Measurement of mercury in human urine

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SYNOPSIS Four methods of determining the concentration of mercury in human urine have been studied. A simple method suitable for general laboratory use is recommended and the requirements for accurate results are defined. The method employs mild oxidation with permanganate and HS_2O_4 followed by dithizone extraction and measurement of absorbance at 485 nm and 620 nm.

No mercury was detected in any of 74 urines from unexposed laboratory controls and hospital patients. A random urine sample seems adequate for the investigation of clinical or industrial mercury poisoning. Two individuals, free of symptoms, but subjected to moderate exposure, excreted 3.0-9.7 μg of mercury per 100 ml of urine.

After the administration of an organic mercurial to two volunteers, urinary excretion was rapid and virtually complete within 48 hours.

The symptoms of mercury poisoning are vague and non-specific, and the clinical signs few (Buckell, Hunter, Milton, and Perry, 1946; Warkany and Hubbard, 1951). Considerable responsibility thus rests upon the laboratory, since the measurement of mercury in the urine offers virtually the only objective method of substantiating the diagnosis.

The techniques proposed for the measurement of mercury in biological materials include electro-deposition (Stock and Zimmerman, 1928), distillation (Kozelka, 1947), vapour detection (Monkman, Maffet, and Doherty, 1956), atomic absorption spectrophotometry (Lindstrom, 1959; Willis, 1962), and radio-activation analysis (Rodger and Smith, 1967). For the analysis of urine, oxidation of organic matter followed by dithizone extraction has been most popular, and earlier work in this field is described by Maren (1943), Gray (1952), Polley and Miller (1955), Rolfe, Russell, and Wilkinson (1955), and Nobel and Nobel (1958). The published figures for 24-hour urinary excretion of mercury in the normal subject show discrepancies, viz, 5-90 μg (Buckell *et al*, 1946), less than 1 μg (Monier-Williams,

1949), 0-50 μg (Warkany and Hubbard, 1951), less than 5 μg (Tompsett and Smith, 1959), less than 30 μg (Nobel and Leifheit, 1961), and less than 20 μg (Berman, 1967).

The object of this study was to establish the method most suitable for general laboratory use and to define the requirements for accurate analytical results. We have confined ourselves to four variants of the dithizone technique since the apparatus required is inexpensive and the analysis is rapid.

Materials and Methods

The bottles used for urine collections and analytical glassware were soaked in 50% (v/v) nitric acid for 24 hours and thoroughly rinsed, first with water and then with a dilute solution of dithizone immediately before use and rejected if any colour change took place in the dithizone remaining in contact with the glass. All reagents were Analar grade or, where possible, 'low in heavy metals'. Dithizone in high-grade crystalline form was obtained from Eastman Kodak. This

proved satisfactory without further purification, as documented by Campbell and Head (1955), Miller and Swanberg (1957), Nobel and Nobel (1958), and Nobel and Leifheit (1961). Other dithizone preparations used in preliminary work not included in this report required purification as described by Milton and Hoskins (1947). Mercuric chloride, after thorough desiccation, served as standard. A brief outline of the methods used follows: adequate details are given in the original publications.

METHOD A (VARLEY, 1963)

Urine is refluxed with sulphuric acid and permanganate using gentle heat. Decolorization with hydroxylamine is followed by extraction into dithizone. It is stated that the dithizone extract may be treated in one of two ways: the absorbance at 490 nm and 620 nm may be determined without further treatment and the mercury content calculated according to the formula of Clarkson and Kench (1956); or it may be washed twice with 5% ammonia as recommended by Milton and Hoskins (1947) to remove free dithizone, after which the absorbance at 480 nm due to mercury dithizonate is measured. Since the absorbance of the dithizone reagent at 490 nm and 620 nm was 1.410 and 3.2 respectively using a 1-cm light path, it is clearly inappropriate to carry out optical measurements on the untreated dithizone extract. We found that a minimum of four successive washes with 5% ammonia were required to remove excess of free dithizone from the extract, during which steps the organic layer became emulsified and required centrifugation before optical measurements could be made.

