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Determination of Mercury in Small Quantities in Biologic Material by a Modified Photometric-Mercury Vapor Procedure

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A modification of the Jacobs-Yamaguchi method of determining mercury is presented. In the original method mercury is extracted with dithizone in chloroform from incompletely digested samples. The dithizone-mercury complex is subjected to heat destruction and the resulting mercury vapor is determined photometrically. This method suffers from some disadvantages among which is a low and uncertain maximum deflection because of a lengthy evaporation period for the mercury vapor during the heat destruction of the dithizone-mercury complex, and in some cases background readings from interfering substances.

In the presented modification the mercury vapor is absorbed on gold and then released again rapidly by mild heating of the gold filter. The method results in comparatively high reading even with amounts of mercury as low as 5 ng. The coefficient of variation of determinations of mercury in a standard solution has been found to be about 30 %, when amounts of mercury varying from 5 to 100 ng were determined. The method has been controlled by comparison with neutron activation analyses and scintillation analyses of radioactive mercury. These comparisons show that analysis of samples containing more than 10 ng of mercury per g can be made with the photometric method to give results of the correct order of magnitude.

Silver or copper will not influence the determinations. Iodide, however, will inhibit the extraction of mercury from the digest solution when present in amounts of the same order of magnitude as the mercury itself.

The most reliable method of analysis of small quantities of mercury in small samples of, *e.g.*, biological origin is most probably the neutron activation analysis method, *e.g.* as developed by Westermark and Sjöstrand.[1] This method is, however, not always the most suitable, since it is rather time-consuming and expensive and needs highly qualified personnel.

An interesting principle of analysis utilises the photometric determination of mercury vapor in air by means of ultraviolet light absorption at 253.7 nm.

Lindström [2] has developed an application of this principle according to which the samples are burned directly in an atomizer burner and the gases are analyzed in a mercury vapor meter.

A promising application of the same principle was developed by Jacobs, Yamaguchi *et al.*[3] Here a blood sample is incompletely digested with sulphuric acid and potassium permanganate at low temperature (50—60°C) and the digested sample extracted with dithizone in chloroform. The chloroform solution is evaporated in an ignition tube. The mercury-dithizone complex remaining is heated with a gas flame and the liberated mercury vapor determined photometrically.

Nielsen Kudsk [4] has modified the Jacobs-Yamaguchi method. According to this modification the ignition tube with the mercury-dithizone complex is closed during the heating so that the destruction of the mercury complex is completed before the mercury vapor is conducted to the photometer.

In this laboratory a modification of the Jacobs-Yamaguchi method has been used, according to which the mercury vapor from the ignition tube is absorbed on gold foil. When the heat destruction of the mercury-dithizone complex is completed, the gold filter is heated to give off the mercury through the photometer. The method has two advantages over the original method. The mercury vapor can be released through the photometer in one very short operation, which gives high and reproducible reading, and the method is more selective for mercury, since most interfering substances will not be absorbed on the gold filter.

MATERIALS AND METHODS

Apparatus. The apparatus is shown in Fig. 1 and comprises an ignition tube connected to a U-tube, containing absorbent cotton, followed by a cold trap. After the cold trap is a quartz tube, 200 mm in length, with an inner diameter of about 5 mm. In the middle of the tube is a constriction. Immediately before the constriction is placed a ball made of strips cut from gold foil, 0.1 mm thick. The length of this gold filter is between 5 and 10 mm. After the quartz tube is a second cold trap and a rotameter. To avoid accumula-

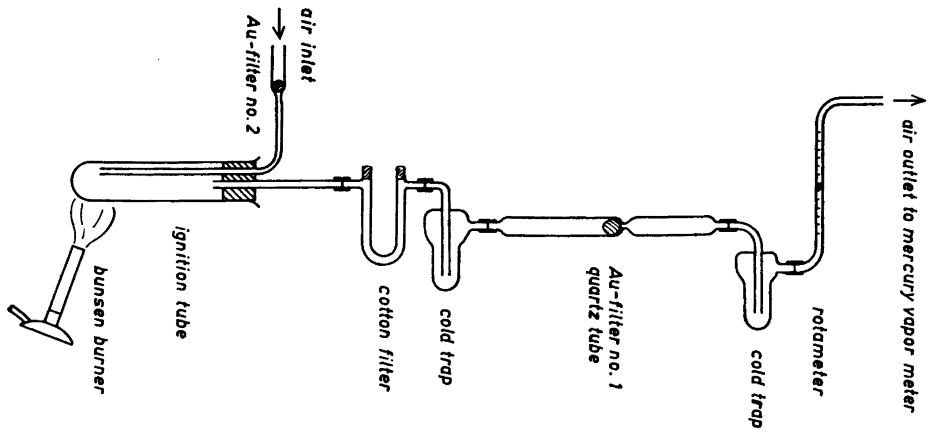

Fig. 1. Sketch of the apparatus for the microdetermination of mercury.

tion of mercury from the atmosphere in the gold filter, the ignition tube is fitted with an inlet tube of quartz, also with a constriction and a second gold filter.

