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THE DETERMINATION OF TRACE METALS IN BLOOD AND URINE BY ATOMIC ABSORPTION SPECTROPHOTOMETRY*

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The determination of the four major metallic constituents of biological systems-sodium, potassium, calcium and magnesium-by atomic absorption spectrophotometry is well established and in routine use in many laboratories. But the atomic absorption method also is ideally suited to the determination of trace metals. More than a year ago Willis published a determination of zinc and cadium in urine (1) requiring basic equipment and a very direct procedure. Using an organic extraction procedure, he determined lead, bismuth, mercury and nickel in urine samples. Another worker, Feldman, also used an extraction technique for determining mercury in urine (2). In addition, chromium, cadmium, cobalt, lead and thallium at toxicological concentrations have all been determined in blood and urine (3).

The procedure is short and direct for metals that occur at relatively high levels in serum and urine. For example, urine samples with metals such as copper, iron and zinc in the range from 0.5 to 2 μg per ml are determined directly with no sample preparation at all. Serum samples must be deproteinized since the protein precipitates in the burner and causes clogging. All three metals can be determined without interferences against standards containing only the desired metal. Excluding protein precipitation, where required, each metal determination in an analysis can be done in less than five minutes.

SERUM IRON

Six serum samples were analyzed for iron. Samples of about 2 ml of blood were diluted 1:1 with 20% trichloroacetic acid and centrifuged. They were then burned in a Perkin-Elmer Model 303 and the absorption recorded at the iron resonance line. The iron concentration in the samples was determined from a working curve prepared from the absorption of iron standards in a dilute acid solution. The results are compared in Table I with colorimetric analyses performed by the Hamsay technique (9) at the Walter Reed Army Institute of Research which supplied the samples.

* Part of this material was presented at the Clinical Chemical Congress, Detroit, Aug. 1963.

TABLE I

Iron Determination in Serum (μg Fe/ml Serum)

Sample	Atomic Absorption	Colorimetry
1	0.4	0.5
2	0.4	0.3
3	0.6	1.0
4	1.4	1.9
5	0.8	0.9
6	1.4	2.3

URINARY COPPER

Recently we were asked to determine urinary copper in patients being treated for Wilson's disease. This disease is characterized by the patient's inability to metabolize ingested copper. The patients were being treated with a chelating agent to cause elimination of the organic copper complex through the urine. Urine samples were collected for a number of days. Copper was determined directly against water standards without any sample treatment. The set of nine samples was completed in about thirty minutes.

On the first day, before treatment with the chelating agent, the urinary copper was very low as shown in Sample 1 of Table II. After ingestion of the drug, the copper rose to an abnormally high level, and then fell off gradually as the body copper level began to return to normal. Similar series of data were obtained on two additional patients.

TABLE II

Copper in Urine*

Sample	Cu (μg/ml)
1	0.35
2	6.9
3	8.2
4	4.9
5	6.5
6	4.9
7	4.8
8	0.65
9	0.17

* Samples courtesy of Dr. Herbert Revin, Sinai Hospital, Detroit.

ZINC

The atomic absorption method is one of the most sensitive determinations of zinc. Many laboratories routinely use atomic absorption to determine zinc in blood, urine and animal tissue (4). In a previous Newsletter (6) we reported a very simple experiment in which zinc was determined in urine without sample preparation steps.

TRACE ANALYSES IN URINE WITHOUT SAMPLE PREPARATION

The atomic absorption method can be utilized for many metals without preliminary sample treatment if a spectrophotometer is used in conjunction with noise suppression circuits. In work reported earlier (3), various trace metals were determined in blood and urine. These determinations and the results of later work are summarized in Table III.

TABLE III

Detection Limits in Urine

Metal	Detection Limit (μg/ml)
Zn	0.005
Cu	0.005
Fe	0.05
Pb	0.15
Cd	0.01
Cr	0.01
Mn	0.01
Ni	0.05
Co	0.15
Tl	0.2
Mo	0.2
Au	0.1
Ag	0.02

The urine samples were determined without preparatory sample treatment in all the analyses. Sample quantities of 5 ml of urine are generally recommended for each determination, although 2 ml are usually sufficient. The entire analytical time for each element varies from 2 to 10 minutes per sample, depending upon the number of samples to be determined.

