Microdetermination of Lead in Biological Material

With Dithizone Extraction at High pH

JACOB CHOLAK, DONALD M. HUBBARD, AND ROLAND E. BURKEY

Kettering Laboratory of Applied Physiology, University of Cincinnati, Cincinnati, Ohio

The use of a procedure for the quantitative extraction of lead with dithizone at high pH has facilitated the determination of lead in biological material. The high pH extraction step has been incorporated in the final estimation step of the Bambach-Burkey method (*1*). The difficulties and advantages of the revised procedure are discussed and analytical data are given to prove the adequate nature of the revised procedure.

A PROCEDURE for the quantitative extraction of lead with dithizone at high pH, which was recently reported by Snyder (*4*), has facilitated the determination of lead in biological material. Following a personal communication early in 1945 (*5*), the procedure was incorporated in the Bambach and Burkey method (*1*) and has been used satisfactorily since then for the routine analysis of all types of samples. The revised procedure represents a significant advance in analytical technique, and is deemed worthy of presentation in order to point out the difficulties and advantages encountered in the application of the high pH extraction procedure to the analysis of biological material. For the convenience of analysts, the revised procedure is described stepwise, but only in so far as it differs from that given by Bambach and Burkey (*1*).

PROCEDURE

Samples are prepared without change, and only the following changes in reagents are required.

Ammonium Hydroxide–Potassium Cyanide Mixture. Dissolve 10 grams of potassium cyanide in 1000 ml. of ammonium hydroxide (specific gravity 0.900). Lead-free ammonium hydroxide of the proper specific gravity may be made by passing tank ammonia into distilled water immersed in an ice bath.

Dithizone Extraction Solution. Only one solution is needed—30 mg. of Eastman dithizone per liter of lead-free (redistilled and stabilized) chloroform.

Isolation of Lead and Removal of Bismuth Interference. The initial isolation of lead is carried out exactly as described by Bambach and Burkey (*1*). Because it is no longer necessary to observe each 5-ml. extraction in order to fit the samples into the three concentration ranges used by Bambach and Burkey (*1*), the extraction may be started with 10, 15, or 20 ml. of dithizone solution as the analyst prefers. The approximate total quantity of lead, however, must be known and this is determined from the total volume of extraction solution used. Each 5 ml. of dithizone extraction solution will extract approximately 40 micrograms of lead (*1*). If the quantity of the dithizone extraction solution used indicates the presence of more than 150 micrograms of lead, an aliquot is removed to bring the quantity of lead to or below 150 micrograms before adding the 50 ml. of the pH 3.4 buffer which is used to separate the lead from the bismuth.

Final Estimation of Lead. To the 50 ml. of pH 3.4 buffer (*1*) containing less than 150 micrograms, add 20 ml. of the ammonium hydroxide–potassium cyanide mixture. (The automatic pipet shown in Figure 1 has been found convenient for this purpose. It was designed and made in this laboratory by W. J. Younker.) Add 15 ml. of dithizone extraction solution and shake for 1 minute, releasing the pressure which develops through the stopper rather than through the stopcock. The colors in an entire series are developed in this way before proceeding to the photometry.

Photometry. Place a small pledget of cotton in the tip of the stem of each funnel, and just before filling the cells, discard 2 to 3 ml. of the chloroform phase by allowing it to pass through the cotton pledget. Fill the cell by filtering the chloroform solution through the pledget and read the density or the transmittancy of the solution at 510 mμ in any suitable photometer. The proper size of cell to use is determined as in Snyder's method (*4*), from the depth of color in the chloroform phase. It is usually possible to make measurements with only two cells, a cell of 50-mm. light path for quantities from 0 to 15 micrograms and a cell of 10-mm. light path for quantities from about 15 to 100 or 110 micrograms of lead. (A cell with a 5-mm. light path must be used when the amount of lead is between 110 and 150 micrograms.) The working curves are prepared from known amounts of lead added to the pH 3.4 buffer. A blank, starting with the pH 3.4 buffer, is run in order to obtain the daily zero points of the calibration curves. A blank of all of the reagents used in the complete extraction must also be determined and subtracted from the values read from the calibration curves.

