TECHNICAL METHODS

THE IDENTIFICATION OF BARBITURATE DRUGS IN GASTRIC CONTENTS AND URINE

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Cases are admitted from time to time to most large hospitals in a state of coma or semi-coma in which the differential diagnosis includes overdosage with the barbiturate series of narcotic drugs. It is often important to confirm or exclude this factor as quickly as possible. Standard methods of analysis (e.g., the Stas-Otto process, Smith, 1934a), although ultimately giving accurate quantitative results, are time-consuming.

An endeavour has been made to evolve a more rapid method of analysis which is sufficiently delicate to give an answer to the question of whether or not barbiturate poisoning plays a significant part in the clinical condition of the patient. The method in our hands takes about three hours to perform and is based upon the facts that (1) the barbiturate drugs, being derived from barbituric acid, are either the acid derivatives, e.g., diethylbarbituric acid (barbitone), or their sodium salts, e.g., sodium diethylbarbiturate (soluble barbitone). (2) When in aqueous solution they can be changed from one form to the other by making the pH acid or alkaline. (3) The barbituric acid derivatives are only slightly soluble in water but are readily soluble in ether, whereas their sodium salts are readily soluble in water and almost insoluble in ether.

As part of any ingested barbituric acid is excreted unchanged in the urine, at least 100 ml. of urine are collected and examined in all cases. Unless the state of coma is known to have lasted for a long time, the gastric contents are aspirated and examined as well.

Method of Extraction

The sample is tested with litmus paper and if necessary made acid with concentrated hydrochloric acid drop by drop and then evaporated to small bulk on a boiling-water bath; e.g., 100 ml. is reduced to 15 ml. Frequent stirring may be necessary to prevent charring of the mucoid deposit on the side of the evaporating dish. The residue is transferred quantitatively with two washings of 2 ml. distilled water to two large centrifuge tubes (20–50 ml. capacity), half in each, and extracted three times with equal volumes of ether. Centrifuge tubes are used, as any emulsion formation can be quickly broken down by spinning and the supernatant ether removed with a Pasteur pipette. The ether extracts, containing the barbituric acid if present, are filtered and evaporated

to dryness. The total residue is stirred with 5–10 ml. *N*-sodium hydroxide to form a solution of barbiturate, the insoluble impurities filtered off, and the filtrate just acidified to litmus paper with concentrated hydrochloric acid. If much fat were present in the ether residue, the alkaline solution is put in the refrigerator, and when cold most of the fat adheres to the sides. The solution is then filtered again when the remainder of the fat adheres to the filter paper. If much barbituric acid is present, a white precipitate appears on acidifying, but faint precipitates often occur with normal extracts. This mixture is extracted with ether as before, the extracts being filtered into a tube containing about 1–2 g. anhydrous sodium sulphate. After mixing well and allowing the sulphate to settle, the dried ether is filtered, together with ether washings of the sulphate, into a tube containing about 0.5 g. activated charcoal. After mixing well and allowing the charcoal to settle, the ether is filtered through a funnel containing a layer of charcoal about 1 cm. deep supported on a cotton-wool wad. The ether filtrate, freed of any pigments, and ether washings of the charcoal are evaporated to dryness. A barbituric acid may appear as white crystals or as a clear, colourless gum which slowly crystallizes into needles or rosettes.

Tests on Ether Residue

Cobalt Test.—A few crystals or a drop of the gum are dissolved in a few drops of absolute ethyl alcohol, 6 drops of 5% isopropylamine in absolute methyl alcohol are added, and then one or two drops of 0.5% cobaltous acetate in absolute methyl alcohol. In the presence of a barbituric acid, a violet colour is obtained, otherwise only a barely discernible pink colour is given. (Thiobarbituric acid derivatives such as pentothal give a paler and pinker colour, the sodium salt giving a green colour.) When only a small amount of residue is left after evaporating the final ether extract, the cobalt test is carried out directly on the residue as a whole. In this way 15 mg. (approximately grains $\frac{1}{4}$) phenobarbitone added experimentally to 100 ml. gastric juice were detected by a weak positive cobalt reaction, a violet colour fading within 30 seconds. Larger amounts gave a deep permanent violet colour.