Considerable prior experience indicates that no interference is expected for the metals in Table III in a urine matrix. Some small possibility of interference exists for chromium and molybdenum; however, in both cases recovery experiments in urine indicate no problem.

TRACE ANALYSES IN BLOOD

Most of the metals listed in Table III have also been determined in blood down to concentration levels approximately double the detection limits shown. Since blood is too viscous

to be aspirated directly, the sample must first be treated. If the metal is not bound to the protein or blood cells, it is particularly convenient to dilute the blood 1:1 in 20% trichloroacetic acid. The sample is then centrifuged and the supernatant run directly on the atomic absorption apparatus. Standards are simple water solutions of the metals being determined.

If the metal of interest can be removed by precipitation, it will be necessary to dissolve the blood in acid, as described in the second paper of this Newsletter. As little as 2 ml of blood will be required for either method of sample preparation.

CHELATION METHODS

A number of workers have reported on the advantages of coupling simple chelation methods with atomic absorption spectrophotometry (1,5). Since the atomic absorption procedure is specific for a particular metal, the chelation method need not be specific and the main source of difficulty when chelation is applied to colorimetry is therefore eliminated. Two important improvements are effected when a metal is chelated in the aqueous solution and extracted into an organic solvent. First, the sensitivity for each metal is enhanced about five times in most organics that are immiscible in water, and second, the extraction procedure can be accomplished conveniently by adjusting the volume ratio between the aqueous and organic phases to concentrate the metal in the organic phase. The sensitivity for urine samples can be increased more than twenty times with this procedure when 25 ml of urine per determination is used. The sensitivity for blood samples can be increased about 10 times when 10 ml of blood is used. Copper and lead can be chelated with ammonium pyrrolidine dithiocarbamate (1, 8) and extracted into an organic solvent that is immiscible in water. Many of the ketones are suitable.

The experimental procedure for urine is quick and very simple. About 25 ml of acidified sample, 1 ml of 5% solution of the chelating agent, and 5 ml of a suitable ketone are put into a separatory funnel. This mixture is shaken by hand for a few minutes, and the phases are allowed to separate by standing for more than an hour. The aqueous phase is withdrawn from the funnel and the organic phase is collected in a test tube. There is no need to collect the organic phase quantitatively since the quantitative aspect is assured by the 5-to-1 ratio of sample to organic solvent. Other metals that co-extract produce no interference in the analysis. The standards are similarly extracted from simple aqueous solutions.

Three replicate extractions for copper, in which the original aqueous solution contained 0.005 ppm copper, are shown in Figure 1. There was a volume ratio of 5:1 between the organic and aqueous phases. Copper concentrations as low as 0.5 ppb can be detected above background. A similar experiment with various concentrations of lead is shown in Figure 2. This extends the detection limit to 0.01 ppm Pb in blood or urine, and the analytical time is about fifteen minutes per determination. The determination can be performed with about 10 ml of blood.

LEAD IN URINE AND BLOOD

An experiment was performed on a group of blood and urine samples from workers routinely exposed to lead. The urine specimens were acidified to about pH 2.8 using dilute HCl and a pH meter as an indicator. Twenty ml of each urine sample were added to separatory funnels. One ml of 5% aqueous solution of ammonium pyrrolidine dithiocarbamate and 10 ml of methyl isobutyl ketone were added. The funnels were shaken by hand for two minutes each. Since an emulsion formed at the interface of the layers, the samples were centrifuged to separate the phases.