The assembly is connected to a Beckman DU spectrophotometer with a mercury lamp and adjusted to 253.7 nm. The instrument is fitted with a gas cell, 100 mm in length and with an inner diameter of about 15 mm. The Beckman DU spectrophotometer is used together with a Sargent recorder, model FRL.

The gas cell is connected to a water suction pump.

Procedure. The reagents and standard solutions are prepared according to Jacobs *et al.*[3] 0.1 – 1 g samples, solid or liquid, are weighed and digested in the sulphuric acid-permanganate solution on a hot plate at about 70°C. The extraction of mercury and also the evaporation of the chloroform solution in the ignition tubes is also done according to Jacobs *et al.*[3] After the evaporation of the chloroform solution in the ignition tube is accomplished, the tube is connected to the apparatus according to Fig. 1. The water suction pump is started and the air flow is adjusted to 1.5 l/min by a screw clip placed on the rubber tube which connects the assembly to the pump. The automatic recorder is started and gold filter No. 1 is heated for 10 sec with a luminous Bunsen flame, to remove any mercury which may have been absorbed from the atmosphere between runs, when gold filter No. 2 is not connected.

The quartz tube with the gold filter is allowed to cool, or is cooled by a stream of air, until it is near room temperature. The ignition tube is now heated with the Bunsen burner for 30 sec. Normally no reading is recorded at this stage. Gold filter No. 1 is now heated for 10 sec with a luminous flame and the reading on the paper is marked with a pencil for identification.

At the beginning of a day's operations gold filter No. 2 is rinsed by heating for 10 sec with the air pump running. After some time both gold filters have to be exchanged for new ones, since the repeated heating makes them less effective as mercury absorbers.

The maximum height of the recorded response of the photometer is taken as being representative of the concentration of mercury. No increase in precision resulted when the area under the curve was used instead.

Standard curve. A chloroform solution of mercury(II)-dithizone complex containing 100 ng of mercury per ml of solution is prepared by extracting a water solution of mercuric chloride with dithizone in chloroform according to Jacobs *et al.*[3] Different volumes of this solution are added with a pipette to ignition tubes, to obtain tubes containing, *e.g.*, 5, 10, 20, 30, 40, 50, and 100 ng. The chloroform is evaporated slowly by placing the ignition tubes in a water bath at room temperature and heating the water up to 80°C.

Table 1. Calibration data.

Amount of mercury ng	Mean reading mm (6 determinations)	Standard deviation	Coefficient of variation %
5	3.8	1.4	37
10	7.6	2.2	29
20	16.4	6.1	37
30	33.1	10.1	31
40	41.3	16.7	40
50	66.3	20.1	30
100	174.2	42.0	24

The evaporation will take about 1 h. The tubes are thereafter inserted in the apparatus, Fig. 1, and heated as described under "Procedure". A set of calibration data is listed in Table 1 and the corresponding curve is plotted in Fig. 2. To obtain the figures in Table 1, one and the same chloroform solution of the mercury dithizone complex has been charged in different amounts to the different ignition tubes.

It appears from Table 1 that there is a rather high incertainty in the single determinations, the coefficient of variation being around 30 %. The standard curve is not a straight

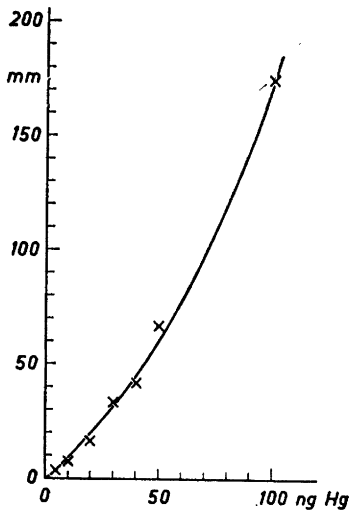

Fig. 2. Standard curve.

line, the readings are proportionally higher at higher amounts of mercury. The reason for this is not clear, but a similar behaviour is found in the paper of Jacobs *et al.*[3]

Control analyses. In order to investigate the practical value of the photometric method as described in this paper a large number of analyses have been made with both the photometric method and neutron activation method on the same samples. A presentation of all the material collected would take too much space; hence a description of the correlation in statistical terms is considered more suitable.