METHOD B (TOMPSETT AND SMITH, 1959)

Urine is refluxed for two hours with a mixture of acids and selenium powder. Permanganate oxidation is then carried out, followed by hydroxylamine treatment. Mercury is extracted into dithizone, the absorption of which is read at 620 nm. The dithizone extract is then treated with acid sodium thiosulphate to split mercury dithizonate and the absorption at 620 nm once more determined. The difference between the two readings is a measure of the mercury present. In theory, the second reading should be greater than or equal to the first reading. In practice this was not always so, and considerable uncertainty exists when measuring small quantities of mercury because of the need to subtract a large value from one only a little greater.

METHOD C (NOBEL AND LEIFHEIT, 1961)

Urine is treated with sulphuric acid, copper sulphate, and hydrazine, followed by sodium hydroxide, sulphuric acid, capryl alcohol, and potassium permanganate; the latter is then

decolorized by adding hydroxylamine. The solution is then washed with chloroform, and extractive titration with dithizone is carried out. This involves the addition of 0.5 ml portions of dithizone in chloroform to the solution followed by shaking, and separation of the organic phase. Titration is complete when the blue-green colour of a portion of the dithizone does not change when added to the solution, shaken, and allowed to separate. The volume of the dithizone solution required to achieve this is a measure of the mercury present.

METHOD D (WALL AND RHODES, 1966)

Since this method was finally adopted, it is described in detail incorporating modifications recommended by the present authors.

Reagents

- 1 Potassium permanganate
- 2 Sulphuric acid 50% (v/v)
- 3 Hydroxylamine hydrochloride 50% (w/v)
- 4 Carbon tetrachloride
- 5 Sodium sulphate (anhydrous)
- 6 Mercuric chloride (desiccated)
- 7 Diphenylthiocarbazone (Dithizone¹) dissolve about 1 mg in approximately 10 ml chloroform and add carbon tetrachloride (approximately 250 ml) to give an absorbance at 485 nm of 0.20 to 0.25 when read against CCl₄ in a 1-cm light path cuvette. Prepare freshly each day. Stopper and keep in the dark when not in use.

Technique

To a 250 ml Erlenmeyer flask add successively 50 ml urine, 3 g KMnO₄ (rough balance), and 10 ml of 50% H₂SO₄. Stand for five to 10 minutes to allow frothing to settle, cover the flask with a watch glass, and simmer on a hot plate set to give a temperature of the flask contents of 50°C ± 2°C.

After 30 minutes carefully add hydroxylamine, 0.5 ml at a time, until decolorization is complete. When all solid matter is completely dissolved, add a further 0.5 ml hydroxylamine. After cooling, add 10 ml dithizone, stopper the flask, and shake vigorously for 30 seconds. Transfer to a separating funnel and run off the organic layer quantitatively to a centrifuge tube.

Unless the green colour of the dithizone is unaltered, add a further 10 ml to the aqueous phase, shake, and separate as before, repeating the process if necessary. One extraction should suffice where the mercury content of the flask is 5 µg or less, and three extractions for a content of 15 to 20 µg. For very high levels, the total volume after the addition of hydroxylamine may be measured and extraction with dithizone carried out on a small portion. To each discrete dithizone extract add anhydrous sodium sul-

¹Eastman Kodak catalogue no. 3092

phate to absorb any water present and centrifuge at $500 \times g$ for two minutes. Read the absorbance at 485 nm and 620 nm using CCl_4 as reference. When more than one extraction is required, it is convenient to pool the extracts from that sample, mix thoroughly, add sodium sulphate to an aliquot, and read after centrifugation.