RESULTS

In Table I are listed typical results chosen at random from approximately one hundred samples, duplicate aliquots of which were analyzed in parallel by the Bambach and Burkey (*1*) method and the modified procedure. In the case of the blood samples, these two sets of results are compared with the spectrographic findings.

The comparative degrees of accuracy and reproducibility of results obtained on two series of samples by the old and new procedures are indicated in Table II. The ash of a series of samples of feces, in the one case, and of urine, in the other, which remained following certain other experimental observations, was composited and then divided into 40 equivalent samples, respectively, for the parallel analyses. The other parallel series consisted of two sets of samples of synthetic urine (*2*) to which known amounts of lead were added.

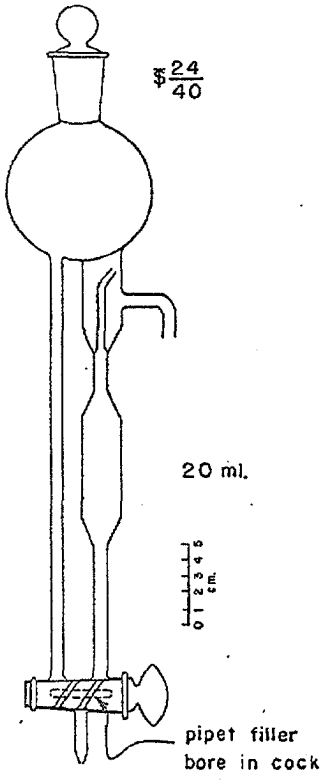

Figure 1. Automatic Dispensing Pipet

DISCUSSION

Inspection of Tables I and II shows that there is little difference in the findings obtained by the two methods. The differences between the respective mean values in Table II, however, are

Table I. Results Obtained on Duplicate Samples by Extracting with Dithizone at pH 9.5 and 11.5

Description		Found by Bambach and Burkey Method (1)	Found by Revised Procedure
		Mg.	*Mg.*
Urine 1185		0.325	0.320
Urine 1282		0.175	0.177
Urine 1299		0.160	0.160
Urine 1487		0.190	0.187
Feces 1216		0.430	0.430
Feces 1438		0.57	0.56
Feces 1442		0.52	0.51
Feces 1368		0.31	0.33
Food (mixed 24 hour total) 1369		0.19	0.19
Food (mixed 24 hour total) 1421		0.18	0.19
Food (mixed 24 hour total) 1576		0.16	0.16
Food (mixed 24 hour total) 1624		0.33	0.33
	Spectrographic (2)	*Mg./100 g.*	*Mg./100 g.*
Blood 2152	0.050	0.055	0.051
Blood 2153	0.060	0.066	0.066
Blood 2185	0.050	0.044	0.044
Blood 2192	0.055	0.061	0.061

statistically significant; slightly superior accuracy is shown by the revised method, particularly in the higher ranges of concentration. The reproducibility of the results, on the other hand, is somewhat superior in the case of the Bambach and Burkey method (*1*).

In the analysis of biological material, special precautions must be taken to prevent interference by bismuth. For this reason, it is necessary to make a preliminary extraction, which isolates lead and bismuth from the extraneous ash, and then to remove the bismuth before proceeding to the final lead step of extraction and photometry. The initial extraction may be made within the range of pH 8.5 and 11.5, but it is best to work at the lower pH (not exceeding 9) if the bismuth separation is to be made by washing the initial extract with the buffer solution at pH 3.4 (*1*). Changes in the buffer at this point will result in low recoveries of lead, and the losses are not reclaimed unless the initial chloroform extract is washed thoroughly with water to remove entrained alkali. When the initial extraction is made at pH 8.5, a single wash of the chloroform extract with 50 ml. of distilled water is sufficient to remove all the entrained alkali. On the other hand, at pH 11.5, interfering quantities of alkali remain even if the chloroform extract is washed with two 50-ml. portions of distilled water, and there may be significant losses of lead. Hence, pH 8.5 is chosen for the initial extraction.

