ph phenobarbitone.

1, extract 1 containing glutethimide.

2, extract 2 containing an intermediate or fast acting barbiturate.

SA mixed standard A, SB mixed standard B.

Thin layer, silica gel.

Solvent, methanol : chloroform (9 : 91).

Time, 16 min.

level was obtained by comparing the size of spots. (The aliquot of extract applied to the plate was altered until the spot corresponded to the standard.)

The spot test for the detection of urinary phenothiazine derivatives was found to give a false positive in one instance. On addition of acid only to the urine a strong purple colour formed immediately, suggesting the presence of an indicator type substance in the urine. In this case chromatography of a urine extract (Cochin and Daly, 1962) gave negative results with iodoplatinate reagent. All positive urinary F.P.N. (ferric chloride perchloric nitric acid) tests are now checked by testing the urine with acid. No false negative results were obtained in 20 cases when both the spot test and chromatography were carried out.

The carbon monoxide method is extremely sensitive. When undiluted blood is used in the unit incubated for only 15 minutes the presence of the gas can be detected at a level of 1% of maximum saturation, the concentration being estimated on the unit incubated for 60 minutes.

The alcohol is measured after 60 minutes incubation by a colour reaction involving brucine. Some time is lost at this stage of the procedure but

if the case is urgent the presence of alcohol is visible to the eye by a change in colour of the dichromate solution in the centre well. This diffusion method is not specific for ethanol, but also detects methanol and acetone.

The normal levels for plasma bromide and salicylate are quoted at <4 mg./100 ml. and <2 mg./100 ml. respectively and are a measure of the errors inherent in the methods at the very low levels.

DISCUSSION

Table I shows the total number of cases investigated for the presence of drugs in 1962 and 1965 indicating the increasing incidence of poisonings and addictions.

TABLE I
SUMMARY OF RESULTS

	Year	
	1962	1965
No. of cases investigated	260	400
Drugs detected:		
Barbiturate	150	210
Bromide	30	69
Glutethimide	3	4
Phenothiazines	6	9
Salicylate	3	6

It is essential to separate the drugs into the neutral and acidic groups before chromatography because of possible interference in identification due to metabolites. This is best illustrated in Figure 1 where the metabolites of glutethimide are shown diagrammatically. If the two extracts were run as one a falsely positive barbiturate identification could be made. The glutethimide metabolite pattern is detected on the plates in less than 24 hours after ingestion. Wallenius, Zaar, and Lausing (1963) noted interference by glutethimide in their colour reaction for the detection of barbiturates.

Carbromal, a brominated mono-ureide, is hydrolysed *in vivo* to produce 2-ethyl-butyl urea and bromide. Shortly after ingestion the carbromal may be located on the plate, but a few hours later it is barely detectable and the bromide ion can then be measured colorimetrically.

There are many methods available for the detection of barbiturates, such as the ingenious spot tests by Wallenius *et al.* (1963), Curry (1963) and Baer (1965), paper chromatography with modifications by Podmore (1962) and Waddell (1965), and thin-layer chromatography by Schmidt (1962) and Lehmann and Karamustafaoglu (1962). These chromatographic techniques provide a more detailed analysis of the barbiturates present than the method described here. However, the separation of the barbiturate derivatives into only two groups, the

slow acting or non-metabolized and the intermediate or fast acting (*i.e.* metabolized) is adequate for our clinical purposes. Lee and Ames (1965) base their decisions for haemodialysis on the group and level of barbiturate present and whether taken concurrently with phenothiazines.

Jackson (1963) describes a system of paper chromatography which covers a considerable number of drugs but the chromatography requires at least two hours. The reverse phase high temperature paper chromatography technique developed by Street (1962) provides a more rapid screen. However, thin-layer chromatography, apart from its rapidity, is extremely robust, giving good resolution of barbiturates over a range of 1 to 150 $\mu\text{g.}$, making it applicable for cases both of addiction and overdose.

The simple method of using one reagent to detect all the neutral and acidic drugs has proved to be rapid, reliable, and sensitive. The plates are easier to read at the low levels frequently encountered than the method of alkalization and viewing under ultra-violet light at 253 m μ . Podmore (1962) reports a sensitivity of 10 to 20 $\mu\text{g.}$ for barbiturates by the latter method.

Sunshine (1963) and Cochin and Daly (1963) also use thin-layer chromatography for the detection of a wide array of compounds, but on the standard 20 cm. plates, thereby increasing the time interval before identification.

The microchromatoplates provide a simple, rapid technique and it was found that, with the system

described, the separation was not improved when the larger plates were used.

We wish to thank Mr. J. T. Wearne for his help in this study.

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