If a good yield of cobalt-reacting crystals or gum is obtained from gastric contents a further purification as described below can be carried out, and the barbituric acid present in the sample estimated roughly as $\frac{3}{2} \times$ weight of the final yield.

Similar extraction rates were obtained with urine samples, but the amount of the barbituric acid excreted in the urine and the rate of the excretion varies considerably with the different compounds, and presumably with the patient's kidney function as well. We have found that the ether residues (without the further purification) could roughly be arranged into three groups as a guide to the quantity of the drug consumed.

(1) Urine collected for about nine hours after a single dose of a barbiturate drug of $1\frac{1}{2}$ –3 grains (approximately 100–200 mg.) gave either a negative result or occasionally a weak positive as above. The cobalt test was performed on the whole of the ether residue, which was usually about 10 mg. in weight and obviously impure. Occasionally larger crystalline residues were obtained, but portions gave negative results and so the test was repeated on the whole.

(2) Doses of barbiturate of 5–10 grains (approximately 300–600 mg.) resulted in residues, sometimes showing characteristic crystal formation, which gave a deep permanent violet colour with the cobalt test.

(3) Doses of 15 grains (approximately 1,000 mg.) and upwards gave yields of 50–100 mg. or more, usually showing the characteristic crystal formation of the particular compound after evaporation of the ether (Turfitt, 1948). Portions of such residues gave positive cobalt reactions. We have so far obtained results such as these on all the four patients admitted during the past eight months in coma, later shown to have taken large quantities of a barbiturate drug. In these cases the urine was collected from the time of admission. In one case this was 36 hours after the onset of coma, and 500 ml. urine, collected by an in-dwelling catheter during 12 hours with intravenous fluid therapy, yielded 80 mg. barbitone.

Selenious Acid Test.—It was found that the selenious acid reaction (Turfitt, 1948) gave positive results with the ether available in this laboratory, similarly the salicyl aldehyde reaction (Smith, 1934b) gave positive results with absolute alcohol: consequently this test could not be used.

Identification of the Extracted Barbituric Acid

When sufficiently large residues are obtained further identification of the barbituric acid derivative is carried out as follows.

(1) The residue is further purified by extraction with N ammonium hydroxide, filtering, acidifying the filtrate, extracting with ether and continuing as before with sodium sulphate and charcoal. With amounts of 100 mg. (approximately gr. 1½) or more of a barbituric acid in 100 ml. gastric juice, the yield of crystals after this purification was 60–70%.

(2) The melting point of resulting crystals is determined. This enabled the substance to be tentatively assigned to one of four groups shown in Table I.

TABLE I

Barbituric Acid	Melting-point of Pure Substance (° C.)	Melting-point of Final Ether Residue (° C.)
Barbitone ("veronal")	189–192	180–182
Phemitone ("prominal")	178–181	169
Phenobarbitone ("luminal")	173–177	170
"Phanodorm"	173	169–170
Allobarbitone ("dial")	171–172	170–171
Thiopentone ("pentothal")	156–159	156
"Amytal"	153–155	151
Hexobarbitone ("evipan")	145–147	135
Pentobarbitone ("nembutal" is sodium salt)	127–130	125
Butobarbitone ("soneryl")	127	118–120

(3) Further evidence is obtained from the three colour reactions (i) vanillin reaction (Turfitt, 1948); (ii) *p*-dimethylaminobenzaldehyde reaction (Turfitt, 1948); (iii) formalin reaction (Smith, 1934b).