Table II. Reproducibility and Accuracy of Two Analytical Procedures Applied in Parallel

Material	No. of Analyses	Method	—Micrograms of Lead— Mean	Std. error	Std. dev.
Ashed feces, composited and subdivided	20	Bambach and Burkey	42.63	±0.064	±0.29
	20	Revised	44.00	±0.123	±0.55
Ashed urine, composited and subdivided	20	Bambach and Burkey	8.69	±0.056	±0.250
	20	Revised	8.90	±0.050	±0.224
Synthetic urine, 8 micrograms Pb added	10	Bambach and Burkey	7.9	±0.024	±0.077
	10	Revised	8.16	±0.073	±0.230
Synthetic urine, 40 micrograms Pb added	10	Bambach and Burkey	38.66	±0.209	±0.66
	10	Revised	39.8	±0.201	±0.64

The extraction at high pH possesses a distinct advantage when carried out in the presence of moderate amounts of phosphate ion. This fact is indicated in Table III, wherein are recorded the results obtained in the analysis of samples containing 50 micrograms of lead buffered at pH 3.4, to which various amounts of phosphate ion were added, following extractions at pH 9.5 and pH 11.5, respectively. Extractions with dithizone were made after the phosphate and lead had been allowed to remain in contact for various periods of time. The extraction of lead with dithizone at pH 11.5 is quantitative even in the presence of 5 mg. of phosphate ion. On the other hand, low recoveries are obtained at pH 9.5 in the presence of 1 mg. of phosphate or less. Actually, other experiments have shown that quantities of phosphate as low as 0.5 mg. in the final solution prevent the complete extraction of lead.

In the Bambach-Burkey method (*1*) solutions of lead dithizonate may be subjected to photometry without preliminary filtration. Although the same procedure may be followed in extracting at high pH, the results so obtained show a lesser degree of reproducibility than do those obtained after filtration. A convenient and rapid method of filtration is provided by the insertion of a small pledget of cotton into the stem of the funnel. No special purification of the cotton is required other than that accomplished by wasting 2 ml. of the chloroform phase through the pledget before filtering the remainder into the measuring cell.

The use of a specific type of cell (Style D, American Instrument Company, or equivalent) permits the employment of small volumes of solution and results in an increased sensitivity of detection. Only 15 ml. are used and only 10 ml. of this amount are required to fill the cell (having a light path of 50 mm.) which is used in the range 0 to 15 micrograms of lead.

Table III. Effect of Phosphate Ion on Extraction of Lead with Dithizone

(50 micrograms of Pb)

Added PO_4^{--}	pH of Final Extraction	Extracted Immediately	Extracted after 10 min.	20 min.	30 min.
Mg.		γ	γ	γ	γ
0	9.5	50	50	..	..
	11.5	49	49.9	..	..
1	9.5	33	42.5	..	..
	11.5	50	49.7	..	..
2	9.5	..	10.5	..	..
	11.5	48	49	..	..
3	9.5	..	8.5	..	..
	11.5	51	50	..	..
4	11.5	49.8	49	48	49
5	11.5	50	50	..	..

As shown by Snyder (*4*), it is no longer necessary to prepare separate standard solutions of dithizone for the three ranges of lead concentration, and the same extraction solution which is used for the initial extraction can be used in the final estimation of color. For the final extraction of lead, the proper quantity of dithizone also may be included in the ammonium hydroxide–potassium cyanide mixture. After adding the mixture, it is only necessary to add 15 ml. of clear chloroform to extract the lead. Such a mixture has been employed in a rapid screening test for lead in urine (*3*). Solutions of this type have been kept apparently unchanged for more than six months. The great convenience associated with the stability of such solutions is obvious, especially in laboratories in which only occasional lead determinations are carried out. Under these conditions, the buffer solution containing the dithizone can be stored in a refrigerator, and preserved further in an unchanged state. The initial extraction solution can then be made up as required and in sufficient amounts to take care of current samples.

LITERATURE CITED

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