[CONTRIBUTION FROM THE MORLEY CHEMICAL LABORATORY OF WESTERN RESERVE UNIVERSITY]

THE DETERMINATION OF TRACES OF MERCURY
II. THE QUANTITATIVE DETERMINATION OF MERCURY IN THE PRESENCE OF ORGANIC MATTER[1,2,3]

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Experimental Part

The object of this investigation was to develop an accurate method for the determination of small amounts of mercury in physiological fluids. Practically all the methods for the determination of traces of mercury in the presence of organic matter include the following operations: (1) oxidation of the organic material; (2) concentration of the mercury; (3) separation of the mercury precipitate from the solution; (4) solution of the collected mercury compound; (5) quantitative determination of the mercury.

Oxidation of the Organic Material.—Although the Fresenius-Babo method of oxidation with hydrochloric acid and potassium chlorate has been most widely used, it was thought desirable to avoid the formation of the volatile mercury halides which this produces. A method introduced by Palme[4] and Lomholt and Christiansen,[5] whereby oxidation is effected by means of potassium permanganate in the presence of sulfuric acid, was therefore adopted with a few modifications.[6] In heating to effect oxidation, an all-glass refluxing apparatus (Fig. 1) with a 3-liter, long-neck, round-bottom, Pyrex flask was employed. This prevents losses due to the action on the cork, or other sealing materials, and completely condenses all mercury vapors before they reach the mouth of the flask.

Lomholt and Christiansen[5] first added potassium permanganate to the

[1] This research was carried out in collaboration with Dr. T. Sollmann and Dr. H. N. Cole of the School of Medicine of Western Reserve University, as a preliminary to a comprehensive study of the absorption and elimination of mercury and mercury compounds by the human body.

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[2] Presented at the Washington Meeting of the American Chemical Society, April, 1924.

[3] For Part I, see THIS JOURNAL, **47**, 2625 (1925).

[4] Palme, *Z. physiol. Chem.*, **89**, 345 (1914).

[5] Lomholt and Christiansen, *Biochem. Z.*, **81**, 356 (1917).

[6] For the study of the oxidation of organic material, solutions containing 1% of gelatin—to simulate the organic matter in urine—to which known amounts of mercury salts were added were used, rather than urine, in order to eliminate in this first study possible interferences which might make it more difficult to evaluate the method. We may add, however, that we subsequently confirmed the method on specimens of urine.

urine, and then concd. sulfuric acid. This causes a great deal of bumping and foaming, which we found could be prevented by adding the sulfuric acid first, heating the mixture to boiling, cooling slightly and then adding the potassium permanganate in 1g. tablets (compressed without a filler) as may be required. Too large an excess of permanganate should be avoided. The mixture is boiled and the oxidation continued until no more organic odors are evolved. The small amount of manganese dioxide which settles out is reduced by the cautious addition of a few drops of "50 volume" hydrogen peroxide, and remains in solution as sulfate. The boiling is then continued for a short time to break down any excess of hydrogen peroxide, which later would oxidize the hydrogen sulfide and liberate free sulfur. Hydrogen peroxide was added rather than oxalic acid because the latter tends to form the insoluble manganese oxalate, which interferes with the later procedure. After the manganese dioxide has been reduced, the condenser is carefully rinsed into the flask, and the clear solution cooled, filtered and transferred to a large beaker for concentration.

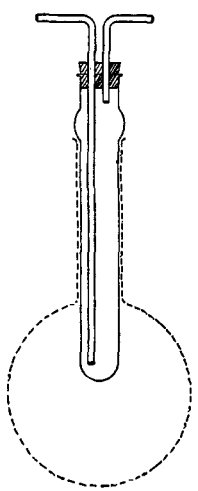

Fig. 1.

Concentration of the Mercury.—Experiment showed that solutions of mercury salts cannot be sufficiently concentrated by evaporation without a partial loss of mercury, nor can all the mercury be volatilized as chloride from the solution even at a high temperature and with a large excess of hydrogen chloride gas present. This precludes the use of evaporation as a method of concentration.