A reagent blank in which water replaces urine, and two standards ($5 \mu\text{g}$ and $10 \mu\text{g}$) made up to 50 ml in water, are processed with each batch of tests. The difference in absorbance at 485 nm between blank and test is compared with the corresponding difference between blank and standard to give the amount of mercury in the 50 ml urine sample. When a pooled extract is measured, the difference in absorbance between test and blank is multiplied by the number of extractions to correct for dilution of the mercury dithizonate by CCl_4 . The absorbance at 620 nm serves to rule out oxidation of dithizone or the presence of other metals as outlined subsequently. Absorbance determinations at single wavelengths were made with the Uvispeck¹ spectrophotometer and absorption curves were drawn with the SP 800 recording spectrophotometer².

Results

METHOD A

When this method was applied according to the description of Varley (1963), the range of values obtained for daily mercury excretion in the urine of 13 unexposed laboratory personnel was 100–650 μg . The spectrum of the pure mercury standard bore little relationship to that of the urine extract (Fig. 1 presents a typical example of each) so that the absorbance of the latter

¹Hilger and Watts, London

²Unicam Instruments, Cambridge, England

Fig. 1 Absorption curves of $10 \mu\text{g}$ mercury standard (A) and a single urine containing $10 \mu\text{g}$ mercury per 50 ml (B) obtained by the method of Varley (1963) after shaking dithizone extract with $4 \times 10 \text{ ml } 5\%$ (v/v) ammonia.

at 480 nm is not a measure of the mercury present.

METHOD B

Duplicate determinations of urines containing 1–19 μg mercury per 100 ml differed by as much as 35%. Poorer results were obtained before we realized the decay in colour that took place in the dithizone extract before and after thio sulphate reversion, even when held in the dark. This decay averaged 1% per minute and was greatly accelerated by exposure to light. As it was desirable to centrifuge the organic phase before reading on each occasion, some decay was inevitable, and doubtless was the major factor contributing to the imprecision of the method.

METHOD C

We are in agreement with Nobel and Leifher (1961) regarding the sensitivity of this method, although we find the technique of extractive titration a tedious procedure in comparison with optical measurements. A more serious objection lies in the accuracy of the technique which depends upon the addition of small aliquots of dithizone until the blue-green colour is unaltered. It is not possible to distinguish adequately between the colour of the mercury dithizonate and that of oxidized dithizone, as described subsequently, so that without spectral analysis one cannot be certain that alteration of the blue-green dithizone is exclusively due to mercury.

METHOD D

This was the most rapid of the four methods and, with the modifications made, proved to be both accurate and precise. The following alterations did not affect the results obtained from urines to which a known amount of mercury was added: varying the amount of KMnO_4 from 3 to 6 g, the volume of H_2SO_4 from 10 to 20 ml, the time of oxidation from 30 to 60 minutes, and the volume of hydroxylamine added after complete solubilization of the permanganate from 0.5 to 3.0 ml. Carrying out the oxidation stage at 100°C using a reflux condenser gave results identical with those obtained at 50°C in one set of experiments; in another set, the results at 100°C were lower by a mean of 12%, possibly due to volatilization of the mercury.

The increase in absorbance at 485 nm bore a linear relationship to mercury added when read on single extracts (Fig. 2, line A) or on pooled extracts (Fig. 2, line D). Although we did not routinely use standards higher than $20 \mu\text{g}$ in 50 ml, much higher concentrations were measurable as in the urine of patients after mersalyl, and we can see no reason why a linear relationship should not hold indefinitely provided the dithizone extractions are continued until the

Fig. 2 Absorbance differences between reagent blank and 50 ml water containing various amounts of mercury. Line A plots the sum of the differences at 485 nm between the blank and individual dithizone extracts (two extractions at each point). Line D plots the difference at 485 nm between the blank and the pooled dithizone extracts. Line B plots the difference at 620 nm between the blank and the pooled dithizone extracts. Line C plots the sum of differences at 485 nm and 620 nm between the blank and the pooled dithizone extracts.

original colour is no longer altered, and the organic material does not exceed the oxidative capacity of the KMnO_4 and H_2SO_4 . In connexion with this last point, the addition of 0.2 to 1.0 ml of human plasma (6.9 g protein/100 ml) to urines containing mercury was without effect.

