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Note on the Behavior of Sulfonamides in the Cobalt Color Tests for Barbiturates

*By Theodore Koppanyi, Melvin W. Green and Charles R. Linegar**

Recently, samples of sulfathiazole contaminated with phenobarbital were found on the open market. In connection with this finding, Dr. Knudson of the Albany Medical College and others called our attention to the fact that the Koppanyi test for barbiturates is also positive to the sulfonamides. In the fear that some unadulterated sulfonamide preparation might be rejected, unfairly, on the basis of this color test, we have investigated the behavior of sulfathiazole, sulfani'amide and sulfapyridine with reference to the cobalt color tests. We are concerned here with two different aspects of the same problem: (1) the differentiation of barbiturates from sulfonamides in pharmaceutical analytical work, and (2) the possible interference of sulfonamides, in the urine and other body fluids, in the diagnosis of barbiturate poisoning.

EXPERIMENTAL

1. Dille and Koppanyi (1) have shown that the cobalt acetate-isopropylamine test may be used to assay pharmaceutical preparations containing barbiturates. In that procedure the alkaline preparation is dissolved in water, acidulated and then shaken out with at least ten volumes of chloroform.

The same procedure was followed using prepared mixtures containing known amounts of phenobarbital and different sulfonamides.

Table I indicates that the presence of sulfonamides does not appreciably interfere with the pharmaceutical assay of barbiturate preparations provided the Dille-Koppanyi procedure is strictly adhered to. The sample containing 100 mg. of sulfonamides with no barbiturates yields less color, in the colorimetric procedure, than does 2.0 mg. of phenobarbital. The presence of phenobarbital or any barbiturate in admixture with any suspected sulfonamide preparation can thus be detected by the following procedure. Weigh out a 100-mg. sample of the suspected sulfonamide, dissolve in 20 cc. of water, acidulate and shake out with 20 volumes of chloroform. Simultaneously, subject a 100-mg. sample of the pure sulfonamide to the same procedure. Evaporate the chloroform extract to dryness and take up the residue in a convenient, measured volume of chloroform. Compare each of these chloroform solutions with suitable standard solutions (0.02 to 0.08 per cent in chloroform) of the barbiturate in question. If the suspected sulfonamide produces a more intense color than the pure sulfonamide itself, the preparation is contaminated. And, moreover, the degree of adulteration can be

Table I.—Recovery of Phenobarbital from Phenobarbital-Sulfonamide Mixtures as Determined by the Cobalt-Isopropylamine Method

Sulfonamides Used	Amount of Sulfonamide in Sample, Mg.	Amount of Phenobarbital in Sample, Mg.	Color Comparison in Terms of Pure Phenobarbital as a Standard, Mg.	Percentage Phenobarbital Recovery, Per Cent
Sulfathiazole	100	0.0	<2.0–	.....
Sulfathiazole	95	5.0	4.6– 5.7	91.2–114
Sulfathiazole	90	10.0	9.2–10.4	92.4–104
Sulfathiazole	50	50.0	54.0–56.7	108.0–113.4
Sulfapyridine	100	0.0	<2.0–	.....
Sulfapyridine	50	50.0	54.5	109.0
Sulfapyridine[a]	50	50.0	58.0	116.0

[a] Determination made in the chloroform solution directly.

* Department of Pharmacology, Georgetown University School of Medicine, Washington, D. C.

readily determined. Since some sulfonamides, particularly sulfathiazole, in chloroform and chloroform-alcoholic solutions give a strong color with the cobalt-isopropylamine reagent, it should be reiterated that the assay must be carried out strictly as directed and not by attempting to dissolve the samples directly in chloroform.

2. It is not likely that toxic amounts of barbiturate would be taken by patients receiving energetic sulfonamide medication, nor is it likely that sulfonamides alone would be taken in sufficiently large doses to produce acute collapse. Nevertheless, the possibility must be faced that an occasional case will present itself to a hospital laboratory where diagnosis of acute sulfonamide poisoning might be complicated by a previous barbiturate medication. In such cases the urine and blood, when properly treated, may give a positive cobalt color test due to the presence of either sulfonamide or barbiturate. Even in these rare cases it should not be difficult to demonstrate the presence or absence of barbiturates.

In saturated chloroform-alcoholic solutions all three of the sulfonamides studied give positive reactions with the barium, lithium and isopropylamine tests (Koppanyi, *et al.* (2)), but positive barium and lithium tests are not obtainable if the sulfonamides are extracted from 2.0 per cent aqueous media with chloroform.

Since the maximum concentration of free sulfonamide to be expected in the urine or other tissue fluids under the most extreme conditions would not exceed 2.0 per cent, it follows that urines from patients receiving sulfonamides alone would be negative with the barium and lithium tests, and positive only with the isopropylamine test. Using the shake-out procedure, if the barium and lithium tests are positive, this indicates that barbiturates are present in the urine in addition to sulfonamides.

It is, therefore, easy to diagnose barbiturate poisoning and distinguish it from sulfonamide intoxication. The barium and lithium tests, which are not only qualitative but also approximately quantitative, should be performed first on body fluids of suspected cases, and only if these tests are positive should one proceed with the more quantitative isopropylamine tests.

Recently, all three tests have been performed on a number of pathological urines of treated and untreated patients. In no case was a positive test obtained unless the patient had received barbiturate medication. These findings will be reported in the near future.

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Umbellatine from *Berberis insignis*, Hook. f.

*By R. Chatterjee**

It is well known that alkaloids occur in the species of *Berberis*, family *Berberidaceæ*. Table I is a résumé of the investigations recorded in literature.

The results show that several other alkaloids together with berberine occur in the species *Berberes*. Though several European varieties have been worked up, few references are found for Himalayan *Berberes*. Chopra and others (7) in their study of the medicinal plants which grow in the Himalayas mention the total alkaloid contents of *B. asiatica*, as a mixture of berberine and oxyacanthine. Because of their yellow stem, the *Berberes* are called in Sanscrit *Daruharidra* (yellow wood), an extract of which was used by the Hindus, Greeks and Arabs for medicinal purposes. The extract was commonly used in India as a remedy against malaria and diarrhea. It was also applied to any external inflammation and in the treatment of oriental sores (7).

Several varieties of Himalayan *Berberres* have been referred to by Chopra (13) as berberine-containing plants. *B. umbellata* Wall., growing in the Himalayas, at an altitude of 9000–11,000 ft. (14), showed the absence of berberine and yielded a new alkaloid, called umbellatine (12). It was suspected that other Himalayan *Berberes* also may not contain berberine at all. The present paper deals with the isolation and identification of umbellatine, in another species of Himalayan *Berberes*, *B. insignis*, Hook. f., which grows at an altitude of 7000–10,000 ft., in the humid forests of the Eastern Himalayas from Nepal to Bhotan (14).

I could procure only stem-barks for the extraction of the alkaloid. However, a gravimetric assay showed the presence of the largest amount of the alkaloid in the roots.

It was in the month of May that I obtained the stem-barks of *B. insignis*, Hook. f. grown at a place near Darjeeling, 6500 ft. above sea level. I could find only one

* Professor of Chemistry, St. Joseph's College Darjeeling, India.