The amalgamation method, according to many investigators, does not give a quantitative separation so that it did not seem worth while to study this method. The precipitation method presented the greatest possibilities for concentration of the mercury, but it was found that with such a small amount of mercury present the sulfide remained in suspension and was difficult to collect on a filter. Raaschou,[7] Palme[4] and Lomholt and Christiansen[5] added copper sulfate to the oxidized solution of the mercury salt and precipitated both the copper and mercury as sulfide. Raaschou thought that the copper sulfide functioned merely to increase the volume of the precipitate. However, the evidence from our experimental work on electrometric titrations of solutions of mercury salts indicates that dilute solutions of mercury salts are hydrolyzed and the mercury is present in part as colloidal mercury compounds. Since this colloid is not ionized, it is not probable that hydrogen sulfide would react with it to precipitate it. Obviously, the copper sulfide adsorbs the colloidal mercury compounds, thus removing both ionized and colloidal mercury salts. A more gelatinous

[7] Raaschou, *Z. anal. Chem.*, **49**, 172 (1910).

compound than copper sulfide, such as manganous hydroxide, would be more effective and proportionately less would be required.

To test this, a series of experiments was conducted to learn whether gelatinous manganous hydroxide, which is easily formed from the manganous sulfate present in the oxidized solution, carries down both suspended mercury sulfide and colloidal mercury compounds.

To 250 cc. of a solution of (1) mercuric chloride and (2) mercuric nitrate, containing 50 mg. of mercury per liter, were added 50 cc. of a 2.5% solution of manganous sulfate and 25 cc. of concd. sulfuric acid. No hydrogen sulfide was added in this case. The solution was made slightly alkaline with sodium hydroxide to precipitate the manganous hydroxide, the solution well stirred and 2 cc. of a 1% sodium hydroxide solution was floated on top. When the manganous hydroxide had settled, the solution was filtered and the filtrate tested for mercury with the electromicroscopic qualitative test.[8] Large amounts of mercury were invariably found in the filtrate, showing that the coagulation method *alone* would not remove completely both the ionized mercury and colloidal mercury compounds. The same experiments on the mercury-manganese solution were then repeated but the ionic mercury was first precipitated as the sulfide in acid solution, and the excess hydrogen sulfide removed by bubbling dust-free air through the solution. This prevents the formation of sodium sulfide later, which would dissolve the mercuric sulfide. The solution was made slightly alkaline, as previously described, allowed to settle and then filtered through a Gooch crucible, using an asbestos mat.

No trace of mercury could be found in the filtrate even after concentration at room temperature of 25 cc. to one drop. Thus there was less than one part of mercury in one billion left in the filtrate. From this we concluded that the gelatinous manganous hydroxide in settling had completely removed all of the suspended mercury sulfide as well as colloidal mercury compounds. This confirms our statement that in dilute solutions of mercury salts, there are present both mercury ions and colloidal mercury compounds and furthermore, that no quantitative method that fails to take this into account can be accurate. The details of the analytical procedure adopted are fully described under *Summary of the New Method.*

Separation of the Mercury Precipitate from the Solution.—The precipitated mass obtained as described above is filtered through a Caldwell-Gooch crucible of 50cc. capacity provided with a disk supporting an asbestos mat. The precipitate is washed with distilled water until free from alkali and then dried for 10 to 12 hours at 110° in an electric oven.

Quantitative Determination.—We first tried dissolving the precipitate on the Gooch filter and determining the amount of mercury present by electrometric titration[9] with a standard sodium chloride solution. In every case the results were from 4% to 10% too low, depending upon the age of the mercury solutions.[10] This indicates the presence of a variable

[8] Booth and Schreiber, Ref. 3.