The absorption curves in Fig. 3 describe the changes occurring during formation of mercury dithizonate. The peak at 620 nm shown by dithizone is reduced by mercury with the simultaneous formation of a peak at 485 nm. The spectrum of the reagent blank was identical with that of dithizone, and the spectrum of mercury dithizonate formed from aqueous standard was identical with that formed in the presence of urine, except for the qualifications to be described. The recovery of mercury standard from 20 urines averaged 98.8% with a SD of $\pm 2.9\%$. When organic mercurial compounds were added to urines over the range 5–20 μg mercury per flask, the increase in E_{485} was proportional to the amount added, and the recovery was satisfactory. These compounds were mercury orange in dichloroethane; mercury dibenzyl in acetone; mercury chloranilate in acetone; mercury diphenyl in ethanol; and *p*-chloromercuribenzoate in dilute NaOH.

The reduction in absorbance at 620 nm was proportional to the amount of mercury present (Fig. 2, line B) and may be used as an index of mercury concentration. It is also possible to increase sensitivity by summing the absorbance differences between test or standard and blank at both 485 nm and 620 nm. This gives a line more than twice as high as that based on measure-

Fig. 3 Absorption curves of reagent blank (A), dithizone saturated with mercury (C), and urine containing 2 μg mercury per 50 ml (B) obtained by the method of Wall and Rhodes (1966).

ment at 485 nm alone (Fig. 2, line C). A number of problems, however, make the use of readings at 620 nm unsatisfactory for analytical purposes. Whereas the absorbance at 485 nm was stable for up to 150 minutes when solutions were held in stoppered tubes kept in the dark, the absorbance at 620 nm declined by about 15% over this period. In bright sunlight and in unstoppered tubes, the fall at 485 nm and 620 nm averaged 8% and 24% respectively over one hour, the decay being directly related to the intensity of sunlight and inversely related to the mercury concentration of the extract. The major source of instability is probably unaltered dithizone which has a value of 3 for the ratio $E_{620} : E_{485}$. Because at 620 nm a large value is subtracted from one a little larger, precision is poor. Twenty duplicate determinations over the range 1–20 μg mercury gave at 485 nm a mean percentage difference and SD of 3.9 ± 1.9 ; at 620 nm the corresponding values were 7.0 ± 2.8 . Precision was much poorer when the absorbance differences at 485 nm and 620 nm between test and blank were summed.

The decrease in absorbance at 620 nm was not specific to mercury whereas the increase at 485 nm was. Addition to urine or to urine laced with mercury of Fe^{++} , Cu^{++} , and Zn^{++} in quantities up to 1 mg, and of Cd^{++} and Pb^{++} in quantities up to 50 μg caused in some instances distortion of the spectrum of dithizone or of mercury dithizonate, usually associated with reduction in absorbance at 620 nm but never with alteration in absorbance at 485 nm.

Some urines caused a reduction in absorbance of dithizone at 485 nm and 620 nm. The recovery of 5 μg mercury from four such urines was 101%, 97%, 82%, and 65% based on absorbance measurements at 485 nm. This problem was never encountered in urines from laboratory controls but occurred not uncommonly in urines from inpatients. Where it was important for diagnostic reasons to exclude mercury poisoning, all drug therapy was stopped and within three days a satisfactory urine sample was obtained. The phenomenon appears to represent oxidation

of dithizone to diphenylthiocarbodiazone by inorganic ions in the urine or organic material surviving permanganate treatment (Barrett, 1956). Addition of excess KMnO_4 and H_2SO_4 in the first stage did not prevent dithizone oxidation; nor did substitution of the wet oxidation techniques of methods A-C. On the other hand the addition of one tablet of aspirin, codeine, paracetamol, and ampicillin to 50 ml normal urine decreased the absorbance of the dithizone extract at 620 nm without decreasing it at 485 nm. Such drugs were among the commonest taken by patients producing urines capable of oxidizing dithizone.