87 samples of human blood were analysed by the photometric technique and by the neutron activation technique of Westermark and Sjöstrand.[1] The activation analyses were run at Isotoptekniska laboratoriet, Stockholm, Sweden.

The activation technique is stated to give a maximal difference between repeated determinations of less than 10 %. The results obtained with this method are here referred to as "true values".

The over-all correlation coefficient between the two methods was found to be 0.85 and the regression coefficient to be 1.11 (results from the photometric analyses taken as the dependent variable). This means that the photometric technique in this series gave somewhat higher values than the "true values".

8 samples were found by the photometric technique to have zero values while the "true values" were between 3 and 9 ng of mercury per gram of sample. 3 samples were found to contain between 92 and 135 ng/g, while the "true values" were between 10 and 12 ng/g.

In no cases were zero values obtained with the photometric technique when the "true values" were $>$ 10 ng/g.

A second series of control analyes was run with the photometric technique against a scintillation technique using radioactive mercury.

Rats were injected with mercury compounds labeled with the radioactive isotope ^{203}Hg, as described by Swensson *et al.*[5] The rats were killed and the organs were taken and analysed by the two methods. The result is listed in Table 2. The difference between repeated determinations with the scintillation technique is only a few percent and these results are here referred to as "true values". As can be seen from Table 2 no systematic difference is found between the two methods. The correlation coefficient is 0.80 in this series and the regression coefficient is 1.00 (as in the first control series the results from the photometric analyses were taken as the dependent variable).

The result of these series may be summarized as follows:

Table 2. Control analyses, ng mercury per g of organ sample, according to two methods.

Scintillation technique	Photometric analysis	Organ (hen)
3900	1 910	kidney
1440	900	rump
3670	5 600	kidney
104	86	breast muscle
4080	3680	» »
1759	655	rump
156	139	breast muscle
287	122	» »
2020	1 590	liver
1056	1 130	»
7080	5 500	»
603	645	»
5180	12 600	»
7620	9 150	»
3950	1860	»
7940	4 700	»
7560	7 400	kidney
$\bar{X}$ = 3436	$\bar{Y}$ = 3 392	
y = 0.803		
b = 1.004		

1) When the true mercury content is below 10 ng/g the photometric method gives very uncertain results.

2) In no case where the true mercury concentration is more than 10 ng/g are zero values found by the photometric technique.

3) In a few cases where the true mercury concentration was low, values about 10 times too high were found with the photometric technique. The reason for this is not known.

4) The correlation between the photometric technique and the control methods is high.

5) No systematic error is found with the photometric technique.

Interfering substances. Nielsen Kudsk [4] states that the determination of the mercury content of digest solutions in the presence of the noble metals, copper, and iodine could

Table 3. Interference from Ag and Cu.

Added amounts of metals in the sample, ng			Mercury found, ng
Hg	Cu	Ag	
20	0	0	25
50	0	0	54
0	20	0	0
0	200	0	0
20	20	0	25
20	200	0	19
50	500	0	48
0	0	20	0
0	0	200	0
20	0	20	24
20	0	200	23

not be done by the method of Jacobs *et al.*[3] In order to check if the determinations are influenced by the metals mentioned when the analyses are run according to the present modification, mercury and copper sulphate or silver nitrate were added to samples of urine, and the samples were analysed in the normal way. The result is presented in Table 3. No influence was found. When the ignition tube was heated and the air passed through the gold filter no reading was noted in any case. In the concentration range used, copper and silver therefore have been found not to influence the analyses, whether a gold filter is used or not.

Iodide, however, inhibits the extraction of mercury from the digest solution as can be concluded from Table 4. This is consistent with the results reported by Nielsen Kudsk.

Table 4. Interference from iodide.

Added mercury ng	Added iodide (KI) ng	Found mercury ng
20	0	25
20	0	19
50	0	54
50	0	52
20	20	16
20	20	12
50	50	30
50	50	32
20	200	6
50	500	6
0	200	0
0	200	0

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