[9] Dutoit et Duboux, "L'Analyse des Vins par Volumetrie Physico-Chimique," R. Rouge et Cie., Éditeurs, Lausanne, **1912.**

[10] Our study of the electrometric titration of mercury salts will be published later as a separate article.

amount of un-ionized, colloidal mercury compounds, probably in the form of colloidal oxy salts. Any quantitative method, therefore, that does not provide for the determination of colloidal mercury compounds as well as of ionic mercury will be inadequate.

The simplest and most definite procedure for including the colloidal as well as ionic mercury is dry decomposition of the sulfide with an oxidizing agent. Bouton and Duschak[11] decomposed mercury ores by heating with lime and condensing the volatilized mercury. Although this method is accurate enough for relatively large amounts of mercury, we found, as a result of many experiments in which we tried numerous modifications, such as substituting magnesium or barium oxide for lime with or without the addition of cupric oxide, iron or zinc, that where only traces of mercury are present the method gives inconsistently low results. Preliminary experiments showed that decomposition of the mercuric sulfide by lead chromate,[12] a denser substance and an oxidizing agent also, was more promising and so was accurately tested as follows.

Fig. 2.—(1) Magnesite. (2) Asbestos. (3) Lead chromate. (4) Dried precipitate ground with lead chromate. (5) Lead chromate. (6) Asbestos. (7) Sodium carbonate. (8) Glass wool. (9) Glass wool. (10) Phosphorus pentoxide.

Weighed amounts (0.4–5.0 mg.) of pure mercury were dissolved in a few drops of concd. nitric acid and the solutions diluted. Sulfuric acid and manganous sulfate were added so as to duplicate the oxidized solution. The mercury was then precipitated as sulfide, coagulated, filtered off and dried, as previously described. The precipitate of manganous hydroxide and mercury sulfide was ground in an agate mortar with 0.5 g. of lead chromate, previously dried at 500°. This mixture was transferred to a small glass tube (inner decomposition tube) sealed at one end and containing 0.2 g. of dried magnesite. A loose plug of glass wool was then inserted to keep the mixture in place. It was found necessary to have this small amount of magnesite present in the end of the tube to furnish a small, steady stream of gas to drive the mercury from the decomposed sulfide into the cool portion of the outer decomposition tube. The inner tube was slid into a larger glass tube (outer decomposition tube), Fig. 2, which was then slightly constricted about 10 cm. from the open end. A small plug of glass wool and 0.2 g. of phosphorus pentoxide were inserted to absorb the evolved moisture, which would otherwise prevent the complete collection of the condensed mercury into one globule. The open end was then drawn out to a long capillary. The tube was placed in the decomposition furnace,[12] with the end containing the magnesite projecting from the back of the furnace, and heated for several hours. The tube was then moved forward so that the magnesite would decompose and drive over the last traces of mercury. The portion of the tube projecting from the front of the furnace was cooled with wet wicking, to in-

[11] Bouton and Duschak, "The Determination of Mercury," *U. S. Dept. Interior Techn. Paper*, **227** (1920).

[12] Zdrahal, *Oesterr. Z.*, **29**, 561 (1881).

duce condensation of the mercury which deposited in a narrow band, 4 to 5 cm. from the end of the furnace. The tube was cooled, and broken halfway between the end of the inner decomposition tube and deposit of mercury, and at the constriction. The mercury was then collected into one globule and measured.

The details of this procedure are described under *Summary of the New Method.*

Micrometric Measurement.—On account of the difficulty of completely drying the mercury obtained from the distillation, it is not, in our opinion, advisable to attempt to weigh it directly. It seems more practical and rapid to apply the method of micrometric measurement properly modified, to the accurate determination of this mercury. Raaschou[7] measured the diameter of the mercury globule under the microscope with a micrometer ocular and calculated the weight of the mercury globule. He found, however, that the tendency of the mercury globule to "flatten out" caused an appreciable error in amounts larger than 2 mg.