STUDIES ON HUMAN SUBJECTS

Twenty-four healthy laboratory staff, including those whose urines had previously been analysed by method A, had no detectable mercury in 50 ml of urine when analysed by method D. The limit of detection was regarded as $0.5 \mu\text{g}$ mercury, corresponding to an absorbance difference between test and blank of $+0.015$ at 485 nm and -0.015 at 620 nm. None of these urines caused oxidation of dithizone and each was part of a 24-hour collection. Future tests were carried out on an overnight sample, positive results being followed by analysis of a 24-hour specimen.

Urines of 50 inpatients not suspected of exposure to mercury gave negative results. In eight, the first sample caused oxidation of dithizone. Eighteen samples from patients in whom the question of mercury toxicity arose were analysed. One positive result was obtained. This was in an industrial worker aware of a ventilation fault in a room where he carried out processes involving use of a mercury still. He was completely symptom-free but desired a check-up. At two, four, five, and six days after termination of exposure, his daily excretion was 149, 64, 45, and $54 \mu\text{g}$ mercury. None of the remaining patients examined (mostly infants) had a history of exposure to mercury, and the analysis was requested towards the end of investigations when more likely possibilities had been excluded.

Urines from 103 industrial workers were examined. Fifteen engaged in work which occasionally involved the use of mercury; the remainder were divided almost equally into those working with metals other than mercury, and those who in the course of their work did not normally come into contact with metals. The laboratory was unaware of the status of the subjects before analysis. Mercury in a concentration of $7 \mu\text{g}/100 \text{ ml}$ was found in the urine of one symptomless subject in the group exposed to mercury. No other positive results were obtained, but eight of the urines from those exposed to metals other than mercury caused oxidation of dithizone. It was subsequently discovered that five of the eight had been taking aspirin, codeine, or paracetamol the night before the sample was taken; the other

three could not be traced at the time of follow-up.

One ml of mersalyl BP (39.7 mg mercury) was given by intramuscular injection to two volunteers. Urine from the first volunteer was collected 12-hourly for three days. During this time, 36.2 mg of mercury was excreted in the urine, 98.9% of this being passed in the first 12-hour sample. By the third day, the total 24-hour excretion was only $32 \mu\text{g}$. Urine from the second volunteer was collected six hourly for 48 hours. During this time 34.1 mg of mercury was excreted, 77.9% of this being passed in the first six-hour sample and 14.8% in the second. The last six-hour sample contained only $9.2 \mu\text{g}$ mercury.

Discussion

The method of Varley (1963) proved to be highly inaccurate and that of Tompsett and Smith (1959) imprecise. Thiosulphate reduces mercury recovery (Campbell and Head, 1955) and its use may contribute to the imprecision of the method of Tompsett and Smith (1959). We noted the instability of the extract in this method, and have learned that the sequence of extraction, first reading, reversion, and second reading should be completed without delay on each digestate individually (Tompsett, personal communication). This raises problems in batch analysis. The sensitivity of the method is less than that of the Wall and Rhodes technique when extracts are read individually: $20 \mu\text{g}$ mercury gave an absorbance difference in a 1-cm light path of 0.200 at 620 nm by the method of Tompsett and Smith (1959) and 0.565 at 485 nm by the method of Wall and Rhodes (1966).

The method of Nobel and Leifheit (1961) is tedious, and extractive titration may give misleading results due to alteration of dithizone by factors other than mercury. The method of Wall and Rhodes (1966) is simple and rapid and employs few reagents, thereby minimizing the risk of contamination and high reagent blanks. Two further problems in the estimation of mercury lie in the volatility of the metal and its compounds, and the necessity of ensuring the correct pH during extraction with dithizone. With regard to the first point, no mercury was lost when the duration of wet oxidation was twice the recommended period, and good recovery of mercury was obtained when inorganic and organic mercurials were added to urine. With regard to the second point, the pH of the solution before the addition of the dithizone always lay within the range -1 to 0.6 as recommended by Irving, Andrew, and Risdon (1949) and it was considered unnecessary to check this routinely. The modifications proposed, apart from more rigorous standardization of the technique, include

