MODIFIED ELECTROLYTIC GUTZEIT METHOD FOR RAPID MICRODETERMINATION OF ARSENIC*

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An investigation requiring the accurate determination of minute amounts of arsenic in cerebrospinal fluid led to the further modification of the electrolytic Gutzeit method previously reported by the senior author (1). The present method allows the determination of 1 to 100 μ of arsenic with an error of 5 per cent or less. The per cent of error is increased to between 5 and 10 per cent when the values dealt with are between 0.1 and 1.0 μ. The apparatus (Fig. 1) and procedures are relatively simple and as many determinations as one has electrolytic chambers for can be made in about an hour and a half.

The method consists of a preliminary sulfuric acid digestion of the sample and reduction with stannous chloride. Electrolysis of the acid solution is utilized to liberate arsine. After passage of the arsine through glass wool impregnated with lead acetate, it stains a mercuric chloride-sensitized string to a length accurately proportionate to the amount of arsenic present.

The mercury bromide-sensitized paper strips previously used have been discarded in favor of the mercuric chloride-sensitized string snugly suspended in a capillary tube as described by How (2). A complete review of the literature is included in his article. How made a careful and thorough study of the various types of sensitizers, materials used for indicators, and the conditions which gave the most satisfactory results. With a few variations, the string is prepared and used as recommended by How. It not only has the advantage of a uniform and sharply demarcated stain but also allows a very broad range of determinations without loss of accuracy. This is made possible through the use of strings of varying degrees of sensitivity. For such minute quantities as 0.1 to 2.0 μ, strings saturated in 0.25 per cent solution of mercuric chloride are used; for 1 to 10 μ, strings saturated in 1

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per cent solution of mercuric chloride are used; and for 5 to 100 \(\gamma\), strings saturated in 5 per cent solution of mercuric chloride give the most satisfactory length of stain.

Reagents completely free of arsenic are desirable and, even if these are available, blanks should be run at frequent intervals, since the minute traces from the glassware, apparatus, and the so-called arsenic-free reagents are detectable with the more sensitive strings. When samples containing relatively large amounts of arsenic, for example 50 to 100 \(\gamma\), are run, detectable traces remain in the chamber despite thorough washing. These can be removed only by continuing electrolysis with blank reagents in the chamber for a number of hours.

Following digestion, How reduced the pentavalent arsenic to the trivalent state by the addition of 0.1 gm. of sodium bisulfite to the sample, heating for 30 minutes on an 80–85° water bath, and then removing the remaining sulfur dioxide by boiling for 3 minutes. The sodium bisulfite is unstable in aqueous solution and therefore 0.1 gm. amounts of the powder must be weighed individually. An additional objection is the relatively rapid exhaustion of the lead acetate scrubber by the traces of remaining hydrogen sulfide which is produced. Equally satisfactory reduction can be obtained by adding 3 drops of a 60 per cent solution of stannous chloride in concentrated hydrochloric acid and boiling for only 3 minutes.

How's method requires meticulous and time-consuming preparation of the zinc alloy generator which, however, is reported to give consistently uniform results. The advantage of the electrolytic chamber lies in its relative simplicity of construction and use. The apparatus can be made by the average glass-blower. It operates on a direct current of 1.0 ampere and 12.0 volts, and any desired number of the electrolytic chambers can be connected in series.

Instead of the V-shaped cathode and anode chambers separated by an alundum disk as previously described, the apparatus now used has a sealed-in porous glass disk at the base of the cathode chamber which is conveniently suspended within the anode chamber. When the apparatus is set up so that the fluid level in the anode chamber is slightly higher than that in the cathode chamber, diffusion of arsenous acid out of the latter is prevented and complete and rapid reduction of the arsenous acid to arsine occurs. The gases evolved from the electrolytic chambers have a high water vapor content but a more uniformly high saturation is insured through the insertion of a water saturation tube as shown in Fig. 1. An actual water trap should not be used with the electrolytic apparatus, since it is not a completely closed system and a trap alters diffusion through the porous glass disk.

The electrolytic chamber rests in a water bath which during operation is
maintained at a temperature of about 5° above the temperature of the capillary absorption tube, which is at room temperature. As emphasized by How, this temperature ratio gives the sharpest stain end-point, a fact probably attributable to water vapor condensation.

**Apparatus and Reagents**

The apparatus consists of Pyrex glass and petrolatum-lubricated ground glass joints of the dimensions noted on the diagram. The cathode consists of a lead tube folded from a sheet, or preferably of a platinum tube, since all lead sheet at our disposal contains traces of arsenic. The anode is a small sheet of platinum bent to conform to the curvature of the chamber. The cathode is fastened to the glass stopper by a sealed-in platinum wire connected through mercury to a copper wire contacting the source of current. Short pieces of insulated copper wire with terminal clamps serve as connec-
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Amperage of the direct current is controlled by a rheostat connected in the circuit.

The electrolytic chambers are placed in a metal rack resting in a water bath with inlet at the bottom and drainage near the top. A thermometer is suspended in the water bath. Adequate temperature control is effected through regulation of the rate of water flow.