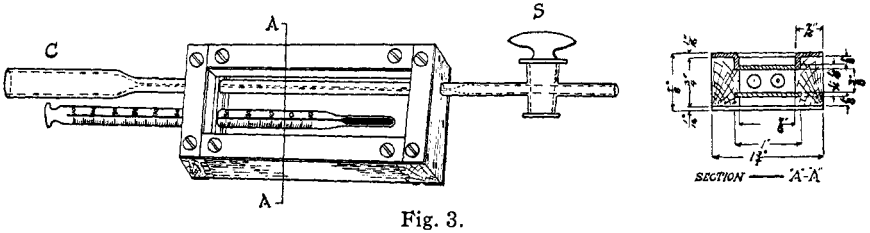

Fig. 3.

It occurred to us that this difficulty might be overcome by transferring the globule of mercury to a fine capillary and measuring the length of the thread of mercury. A piece of capillary tubing about 50 cm. long and of 0.272mm. bore, which appeared uniform, was selected. A globule of mercury was drawn into the tubing about 4 cm. from one end, and its length measured with a calibrated cathetometer. The mercury globule was then drawn a few millimeters further along the tube and the length again measured. This was continued until a 10cm. section of uniform bore was found. To one end of this capillary was sealed a capillary stopcock (S), Fig. 3, and to the other, a short piece of glass tubing (C) (8 mm. internal diameter) to guide the globule of mercury into the capillary. This capillary buret was filled with constant-boiling hydrochloric acid[13] and all air bubbles were removed. The globule of mercury was transferred to the small cup (C), the stopcock (S) opened and the mercury drawn down to the middle of the capillary. The stopcock was closed, the excess of acid removed from the cup, and the length of the mercury thread measured with a com-

[13] Constant-boiling hydrochloric acid is used because this concentration is easily arrived at and maintained, and it was found that the mercury globule would slide down the capillary more easily in hydrochloric acid than in air or water. The hydrochloric acid also removes any foreign substances that have adhered to the surface of the mercury.

parator.[14] With this instrument the end of one meniscus can be brought into line with the cross hairs at the optic axis (care being taken to avoid backlash), the reading taken on the millimeter scale and Vernier, and the body tube slowly moved across the length of mercury until the end of the other meniscus coincides with the cross hairs, and the reading again taken.

The thick glass capillary tubing, however, served as a cylindrical lens, giving a distorted image of the mercury thread and making accurate reading impossible. This lens effect was obviated by immersing the capillary in a medium of the same refractive index as glass, the whole contained in a glass box so constructed that the upper and lower sides would be in parallel planes. The case (Fig. 3) placed horizontally with the capillary buret and thermometer in position, was filled with pale yellow Canada balsam and the cover slide slowly lowered into place to avoid the inclusion of air bubbles. After the balsam had hardened, the brass strips which hold the top glass slide firmly in place were tightly screwed down. Optically, the resulting effect is the same as if a capillary tube were in the center of a glass slab with parallel faces. This device completely eliminates the optical distortion obtained with the capillary tube alone, and at the same time serves to maintain the mercury at a constant temperature in the tube. The resulting mercury thread as seen through the comparator has a convex end, extremely clean-cut, easily observed and measured. According to Kohlrausch[15] and Lohenstein,[16] the mercury meniscus under these conditions is not a hemisphere and the height of the meniscus is so small it is almost negligible. The simplest method of measurement, therefore, seemed to be to plot the weight of a globule of mercury against the length of the mercury thread as measured in the microburet.

Calibration of Microburet and Method of Use.—Various amounts of mercury (0.3 mg. to 6.2 mg.) which had been purified by vacuum distillation, were carefully weighed on a Rueprecht precision balance by methods of substitution and swings. The mercury globule was transferred to the capillary buret in the viewing case, the temperature observed and the length of the column measured.