the addition of anhydrous sodium sulphate as used by Milton and Hoskins (1947) followed by centrifugation to remove traces of moisture from the organic phase; pooling of multiple extracts from positive samples instead of reading them individually, a procedure validated by Campbell and Head (1955); reading against CCl_4 instead of against dithizone, and at 620 nm as well as at 485 nm. These last modifications were essential in order to guard against changes in dithizone during a working day, although it was always freshly made and held in the dark, and to detect oxidation of dithizone by individual urine samples.

We have been unable to find an explanation for this phenomenon, or to confirm our suspicions that it may be related to drug metabolites, since administration of aspirin, codeine, paracetamol, and barbiturates to healthy controls did not lead to the production of such urines. Since chloride ions have been reported to decrease recovery of mercury (Kozelka, 1947; Campbell and Head, 1955) we substituted hydroxylamine sulphate for hydroxylamine chloride, without improvement; nor could we demonstrate a raised chloride content in such urines. Other wet oxidation techniques gave no better results. Miller and Swanberg (1957) have drawn attention to the similarity in colour between mercury dithizonate and diphenylthiocarbodiazone, the oxidation product of dithizone; they were unable to prevent formation of the latter in certain urines by the addition of a large number of reducing agents. Fats and fatty acids can survive most of the digestion techniques used in the preparation of organic materials for mercury analysis and are able to oxidize dithizone and the related compound di-beta-naphthylthiocarbazon (Cholak and Hubbard, 1946). It is thus apparent that previous workers have encountered problems due to oxidation of dithizone by urine and other biological materials, and that no solution has yet emerged. It is probable, moreover, that other workers have met the problem without recognizing it. This hazard is especially great with methods utilizing absorbance measurements at 620 nm, since reduction in absorbance due to oxidation could be mistakenly attributed to mercury. This error is unlikely when absorbance at 485 nm is also determined. Fortunately, the problem does not often arise, and satisfactory urines were obtained from these patients after stopping all drugs, although this is not proof that drugs were related to the phenomenon in the first instance.

Our work supports the validity of a random urine sample for the diagnosis of mercury poisoning (Nobel and Leifheit, 1961); this was usually the first sample passed on waking. We are also in agreement with previous workers who have been unable to detect mercury in the urine of normal human subjects (Kozelka, 1947; Monier-Williams, 1949; Monkman *et al*,

1956; Tompsett and Smith, 1959). The recovery of mercury injected in the form of mersalyl from the urine of two subjects exceeded 90% of the administered dose. This provides further validation of the method, since no allowance was made for excretion via the skin, salivary gland and intestine, or possible retention in bone. The finding that half the dose administered appears in the urine within a few hours and most of the remainder within 24 hours agrees with the reports of earlier workers (Burch, Ray, Threefoot, Kelly, and Svedberg, 1950; Grossman, Weston, Lehman, Halperin, Ullmann, and Leiter, 1951).