The lead acetate-impregnated glass wool for removal of hydrogen sulfide gases is prepared by soaking the glass wool for several hours in 10 per cent solution of lead acetate. The excess solution is expressed and the glass wool placed on a towel to dry in the air. Strands of the glass wool are then loosely packed into the long side arm stem.

The water vapor chamber should contain 0.2 cc. of distilled water and can be filled from the top with a pipette.

The string (No. 8 knitting cotton) can be prepared by winding 20 to 50 feet of it in closely spaced spirals around a glass cylinder or tube. A 500 cc. cylinder is satisfactory. The alcoholic solution of mercuric chloride of the desired concentration (0.25, 1.0, or 5.0 per cent) is prepared with 95 per cent alcohol. The wound tube is immersed in the solution which has been poured into a cylinder large enough to accommodate the wound tube. Although How recommended soaking for many hours, we have found 1 hour of soaking to be adequate, although longer soaking does no harm. Upon removal of the wound tube from the solution, the entire string is drawn off, held slightly taut, and rotated in a horizontal position while it dries for 5 minutes. With a minimal amount of handling the string is cut into 10 to 12 cm. pieces, which are stored in a covered glass tube or chamber protected from the light with black paint or paper. Despite apparent uniformity of technique in preparation of strings of the same concentration, slight differences in sensitivity occur between the different lots, so that for greatest accuracy each lot must be standardized individually.

A 2 per cent solution of silver nitrate in a 10 per cent solution of ammonium hydroxide is used to develop the black stain on the exposed end of the string.

The 60 per cent solution of stannous chloride solution is prepared by dissolving 30 gm. of the crystals in concentrated hydrochloric acid made up to 50 cc.

12.5 per cent sulfuric acid is used to half fill the anode chamber for electrolysis.

Concentrated sulfuric acid and a 10 per cent solution of copper sulfate are used in the digestion process.

Procedure

For the analysis of a biological fluid such as spinal fluid, add 5 cc. of concentrated sulfuric acid and 0.5 cc. of 10 per cent copper sulfate solution to
2 cc. of spinal fluid in a Kjeldahl flask. Boil in a hood or on a Kjeldahl digestion rack for 30 minutes and then cool. If blood, feces, skin, or other tissues are being analyzed, use 10 to 20 cc. of sulfuric acid and 1 cc. of copper sulfate solution and continue the digestion for an hour.

Dilute the digested mixture to 50 to 100 cc. and use an aliquot part for electrolysis, the quantity of the aliquot depending on the amount of arsenic expected to be present. A 20 or 25 cc. aliquot part is usually satisfactory.

Add 3 drops of the 60 per cent stannous chloride solution and two Pyrex glass beads to the aliquot part in a 100 cc. Erlenmeyer flask and boil for 3 minutes. Cool.

Fill the anode chamber with 12.5 per cent sulfuric acid to about the half full mark. Place the cathode chamber down in the anode chamber and after 1 to 2 cc. of acid have diffused up through the porous glass disk pour the contents of the flask into the cathode chamber. Rinse the flask with 1 to 2 cc. of distilled water from a wash bottle and add the washing to the cathode chamber. Ideally at this point the fluid level in the outer anode chamber should be about 1 cm. higher than in the inner cathode chamber. The cathode and its glass stopper are lowered into place, sealing the chamber which is placed in the water bath.

The capillary absorption tube containing the sensitized string, previously placed in position by applying slight suction, is now affixed to the water vapor chamber which in turn can be fitted into its joint at the top of the long side arm. The direct current terminals are clamped to the electrodes and the electrolysis continued for 30 minutes.

After this time the sensitized string is withdrawn from its tube and the lower end is dipped into the 2 per cent solution of ammoniacal silver nitrate. A deep, black, sharply demarcated stain instantly develops over the end of the string. The length of stain is measured with calipers or with a millimeter rule against a white background.

Preliminary tests with known amounts of arsenic give data for tables or curves, so that the calculation of the amount of arsenic in the sample depends merely on the amount of original material digested, the amount of dilution, and the size of the aliquot part. For example, 2 cc. of spinal fluid diluted after digestion to 50 cc., of which 25 cc. have been used as the aliquot part, give a stain 5.0 mm. long on a string saturated in 0.25 per cent solution of mercuric chloride. Tabulated data from preliminary "known" analyses show that a 5.0 mm. stain on the 0.25 per cent string of that lot is equivalent to 1.0 γ of arsenic. The sample of spinal fluid therefore contained 1.0 γ of arsenic per cc.

Representative samples of sensitized string saturated with a 1 per cent solution of mercuric chloride have in our hands given the following lengths of coloration with the various quantities of arsenic: 1 γ, 1.0 mm.; 3 γ, 3.0 mm.; 7 γ, 6.8 mm.; 10 γ, 9.8 mm.
SUMMARY

We have described an apparatus and technique which combine the use of an electrolytic Gutzeit device with certain features of the How method for the determination of minute amounts of arsenic in biological material. The accuracy of the method is of the order of 10 per cent for amounts of arsenic from 0.1 to 1.0 γ and is increased to 5 per cent with amounts of arsenic from 1.0 to 100 γ.

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