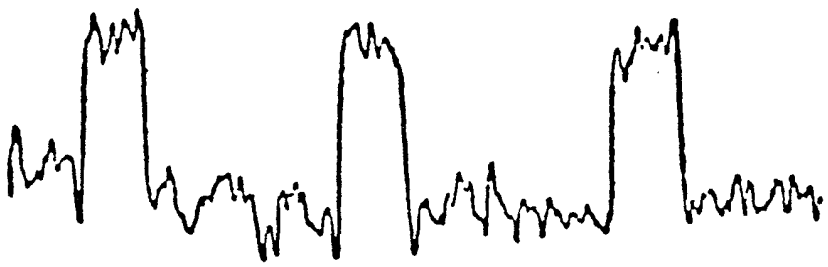

Fig. 1 - Absorption of a Solution of 0.005 ppm Copper

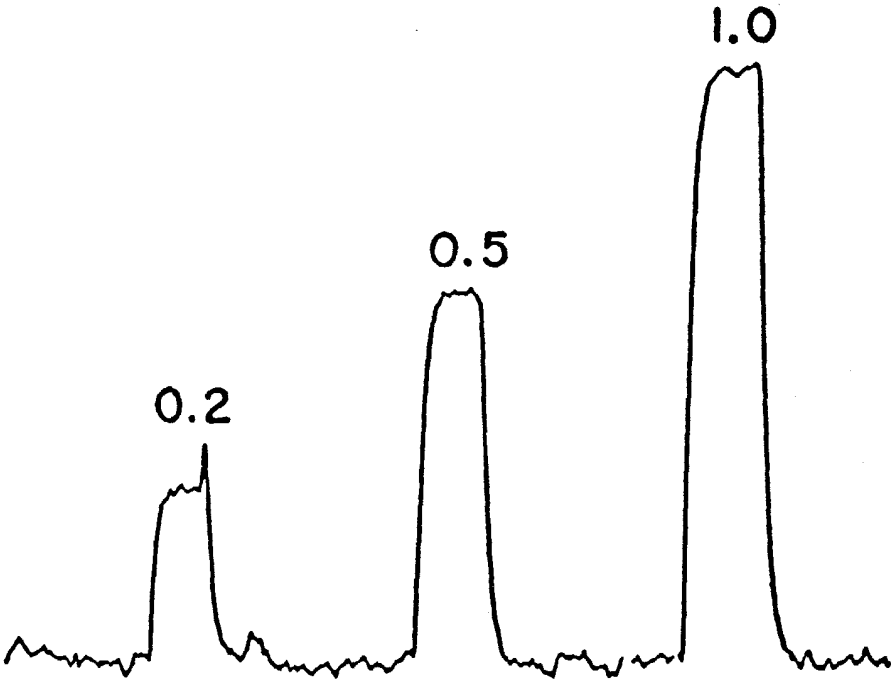

Fig. 2 - Absorptions of Solutions of 1.0, 0.5, and 0.2 ppm Lead

Each sample contained about 6 ml of blood. An equal volume of 8% trichloroacetic acid was added and the sample centrifuged. Six ml of the supernatant solution were brought to pH 2.8 by dropwise addition of dilute NaOH. One ml of the chelating solution and 3 ml of ketone were added to the funnels. The samples were shaken and centrifuged to separate the phases.

The values obtained for the urine and blood samples are given below.

Sample	Urine	Blood
1	<0.04 μg/ml	0.34
2	0.14	1.8
3	<0.04	

There may be errors in these analyses. For instance, the chelating agent may not recover all the organically bound lead. This error would not be uncovered by Willis' experiments (1) by recovery. Also, it is quite possible that in the blood samples lead was lost in the precipitate. This potential error would have been avoided if the bloods had been dissolved in nitric acid. We have not yet evaluated these errors.

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REFERENCES

1. J.B. Willis, Anal. Chem 34, 614 (1962).
2. C. Feldman and R.K. Dhumwad, Office Tech. Services, TID-7655, Washington, DC, 1963.
3. W. Slavin, S. Sprague, F. Rieders, V. Cordova, J. Forensic Sciences, (to appear), reprinted in this Newsletter.
4. H.E. Parker, Perkin-Elmer Newsletter 13, May 1963.
5. E. Berman, Clinical Chemical Congress, Detroit, 1963.
6. Perkin-Elmer Newsletter No. 11, March 1963.
7. H. Kahn and W. Slavin, Appl. Optics 2, 931 (1963).
8. J.E. Allan, Spectrochim. Acta 17, 459 (1961).
9. Ramsay, W.N.M., Biochem. J. 53, 227 (1953).