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References

- Barrett, F. R. (1956). Micro-determination of mercury in biological materials. *Analyst*, **81**, 294-298.
- Berman, E. (1967). Determination of cadmium, thallium and mercury in biological materials by atomic absorption. *Perkin-Elmer Atom. Absorp. Newsl.* **6**, 57-60.
- Buckell, M., Hunter, D., Milton, R., and Perry, K. M. A. (1946). Chronic mercury poisoning. *Brit. J. industr. Med.*, **3**, 55-63.
- Burch, G., Ray, T., Threefoot, S., Kelly, F. J., and Svedberg, A. (1950). The urinary excretion and biologic decay periods of radiomercury labelling a mercurial diuretic in normal and diseased man. *J. clin. Invest.*, **29**, 1131-1138.
- Campbell, E. E., and Head, B. M. (1955). The determination of mercury in urine—single extraction method. *Industr. Hyg. Quart.*, **16**, 275-279.
- Cholak, J., and Hubbard, D. M. (1946). Microdetermination of mercury in biological material. *Ind. Eng. Chem. Anal. Ed.*, **18**, 149-151.
- Clarkson, T. W., and Kench, J. E. (1956). Urinary excretion of amino acids by men absorbing heavy metals. *Biochem. J.*, **62**, 361-372.
- Gray, D. J. S. (1952). The determination of traces of mercury in urine by the reversion technique. *Analyst*, **77**, 436-438.
- Grossman, J., Weston, R. E., Lehman, R. A., Halperin, J. P., Ullmann, T. D., and Leiter, L. (1951). Urinary and fecal excretion of mercury in man following administration of mercurial diuretics. *J. clin. Invest.*, **30**, 1208-1227.
- Irving, H., Andrew, G., and Risdon, E. J. (1949). Studies with dithizone. Part I. The determination of traces of mercury. *J. chem. Soc.*, pp. 541-547.
- Kozelka, F. L. (1947). Determination of mercury in biological material. *Anal. Chem.*, **19**, 494-496.
- Lindstrom, O. (1959). Rapid microdetermination of mercury by spectrophotometric flame combustion. *Anal. Chem.*, **31**, 461-467.
- Maren, T. A. (1943). A simple and accurate method for the determination of mercury in biological material. *J. Lab. clin. Med.*, **28**, 1511-1514.
- Miller, V. L., and Swanberg, F. (1957). Determination of mercury in urine. *Anal. Chem.*, **29**, 391-393.
- Milton, R. F., and Hoskins, J. L. (1947). The estimation of traces of mercury in urine. *Analyst*, **72**, 6-10.
- Monier-Williams, G. W. (1949). *Trace Elements in Food*, p. 453. Chapman and Hall, London.
- Monkman, J. L., Maffett, P. A., and Doherty, T. F. (1965). The determination of mercury in air samples and biological materials. *Industr. Hyg. Quart.*, **17**, 418-420.
- Nobel, S., and Leifheit, H. C. (1961). In *Standard Methods of Clinical Chemistry*, edited by D. Seligson, vol. 3, p. 176. Academic Press, New York.
- Nobel, S., and Nobel, D. (1958). Determination of mercury in urine. *Clin. Chem.*, **4**, 150-158.

- Polley, D., and Miller, V. L. (1955). Rapid microprocedure for determination of mercury in biological and mineral materials. *Anal. Chem.*, **27**, 1162-1164.
- Rodger, W. J., and Smith, H. (1967). Mercury absorption by fingerprint officers using 'grey powder'. *J. forens. Sci. Soc.*, **7**, 86-89.
- Rolfe, A. C., Russell, F. R., and Wilkinson, N. T. (1955). The absorptiometric determination of mercury in urine. *Analyst*, **80**, 523-530.
- Stock, J., and Zimmerman, W. (1928). *Z. angew. Bäder-u. Klimahelk. Chem.*, **41**, 546, cited by Milton, R. F., and Hoskins, J. L. (1947). (See above).
- Tompsett, S. L., and Smith, D. C. (1959). Mercury in biological materials. *J. clin. Path.*, **12**, 219-221.
- Varley, H. (1963). *Practical Clinical Biochemistry*, 3rd edn, p. 642. Heinemann, London.
- Wall, H., and Rhodes, C. (1966). The effect of bacterial contamination and ageing on the volatility of mercury in urine specimens. *Clin. Chem.*, **12**, 837-843.
- Warkany, J., and Hubbard, D. M. (1951). Adverse mercurial reactions in the form of acrodynia and related conditions. *Amer. J. Dis. Child.*, **81**, 335-373.
- Willis, J. B. (1962). Determination of lead and other heavy metals in urine by atomic absorption spectroscopy. *Anal. Chem.*, **34**, 614-617.

The February 1970 Issue

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