From the formula $L_0 = L_T/(\alpha T + 1)$, the length of the thread of mercury was corrected to 0° where L_0 is the length of mercury thread at 0°,

[14] A comparator with a fixed stage and traveling body tube, rather than an ocular micrometer, is required for greater accuracy. With this instrument, measurements are made only in the center of the field, thus eliminating the optical errors possible in the outer edges of the field in an ocular micrometer. It is also possible to measure accurately a longer thread than with an ocular micrometer. The Model M1200 Special, supplied by Gaertner and Co., Chicago, was found satisfactory for this purpose.

[15] Kohlrausch, "Lehrbuch der praktischen Physik," p. 104, Teubner, Leipzig and Berlin, **1905**.

[16] Lohenstein, *Ann. Physik*, **33**, 296 (1910).

L_T is the length of mercury thread at $T°$ and α is the coefficient of expansion of mercury (0.0001815—Regnault).

The weights of mercury were plotted as abscissas and the corresponding lengths at 0° as ordinates, and a curve was drawn through the points. After such a straight-line graph is plotted, only the length of the mercury thread need be measured, corrected to 0°, and then the corresponding weight may be read directly from the graph. After one becomes familiar with the manipulation there is no difficulty in obtaining an accuracy of 0.01 mg.

Test of the Complete Method.—After the individual parts of the method had been carefully tested, it was thought advisable to run a series of complete analyses, using various quantities of mercury. In order to simulate the actual conditions met with in analyzing urine, feces and other physiological materials, samples of mercury, accurately measured in the capillary buret were dissolved in nitric acid, the solutions diluted to one liter and gelatin was added in various amounts. Since the accuracy of this method of measurement of mercury had been proved, it was thought advisable to measure the sample and final weight of product in the same manner. These solutions were then put through the whole analytical procedure. The result of ten analyses made after this fashion, using amounts of mercury varying from 4.21 mg. to 0.27 mg., are recorded in Table I.

TABLE I

TEST OF COMPLETE METHOD USING SOLUTIONS OF MERCURIC NITRATE AND GELATIN

Length of mercury taken, corr. to 0°, mm.	Calcd. wt. of mercury taken, mg.	Length of mercury found, corr. to 0°, mm.	Calcd. wt. of mercury found, mg.	Error, mg.
0.348	0.27	0.358	0.28	+0.01
0.398	0.30	0.375	0.29	− .01
1.210	0.95	1.158	0.92	− .03
1.651	1.30	1.623	1.28	− .02
2.407	1.89	2.376	1.88	− .01
2.814	2.21	2.780	2.19	− .02
2.844	2.25	2.803	2.22	− .03
3.880	3.08	3.859	3.06	− .02
4.267	3.39	4.244	3.37	− .02
5.341	4.21	5.317	4.19	− .02

The "error" is seen to consist (with a single exception) in a loss of 0.01 to 0.03 mg., with a median of 0.02 mg., and this is independent of the amount taken. It would, therefore, be justifiable to correct the analytical results by increasing them by 0.02 mg., but as the error constitutes only 2% of 1 mg., or 0.4% of 5 mg., we prefer to use the uncorrected values.

Summary of the New Method

One liter of a solution containing a mercury salt in the presence of organic matter is transferred to a 3-liter, round-bottom, long-neck, Pyrex

flask; 100 cc. of concd. sulfuric acid is added and the solution boiled for 15 minutes with the internal reflux condenser in place. After the solution has slightly cooled, compressed 1g. tablets of potassium permanganate are added gradually as needed and the mixture is boiled until organic odors are no longer evolved and the solution is clear and colorless. A few drops of 50 volume hydrogen peroxide are added and the solution is boiled until the manganese dioxide is reduced and the excess of hydrogen peroxide decomposed. The condenser is carefully rinsed and removed, the solution cooled and filtered and transferred to a 2-liter beaker.