It was found necessary to set up a full series of control tests with pure samples of the barbituric acids whenever these reactions were tried, as the shades of the colours seemed to vary with the rate and duration of heating. The formalin reaction

TABLE II

Barbituric Acid							Formalin Colour Reaction
Barbitone	..	..	..	..	..	..	Colourless
Phemitone	..	..	..	..	..	..	Red-brown
Phenobarbitone	..	..	..	..	..	..	Pink
"Phanodorm"	..	..	..	..	..	..	Orange
Allobarbitone	..	..	..	..	..	..	Yellow
Thiopentone	..	..	..	..	..	..	Pale yellow
"Amytal"	..	..	..	..	..	..	Deep yellow
Hexobarbitone	..	..	..	..	..	..	Deep yellow
Pentobarbitone	..	..	..	..	..	..	Yellow
Butobarbitone	..	..	..	..	..	..	Very pale yellow
Reagent control	..	..	..	..	..	..	Colourless

was originally described to distinguish phenobarbitone from barbitone and phemitone, but was found useful as a general colour reaction as shown in Table II.

For the test about 1 mg. of crystals are heated with 0.5 ml. formalin and 4 ml. concentrated sulphuric acid in a boiling-water bath for one minute. The crystalline forms of the barbituric acids are also described by Turfitt, and these provide additional evidence of identity.

(4) Final evidence of identity can be obtained by preparing a pure crystalline sample by crystallization from absolute alcohol or micro-sublimation. The melting point and mixed melting point (with known pure crystals) are then determined.

The barbituric acids extracted can usually be identified by the preliminary melting points and the colour reactions before proceeding to this final test.

Medicinal Tablets or Capsules

A tablet ground up or the powder from a capsule is stirred with a few millilitres of dilute hydrochloric acid and the mixture, after checking it to be acid to litmus, is extracted with an equal volume of ether. A portion of the filtered extract is tested by the cobalt test for the presence of a barbituric compound.

If a barbiturate is present, a pure sample is obtained by stirring the powder from a few tablets or capsules with a few millilitres of dilute hydrochloric acid, making certain that the mixture is acid, and extracting with ether. The extract is filtered, evaporated to dryness, and the residue treated as described above.

Specificity of the Cobalt Test

Parri (1924) described this reaction using cobalt and ammonia solution. We have used Koppanyi's method with *iso*-propylamine as it gives a more distinctive violet colour which does not disappear with excess *iso*-propylamine; excess ammonia removes the violet colour. Lapiere (1947) found that certain sulphonamides react with Parri's test. We found that sulphathiazole gave the characteristic violet colour, but not so deeply as the barbiturates, whilst sulphanilamide, sulphapyridine, and sulphadiazine gave a pale pinkish-violet colour. But sulphonamides

are soluble in acid solutions, and insoluble or only slightly soluble in ether, so that the extraction methods described above do not remove sulphonamides from the test material in sufficient quantity to give positive cobalt reactions. This has been checked by extracting 100 ml. gastric juice containing 5 g. sulphathiazole, and medicinal tablets containing 0.5-g. amounts of these four sulphonamides with negative results. Urine containing both free and acetylated sulphonamide also gave negative results; the acetylated compound is hydrolysed to the free compound by the preliminary heating in acid solution.

Summary

A method of detecting barbituric acid derivatives in gastric contents, urine, and medicinal preparations is described. The method has been kept as simple and as rapid as possible while retaining sufficient sensitivity to enable it to be used in confirming the diagnosis in cases of coma with suspected barbiturate poisoning.

A scheme is presented for obtaining rough estimates of the amounts of barbiturate drug consumed. Therapeutic doses (up to 3 gr. or 200 mg.) by mouth gave negative or very weak positive results on urine analysis; doses of 5–10 gr. (approximately 300–600 mg.) gave consistent positive results; higher dosage gave good yields allowing identification of the particular compound. The method detects gr. $\frac{1}{4}$ or 15 mg. barbiturate in 100 ml. of urine or gastric contents, and with the presence of more than $1\frac{1}{2}$ gr. or 100 mg. per 100 ml. the yield after further purification was 60–70%.

The identification of ten barbituric acid compounds in common use is described.

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