Hydrogen sulfide is bubbled through the solution for 20 minutes and the excess of hydrogen sulfide removed by bubbling pure, dust-free air through the solution. While the latter is still being slightly agitated with air, a 50% solution of sodium hydroxide is added until a few flocks of manganese hydroxide are formed. These are allowed to settle overnight. The precipitate is filtered on a Caldwell-Gooch crucible fitted with an asbestos mat, washed with water until free from alkali and then dried for ten to twelve hours in an oven at 110°.

The precipitate of mercury sulfide and manganese hydroxide, together with the asbestos, is transferred from the Gooch crucible to an agate mortar and ground thoroughly with 0.5 g. of "*precipitated*" lead chromate, previously dried at 500°. The mixture is transferred through a very small funnel into a glass tube 7 cm. long and 0.6 cm. in diameter (see Fig. 2), sealed at one end and containing in the bottom 0.2 g. of dried, powdered magnesite, then a small layer of asbestos and a 0.2 cm. layer of lead chromate. A small amount of lead chromate is ground in the agate mortar to remove the last traces of the precipitate and this is transferred to the tube above the decomposition mixture. Then a layer of 0.1 g. of dried sodium carbonate is added and the tube is closed with a *loose* plug of glass wool. This tube is placed in a glass tube (36 cm. long) open at both ends, and dried in the tube furnace at 200° for one-half hour. Most of the moisture present is thus condensed in the long, cool portion extending from the front end of the furnace. The small inner tube is then removed through the dry end and slid into a previously dried, outer decomposition tube one end of which is sealed. The decomposition tube is slightly constricted 10 cm. from the open end. A small plug of glass wool is placed outside this constriction and a layer of 0.2 g. of phosphorus pentoxide added. The open end is drawn out to a capillary 30 cm. long. The tubes are placed in the tube furnace with the end containing the magnesite projecting from the back of the furnace. A wicking is wrapped around the outer tube about 2 cm. from the front of the furnace and is kept constantly cool by dripping cold water on it during the decomposition. The tubes are gradually heated to 350° and this temperature maintained for two hours. The furnace is cooled to 200° and the tube moved along so that the end containing the

magnesite will be heated and the magnesite decomposed. The temperature is gradually raised to 520–550° and the heating continued for two hours. The tubes are then cooled, the decomposition tube is broken at the constricted part and again half way between the inner tube and the deposit of mercury. This section of tubing is placed in a vacuum desiccator over phosphorus pentoxide and dried for two hours. The deposit of mercury is then collected into one globule with the aid of a slender glass rod, drawn out to a fine hair.

The buret (see Fig. 3) is filled with constant-boiling hydrochloric acid and the globule of mercury transferred to the small cup. The stopcock is opened and the mercury allowed to run down to the middle of the capillary. The stopcock is closed, the excess of hydrochloric acid removed from the cup and the buret placed on the stage of the comparator. The stopcock is opened and when the thread of mercury has come to rest, the length is measured and the temperature observed. The length of mercury is then corrected to 0° by the formula, $L_0 = L_T/(\alpha T + 1)$, and the corresponding weight of mercury read from the graph.

The authors desire to express their appreciation of the kindness of Mr. M. J. Rentschler of the J. H. R. Products Co., Willoughby, Ohio, for furnishing the 50-volume hydrogen peroxide specially prepared so as to be free from interfering impurities.

Summary

1. It is shown that very dilute solutions of mercury salts contain not only mercuric ion but also colloidal mercury compounds and that no method for the quantitative determination of mercury that does not make provisions for the simultaneous determination of ionic mercury and colloidal mercury compounds can be accurate.

2. A method is described which provides for the determination of both ionic and colloidal-mercury compounds and which permits the quantitative determination of small quantities of mercury in the presence of organic matter with a loss of 0.01 mg. to 0.02 mg. of mercury in 5 mg., or less, in a liter of solution.

3. A simplified procedure is given for the oxidation of organic material, which prevents loss of mercury due to volatilization.

4. A new micrometric method for measuring small amounts of metallic mercury is described.

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